Calcium Phosphate and Bioglass Reinforced PLA Thin Film Biocomposites for Slow Drug Delivery Applications

Innocent Jacob Macha

B.Sc., (UDSM) M.Sc (KTH)

Submitted in total fulfilment of the requirements of the degree of Doctor of Philosophy

February 2016

Faculty of Science,

University of Technology Sydney (UTS)

CERTIFICATE OF ORIGINAL AUTHORSHIP

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

_____ _____

Innocent Jacob Macha Date

Acknowledgement

First and foremost, I would like to thank God whose everlasting loving kindness and many blessings have transformed my life and made me who I am today. Only my name appears on the cover of this document, however, there are several great people with whom I am indebted for their valuable contribution in the research, study and writing of this thesis.

I am forever grateful to my supervisors, Professor Besim Ben-Nissan and Professor Bruce Milthorpe. They were the pillars of my research and among the smartest people I have ever known. As busy as dean of faculty could be, Prof. Milthorpe was always there when I needed him. Both supervisors provided insightful discussion about the research gave the guidelines and have given me the freedom to pursue various projects without objection. Prof. Ben-Nissan was more than a library to me. I gained immensely, both knowledge and wisdom from his mentorship. His encouragements, hospitality, scientific advice, knowledge and many insightful discussions and suggestions are totally priceless. I don't know if I could be him in the story!

The members of the Advanced Tissue Regeneration and Drug Delivery Group have also contributed immensely to my personal and professional time at UTS. I would also like to thank A/Professor Sophie Cazalbou and A/Professor David Grossin of the University of Toulouse, France, A/Professor Cynthia Witchurch and Dr Rosy Cavaliere of iThree Institute, UTS and Prof. R. J. Chung of the National Taipei University of Technology, Taiwan for their collaborative efforts. Their assistance, insightful comments and constructive criticisms were thought provoking and they helped me focus my ideas.

I would like to thank these individuals: Ariela Ben-Nissan - your motherly love and kindness made my stay in Australia enjoyable and memorable. Gai Parkinson - you and your family treated my family as yours, you were like a mother to me, your love is something that I cannot forget and I will always treasure that.

I would also like to thank all of my friends who supported me in writing, and gave me the incentive to strive towards my goal. My sincere gratitude to Glyn and Donna Jones for editing my thesis and correcting my broken English, your love and kindness is priceless, thank you.

Lastly, I am grateful to my wife Sayuni Mbwilo and son Joshua Macha. There are no words to convey how much I love you. Sayuni has been a true and great supporter and has unconditionally loved me throughout my academic trajectory despite the isolation that came as a result of me pursuing my goals. She has faith in me and my intellect even when I felt like digging a hole and crawling into it because I didn't have faith in myself. Your prayers for me are what has sustained me thus far.

Preface

The work presented in this PhD thesis was discussed and/or carried out after consultation with my supervisors, Professor Bruce Milthorpe and Professor Besim Ben-Nissan (School of Chemistry and Forensic Science, University of Technology Sydney).

Abstract

The rise in the number of musculoskeletal disorders (MSDs) due to the increase in aging population and advancement in medical technology has led to an increasing demand for medication to prevent and treat these diseases. The development of new drugs or formulations to allow treatment of these diseases in their very early stages is only increasing. Local direct and multidelivery of medication and key minerals to support bone repair and regeneration at the defect site, from flexible degradable devices at the rate within the therapeutic window, seems to be an effective strategy. However current drug delivery vehicles are neither flexible and degradable, nor able to deliver both medication and minerals effectively. Using a simple "solution casting" method, preparation of medical devices with such potential for slow drug delivery for biomedical applications served as the research objective.

Polylactic acid (PLA) and hydroxyapatite-hydrothermally converted coral were used to develop PLA thin film composites as drug delivery systems. PLA provided flexibility and biodegradability of the systems, while coralline hydroxyapatite provided a unique architecture with its porous and bioactive nature, which is suitable for drug loading and slow drug release. Two drugs, gentamicin (antibiotic) and bisphosphonate were loaded into the device and their release profiles and activities were studied for the treatment of medical-implant related infection and osteoposis respectively. The biocompatibility study on human adipose derived stem cells (hADSC) and biofilm formation behaviour of both gram-negative (*Pseudomonas aeruginosa*) and gram-positive bacterial (*Staphylococcus aureus*) were studied on PLA thin film composites loaded with gentamicin. The mechanical properties of PLA-surface treated bioglass for tissue engineering applications was also studied. An alternative conversion method of coralline materials and other natural materials such as sea mussel and ostrich eggshells to calcium phosphate materials were also evaluated. Although nanosurface bioglass treated with 1% (3-Aminopropyl)triethoxysilane (APTES) suggested effective improvement in elongation at the break of PLA/bioglass composites, they lacked the required drug release efficiency.

However, the PLA thin film composites displayed ability for potential applications in biomedical field as drug delivery systems. The flexibility they provide allows them to conform to any desired clinical shape and size. Incorporation of hydroxyapatite in the matrix, has the added advantages of controlled release, improved encapsulation efficiency, increased drug stability and maintenance of bioactivity and continuous supply of calcium Ca^{2+} and phosphate PO_4^{2-} ions, which can assist in bone regeneration and repair. Gentamicin release profiles, exhibited a steady state release rate, with significant antimicrobial activity even at high concentrations of bacteria. The systems also showed the potential for prolonged release of both antibiotic and bisphosphonate. The loading of the drug onto HAp particles induces a significant decrease of the release rate and period, for both gentamicin and bisphosphonate permitting the therapeutic efficacy of composite biomaterial locally to be extended. hADSC showed attachment and proliferation on PLA thin film-HAp composites signifying the increase in osteointegration due to the presence of HAp.

Mechano-chemical conversion methods proved to be an effective alternative to the hydrothermal technique for coral conversion to calcium phosphate materials at moderate temperature conditions. The modified composites may have a wide range of biomedical applications in tissue engineering with improved elastic properties.

Table of Contents

List of Figures and Tables

List of Publications

Peer reviewed Journals

1. Macha, I.J., Cazalbou, S., Shimmon, R., Ben-Nissan, B. & Milthorpe, B. 2015, 'Development and dissolution studies of bisphosphonate (clodronate)-containing hydroxyapatite–polylactic acid biocomposites for slow drug delivery', *Journal of Tissue Engineering and Regenerative Medicine*,. DOI: 10.1002/term.2066, (IF: 5.199)
2. Macha, I.J., Ozyegin, L.S., Oktar, F.N. & Ben-Nissan, B. 2015, 'Conversion of Ostrich Eggshells (Struthio camelus) to Calcium Phosphates', *Journal of The Australian Ceramic Society*, vol. 51, no. 1, p. 9. (IF: 0.658)
3. Macha, I.J., Cazalbou, S., Ben-Nissan, B., Harvey, K.L. & Milthorpe, B. 2015, 'Marine structure derived calcium phosphate-polymer biocomposites for local antibiotic delivery', *Mar Drugs*, vol. 13, no. 1, pp. 666-80. (IF: 2.853)
4. Macha, I.J., Boonyang, U., Cazalbou, S., Ben-Nissan, B., Charvillat, C., Oktar, F.N. & Grossin, D. 2015, 'Comparative study of Coral Conversion, Part 2: Microstructural evolution of calcium phosphate', *Journal of the Australian Ceramic Society* vol. 51, no. 2, pp. 149-59. (IF: 0.658)
5. Macha, I.J., Ben-Nissan, B. & Milthorpe, B. 2014, 'Improvement of Elongation in Nanosurface Modified Bioglass/PLA Thin Film Composites', *Current Nanoscience*, vol. 10, no. 2, pp. 200-4. (IF: 1.096)
6. Cegla, R.-N.R., Macha, I.J., Ben-Nissan, B., Grossin, D., Heness, G. & Chung, R.-J. 2014, 'Comparative Study of Conversion of Coral with Ammonium Dihydrogen Phosphate and Orthophosphoric Acid to Produce Calcium Phosphates', *Journal of the Australian Ceramics Society*, vol. 50, no. 2, pp. 154-61. (IF: 0.658)
7. Macha, I.J., Ozyegin, L.S., Chou, J., Samur, R., Oktar, F.N. & Ben-Nissan, B. 2013, 'An Alternative Synthesis Method for Di Calcium Phosphate (Monetite) Powders from Mediterranean Mussel (Mytilus galloprovincialis) Shells', *Journal of The Australian Ceramic Society*, vol. 49, no. 2, pp. 122-8. (IF: 0.658)
8. Macha, I.J., Charvillat, C., Cazalbou, S., Grossin, D., Boonyang, U., & Ben-Nissan, B. 2016, 'Comparative study of Coral Conversion, Part 3:

Intermediate products in the first half an hour', *Journal of the Australian Ceramic Society* vol. 52, no. 21 pp. 177-82. (IF: 0.658)

Book Chapter

9. Choi, A., Ben-Nissan, B., Conway, R. & Macha, I. 2014, 'Advances in Calcium Phosphate Nanocoatings and Nanocomposites', in B. Ben-Nissan (ed.), *Advances in Calcium Phosphate Biomaterials*, vol. 2, Springer Berlin Heidelberg, pp. 485-509.
10. Ben-Nissan, B., Choi, A., Macha, I.J. and Cazalbou, S. 2015, 'Sol-gel Nanocoatings of Bioceramics', in I.V. Antoniac (ed.), *Handbook of Bioceramics and Biocomposites,* Springer Berlin Heidelberg. Ch. 33 (DOI 10.1007/978-3-319-09230-0_33-1)

Articles in Print

11. Macha, I.J., Ben-Bissan, B., Cazalbou, S., Santos, J., Milthorpe, B. 2015, 'Hydroxyapatite/PLA biocomposite thin films for slow drug delivery of antibiotics for the treatment of bone and implant-related infections', Key Engineering Materials (in print).
12. Macha, I.J., Grossin, D., Ben-Nissan, B. 2015, 'Conversion of marine structures to calcium phosphate materials: Mechanisms of conversion using two different phosphate solutions.' Key Engineering Materials (in print)
13. Ben-Nissan, B., Macha, I.J., Cazalbou, S., Choi, A. 2015, 'Calcium Phosphate Nanocoatings and Nanocomposites, Part II: Thin Films For Slow Drug Delivery and Osteomyelitis, *Nanomedicine* (in print).

GENERAL INTRODUCTION

Adverse events associated with medical implant-tissue infections and clinical conventional therapies have been reported and are a public health concern and economic burden for many countries. Significant efforts have been focused to either discover new drugs or improve the clinical outcomes of current drugs and practices by using new formulations (Gao et al. 2011). Currently, clinical therapies are based on intermittent oral or intravenous administration of the drug, which provide a high level of drug in the blood immediately after the dose is administered. However, the drug level in the bloodstream quickly decreases below the therapeutic window. Drug delivery technology presents an interesting interdisciplinary field aimed to address challenges for pharmaceutical, chemical engineering, biomaterials and medical communities (Rao 2002). The key issue that has been explored widely in recent times in regard to these treatments is the ability to direct drugs to specific organs and/or affected sites. Extensive studies have been conducted attempting to develop the ideal drug carrier systems; and many factors affecting the properties of drug delivery systems, including porosity, stiffness, strength and material itself have also been addressed. Clinical applications have demonstrated the advantages of an extended drug release system to treat bone-related disease such as osteoporosis or implant-related infections.

Generally, a biomaterial that will act as a drug carrier must have the ability to incorporate a drug, to retain it in a specific site, and to deliver it progressively over time to the surrounding tissues. Furthermore, it would be advantageous if the material is injectable, or alternatively coatable, on an implant and most importantly biodegradable to give the extended release (Choi, Cazalbou & Ben-Nissan 2015). Implantable medical devices must be fine-tuned to the biological environment they are in. A number of procedures have been established using in-vitro laboratory settings to avoid the risks of danger for patients and to also avoid unnecessary animal experiments. After implantation, the implant must elicit a negligible immune reaction in order to prevent it causing such a severe inflammatory response that it might reduce healing, or cause rejection by the body.

Biodegradable polymer films loaded with gentamicin have been developed to serve as "coatings" for fracture fixation devices to prevent implant-associated infections (Aviv, Berdicevsky & Zilberman 2007). The use of biodegradable polymer films is advantageous due to their propensity to uptake and release clinical active substances, as a consequence of their degradability. A combination of two or more materials in this respect allows the optimization of required properties in the materials, interactions with their biological environment and controlled drug delivery systems. Biodegradable polymer-bioceramic composites would be ideal in this endeavour because of the bioactive nature of ceramic materials, which promote tissue growth. Incorporation of bioceramics in these films will improve not only controlled drug release but also bioactivity and tissue regeneration, especially in orthopaedic and maxillofacial applications. Talal et al. (Talal et al. 2009) suggest that HA-PLA-PLA fibre composite membranes display superior protein absorption kinetics and sustained release of protein compared with PLA-PLA fibre membranes due to the presence of HAp. They also suggest that these composite membranes could be useful therapeutically as a delivery system for bioactive proteins.

It has been demonstrated by Wang and his co-workers (Wang et al. 2004) that PLA microcapsules could release more than 80% of loaded gentamicin sulphate within 3 weeks for the treatment of osteomyelitis. Drug release time and the shape of the release systems would probably limit their wide application especially for prolonged release. Bioerodable, polyanhydride-gentamicin beads were used *in vivo* study for the treatment of Osteomyelitis (Guo et al. 2007). They reported to have reduced osteomyelitis by 93% after 4 weeks of implantation for 20% gentamicin loaded beads. However most of the past studies have shown that antibiotics have been ototoxic and nephrotoxic at high dosages (Shields, Martello & Potoski 2009). Optimum properties of drug delivery systems could be achieved by varying the combination of materials and their properties and experts are making progress toward pinpointing the ideal combination of factors for localized and sustained drug release applications.

Main Objective

The clinical demand for sustained release devices with multifunctional advantages is just increasing. The research efforts were focused on the development of bioactive drug delivery PLA calcium phosphate thin film composites for biomedical applications.

Specific objectives

i. To establish the effects of drying methods to mechanical properties of PLA thin film
ii. To improve the interfacial properties of bioactive ceramics and PLA in PLA thin film composites
iii. To assess the drug release profiles from drug loaded PLA ceramic thin film composites
iv. To assess the biocompatibility in vitro and biofilm behavior on the surfaces of PLA thin film composites

Significance of study

i. To give scientific explanations on the materials' combination factors for achieving improved mechanical properties.
ii. Contribute in the development of thin film medical devices for tailored slow drug delivery

Hypothesis

It was hypothesized that surface modified PLA/ calcium phosphate flexible thin film biocomposites could be easily produced and applied as a reliable slow drug delivery system.

Thesis layout

This thesis consists of eight (8) **Chapters,** written based on the facts available in literature, but mostly based on the findings from this research; of which some are published and some are under consideration to be published. They are stand-alone complete chapters with their layout similar to published scientific manuscripts,

aimed to address the clinical need for flexible thin film composites as drug delivery systems. Chapter one gives a state-of-the-art background on biomaterials and their applications in the medical field. Chapter two to chapter seven present the experimental findings of this research from the productions and characterizations of PLA thin film composites, improvement of interfacial properties of PLA and ceramics to drug loading and dissolution, and bacterial biofilm behaviour on the surfaces. Chapter eight contains the general concluding remarks of the study and suggests a way forward.

References

Aviv, M., Berdicevsky, I. & Zilberman, M. 2007, 'Gentamicin-loaded bioresorbable films for prevention of bacterial infections associated with orthopedic implants', *J Biomed Mater Res A*, vol. 83, no. 1, pp. 10-9.

Gao, P., Nie, X., Zou, M., Shi, Y. & Cheng, G. 2011, 'Recent advances in materials for extended-release antibiotic delivery system', *J Antibiot (Tokyo)*, vol. 64, no. 9, pp. 625-34.

Guo, W., Shi, Z., Guo, R. & Sun, R. 2007, '[Preparation of gentamicin sulfate-polyanhydride sustained-release beads and in vitro bacteriostatic activity studies]', *Sheng Wu Yi Xue Gong Cheng Xue Za Zhi*, vol. 24, no. 2, pp. 360-2, 84.

Rao, M.S.a.K.P. 2002, 'Preparation, characterization and in vitro release of gentamicin from coralline hydroxyapatite-gelatin composite microspheres', *Biomaterials*, vol. 23, no. 15, pp. 3175-81.

Talal, A., Waheed, N., Al-Masri, M., McKay, I.J., Tanner, K.E. & Hughes, F.J. 2009, 'Absorption and release of protein from hydroxyapatite-polylactic acid (HA-PLA) membranes', *J Dent*, vol. 37, no. 11, pp. 820-6.

Wang, G., Liu, S.-J., Ueng, S.W.-N. & Chan, E.-C. 2004, 'The release of cefazolin and gentamicin from biodegradable PLA/PGA beads', *International Journal of Pharmaceutics*, vol. 273, no. 1–2, pp. 203-12.

CHAPTER 1

LITERATURE BACKGROUND

CHAPTER 1: LITERATURE BACKGROUND

1.1 Biomaterials

1.1.1 Historical background

Biomaterials have made significant improvements in recent years to the lives of most individuals due to the impact on their recovery and long-term well being. History shows that synthetic implants were used thousands of years ago with varying degrees of success. Egyptians, Romans, Chinese, and Aztec's used gold in dentistry more than 2000 years ago. The earliest known example of dental implant tooth replacements dating back to 600 A.D. was discovered in Mayan civilization. Possibly the first systematic research on biomaterials started in 1829 by H.S. Levert who studied canine responses to implanted metals *in-vivo* in the living body. The introduction of aseptic surgical techniques by British surgeon Joseph Lester improved the application of biomaterials research in medicine. The increase in biomaterials usage was noted in 1886 when German surgeon Hansmann who used metal plates for internal fixation for the first time (RM, AR & A. 2011). Since then, many new materials and orthopaedic techniques have been developed, especially since WWII.

Mostly, metals were used because only a few plastics existed. Polyethylene (PE), Poly(methyl methacrylate) (PMMA) and Nylon began to be used safely within human tissues after extensive animal experimentation and clinical trials. PMMA has been also used as cement to anchor metallic prostheses to bone, and to make permanent orthopedic implants. Ingraham and his colleagues published for the first time in 1947 on polyethylene as a relative new synthetic plastic which can be made into flexible tubes and thin or pliable sheets for use in surgery (Ingraham, Alexander & Matson 1947).

Currently, the demand for biomaterials in both tissue engineering and medical devices is growing rapidly, due to the increase in ageing populations, and the demand for a new type of biomaterials that could last longer once implanted. These have necessitated the increase in research activities to address most of the

current challenges facing these materials, in-depth understanding of the physical and chemical functioning and the human tissue response to these biomaterials.

During the last 40 years, developments in the field of biomaterials have successfully introduced more than 500 different medical materials, ceramic, metal or polymeric materials, either singly or in a specially designed combinations to replace, repair or augment more than 40 different parts of the body (Hench & Thompson 2010). Tens of millions of individuals have had their quality of life enhanced for up to 25 years or more by the use of implants made from biomaterials.

When a biomaterial is nearly inert and the interface is not chemically or biologically bonded, there is relative movement and the possibility of progressive development of a non-adherent fibrous capsule in both soft and hard tissues, which eventually leads to deterioration in function of the implant or the tissue at the interface or both (Hench 1991). Therefore, the field of biomaterials began to shift in emphasis from achieving a bio-inert tissue response exclusively, to instead producing bioactive components that could elicit a controlled action and reaction in the physiological environment (Hench & Wilson 1984).

Both first and second-generation biomaterials have been improved over the years. However, they are partly limited in application because they cannot respond to changing physiological load or biochemical stimuli like living tissues. This limits the lifetime of artificial body implants and paved the way to consider an alternative, which is more biologically based method for the repair and regeneration of tissues (Hench 1980).

Whereas second generation biomaterials were designed to be either resorbable or bioactive, currently the third generation of biomaterials is combining these two properties, with the aim of developing materials that once implanted, will help the body heals itself (Hench & Polak 2002; Hench et al. 2003). Several studies have shown that the combination of the concepts of bioactive materials and resorbable materials has brought a breakthrough in biomedical fields such as tissue

engineering, orthopaedics, gene control and drug delivery (Zhou et al. 2007; Brown & Farrar 2008; Vedantham et al. 2012) in which bioactive materials are being made resorbable.

In this venture, biomaterials are being designed to stimulate specific cellular responses at the molecular level. Moreover, molecular modifications of resorbable materials elicit specific interactions with cell integrins and thereby direct cell proliferation, differentiation, and extracellular matrix production and organization. In their recent review, Hench and Thompson (Hench & Thompson 2010) indicated that a significant body of work has been directed towards improving third-generation biomaterials; but still there are challenges to be overcome in order to design a new generation of gene-activating biomaterials tailored for specific patients and disease states.

1.1.2 Classifications of biomaterials

Biomaterials are generally defined by their applications and not by their chemical make-up. The American National Institute of Health defines biomaterials as "any substance or combination of substances other than drugs, synthetic or natural in origin, which can be used in any period of time, which augments or replaces partially or totally any tissue, organ or function of the body, in order to maintain or improve the quality of life of the individual". Based on the reaction of biological systems to biomaterials when implanted, they are grouped into three classes; bio-inert, bioactive and bio-resorbable.

Bio-inert materials form a weak bond at the biomaterial-bone interface because they stimulate formation of fibrous tissue and therefore do not directly bond to bone. They include metals (e.g., titanium or titanium alloys, stainless steel, cobalt-chromium, alloys), some synthetic polymers (e.g., PEEK, Teflon-type), and some ceramics (e.g., alumina, zirconia, carbon).

Bioactive materials stimulate bone tissue formation and therefore directly bond with bone and thus form a uniquely strong biomaterial-bone interface. Hench and his colleagues initiated the bioactive glass material in the early 1970s. They

discovered bioglass systems contain Na_2O, CaO, SiO_2 and P_2O_5 which formed a bond with bone so strong that it could not be removed without breaking the bone (Hench et al. 1971). Since the invention, the composition of bioglass has been varied and many different new bioactive biomaterials and products have been developed. Bioactive materials include metals, polymers, ceramics, glasses and composites; which include bioactive glasses (silica and non-silica based), natural polymers, calcium phosphates (synthetic or derived from biological origin materials such as corals, marine shells, bovine bone), bioactive composites and coatings which bond to living tissues (LeGeros 2008a).

Bioresorbables, they are designed to degrade/dissolve gradually over a period of time and be replaced by tissues. They represent an optimal solution to biomaterials failures, if short-term performance and strength requirements are met. The challenges facing this group are the maintenance of strength during resorption and matching resorption rates to cellular metabolism. Although their usage is increasing, these restrictions impose considerable limitations on the design of resorbable biomaterials, which is the reason why very few are being used clinically.

1.1.3 Metallic Biomaterials

Metallic medical implants continue to be used extensively due to their excellent mechanical properties. The outstanding mechanical properties of these materials provide them with reliability for long-term implant performance in major load-bearing applications such as artificial hip and knee joints, bone plates, and dental implants. Apart from mechanical properties, metallic biomaterials have to display adequate corrosion and wear resistance and biocompatibility. A few metals and their alloys like stainless steels, Co-Cr alloys, and Ti and its alloys are mainly used in the fabrication of metallic biomaterials because they have the required properties. The American Society of Testing and Materials (ASTM) recommended type 316L stainless steel (18Cr-14Ni-2.5Mo) which is a vacuum-melted low-carbon variant of the standard type 316 composition for implant fabrications. 316L is designed to maximize the pitting corrosion resistance and provide a ferrite-free microstructure (Davis 2003). The Cr has affinity to oxygen and it forms a passive

layer of chromium oxide on the surface of steel which prevents steel from corrosion (Navarro et al. 2008).

Corrosion of metallic biomaterials is considered to pose detrimental effects on biocompatibility during in vivo use. A number of studies show that 316L implants often degrade due to pitting, crevice, corrosion fatigue, fretting corrosion, stress corrosion cracking, and galvanic corrosion in the body (Singh & Dahotre 2007; Hryniewicz, Rokicki & Rokosz 2008; Xu et al. 2008). On the other hand it has been shown that 316L stainless steel releases nickel ions into the human body which causes a danger of allergic reaction which appears in a significant number of patients (Yamamoto, Honma & Sumita 1998; Yamamoto, Kohyama & Hanawa 2002; Navarro et al. 2008; Talha, Behera & Sinha 2013). This has promoted the development of Ni-free nitrogen containing austenitic stainless steels. Nitrogen not only replaces nickel for austenitic structure stability but also much improves steel's properties. It has been shown that nickel-free high nitrogen austenitic stainless steel has high corrosion resistance in vivo (Kuroda et al. 2002). Although these improvements are effective in orthopaedics, for total hip and knee implants, 316L is not being used.

Cobalt-Chromium alloys are used for making surgical implants and are recommended as stems of prostheses for heavily loaded joints such as the knee and hip. These materials are highly resistant to corrosion even in a chloride environment and have excellent mechanical properties. Although still controversial, studies show that corrosion products of Co-Cr-Mo are more toxic than those of stainless steel 316L (Vidal & Muñoz 2009).

Titanium alloys have become the most attractive metallic materials for biomedical applications due to their lightness, good mechano-chemical properties and non-allergic reaction. Commercially pure titanium (Ti) and Ti-6Al-4V are the two most common titanium base implant biomaterials. However, for permanent implant applications the alloy shows a toxic effect resulting from the release of vanadium and aluminium (Yang et al. 2007). Research attention has been focused on

developing a vanadium and aluminum-free alloys for application as a medical implants (Li et al. 2014).

Nickel Titanium alloy known as Nitinol displays a shape-memory effect as well as good corrosion resistance and has been used in orthopedic, dental and cardiovascular applications. There has been concern in the medical industry about the release of Ni, which has an allergic effect. However, it has been reported that when properly treated, Nitinol forms a very stable protective layer of TiO_2 which reduces the release of Ni to the minimum (Ha & Gardella 2005). It has been shown that fully covered Nitinol vascular, self-expandable metallic stents, show no evidence of corrosion or nickel release in patients (Elbert & Hubbell 1996).

1.1.4 Polymeric Biomaterials

Polymeric biomaterials form one of the most important material groups in biomedical engineering. They also have a wide range of application in drug delivery and wound healing (Wiegand & Hipler 2010; Agrawal et al. 2014). Synthetic polymers like α-hydroxy acids, which include poly (glycolic acid), poly (lactic acid) and their co-polymers, polyanhydrides and naturally occurring polymers like chitosan and hyaluronan have been extensively used in medical devices and the pharmaceutical industry.

The combination effect of polymeric biomaterials, cells and bioactive molecules has become the main focus of scientists and industry in the cause of regenerating damaged tissues (Sokolsky-Papkov et al. 2007). Based on their structure, most polymeric biomaterials display weakness when utilized in load-bearing medical devices. Ultra-high molecular weight polyethylene (UHMWPE) is one of the polymeric materials, which has the highest impact strength and is one of the best available orthopaedic polymeric materials. Under biomechanical functional loads such as knee or hip implants they can survive around 15 years before deterioration (Ben-Nissan & Pezzotti 2002).

The propensity of some of the polymeric biomaterials to uptake and release active substances as the consequence of their degradation addresses the significant

healthcare costs involved and the deaths associated with many clinical complications. Degradable polymeric biomaterials have been used in this endeavor. Many attempts have been successfully made to incorporate a drug into implantable polymeric devices for a sustainable and controlled release.

Interfacial interaction between any biomaterials and biological systems are crucial; and for polymeric materials a number of studies has been conducted on the surface characterization (Elbert & Hubbell 1996; Tirrell, Kokkoli & Biesalski 2002; Ha & Gardella 2005) and the cellular response in vitro and in vivo experiments (Tirrell, Kokkoli & Biesalski 2002). Apart from improving biocompatibility, surface modifications are necessary to improve materials' degradability, which controls drug release kinetics, or to improve wettability and adhesion.

Hydrogels are constructed of a network of hydrophilic cross-linked polymer chains capable of retaining large amounts of water and maintaining their three-dimensional structure (Vermonden, Censi & Hennink 2012). They have been studied and used for a wide range of biomedical applications from tissue engineering to diagnostic and drug delivery systems (Nam, Watanabe & Ishihara 2004). Being biocompatible, hydrogels can also be made biodegradable by choosing the building blocks and cross-link agents properly. Their soft nature when swollen is similar to natural extracellular matrices, which render them extremely suitable for delivery of active substances like proteins and peptides, where preparation methods are detrimental (Vermonden, Censi & Hennink 2012). Hydrogels and other medical systems have been administered by surgical intervention, which is costly, and a terrible ordeal for patients. Efforts have been focused on developing systems like injectable hydrogels that can be administered by minimal invasive methods. The United States Food and Drug Administration (FDA) has recently approved a less invasive medical device for the treatment of benign prostatic hyperplasia (BPH) (FDA 2013).

1.1.5 Ceramic Biomaterials

Ceramic biomaterials or bioceramics are the class of ceramics used in the biomedical field to repair and reconstruct the deceased and damaged tissue of

musculo-skeletal systems (Hench 1991). Alumina (Al_2O_3) and Zirconia (ZrO_2) are termed bioinert, bioglass and glass ceramic are bioactive, while calcium phosphate ceramics (CPC) are categorized as bioactive and bio-resorbable.

High density alumina (α-Al_2O_3) is high strength alumina with an excellent corrosion resistance, good biocompatibility and high wear resistance. It was the first ceramic biomaterial to be widely used clinically (Lemons 1996). Related to alumina is zirconia (ZrO_2), or more accurately partially stabilized zirconia (PSZ), which has a high mechanical strength and fracture toughness. Both are currently used in orthopaedic and maxillofacial surgical implants as alumina and zirconia toughened alumina forms.

Calcium phosphate ceramics (CPC) are used for augmenting and replacing bone tissue. The most widely used are hydroxyapatite (HAp) and β-tricalcium phosphate (β-TCP). Different calcium phosphate ceramics with their chemical formulae are presented in Table 1. Most CPCs are prepared by either the wet chemical method or a solid-state reaction. Due to an increase in medical expenses, efforts have been directed towards reducing the cost of bioceramic devices by producing high-purity bioceramic materials from low cost raw materials. A number of attempts have been successful using biological origin materials such as enamel (Oktar et al. 2010), sea shells (Macha et al. 2013), coral (Ben-Nissan 2003a; Chou et al. 2007), and land snail (Kel et al. 2012) to mention a few to synthesize calcium phosphate ceramic materials because of their chemical composition and unique structure.

Though bioceramics are widely used as implants in orthopaedics, maxillofacial surgery and for dental implants, more developments are in progress for extending their applications and achieving improvements in their performance and reliability. Metal implants like titanium and its alloys have a long-term problem of loosening after being implanted, due to a lack of sufficient bioactivity on the surface over time (Cook et al. 1988; Suchanek & Yoshimura 1998). Ben-Nissan (Chai et al. 1995; Ben-Nissan & Choi 2006) developed sol-gel crystalline nanocoatings of hydroxyapatite on different substrates of medical implants. Using hydroxyapatite, which is chemically similar to the mineral component of natural

bone as a coating, has the added advantage that bioinert implant materials like titanium and cobalt chromium alloys and alumina can be given bioactive coatings with an improvement of their osteointegration.

Table 1: Existing calcium orthophosphates (Macha et al. 2013)

Mineral name		**Short name**	**Empirical formulas**	**Ca/P**
Dicalcium phosphate dehydrate	Brushite	DCPD	$CaHPO_4.2H_2O$	1.00
Dicalcium phosphate	Monetite	DCPA	$CaHPO_4$	1.00
Octacalcium phosphate		OCP	$Ca_8H_2(PO_4)_6.5H_2O$	1.33
β-Tricalcium phosphate	Whitlockite	β-TCP	$\beta\text{-}Ca_3(PO_4)_2$	1.50
Hydroxyapatite		HAp	$Ca_{10}(PO_4)_6(OH)_2$	1.67
Tetracalcium phosphate monoxide		TTCPM	$Ca_4(PO_4)_2O$	2.0
Defect apatite		DA	$Ca_{10-x}(HPO_4)_x(PO_4)_{6-x}(OH)_{2-x}$ $0 < x < 2$	(10-x):6

Other coating techniques like chemical vapour deposition, plasma spray, electrophoretic and electrochemical deposition have also been used. The plasma spraying technique is the most commonly used and the only one approved by The U.S Food and Drug Administration (FDA) for calcium phosphate coatings to implant surfaces (Campbell 2003). Plasma techniques involve the use of high temperature which may have deleterious effects such as evaporation, phase alteration, residual stress, de-bonding, and gas release, which commonly occur in these coatings (Choudhuri, Mohanty & Karthikeyan 2009). Research efforts are currently focused on developing an optimal technique for ceramic coating application by modifying the chemical composition, or the plasma spraying techniques.

1.2 Bioglass

For more than four decades, bioglasses have attracted the attention of many researchers because of their unique properties which can easily be tailored by manipulating their composition. The pioneering work of Hench (Hench 2006) led to the first development of a bioactive silicate glass, called 45S5, of composition: 45

wt% SiO_2, 24.5 wt% Na_2O, 24.5 wt% CaO and 6 wt% P_2O_5, commercially available as Bioglass®, which provided for the first time an alternative interfacial bonding of an implant with host tissues. This group of glasses has become known as bioactive glasses based on the following definition: *"A bioactive material is one that elicits a specific biological response at the interface of the material that results in the formation of bond between the tissues and the material"* (Hench & Andersson 1993). Over the years, many questions concerning its interactions with both hard and soft tissues have been answered with a multi-disciplinary team of materials scientists, orthopaedic surgeons, dental researchers, biomechanics experts and biologists. Many clinical Bioglass® devices like MEP®, ERMI®, HAPEX®, NovaBone®, NovaMin® and NovaThera®, are being used as a substitute for bone augmentation and restoration; in orthorpaedic, dental and maxillofacial surgery; and in the field of tissue engineering (Cacciotti et al. 2012). They have proved to be efficient, some outperforming other bioceramic and metal prostheses (Merwin 1990; Hench 2006).

Bioactive glasses have been produced using conventional glass technology. The glass components, in the form of grains of oxides or carbonates, are mixed and then melted and homogenized at high temperatures: 1250-1400°C (Li, Clark & Hench 1991). A number of processes have been employed in the production of bioglass nanoparticles, which include the microemulsion technique (Karagiozov & Momchilova 2005; Sun et al. 2007), the laser spinning technique (Quintero, Pou, et al. 2007), gas phase synthesis (Pratsinis 1998), and the sol-gel method (Hench & West 1990). Among all these techniques, the sol-gel method has gained a lot of research interest in the last two decades because it can be prepared from gels by sintering at relatively low temperatures (600 – 700 ^oC), which nullify most of the disadvantages of high-temperature processing, with much higher control over purity. In addition, sol-gel processing offers potential advantages of ease of powder production, a broader range of bioactivity, and a better control of bioactivity; by changing either the composition or the microstructure through processing parameters (Sakka 1985). Li and his colleagues (Li, Clark & Hench 1991) showed that SiO_2-CaO-P_2O_5 powders produced by this technique are more bioactive than the melt-derived glasses of the same composition. Moreover, Sepulveda and his co-

workers studied the dissolution rates and rates of surface layer formations on melt derived and sol gel bioglass and the results suggested that 45S5 melt derived bioglass exhibited lower rates than 58S sol gel bioglass powder (Sepulveda, Jones & Hench 2001). The high bioactivity of the sol-gel derived materials is related to the textural features of the gels, i.e., pore size and pore volume associated with the large surface area, higher rate of dissolution, and the negative surface charge (Pereira & Hench 1996). In addition, bioactive sol-gel glasses have been proposed as alternatives to glasses produced by melt and quenching methods, because they exhibit higher rates of apatite-layer formation, more rapid bone bonding, improved homogeneity and purity, and excellent degradation/resorption properties.

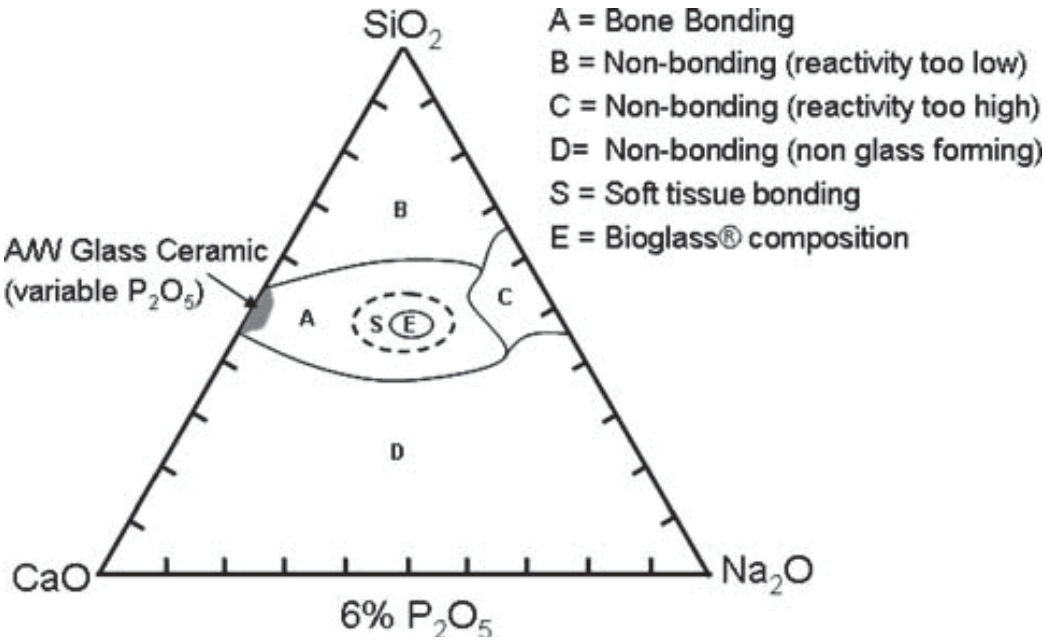

Figure 1: Compositional diagram for bone bonding. Note regions A, B, C, D. Region S is a region of Class A bioactivity where bioactive glasses bond to both bone and soft tissues and are gene activating (Hench 2006).

Bioactive glasses are composed of SiO_2, Na_2O, CaO and P_2O_5. The first and most well studied composition, named Bioglass® 45S5 contains: 45% SiO_2, 24.5% Na_2O, 24.4% CaO and 6% P_2O_5 all in weight percent (Hench & Andersson 1993). Hench and colleagues have studied a series of glasses in these four-component systems

with a constant 6% P_2O_5 content as summarized in the ternary SiO_2-Na_2O-CaO diagram shown in Figure 1.

The boundaries in figure 1 are kinetic boundaries and not phase equilibrium boundaries. The diagram represents the composition boundaries and their respective reaction mechanism. Glasses with the highest level of bioactivity and rapid bone bonding lie in the middle of the Na_2O-CaO-SiO_2 diagram (Region E); all compositions contain a constant 6 weight percent of P_2O_5. Compositions that exhibit slower rates of bonding lie between 52 to 60% by weight of SiO_2 in the glass. Compositions with greater than 60% SiO_2 (Region B) do not bond and are bio-inert. However, a series of studies have shown that bioactive glasses can compose either less or more than these oxides, or be substituted with other oxides without significantly alter bone-bonding behavior (Zhang et al. 2009; Zhou et al. 2012). Greenspan and Hench suggested that increasing the surface area of the glass by making a particulate or a nanoporous solgel derived glass, extends the bone bonding compositions to higher percentages of SiO_2 in the glass (Greenspan & Hench 1976)

The rapid rate surface reaction of bioactive glasses which leads to fast tissue bonding is one of the primary advantages of this material. Bioactive glasses have been used successfully as bone-filling materials in orthopedic and dental surgery, but their poor mechanical strength limits their applications in load-bearing positions (Merwin 1990; Cai & Zhou 2005; Hench 2006). This motivates researchers to combine excellent mechanical properties of metals or polymers with a bioactive phase of either particles or fibres, to produce a bioactive composite with optimized properties. The tensile bending strength of most of the bioactive glass compositions is in the range 40 – 60 MPa with a low modulus of elasticity in the range 30 – 35 GPA.

Bioactive glasses are a category of biomaterials that bond to living tissues, both soft and hard, through the formation of a hydroxy carbonate apatite (HCA) layer on their surfaces (Hench et al. 1972; Hench & Wilson 1984; Gross et al. 1988). However, it has been shown that the key phenomenon for bioactivity, or bond

formation, is the controlled rates of release of ionic dissolution products from the bioglass surface, especially critical concentrations of soluble silica and calcia ions (Hench et al. 2000). Reactions occurring on the surface of the glass lead to the formation of a silica gel layer and subsequent crystallization of HCA. The physical chemical mechanisms involved in forming a bioactive bond to tissues are now well established and represented in Figure 2.

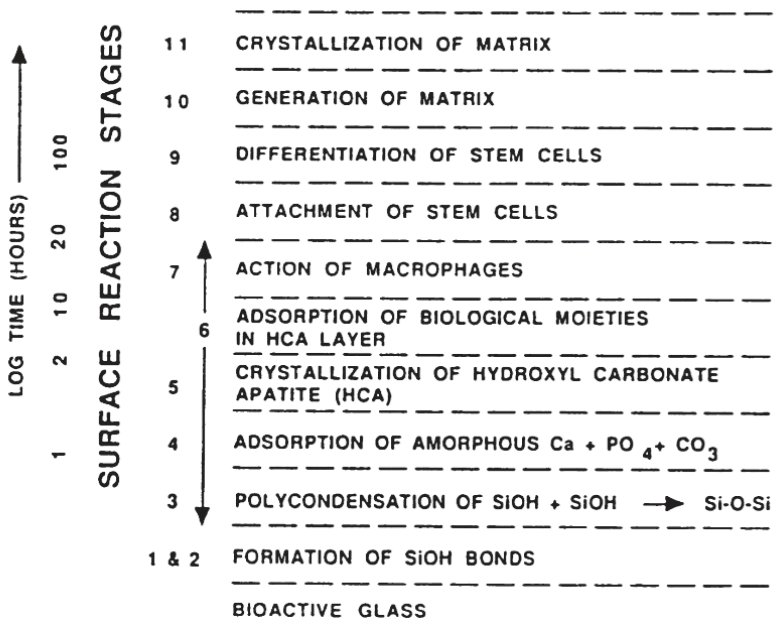

Figure 2: Sequence of interfacial reactions involved in forming a bond between tissue ad bioactive ceramic (Hench 1998)

Using bioactive glasses as regenerative materials for soft and hard tissue will bring forth a biomaterials revolution in the medical field by addressing the limitations of non-regenerative medical devices, with a fresh approach and a shift in priorities.

1.3 Biodegradable polymers

1.3.1 Naturally occurring polymers

Naturally occurring polymers are biodegradable and have excellent biological properties, for they provide a natural substrate for cellular growth, cell proliferation and differentiation and are ideal biomaterials for tissue engineering applications (Seal, Otero & Panitch 2001). However, they have poor mechanical properties and varying physical properties which have hindered their wider application. On the other hand, with the rapid advancement of science, more

understanding of fundamental biosynthesis pathways have come to light with the possibility of genetically manipulating or tailoring these pathways for specialty biopolymers. Polyhydroxyalkanoates (PHAs), are linear polyesters produced in nature by bacterial fermentation of sugar or lipids under unbalanced conditions (Doi, Kitamura & Abe 1995). A combination of monomers of the PHAs family can dramatically give materials extremely different properties. PHAs have been attractive to tissue engineering due to their versatile properties. The main drawbacks of PHAs include very high hydrophobicity, slow degradations under physiological conditions and lack of chemical functionalities. Different modification techniques have been extensively reviewed by Kai and Loh (Kai & Loh 2013).

1.3.2 Synthetic polymers

Biodegradable polymeric materials have had a significant impact on medical technology, greatly enhancing the efficacy of many existing drugs and enabling the construction of entirely new therapeutic modalities (LaVan, McGuire & Langer 2003). Recently, synthetic biopolymers have become attractive alternatives for biomedical applications for the following reasons: (1) although most biologically derived biodegradable polymers possess good biocompatibility, some may trigger an immune response in the human body, possibly one that could be avoided by the use of an appropriate synthetic biopolymer; (2) chemical modifications to biologically derived biodegradable polymers are difficult; (3) chemical modifications are likely to cause the alteration of the bulk properties of biologically derived biodegradable polymers. A variety of properties can be obtained and further modifications are possible with properly designed synthetic biopolymers without altering the bulk properties (Tian et al. 2012). Aliphatic polyesters such as polylactide (PLA), polyglycolide (PGA) and their copolymers have gained widespread biomedical applications because of their biodegradability and biocompatibility properties in the human body (Griffith 2000). In addition, Lou and his colleagues (Lou, Detrembleur & Jérôme 2003) showed that synthetic aliphatic biopolymers have tunable properties, including features such as hydrophilicity, biodegradation rates, bioadhesion and drug/targeting moiety attachment.

1.4 Biodegradable polymer composites

It has been shown that the release of acidic degradation products from polymeric materials causes inflammatory reactions (Bergsma et al. 1993), thus the degradation products of calcium phosphate materials could possibly buffer the acidic products from polymers and reduce the significantly inflammatory effects (Chen, Roether & Boccaccini 2008). In order to upgrade structural and functional properties of these polymers, researchers have been introducing organic and inorganic nanofibres into biodegradable polymers to make composite materials for more than two decades (Boccaccini et al. 2010). Tjong showed that nanocomposite materials often show an excellent balance between strength and toughness and usually improved characteristics compared to their individual components (Tjong 2006). The combination of biodegradable polymer and nanopowders opens a new perspective in the nano-devices for biomedical applications, with tunable mechanical, thermal, morphological and biological properties. From this point of view, nanocomposite materials based on hydroxyapatite, metal and ceramic nanoparticles or carbon nanostructures could be excellent choices for biomedical applications. The major challenge is to optimize biological and mechanical properties of the biodegradable polymer – bioceramics composites due to poor interfacial bonding between particles and the polymeric matrix. To achieve excellent properties, surface modifications of bioceramics particles have been attempted using silane coupling agents, titanate and zirconates in order to improve interfacial bonding between inorganic particles and the matrix (Gomes et al. 2001; Macha, Ben-Nissan & Milthorpe 2014).

1.5 Medical Implants infection

Invasive medical devices are widely used in the medical field to replace and repair damaged tissue or for diagnostic purposes. A significant proportion of these devices; which are especially used in central venous catheters, neurosurgical ventricular shunts, implantable neurological stimulators, cochlear implants, intraocular lenses, heart valves, breast implants, ventricular assist devices, coronary stents, arthro-prostheses, fracture-fixation devices, inflatable penile implants and dental implants; is associated with medical device associated infection (Costerton, Montanaro & Arciola 2005). Increasing evidence suggests that

bacteria biofilm is the leading course of implants failure in device-associated infections, which also lead to significant morbidity and mortality (Klevens et al. 2007). It was reported that more than 5 million central venous catheters alone are implanted annually in the USA, of which 5 to 26 percentage lead to catheter-related infectious complication (Merrer et al. 2001; McGee & Gould 2003). Tacconelli et al. estimated the clinical outcomes and costs associated with catheter-related bloodstream infections (CRBSIs) in four European countries (Tacconelli et al. 2009). Their results suggest that there are more than 1000 deaths per year with an associated cost of €35 to €164 million per year, per country. Biofilm is a microbial derived sessile community, characterized by cells that are irreversibly attached to a substratum or interface to each other, embedded in a matrix of extracellular polymeric substances that they have produced.

According to IUPAC, biofilm is "an aggregate of microorganisms in which cells that are frequently embedded within a self-produced matrix of extracellular polymeric substance (EPS) adhere to each other and/or to a surface" (Vert et al. 2012). The three dimensional extracellular polymeric substances protect bacteria from the external environment so they become more resistant to antimicrobial stress and to the immune system. Treatment of biofilm is difficult after its formation - most of the contaminated devices have to be removed, as the only clinical alternative, ideal option to deal with biofilm is the development of medical devices with surfaces or materials that prevent microbial surface adhesion or viability. Appropriate designs of medical and material selections have not yet been realized because of the lack of in-depth understanding of the mechanisms of bacterial adhesion. The classical Derjaguin–Landau–Verwey–Overbeek (DLVO) theory of colloid stability explains the mechanism based on free energy changes involved in microbial adhesion (Chung et al. 2011). These theories are rather limited as there are other factors, like the biological aspect of adhesion that are not considered. An extensive review of strategies to disrupt or inhibit biofilm using scientific knowledge on structural molecules, on the synthesis and genetics of biofilm was conducted by (Arciola et al. 2012). They suggested that the promising biofilm mitigating strategy would be the one that avoids the spread of antibacterial substances in the neighbouring tissues with the consequent risk of inducing bacterial resistance.

1.6 *In vitro* Biocompatibility (Cell culture studies)

The most important requirement for any biomaterial to be used in biomedical applications is its biocompatibility in a specific environment, together with no-cytotoxicity of its degradation products (Gomes & Reis 2004). Apart from its biocompatibility, any modifications, additives or processing technologies required to obtain different properties should not interfere with the biocompatible behaviour of the resulting materials. Cytotoxicity assays are widely used in drug and medical implants research as initial stages to test the biocompatibility to predict which lead compounds might have safety concerns in humans before significant time and expense are incurred in their development. The assessment of cytotoxicity is performed where cells are exposed to the materials and observation is made on them if they undergo necrosis, in which they lose membrane integrity and die rapidly as a result of cell lysis; or stop growing and dividing; or activate a genetic program of controlled cell death, termed apoptosis. The absence of cytotoxicity, however, does not confer knowledge about the biocompatibility of a biomaterial (Kooten et al. 1997). Assessments beyond cytotoxicity are usually performed to evaluate overall biofunctionality of biomaterials, which focus on establishment of reproducible and quantifiable assays relevant to biomaterials applications. Each application needs to be tested in the proper settings reflecting the natural environment, and the demands posed on the application, in order to study the more specific aspects of biocompatibility. This means that for bone prostheses, for example, osteoblast cells should be used to see if they deposit a proper bone matrix with all the natural constituents balanced. Yamamoto and his colleagues state that cell adhesion is one of the important aspects of cell interactions with biomaterials. Their findings suggest that it is possible to compare the materials affinity for cells using their system, if the cells would be seeded at the same time onto different materials (Yamamoto et al. 1998).

1.7 Calcium phosphate and drug delivery

Calcium orthophosphates, including hydroxyapatite (HAp), octacalcium phosphate (OCP), and dicalcium phosphate dehydrate (DCPD), are biologically familiar as they are produced in normal and pathological mineralization (Elliott 1994). The

compositions and chemical formulas of calcium orthophosphates are shown in Table 1.

For many years the principal biomedical applications for calcium phosphates has been as hard tissue analogs. They are also being explored as drug delivery systems due to their rapid dissolution of either $CaHPO_4.2H_2O$ or its mixtures with $CaSO_4.2H_2O$ to deliver drugs occluded in the pore structures of these solids. Principal, among the calcium phosphates of importance in biomedical application is HAp, the main inorganic component of natural bone. HAp has been extensively investigated since it is recognized as a ceramic material that significantly simulates the composition and mineralogical structure of bone (Albee & Morrison 1920) The composition of HAp is never fixed. The ratio of Ca/P varies from 1.67 (stoichiometric) to approximately 1.5 (fully calcium deficient). In this case a generic formula for HAp composition may be expressed as $Ca_{(10-x)}(HPO_4)_x(PO_4)_{(6-x)}(OH)_{(2-x)}$ where x = 0 – 1. The compositional variability of HAp can be used to control the bioactivity of HAp-based preparation. Gustavsson and colleagues (Gustavsson et al. 2011) studied ion reactivity of calcium deficient HAp in different standard culture media. The results suggested that different compositions of the aqueous environment may provoke opposite ion reactivity of calcium deficient HAp, and this must be carefully considered when evaluating the osteoconductivity of the material.

HAp in monolithic form can be produced by solid-state, sintering reactions at high temperatures below their dissociation temperatures. A preparative method developed by Brown and Chow (1988) demonstrated that monolithic HAp could be formed at moderate temperatures. In particular, it was demonstrated that HAp could be formed by an acid-based reaction between calcium phosphate precursors. Equation 1 shows the formation of HAp from the reaction between dicalcium phosphates dehydrate and tetracalcium phosphate.

$$2CaHPO_4.2H_2O + Ca_4(PO_4)_2O \xrightarrow{H_2O} Ca_{10}(PO_4)_6(OH)_2 + H_2O \quad (1)$$

Basic engineering principles for micro-fabrication can be learned through understanding the phenomenon of molecular self-assembly which is ubiquitous in the natural world. Coral and bone formation are good examples of these biomimetic processes. Coralline apatites can be derived from sea coral, which is composed of calcium carbonate in the form of aragonite and is a naturally occurring material that has optimal strength and structural characteristics (Ben-Nissan 2004). The pore structure of calcium phosphate converted from calcium carbonate produced by a certain species such as corals is similar to human cancellous bone, making it a suitable material for bone graft application. An Australian coral was converted to monophasic HAp using a two-stage process in which the hydrothermal conversion method was followed by the patented HAp sol-gel nanocoating process (Ben-Nissan et al. 2003). The calcium phosphate formed depends on the polymorph of the parent $CaCO_3$ used and thus on the species of coral. The coral *porites* form the aragonite polymorph, which under hydrothermal treatment with dibasic ammonium phosphate, converts to HAp, Equation 2.

$$\underset{\text{(Aragonite)}}{10CaCO_3} + 6(NH_4)_2HPO_4 + 2H_2O \longrightarrow \underset{\text{(HAp)}}{Ca_{10}(PO_4)_6(OH)_2} + 6(NH_4)_2CO_3 + 4H_2CO_3 \quad (2)$$

It has been reported that a dense monolithic HAp implant has low resorption rates that hinder bone in-growth, resulting in chemical bonding only at the interface between the bone and the HAp implant (Jarcho 1981; Kivrak & Taş 1998). This low biodegradability is the drawback of dense monolithic HAp ceramics, which limits the wide application of bulk HAp. Unlike HAp, tri-calcium phosphate (TCP) is considered a resorbable bioceramic (Jarcho 1981). It has been reported that TCP ceramics have been developed as a material that display ideal biodegradability and tend to be replaced by bone as they degrade. It has three polymorphs, low temperature phase β-TCP which is stable below 1180 °C, α-TCP which is stable in the temperature range 1180–1400 ° C and α′-TCP which is observed above 1470 °C (Lin et al. 1998; Ryu et al. 2002). Lin and colleagues reported that the phase transformation is accompanied by density changes for calcium phosphate materials and the density decreases with phase transformation in the following

sequence when heat-treated to higher temperatures (Lin et al. 1998). Hap (ρ = 3.14 gcm^{-3})> β-TCP (ρ = 3.07 gcm^{-3}> α-TCP (ρ =2:77 gcm^{-3}>α′-TCP.

Among the three allotropic forms of TCP, β-TCP is preferred as a bio-ceramic because of its mechanical strength, good tissue compatibility, and ability to bond directly to tissue to regenerate bone without any intermediate connective tissue. Moreover, its fast bone regeneration and proper bio-resorption rate are other additional attributes of β-TCP (Lin et al. 1998). It has been mentioned that the dissolution rate of β-TCP is 3–12 times faster than stoichiometric HAp . Although the porosity size, interconnectivity and pore amounts will influence their dissolution rates due to the surface area change. In vitro studies revealed that α-TCP exhibits a higher dissolution rate than β-TCP (LeGeros et al. 1995; Lin et al. 2001). The order of relative solubility is α-TCP>β-TCP≫HAp (Lin et al. 2001).

In drug delivery, the primary aim is to target drugs to specific sites within the body. In addition to reducing toxicity to non-diseased cells, the use of ceramic systems has the potential benefit of increasing drug efficiency, which translates to a significant cost saving for many of the expensive drug treatments now being engineered. Kundu and his colleagues investigated in vitro ability of HAp and beta-TCP scaffolds to release drugs suitable for osteomyelitis. The results of the study indicated that HAp exhibited a better drug release profile than β-TCP when the drug was used alone, indicating the high influence of the carrier material. However, this restriction got relaxed when a bilayered scaffold was formed using chitosan along with the drug (Kundu et al. 2010). One of the factors that control the release of drugs from the drug carrier is the physical-chemical interaction between them. Baradari et al., after investigating the use of porous β-TCP as an anti- inflammatory drug carrier, found that adsorption isotherm fitted to the Freundlich model, suggests that the interaction between ibuprofen and β-TCP is weak (Baradari et al. 2011). Tailoring better properties for drug release systems can be carefully achieved by the combination of more than two components to make composite systems. It has been reported recently that composite drug delivery systems composed of silica nanoparticles coated with β-TCP and bioactive glass showed high performance in the local and extremely sustained delivery of the bi-

component anti-tubercular drugs and excellent biocompatibility (Zhu et al. 2011). The most studied low molecular weight drugs incorporated in calcium phosphate are antibiotics, while osteoporotic, anticancer and other drugs have also been evaluated; showing in most cases profiles with burst release initially fitting the Higuchi model. It has been shown that these drugs remain active after their incorporation into the cements. However, given the dynamic nature of the setting process, and to approach the reality of the surgical room, it was suggested that it would be of interest to increase the number of release studies from unset drug-containing cements (Ginebra et al. 2012).

Next to calcium phosphate ceramics, calcium phosphate cements are of interest for bone tissue engineering purposes. Calcium phosphate cements consist of a powder phase of calcium and/or phosphate salts, that together with an aqueous phase, react at room/body temperature to form a calcium phosphate precipitate that sets by the entanglement of crystals (Habraken, Wolke & Jansen 2007). The uses of β-TCP cement as a drug delivery device have been reported. The hardening of the calcium phosphate cement takes place at room or body temperature. This fact, together with their intrinsic porosity allows the incorporation of drugs, biologically active molecules or even cells, without thermal denaturalization or loss of activity during preparation or implantation (Ginebra et al. 2012). Generally, ceramic materials are good drug carriers, in which release patterns are strongly dependent on the chemical consistency of the ceramic, type of drug and drug loading. Biodegradable polymers like polylactic acid, gelatin or chitosan are used as matrices for ceramic particles or as adjuvant to calcium phosphate cements. The use of these polymers can introduce a tailored biodegradation/drug release to the ceramic material (Habraken, Wolke & Jansen 2007).

1.8 References

CDC, 2015, *The National and State Healthcare-Associated Infections Progress Report (HAI Progress Report)*, Centers for Disease Control and Prevention (CDC), USA.

Adrover, A. & Nobili, M. 2015, 'Release kinetics from oral thin films: Theory and experiments', *Chemical Engineering Research and Design*, vol. 98, no. 0, pp. 188-201.

Agarwal, M., Koelling, K.W. & Chalmers, J.J. 1998, 'Characterization of the degradation of polylactic acid polymer in a solid substrate environment', *Biotechnol Prog*, vol. 14, no. 3, pp. 517-26.

Agrawal, C.M., Athanasiou, K.A. & Heckman, J.D. 1997, 'Biodegradable PLA-PGA polymers for tissue engineering in orthopaedics', *Porous Materials for Tissue Engineering*, vol. 250, pp. 115-28.

Agrawal, P., Soni, S., Mittal, G. & Bhatnagar, A. 2014, 'Role of polymeric biomaterials as wound healing agents', *Int J Low Extrem Wounds*, vol. 13, no. 3, pp. 180-90.

Alam, P. 2010, 'A mixtures' model for porous particle–polymer composites', *Mechanics Research Communications*, vol. 37, no. 4, pp. 389-93.

Albee, F.H. & Morrison, H.F. 1920, 'Studies in bone growth: Triple CaP as a stimulus to osteogenesis', *Annals of Surgery*, vol. 71, no. 1, pp. 32-9.

Alvarez-Barreto, J.F., Landy, B., VanGordon, S., Place, L., DeAngelis, P.L. & Sikavitsas, V.I. 2011, 'Enhanced osteoblastic differentiation of mesenchymal stem cells seeded in RGD-functionalized PLLA scaffolds and cultured in a flow perfusion bioreactor', *J Tissue Eng Regen Med*, vol. 5, no. 6, pp. 464-75.

An, Y.H. & Friedman, R.J. 1998, 'Concise review of mechanisms of bacterial adhesion to biomaterial surfaces', *Journal of Biomedical Materials Research*, vol. 43, no. 3, pp. 338-48.

Arciola, C.R., Campoccia, D., Speziale, P., Montanaro, L. & Costerton, J.W. 2012, 'Biofilm formation in Staphylococcus implant infections. A review of molecular mechanisms and implications for biofilm-resistant materials', *Biomaterials*, vol. 33, no. 26, pp. 5967-82.

Arcos, D., Ragel, C.V. & Vallet-Regi, M. 2001, 'Bioactivity in glass/PMMA composites used as drug delivery system', *Biomaterials*, vol. 22, no. 7, pp. 701-8.

Arriagada, F.J. & Osseo-Asare, K. 1999, 'Synthesis of Nanosize Silica in a Nonionic Water-in-Oil Microemulsion: Effects of the Water/Surfactant Molar Ratio and Ammonia Concentration', *Journal of Colloid and Interface Science*, vol. 211, no. 2, pp. 210-20.

Asandei, A.D., Erkey, C., Burgess, D.J., Saquing, C., Saha, G. & Zolnik, B.S. 2004, 'Preparation of drug delivery biodegradable nanocomposites by rapid expansion from supercritical solutions.', *Abstracts of Papers of the American Chemical Society*, vol. 228, pp. U345-U.

Athanassiou, E.K., Grass, R.N. & Stark, W.J. 2010, 'Chemical Aerosol Engineering as a Novel Tool for Material Science: From Oxides to Salt and Metal Nanoparticles', *Aerosol Science & Technology*, vol. 44, no. 2, pp. 161-72.

Aviv, M., Berdicevsky, I. & Zilberman, M. 2007, 'Gentamicin-loaded bioresorbable films for prevention of bacterial infections associated with orthopedic implants', *J Biomed Mater Res A*, vol. 83, no. 1, pp. 10-9.

Baradari, H., Damia, C., Dutreih-Colas, M., Champion, E., Chulia, D. & Viana, M. 2011, 'beta-TCP porous pellets as an orthopaedic drug delivery system: ibuprofen/carrier physicochemical interactions', *Science and Technology of Advanced Materials*, vol. 12, no. 5.

Baro, M., Sánchez, E., Delgado, A., Perera, A. & Evora, C. 2002, 'In vitro- in vivo characterization of gentamicin bone implants', *J Control Release.*, vol. 83, no. 3, pp. 353-64.

Batycky, R.P., Hanes, J., Langer, R. & Edwards, D.A. 1997, 'A theoretical model of erosion and macromolecular drug release from biodegrading microspheres', *J Pharm Sci*, vol. 86, no. 12, pp. 1464-77.

Ben-Nissan, B. 2003, 'Natural bioceramics: from coral to bone and beyond', *Current Opinion in Solid State and Materials Science*, vol. 7, no. 4–5, pp. 283-8.

Ben-Nissan, B. 2004, 'Nanoceramics in Biomedical Applications', *MRS Bulletin*, vol. 29, no. 1, pp. 28-32.

Ben-Nissan, B. & Choi, A.H. 2006, 'Sol-gel production of bioactive nanocoatings for medical applications. Part 1: an introduction', *Nanomedicine (Lond)*, vol. 1, no. 3, pp. 311-9.

Ben-Nissan, B. & Green, D. 2014, 'Marine Structures as Templates for Biomaterials', in B. Ben-Nissan (ed.), *Advances in Calcium Phosphate Biomaterials*, vol. 2, Springer Berlin Heidelberg, pp. 391-414.

Ben-Nissan, B., Milev, A., Green, D., Conway, R.M., Kannangara, G.S.K., Russel, J., Hu, J., Gillott, G. & Trefry, C. 2003, *Sol-gel coating*.

Ben-Nissan, B. & Pezzotti, G. 2002, 'Bioceramics: Processing Routes and Mechanical Evaluations', *Journal of the Ceramic Society of Japan*, vol. 110, no. 7, pp. 601 - 8

Benninga, H. 1990, 'A History of Lactic Acid Making: A Chapter in the History of Biotechnology', *The British Journal for the History of Science*, vol. 24, pp. 424 - 75.

Bergsma, E.J., Rozema, F.R., Bos, R.R.M. & Bruijn, W.C.D. 1993, 'Foreign body reactions to resorbable poly(l-lactide) bone plates and screws used for the fixation of unstable zygomatic fractures', *Journal of Oral and Maxillofacial Surgery*, vol. 51, no. 6, pp. 666-70.

Best, S.M., Porter, A.E., Thian, E.S. & Huang, J. 2008, 'Bioceramics: Past, present and for the future', *Journal of the European Ceramic Society*, vol. 28, no. 7, pp. 1319-27.

Bianco, A., Cacciotti, I., Lombardi, M. & Montanaro, L. 2009, 'Si-substituted hydroxyapatite nanopowders: Synthesis, thermal stability and sinterability', *Materials Research Bulletin*, vol. 44, no. 2, pp. 345-54.

Blaker, J.J., Nazhat, S.N. & Boccaccini, A.R. 2004, 'Development and characterisation of silver-doped bioactive glass-coated sutures for tissue engineering and wound healing applications', *Biomaterials*, vol. 25, no. 7-8, pp. 1319-29.

Boccaccini, A.R., Chen, Q., Lefebvre, L., Gremillard, L. & Chevalier, J. 2007, 'Sintering, crystallisation and biodegradation behaviour of Bioglass-derived glass-ceramics', *Faraday Discuss*, vol. 136, pp. 27-44; discussion 107-23.

Boccaccini, A.R., Erol, M., Stark, W.J., Mohn, D., Hong, Z.K. & Mano, J.F. 2010, 'Polymer/bioactive glass nanocomposites for biomedical applications: A review', *Composites Science and Technology*, vol. 70, no. 13, pp. 1764-76.

Boccaccini, A.R. & Maquet, V. 2003, 'Bioresorbable and bioactive polymer/Bioglass® composites with tailored pore structure for tissue engineering applications', *Composites Science and Technology*, vol. 63, no. 16, pp. 2417-29.

Bonfield, W., Grynpas, M.D., Tully, A.E., Bowman, J. & Abram, J. 1981, 'Hydroxyapatite reinforced polyethylene--a mechanically compatible implant material for bone replacement', *Biomaterials*, vol. 2, no. 3, pp. 185-6.

Bosetti, M., Zanardi, L., Hench, L. & Cannas, M. 2003, 'Type I collagen production by osteoblast-like cells cultured in contact with different bioactive glasses', *Journal of Biomedical Materials Research Part A*, vol. 64A, no. 1, pp. 189-95.

Brown, M. & Farrar, D. 2008, 'Development of bioresorbable polymers in orthopaedics', *Plastics Rubber and Composites*, vol. 37, no. 2-4, pp. 46-9.

Buzarovska, A. & Grozdanov, A. 2012, 'Biodegradable Poly(L-lactic acid)/TiO2 Nanocomposites: Thermal Properties and Degradation', *Journal of Applied Polymer Science*, vol. 123, no. 4, pp. 2187-93.

Cabezas, L.I., Gracia, I., de Lucas, A. & Rodríguez, J.F. 2014, 'Novel Model for the Description of the Controlled Release of 5-Fluorouracil from PLGA and PLA Foamed Scaffolds Impregnated in Supercritical CO2', *Industrial & Engineering Chemistry Research*, vol. 53, no. 40, pp. 15374-82.

Cacciotti, I., Lombardi, M., Bianco, A., Ravaglioli, A. & Montanaro, L. 2012, 'Sol-gel derived 45S5 bioglass: synthesis, microstructural evolution and thermal behaviour', *J Mater Sci Mater Med*.

Cai, Y.R. & Zhou, L. 2005, 'Effect of thermal treatment on the microstructure and mechanical properties of gel-derived bioglasses', *Materials Chemistry and Physics*, vol. 94, no. 2-3, pp. 283-7.

Calafiori, A.R., Di Marco, G., Martino, G. & Marotta, M. 2007, 'Preparation and characterization of calcium phosphate biomaterials', *J Mater Sci Mater Med*, vol. 18, no. 12, pp. 2331-8.

Calandrelli, L., Annunziata, M., Della Ragione, F., Laurienzo, P., Malinconico, M. & Oliva, A. 2010, 'Development and performance analysis of PCL/silica nanocomposites for bone regeneration', *J Mater Sci Mater Med*, vol. 21, no. 11, pp. 2923-36.

Campbell, A.A. 2003, 'Bioceramics for implant coatings', *Materials Today*, vol. 6, no. 11, pp. 26-30.

Carothers, W.H., Dorough, G.L. & Natta, F.J.v. 1932, 'Studies of polymerization and ring formation. X. The reversible polymerization of six-membered cyclic esters', *Journal of the American Chemical Society*, vol. 54, no. 2, pp. 761-72.

Cataife, G., Weinberg, D.A., Wong, H.H. & Kahn, K.L. 2014, 'The effect of Surgical Care Improvement Project (SCIP) compliance on surgical site infections (SSI)', *Med Care*, vol. 52, no. 2 Suppl 1, pp. S66-73.

Cecchinato, F., Karlsson, J., Ferroni, L., Gardin, C., Galli, S., Wennerberg, A., Zavan, B., Andersson, M. & Jimbo, R. 2015, 'Osteogenic potential of human adipose-derived stromal cells on 3-dimensional mesoporous TiO2 coating with magnesium impregnation', *Materials Science and Engineering: C*, vol. 52, no. 0, pp. 225-34.

Cegla, R.-N.R., Macha, I.J., Ben-Nissan, B., Grossin, D., Heness, G. & Chung, R.-J. 2014, 'Comparative Study of Conversion of Coral with Ammonium Dihydrogen Phosphate and Orthophosphoric Acid to Produce Calcium Phosphates', *Journal of the Australian Ceramics Society*, vol. 50, no. 2, pp. 154-61.

Cerca, N., Gomes, F., Pereira, S., Teixeira, P. & Oliveira, R. 2012, 'Confocal laser scanning microscopy analysis of S. epidermidis biofilms exposed to farnesol, vancomycin and rifampicin', *BMC Res Notes*, vol. 5, p. 244.

Chai, C., Ben-Nissan, B., Pyke, S. & Evans, L. 1995, 'Sol-Gel Derived Hydroxylapatite Coatings for Biomedical Applications', *Materials and Manufacturing Processes*, vol. 10, no. 2, pp. 205-16.

Chen, C.-W., Riman, R.E., TenHuisen, K.S. & Brown, K. 2004, 'Mechanochemical–hydrothermal synthesis of hydroxyapatite from nonionic surfactant emulsion precursors', *Journal of Crystal Growth*, vol. 270, no. 3–4, pp. 615-23.

Chen, Q., Liang, S. & Thouas, G.A. 2013, 'Elastomeric biomaterials for tissue engineering', *Progress in Polymer Science*, vol. 38, no. 3–4, pp. 584-671.

Chen, Q., Roether, J.A. & Boccaccini, A.R. 2008, 'Tissue Engineering Scaffolds from Bioactive Glass and Composite Materials', in N. Ashammakhi, R. Reis & F. Chiellini (eds), *Topics in Tissue Engineering*, Oulu University, Oulu, Finland.

Chen, Q.Z. & Boccaccini, A.R. 2006, 'Poly(D,L-lactic acid) coated 45S5 Bioglass-based scaffolds: processing and characterization', *J Biomed Mater Res A*, vol. 77, no. 3, pp. 445-57.

Chen, Q.Z., Thompson, I.D. & Boccaccini, A.R. 2006, '45S5 Bioglass-derived glass-ceramic scaffolds for bone tissue engineering', *Biomaterials*, vol. 27, no. 11, pp. 2414-25.

Chen, Q.Z. & Thouas, G.A. 2011, 'Fabrication and characterization of sol-gel derived 45S5 Bioglass (R)-ceramic scaffolds', *Acta Biomaterialia*, vol. 7, no. 10, pp. 3616-26.

Chen, X., Li, S. & Huang, Z. 1995, '[The applications of machinable bioactive glass ceramics in maxillofacial augmentation]', *Zhonghua Zheng Xing Shao Shang Wai Ke Za Zhi*, vol. 11, no. 6, pp. 419-20.

Chou, J., Austin, C., Doble, P., Ben-Nissan, B. & Milthorpe, B. 2014, 'Trace elemental imaging of coralline hydroxyapatite by laser-ablation inductively coupled plasma-mass spectroscopy', *J Tissue Eng Regen Med*, vol. 8, no. 7, pp. 515-20.

Chou, J., Ben-Nissan, B., Choi, A., Green, D. & Wuhrer, R. 2007, 'Conversion of coral sand to calcium phosphate for biomedical applications', *Journal of the Australian Ceramic Society* vol. 43, no. 1, p. 5.

Chou, J., Ben-Nissan, B., Green, D.W., Valenzuela, S.M. & Kohan, L. 2011, 'Targeting and Dissolution Characteristics of Bone Forming and Antibacterial Drugs by Harnessing the Structure of Microspherical Shells from Coral Beach Sand', *Advanced Engineering Materials*, vol. 13, no. 1-2, pp. 93-9.

Chou, J., Valenzuela, S., Green, D.W., Kohan, L., Milthorpe, B., Otsuka, M. & Ben-Nissan, B. 2014, 'Antibiotic delivery potential of nano- and micro-porous marine structure-derived beta-tricalcium phosphate spheres for medical applications', *Nanomedicine (Lond)*, vol. 9, no. 8, pp. 1131-9.

Choudhuri, A., Mohanty, P.S. & Karthikeyan, J. 2009, 'Bio-ceramic Composite Coatings by Cold Spray Technology ', *International Thermal Spray Conference*, ASM International, pp. 391-6.

Chu, G.C., Tomita, T., Sonnichsen, F.D., Yoshida, T. & Ikeda-Saito, M. 1999, 'The heme complex of Hmu O, a bacterial heme degradation enzyme from Corynebacterium diphtheriae. Structure of the catalytic site', *J Biol Chem*, vol. 274, no. 35, pp. 24490-6.

Chung, E.J., Kodali, P., Laskin, W., Koh, J.L. & Ameer, G.A. 2011, 'Long-term in vivo response to citric acid-based nanocomposites for orthopaedic tissue engineering', *J Mater Sci Mater Med*, vol. 22, no. 9, pp. 2131-8.

Cook, S.D., Thomas, K.A., Kay, J.F. & Jarcho, M. 1988, 'Hydroxyapatite-coated titanium for orthopedic implant applications', *Clin Orthop Relat Res*, no. 232, pp. 225-43.

Costa, P. 2001, 'An alternative method to the evaluation of similarity factor in dissolution testing', *International Journal of Pharmaceutics*, vol. 220, no. 1–2, pp. 77-83.

Costa, P. & Sousa Lobo, J.M. 2001, 'Modeling and comparison of dissolution profiles', *Eur J Pharm Sci*, vol. 13, no. 2, pp. 123-33.

Costerton, J.W., Montanaro, L. & Arciola, C.R. 2005, 'Biofilm in implant infections: its production and regulation', *Int J Artif Organs*, vol. 28, no. 11, pp. 1062-8.

Covani, U., Giacomelli, L., Krajewski, A., Ravaglioli, A., Spotorno, L., Loria, P., Das, S. & Nicolinil, C. 2007, 'Biomaterials for orthopedics: A roughness analysis by atomic force microscopy', *Journal of Biomedical Materials Research Part A*, vol. 82A, no. 3, pp. 723-30.

Dash, S., Murthy, P.N., Nath, L. & Chowdhury, P. 2010, 'Kinetic modeling on drug release from controlled drug delivery systems', *Acta Pol Pharm*, vol. 67, no. 3, pp. 217-23.

Davis, J.R. 2003, 'Metallic Materials', in J.R. Davis (ed.), *Handbook of Materials for Medical Devices*, ASM International, US, pp. 21-50.

Day, R.M. 2005, 'Bioactive glass stimulates the secretion of angiogenic growth factors and angiogenesis in vitro', *Tissue Engineering*, vol. 11, no. 5-6, pp. 768-77.

Day, R.M., Maquet, V., Boccaccini, A.R., Jerome, R. & Forbes, A. 2005, 'In vitro and in vivo analysis of macroporous biodegradable poly(D,L-lactide-co-glycolide) scaffolds containing bioactive glass', *Journal of Biomedical Materials Research Part A*, vol. 75A, no. 4, pp. 778-87.

De Santis, P. & Kovacs, A.J. 1968, 'Molecular conformation of poly(S-lactic acid)', *Biopolymers*, vol. 6, no. 3, pp. 299-306.

de Tayrac, R., Oliva-Lauraire, M.C., Guiraud, I., Henry, L., Vert, M. & Mares, P. 2007, 'Long-lasting bioresorbable poly(lactic acid) (PLA(94)) mesh: A new approach for soft tissue reinforcement based on an experimental pilot study', *International Urogynecology Journal*, vol. 18, no. 9, pp. 1007-14.

Desrousseaux, C., Sautou, V., Descamps, S. & Traore, O. 2013, 'Modification of the surfaces of medical devices to prevent microbial adhesion and biofilm formation', *J Hosp Infect*, vol. 85, no. 2, pp. 87-93.

Desrousseaux, C., Sautou, V., Descamps, S. & Traoré, O. 2013, 'Modification of the surfaces of medical devices to prevent microbial adhesion and biofilm formation', *Journal of Hospital Infection*, vol. 85, no. 2, pp. 87-93.

Doi, Y., Kitamura, S. & Abe, H. 1995, 'Microbial Synthesis and Characterization of Poly(3-hydroxybutyrate-co-3-hydroxyhexanoate)', *Macromolecules*, vol. 28, no. 14, pp. 4822-8.

Dou, Y., Lin, K. & Chang, J. 2011, 'Polymer nanocomposites with controllable distribution and arrangement of inorganic nanocomponents', *Nanoscale*, vol. 3, no. 4, pp. 1508-11.

Drnovsek, N., Novak, S., Dragin, U., Ceh, M., Gorensek, M. & Gradisar, M. 2012, 'Bioactive glass enhances bone ingrowth into the porous titanium coating on orthopaedic implants', *International Orthopaedics*.

Drumright, R.E., Gruber, P.R. & Henton, D.E. 2000, 'Polylactic Acid Technology', *Advanced Materials* vol. 12, no. 23, pp. 1841–6.

Ducheyne, P. 1985, 'Bioglass coatings and bioglass composites as implant materials', *Journal of Biomedical Materials Research*, vol. 19, no. 3, pp. 273-91.

Dunn, A.S., Campbell, P.G. & Marra, K.G. 2001, 'The influence of polymer blend composition on the degradation of polymer/hydroxyapatite biomaterials', *Journal of Materials Science: Materials in Medicine*, vol. 12, no. 8, pp. 673-7.

Dvir, T., Benishti, N., Shachar, M. & Cohen, S. 2006, 'A novel perfusion bioreactor providing a homogenous milieu for tissue regeneration', *Tissue Eng*, vol. 12, no. 10, pp. 2843-52.

E, M.K., N, V.V., G, K.B., Carson, M.J., A, L.R. & J, G.A. 2003, ' Adsorption-induced conformational changes in fibronectin due to interactions with well-defined surface chemistries ', *Langmuir*, vol. 19, no. 19, pp. 8033-40.

Ebelmen, M. 1846, 'On the synthesis of silica gels from alkoxides ', *Annales de chimie et de physique* vol. 16, p. 129.

Elbert, D.L. & Hubbell, J.A. 1996, 'Surface Treatments of Polymers for Biocompatibility', *Annual Review of Materials Science*, vol. 26, no. 1, pp. 365-94.

Elliott, J.C. 1994, 'Structure and Chemistry of the Apatites and Other Calcium Orthophosphates', *Elsevier*.

Elmore, K.L. & Farr, T.D. 1940, 'Equilibrium in the System Calcium Oxide–Phosphorus Pentoxide–Water', *Industrial & Engineering Chemistry*, vol. 32, no. 4, pp. 580-6.

Erdemli, O., Captug, O., Bilgili, H., Orhan, D., Tezcaner, A. & Keskin, D. 2010, 'In vitro and in vivo evaluation of the effects of demineralized bone matrix or calcium sulfate addition to polycaprolactone-bioglass composites', *J Mater Sci Mater Med*, vol. 21, no. 1, pp. 295-308.

Fambri, L., Pegoretti, A., Fenner, R., Incardona, S.D. & Migliaresi, C. 1997, 'Biodegradable fibres of poly(l-lactic acid) produced by melt spinning', *Polymer*, vol. 38, no. 1, pp. 79-85.

FDA 2013, '888-INFO-FDA, New medical device treats urinary symptoms related to enlarged prostate', in M. Liscinsky (ed.).

Ford Versypt, A.N., Pack, D.W. & Braatz, R.D. 2013, 'Mathematical modeling of drug delivery from autocatalytically degradable PLGA microspheres--a review', *J Control Release*, vol. 165, no. 1, pp. 29-37.

Francolini, I. & Donell, G. 2010, 'Prevention and control of biofilm-based medical-device-related infections', *FEMS Immunology & Medical Microbiology*, vol. 59, no. 3, p. 12.

Fredenberg, S., Reslow, M. & Axelsson, A. 2005, 'Measurement of Protein Diffusion Through Poly(d,l-Lactide-Co-Glycolide)', *Pharmaceutical Development and Technology*, vol. 10, no. 2, pp. 299-307.

Gao, P., Nie, X., Zou, M., Shi, Y. & Cheng, G. 2011, 'Recent advances in materials for extended-release antibiotic delivery system', *J Antibiot (Tokyo)*, vol. 64, no. 9, pp. 625-34.

Gao, Y. & Chang, J. 2009, 'Surface modification of bioactive glasses and preparation of PDLLA/bioactive glass composite films', *J Biomater Appl*, vol. 24, no. 2, pp. 119-38.

Garlotta, D. 2001, 'A Literature Review of Poly(Lactic Acid)', *Journal of Polymers and the Environment*, vol. 9, no. 2, pp. 63-84.

Garrett, T.R., Bhakoo, M. & Zhang, Z. 2008, 'Bacterial adhesion and biofilms on surfaces', *Progress in Natural Science*, vol. 18, no. 9, pp. 1049-56.

Gatti, A.M., Simonetti, L.A., Monari, E., Guidi, S. & Greenspan, D. 2006, 'Bone augmentation with bioactive glass in three cases of dental implant placement', *Journal of Biomaterials Applications*, vol. 20, no. 4, pp. 325-39.

Gatti, A.M., Valdre, G. & Andersson, O.H. 1994, 'Analysis of the in vivo reactions of a bioactive glass in soft and hard tissue', *Biomaterials*, vol. 15, no. 3, pp. 208-12.

Gerhardt, L.-C. & Boccaccini, A.R. 2010, 'Bioactive Glass and Glass-Ceramic Scaffolds for Bone Tissue Engineering', *Materials*, vol. 3, no. 7, pp. 3867-910.

Gillette, R.L., Swaim, S.F., Sartin, E.A., Bradley, D.M. & Coolman, S.L. 2001, 'Effects of a bioactive glass on healing of closed skin wounds in dogs', *Am J Vet Res*, vol. 62, no. 7, pp. 1149-53.

Ginebra, M.-P., Canal, C., Espanol, M., Pastorino, D. & Montufar, E.B. 2012, 'Calcium phosphate cements as drug delivery materials', *Advanced Drug Delivery Reviews*, vol. 64, no. 12, pp. 1090-110.

Ginebra MP, T.T., Planell JA. 2006, 'Calcium phosphate cements as bone drug delivery systems: A review', *J Control Release.* , vol. 113, no. 2, pp. 102-10.

Gomes, M.E. & Reis, R.L. 2004, 'Biodegradable Polymers and Composites in Biomedical Applications: From Catgut to Tissue Engineering: Part 1 - Available Systems and Their Properties', *International Materials Reviews*, vol. 49, no. 5, p. 13.

Gomes, M.E., Reis, R.L., Cunha, A.M., Blitterswijk, C.A. & de Bruijn, J.D. 2001, 'Cytocompatibility and response of osteoblastic-like cells to starch-based polymers: effect of several additives and processing conditions', *Biomaterials*, vol. 22, no. 13, pp. 1911-7.

Gonzalez, P., Serra, J., Liste, S., Chiussi, S., Leon, B., Perez-Amor, M., Martinez-Fernandez, J., de Arellano-Lopez, A.R. & Varela-Feria, F.M. 2003, 'New biomorphic SiC ceramics coated with bioactive glass for biomedical applications', *Biomaterials*, vol. 24, no. 26, pp. 4827-32.

Graham, T. 1864, 'XXXV.-On the properties of silicic acid and other analogous colloidal substances', *Journal of the Chemical Society*, vol. 17, pp. 318-27.

Green, D.W. & Ben-Nissan, B. 2010, 'Biomimetic Applications in Regenerative Medicine: Scaffolds, Transplantation Modules, Tissue Homing Device and Stem Cells', in V. Tochilin & M. Amiji (eds), *Handbook of Materials for Nanomedicine*, Pan Stanford Publishing Pte Ltd, Singapore, pp. 821-50.

Greenhalgh, E.S. 2009, *Failure analysis and fractographiy of polymer composites*, Woodhead Publishing Limited, Cambridge, UK.

Greenspan, D.C. & Hench, L.L. 1976, 'Chemical and mechanical behavior of bioglass-coated alumina', *J Biomed Mater Res*, vol. 10, no. 4, pp. 503-9.

Greenspan, D.C., Zhong, J.P., Chen, X.F. & LaTorre, G.P. 1997, 'The evaluation of degradability of melt and sol-gel derived Bioglass (R) in-vitro', *Bioceramics, Vol 10*, pp. 391-4.

Griffith, L.G. 2000, 'Polymeric biomaterials', *Acta Materialia*, vol. 48, no. 1, pp. 263-77.

Grijpma, D.W., Nijenhuis, A.J., Wijk, P.G.T. & Pennings, A.J. 1992, 'High impact strength as-polymerized PLLA', *Polymer Bulletin*, vol. 29, no. 5, pp. 571-8.

Gross, U., Kinne, R., Schmitz, H.J. & Strunz, V. 1988, 'The response of bone to surface active glasdglassceramics', *CRC Crit. Rev. Biocomput.*, vol. 4, pp. 2-15.

Gruber, P.R., Hall, E.S., Kolstad, J.J., Iwen, M.L., Benson, R.D. & Borchardt, R.L. 2001, *Continuous process for the manufacture of lactide and lactide polymers*, 6,326,458.

Gu, J.H., Huang, Y.J., Beekman, A. & Goldenberg, M. 2010, 'Simplified preparation of low polydispersity, low molecular weight poly(D,L-lactic acid) by liquid/liquid phase separation for drug delivery systems with discussion of molecular weight characterization', *Polymer International*, vol. 59, no. 12, pp. 1571-7.

Guo, W., Shi, Z., Guo, R. & Sun, R. 2007, '[Preparation of gentamicin sulfate-polyanhydride sustained-release beads and in vitro bacteriostatic activity studies]', *Sheng Wu Yi Xue Gong Cheng Xue Za Zhi*, vol. 24, no. 2, pp. 360-2, 84.

Gustavsson, J., Ginebra, M.P., Engel, E. & Planell, J. 2011, 'Ion reactivity of calcium-deficient hydroxyapatite in standard cell culture media', *Acta Biomaterialia*, vol. 7, no. 12, pp. 4242-52.

Ha, C.S. & Gardella, J.A., Jr. 2005, 'Surface chemistry of biodegradable polymers for drug delivery systems', *Chem Rev*, vol. 105, no. 11, pp. 4205-32.

Habibe, A.F., Maeda, L.D., Souza, R.C., Barboza, M.J.R., Daguano, J.K.M.F., Rogero, S.O. & Santos, C. 2009, 'Effect of bioglass additions on the sintering of Y-TZP bioceramics', *Materials Science and Engineering: C*, vol. 29, no. 6, pp. 1959-64.

Habraken, W.J.E.M., Wolke, J.G.C. & Jansen, J.A. 2007, 'Ceramic composites as matrices and scaffolds for drug delivery in tissue engineering', *Advanced Drug Delivery Reviews*, vol. 59, no. 4–5, pp. 234-48.

Hajiali, H., Karbasi, S., Hosseinalipour, M. & Rezaie, H.R. 2010, 'Preparation of a novel biodegradable nanocomposite scaffold based on poly (3-hydroxybutyrate)/bioglass nanoparticles for bone tissue engineering', *J Mater Sci Mater Med*, vol. 21, no. 7, pp. 2125-32.

Heidemann, W., Jeschkeit, S., Ruffieux, K., Fischer, J.H., Wagner, M., Krüger, G., Wintermantel, E. & Gerlach, K.L. 2001, 'Degradation of poly(d,l)lactide implants with or without addition of calciumphosphates in vivo', *Biomaterials*, vol. 22, no. 17, pp. 2371-81.

Hench, L.L. 1980, 'Biomaterials', *Science*, vol. 208, no. 4446, pp. 826-31.

Hench, L.L. 1991, 'Bioceramics: From Concept to Clinic', *Journal of the American Ceramic Society*, vol. 74, no. 7, pp. 1487-510.

Hench, L.L. 1998, 'Biomaterials: a forecast for the future', *Biomaterials*, vol. 19, no. 16, pp. 1419-23.

Hench, L.L. 2006, 'The story of Bioglass', *J Mater Sci Mater Med*, vol. 17, no. 11, pp. 967-78.

Hench, L.L. & Andersson, A. 1993, 'Bioactive Glasses', in L.L. Hench & J. Wilson (eds), *An Introduction of Bioceramics*, vol. 1, World Scientific Publishing Co. Pty. Ltd, Singapore, pp. 41-62.

Hench, L.L., Boccaccini, A.R., Day, R.M. & Gabe, S.M. 2003, 'Third-generation gene-activating biomaterials', *Thermec'2003, Pts 1-5*, vol. 426-4, pp. 179-84.

Hench, L.L. & Polak, J.M. 2002, 'Third-generation biomedical materials', *Science*, vol. 295, no. 5557, pp. 1014-+.

Hench, L.L., Polak, J.M., Xynos, I.D. & Buttery, L.D.K. 2000, 'Bioactive materials to control cell cycle', *Materials Research Innovations*, vol. 3, no. 6, pp. 313-23.

Hench, L.L., Splinter, R.J., Allen, W.C. & Greenlee, T.K. 1972, 'Bonding mechanisms at the interface of ceramic prosthetic materials', *J. Biomed. Muter. Res*, vol. 2.

Hench, L.L., Splinter, R.J., Allen, W.C. & T. K. Greenlee, J. 1971, 'Bonding Mechanisms at the Interface of Ceramic Prosthetic Materials', *J. Bi-omed. Maters. Res.*, vol. 2, no. 1, pp. 117-41.

Hench, L.L. & Thompson, I. 2010, 'Twenty-first century challenges for biomaterials', *Journal of the Royal Society Interface*, vol. 7, pp. S379-S91.

Hench, L.L. & West, J.K. 1990, 'The sol-gel process', *Chemical Reviews*, vol. 90, no. 1, pp. 33-72.

Hench, L.L. & Wilson, J. 1984, 'Surface-Active Biomaterials', *Science*, vol. 226, no. 4675, pp. 630-6.

Heness, G. & Ben-Nissan, B. 2004, 'Innovative Bioceramics', *Materials Forum* vol. 27, pp. 104-14.

Heydorn, A., Nielsen, A.T., Hentzer, M., Sternberg, C., Givskov, M., Ersboll, B.K. & Molin, S. 2000, 'Quantification of biofilm structures by the novel computer program COMSTAT', *Microbiology*, vol. 146 (Pt 10), pp. 2395-407.

Hoiby, N., Bjarnsholt, T., Givskov, M., Molin, S. & Ciofu, O. 2010, 'Antibiotic resistance of bacterial biofilms', *Int J Antimicrob Agents*, vol. 35, no. 4, pp. 322-32.

Hong, Z., Reis, R.L. & Mano, J.F. 2008, 'Preparation and in vitro characterization of scaffolds of poly(L-lactic acid) containing bioactive glass ceramic nanoparticles', *Acta Biomater*, vol. 4, no. 5, pp. 1297-306.

Hong, Z., Reis, R.L. & Mano, J.F. 2009, 'Preparation and in vitro characterization of novel bioactive glass ceramic nanoparticles', *J Biomed Mater Res A*, vol. 88, no. 2, pp. 304-13.

Howard, D., Partridge, K., Yang, X., Clarke, N.M.P., Okubo, Y., Bessho, K., Howdle, S.M., Shakesheff, K.M. & Oreffo, R.O.C. 2002, 'Immunoselection and adenoviral genetic modulation of human osteoprogenitors: in vivo bone formation on PLA scaffold', *Biochemical and Biophysical Research Communications*, vol. 299, no. 2, pp. 208-15.

Hryniewicz, T., Rokicki, R. & Rokosz, K. 2008, 'Corrosion Characteristics of Medical-Grade AISI Type 316L Stainless Steel Surface After Electropolishing in a Magnetic Field', *Corrosion*, vol. 64, no. 8, pp. 660-5.

Huadong, S., Xiaohong, L. & Zhijun, Z. 2008, 'Surface Modified Nano-Silica and Its Action on Polymer', *Progress in Chemistry*, vol. 20, no. 10, pp. 1509-14.

Ilharreborde, B., Morel, E., Fitoussi, F., Presedo, A., Souchet, P., Pennecot, G.F. & Mazda, K. 2008, 'Bioactive Glass as a Bone Substitute for Spinal Fusion in

Adolescent Idiopathic Scoliosis A Comparative Study With Iliac Crest Autograft', *Journal of Pediatric Orthopaedics*, vol. 28, no. 3, pp. 347-51.

Imola, M.J. & Schramm, V.L. 2002, 'Resorbable internal fixation in pediatric cranial base surgery', *Laryngoscope*, vol. 112, no. 10, pp. 1897-901.

Ingraham, F.D., Alexander, E., Jr. & Matson, D.D. 1947, 'Polyethylene, a new synthetic plastic for use in surgery; experimental applications in neurosurgery', *J Am Med Assoc*, vol. 135, no. 2, pp. 82-7.

Jacobsen, S. & Fritz, H.G. 1999, 'Plasticizing polylactide—the effect of different plasticizers on the mechanical properties', *Polymer Engineering & Science*, vol. 39, no. 7, pp. 1303-10.

Jahnen-Dechent, W. & Ketteler, M. 2012, 'Magnesium basics', *Clinical Kidney Journal*, vol. 5, no. Suppl 1, pp. i3-i14.

Jarcho, M. 1981, 'Calcium phosphate ceramics as hard tissue prosthetics', *Clinical orthopaedics and related research*, vol. 157, pp. 259-78.

Jie, Q., Lin, K.L., Zhong, J.P., Shi, Y.F., Li, Q., Chang, J.A. & Wang, R.D. 2004, 'Preparation of macroporous sol-gel bioglass using PVA particles as pore former', *Journal of Sol-Gel Science and Technology*, vol. 30, no. 1, pp. 49-61.

Kai, D. & Loh, X.J. 2013, 'Polyhydroxyalkanoates: Chemical Modifications Toward Biomedical Applications', *ACS Sustainable Chemistry & Engineering*, vol. 2, no. 2, pp. 106-19.

Karagiozov, C. & Momchilova, D. 2005, 'Synthesis of nano-sized particles from metal carbonates by the method of reversed mycelles', *Chemical Engineering and Processing: Process Intensification*, vol. 44, no. 1, pp. 115-9.

Kargupta, R., Bok, S., Darr, C.M., Crist, B.D., Gangopadhyay, K., Gangopadhyay, S. & Sengupta, S. 2014, 'Coatings and surface modifications imparting antimicrobial activity to orthopedic implants', *Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology*, vol. 6, no. 5, pp. 475-95.

Kel, D., Gökçe, H., Bilgiç, D., Ağaoğulları, D., Duman, I., Öveçoğlu, M.L., Kayalı, E.S., AKıyıcı, I., Agathopoulos, S. & Oktar, F.N. 2012, 'Production of Natural HA from Land Snails', *Key Eng. Materials* vol. 493, pp. 287-92.

Keledi, G., Hari, J. & Pukanszky, B. 2012, 'Polymer nanocomposites: structure, interaction, and functionality', *Nanoscale*, vol. 4, no. 6, pp. 1919-38.

Kenane, M. & Benzeggagh, M.L. 2009, 'Characterization of Mixed Mode Delamination Growth and Thresholds', in T. Boukharouba, M. Elboujdaini & G. Pluvinage (eds), *Damage and Fracture Mechanics*, Springer Netherlands, pp. 523-30.

Keselowsky, B.G., Collard, D.M. & Garcia, A.J. 2003, 'Surface chemistry modulates fibronectin conformation and directs integrin binding and specificity to control cell adhesion', *J Biomed Mater Res A*, vol. 66, no. 2, pp. 247-59.

Kickelbick, G. 2003, 'Concepts for the incorporation of inorganic building blocks into organic polymers on a nanoscale', *Progress in Polymer Science*, vol. 28, no. 1, pp. 83-114.

Kim, D., Lee, J.S., Barry, C.M.F. & Mead, J.L. 2007, 'Effect of fill factor and validation of characterizing the degree of mixing in polymer nanocomposites', *Polymer Engineering & Science*, vol. 47, no. 12, pp. 2049-56.

Kim, H.W., Lee, H.H. & Chun, G.S. 2008, 'Bioactivity and osteoblast responses of novel biomedical nanocomposites of bioactive glass nanofiber filled poly(lactic acid)', *J Biomed Mater Res A*, vol. 85, no. 3, pp. 651-63.

Kim, H.W., Song, J.H. & Kim, H.E. 2006, 'Bioactive glass nanofiber-collagen nanocomposite as a novel bone regeneration matrix', *J Biomed Mater Res A*, vol. 79, no. 3, pp. 698-705.

Kimura, H., Ogura, Y., Moritera, T., Honda, Y., Wada, R., Hyon, S.H. & Ikada, Y. 1992, 'Injectable microspheres with controlled drug release for glaucoma filtering surgery', *Invest Ophthalmol Vis Sci*, vol. 33, no. 12, pp. 3436-41.

Kivrak, N. & Taş, A.C. 1998, 'Synthesis of Calcium Hydroxyapatite-Tricalcium Phosphate (HA-TCP) Composite Bioceramic Powders and Their Sintering Behavior', *Journal of the American Ceramic Society*, vol. 81, no. 9, pp. 2245-52.

Klevens, R.M., Edwards, J.R., Richards, C.L., Jr., Horan, T.C., Gaynes, R.P., Pollock, D.A. & Cardo, D.M. 2007, 'Estimating health care-associated infections and deaths in U.S. hospitals, 2002', *Public Health Rep*, vol. 122, no. 2, pp. 160-6.

Kontonasaki, E., Papadopoulou, L., Zorba, T., Pavlidou, E., Paraskevopoulos, K. & Koidis, P. 2003, 'Apatite formation on dental ceramics modified by a bioactive glass', *J Oral Rehabil*, vol. 30, no. 9, pp. 893-902.

Kooten, T.G.V., Klein, C.L., Kohler, H., Kirkpatrick, C.J., Williams, D.F. & Eloy, R. 1997, 'From cytotoxicity to biocompatibility testing in vitro: cell adhesion molecule expression defines a new set of parameters', *Journal of Materials Science: Materials in Medicine*, vol. 8, no. 12, pp. 835-41.

Kreth, J., Hagerman, E., Tam, K., Merritt, J., Wong, D.T., Wu, B.M., Myung, N.V., Shi, W. & Qi, F. 2004, 'Quantitative analyses of Streptococcus mutans biofilms with quartz crystal microbalance, microjet impingement and confocal microscopy', *Biofilms*, vol. 1, no. 4, pp. 277-84.

Kuhne, J.H., Bartl, R., Frisch, B., Hammer, C., Jansson, V. & Zimmer, M. 1994, 'Bone formation in coralline hydroxyapatite. Effects of pore size studied in rabbits', *Acta Orthop Scand*, vol. 65, no. 3, pp. 246-52.

Kundu, B., Lemos, A., Soundrapandian, C., Sen, P.S., Datta, S., Ferreira, J.M.F. & Basu, D. 2010, 'Development of porous HAp and beta-TCP scaffolds by starch consolidation with foaming method and drug-chitosan bilayered scaffold based drug delivery system', *Journal of Materials Science-Materials in Medicine*, vol. 21, no. 11, pp. 2955-69.

Kuroda, D., Hiromoto, S., Hanawa, T. & Takada, Y. 2002, 'Corrosion Behaviour of Nickel-Free Nitrogen Austenitic Stainless Steel in Simulated Biological Environments', *Materials Transsactions*, vol. 43, no. 12, p. 5.

Langer, R. 1990, 'New methods of drug delivery', *Science*, vol. 249, no. 4976, pp. 1527-33.

Lasprilla, A.J.R., Martinez, G.A.R., Lunelli, B.H., Jardini, A.L. & Filho, R.M. 2012, 'Poly-lactic acid synthesis for application in biomedical devices — A review', *Biotechnology Advances*, vol. 30, no. 1, pp. 321-8.

LaVan, D.A., McGuire, T. & Langer, R. 2003, 'Small-scale systems for in vivo drug delivery', *Nat Biotech*, vol. 21, no. 10, pp. 1184-91.

Lee, H.H., Sang Shin, U., Lee, J.H. & Kim, H.W. 2011, 'Biomedical nanocomposites of poly(lactic acid) and calcium phosphate hybridized with modified carbon nanotubes for hard tissue implants', *J Biomed Mater Res B Appl Biomater*, vol. 98B, no. 2, pp. 246-54.

Lee, H.H., Yu, H.S., Jang, J.H. & Kim, H.W. 2008, 'Bioactivity improvement of poly(epsilon-caprolactone) membrane with the addition of nanofibrous bioactive glass', *Acta Biomater*, vol. 4, no. 3, pp. 622-9.

Lee, S. 1997, 'Mode II delamination failure mechanisms of polymer matrix composites', *Journal of Materials Science*, vol. 32, no. 5, pp. 1287-95.

LeGeros, R.Z. 1988, 'Calcium phosphate materials in restorative dentistry: a review', *Adv Dent Res*, vol. 2, no. 1, pp. 164-80.

LeGeros, R.Z. 2002, 'Properties of osteoconductive biomaterials: calcium phosphates', *Clin Orthop Relat Res*, no. 395, pp. 81-98.

LeGeros, R.Z. 2008, 'Calcium phosphate-based osteoinductive materials', *Chem Rev*, vol. 108, no. 11, pp. 4742-53.

LeGeros, R.Z., LeGeros, J.P., Daculsi, G. & Kijkowaka, R. 1995, *Encyclopedic Handbook of Biomaterials and Bioengineering*, vol. 2, Marcel Dekker, New York.

Lei, B., Shin, K.H., Noh, D.Y., Koh, Y.H., Choi, W.Y. & Kim, H.E. 2012, 'Bioactive glass microspheres as reinforcement for improving the mechanical properties and biological performance of poly(e-caprolactone) polymer for bone tissue regeneration', *Journal of Biomedical Materials Research Part B-Applied Biomaterials*, vol. 100B, no. 4, pp. 967-75.

Lemons, J.E. 1996, 'Ceramics: past, present, and future', *Bone*, vol. 19, no. 1 Suppl, pp. 121s-8s.

Li, G., Feng, S. & Zhou, D. 2011, 'Magnetic bioactive glass ceramic in the system CaO-P2O5-SiO2-MgO-CaF2-MnO2-Fe2O3 for hyperthermia treatment of bone tumor', *J Mater Sci Mater Med*, vol. 22, no. 10, pp. 2197-206.

Li, H., Chen, S., Wu, Y., Jiang, J., Ge, Y., Gao, K., Zhang, P. & Wu, L. 2012, 'Enhancement of the osseointegration of a polyethylene terephthalate artificial ligament graft in a bone tunnel using 58S bioglass', *International Orthopaedics*, vol. 36, no. 1, pp. 191-7.

Li, R., Clark, A.E. & Hench, L.L. 1991, 'An investigation of bioactive glass powders by sol-gel processing', *Journal of Applied Biomaterials*, vol. 2, no. 4, pp. 231-9.

Li, S., Ma, W., Zhou, Y., Chen, X., Ma, M., Xu, Y., Ding, Z. & Wu, X. 2013, '3-aminopropyltriethoxysilanes Modified Porous Silicon as a Voltammetric Sensor for Determination of Silver Ion', *International Journal of Electrochemical Science*, vol. 8, no. 2, p. 11.

Li, Y., Liu, Y.Z., Long, T., Yu, X.B., Tang, T.T., Dai, K.R., Tian, B., Guo, Y.P. & Zhu, Z.A. 2013, 'Mesoporous bioactive glass as a drug delivery system: fabrication,

bactericidal properties and biocompatibility', *J Mater Sci Mater Med*, vol. 24, no. 8, pp. 1951-61.

Li, Y., Yang, C., Zhao, H., Qu, S., Li, X. & Li, Y. 2014, 'New Developments of Ti-Based Alloys for Biomedical Applications', *Materials*, vol. 7, no. 3, pp. 1709-800.

Lie, S.A., Pratt, N., Ryan, P., Engesaeter, L.B., Havelin, L.I., Furnes, O. & Graves, S. 2010, 'Duration of the increase in early postoperative mortality after elective hip and knee replacement', *J Bone Joint Surg Am*, vol. 92, no. 1, pp. 58-63.

Liechty, W.B., Kryscio, D.R., Slaughter, B.V. & Peppas, N.A. 2010a, 'Polymers for Drug Delivery Systems', *Annual Review of Chemical and Biomolecular Engineering*, vol. 1, no. 1, pp. 149-73.

Liechty, W.B., Kryscio, D.R., Slaughter, B.V. & Peppas, N.A. 2010b, 'Polymers for drug delivery systems', *Annu Rev Chem Biomol Eng*, vol. 1, pp. 149-73.

Liesegang, R.E. 1896, 'Ueber einige Eigenschaften von Gallerten', *Naturwissenschaftliche Wochenschrift,*, vol. 11, no. 30, pp. 353 - 62.

Lim, G.K., Wang, J., Ng, S.C. & Gan, L.M. 1996, 'Processing of fine hydroxyapatite powders via an inverse microemulsion route', *Materials Letters*, vol. 28, no. 4–6, pp. 431-6.

Lim, G.K., Wang, J., Ng, S.C. & Gan, L.M. 1999, 'Formation of Nanocrystalline Hydroxyapatite in Nonionic Surfactant Emulsions', *Langmuir*, vol. 15, no. 22, pp. 7472-7.

Lin, C., Mao, C., Zhang, J., Li, Y. & Chen, X. 2012, 'Healing effect of bioactive glass ointment on full-thickness skin wounds', *Biomedical Materials*, vol. 7, no. 4, p. 045017.

Lin, F.-H., Liao, C.-J., Chen, K.-S. & Sun, J.-S. 1998, 'Preparation of high-temperature stabilized β-tricalcium phosphate by heating deficient hydroxyapatite with Na4P2O7 · 10H2O addition', *Biomaterials*, vol. 19, no. 11–12, pp. 1101-7.

Lin, F.-H., Liao, C.-J., Chen, K.-S., Sun, J.-S. & Lin, C.-P. 2001, 'Petal-like apatite formed on the surface of tricalcium phosphate ceramic after soaking in distilled water', *Biomaterials*, vol. 22, no. 22, pp. 2981-92.

Lindfors, N.C. 2009, 'Treatment of a recurrent aneurysmal bone cyst with bioactive glass in a child allows for good bone remodelling and growth', *Bone*, vol. 45, no. 2, pp. 398-400.

Lindfors, N.C., Hyvonen, P., Nyyssonen, M., Kirjavainen, M., Kankare, J., Gullichsen, E. & Salo, J. 2010, 'Bioactive glass S53P4 as bone graft substitute in treatment of osteomyelitis', *Bone*, vol. 47, no. 2, pp. 212-8.

Liu, A., Hong, Z., Zhuang, X., Chen, X., Cui, Y., Liu, Y. & Jing, X. 2008, 'Surface modification of bioactive glass nanoparticles and the mechanical and biological properties of poly(L-lactide) composites', *Acta Biomater*, vol. 4, no. 4, pp. 1005-15.

Liu, C., Huang, Y., Shen, W. & Cui, J. 2001, 'Kinetics of hydroxyapatite precipitation at pH 10 to 11', *Biomaterials*, vol. 22, no. 4, pp. 301-6.

Liu, H. & Webster, T.J. 2010, 'Ceramic/polymer nanocomposites with tunable drug delivery capability at specific disease sites', *Journal of Biomedical Materials Research Part A*, vol. 93A, no. 3, pp. 1180-92.

Liu, P., Luo, Q., Guan, Y. & Zhang, Y. 2010, 'Drug release kinetics from monolayer films of glucose-sensitive microgel', *Polymer*, vol. 51, no. 12, pp. 2668-75.

Liu, X., Xie, Z.P., Zhang, C.Q., Pan, H.B., Rahaman, M.N., Zhang, X., Fu, Q. & Huang, W.H. 2010, 'Bioactive borate glass scaffolds: in vitro and in vivo evaluation for use as a drug delivery system in the treatment of bone infection', *Journal of Materials Science-Materials in Medicine*, vol. 21, no. 2, pp. 575-82.

Lou, X., Detrembleur, C. & Jérôme, R. 2003, 'Novel Aliphatic Polyesters Based on Functional Cyclic (Di)Esters', *Macromolecular Rapid Communications*, vol. 24, no. 2, pp. 161-72.

Lovell-Badge, R. 2007, 'Many ways to pluripotency', *Nat Biotech*, vol. 25, no. 10, pp. 1114-6.

Ma, J., Chen, C., Wang, D., Meng, X. & Shi, J. 2010, 'In vitro degradability and bioactivity of mesoporous CaO-MgO-P₂O₅-SiO₂ glasses synthesized by sol–gel method', *Journal of Sol-Gel Science and Technology*, vol. 54, no. 1, pp. 69-76.

Macha, I.J., Ben-Nissan, B. & Milthorpe, B. 2014, 'Improvement of Elongation in Nanosurface Modified Bioglass/PLA Thin Film Composites', *Current Nanoscience*, vol. 10, no. 2, pp. 200-4.

Macha, I.J., Boonyang, U., Cazalbou, S., Ben-Nissan, B., Charvillat, C., Oktar, F.N. & Grossin, D. 2015, 'Comparative study of Coral Conversion, Part 2: Microstructural evolution of calcium phosphate', *Journal of the Australian Ceramic Society* vol. 52, no. 2.

Macha, I.J., Cazalbou, S., Ben-Nissan, B., Harvey, K.L. & Milthorpe, B. 2015, 'Marine structure derived calcium phosphate-polymer biocomposites for local antibiotic delivery', *Mar Drugs*, vol. 13, no. 1, pp. 666-80.

Macha, I.J., Cazalbou, S., Shimmon, R., Ben-Nissan, B. & Milthorpe, B. 2015, 'Development and dissolution studies of Bisphosphonate (Clodronate) containing hydroxyapatite/PLA biocomposites for slow drug delivery ', *Journal of Tissue Engineering and Regenerative Medicine*.

Macha, I.J., Ozyegin, L.S., Chou, J., Samur, R., Oktar, F.N. & Ben-Nissan, B. 2013, 'An Alternative Synthesis Method for Di Calcium Phosphate (Monetite) Powders from Mediterranean Mussel (Mytilus galloprovincialis) Shells', *Journal of The Australian Ceramic Society*, vol. 49, no. 2, pp. 122-8.

Macha, I.J., Ozyegin, L.S., Oktar, F.N. & Ben-Nissan, B. 2015, 'Conversion of Ostrich Eggshells (Struthio camelus) to Calcium Phosphates', *Journal of The Australian Ceramic Society* vol. 51, no. 2, pp. 125-33.

Mah, T.-F., Pitts, B., Pellock, B., Walker, G.C., Stewart, P.S. & O'Toole, G.A. 2003, 'A genetic basis for Pseudomonas aeruginosa biofilm antibiotic resistance', *Nature*, vol. 426, no. 6964, pp. 306-10.

Marshall, B.C. & Hazl, L. 2011, *Cystic Fibrosis Patient Registry Annual Data Report*, Cystic Fibrosis Foundation, Bethesda, Maryland USA.

Mauger, J.W., Chilko, D. & Howard, S. 1986, 'On the Analysis of Dissolution Data', *Drug Development and Industrial Pharmacy*, vol. 12, no. 7, pp. 969-92.

McCutcheon, A.L., Kannangara, G.S.K., Wilson, M.A. & Ben-Nissan, B. 2004, 'Preliminary analysis of pore distributions using NMR in natural coral and hydrothermally prepared hydroxyapatite', *Journal of Materials Science*, vol. 39, no. 18, pp. 5711-7.

McGee, D.C. & Gould, M.K. 2003, 'Preventing Complications of Central Venous Catheterization', *New England Journal of Medicine*, vol. 348, no. 12, pp. 1123-33.

Meejoo, S., Maneeprakorn, W. & Winotai, P. 2006, 'Phase and thermal stability of nanocrystalline hydroxyapatite prepared via microwave heating', *Thermochimica Acta*, vol. 447, no. 1, pp. 115-20.

Mehdikhani-Nahrkhalaji, M., Fathi, M.H., Mortazavi, V., Mousavi, S.B., Hashemi-Beni, B. & Razavi, S.M. 2012, 'Novel nanocomposite coating for dental implant applications in vitro and in vivo evaluation', *J Mater Sci Mater Med*, vol. 23, no. 2, pp. 485-95.

Merrer, J., De Jonghe, B., Golliot, F. & et al. 2001, 'Complications of femoral and subclavian venous catheterization in critically ill patients: A randomized controlled trial', *JAMA*, vol. 286, no. 6, pp. 700-7.

Merwin, G.E. 1990, 'Review of Bioactive Materials for Otologic and Maxillofacial Applications', in T. Yamamuro, L.L. Hench & J.Wilson (eds), *Handbook of Bioactive Ceramics*, vol. 1, CRC Press, Boca Raton, Florida, p. 323.

Middleton, J.C. & Tipton, A.J. 2000, 'Synthetic biodegradable polymers as orthopedic devices', *Biomaterials*, vol. 21, no. 23, pp. 2335-46.

Milev, A., Kannangara, G.S.K. & Ben-Nissan, B. 2003, 'Morphological Stability of Plate-Like Hydroxyapatite', *Key Engineering Materials*, vol. 240-242, pp. 481-4.

Mohammadi, G., Barzegar-Jalali, M., Valizadeh, H., Nazemiyeh, H., Barzegar-Jalali, A., Siahi Shadbad, M.R., Adibkia, K. & Zare, M. 2010, 'Reciprocal powered time model for release kinetic analysis of ibuprofen solid dispersions in oleaster powder, microcrystalline cellulose and crospovidone', *J Pharm Pharm Sci*, vol. 13, no. 2, pp. 152-61.

Mohn, D., Zehnder, M., Imfeld, T. & Stark, W.J. 2010, 'Radio-opaque nanosized bioactive glass for potential root canal application: evaluation of radiopacity, bioactivity and alkaline capacity', *International Endodontic Journal*, vol. 43, no. 3, pp. 210-7.

Moncada, E., Quijada, R. & Retuert, J. 2007, 'Nanoparticles prepared by the sol–gel method and their use in the formation of nanocomposites with polypropylene', *Nanotechnology*, vol. 18, no. 33, p. 335606.

Mota, J., Yu, N., Caridade, S.G., Luz, G., Gomes, M.E., Reis, R.L., Jansen, J.A., Walboomers, X.F. & Mano, J.F. 2012, 'Chitosan/bioactive glass nanoparticle composite membranes for periodontal regeneration', *Acta Biomater*.

Muller, U. 2008, 'In vitro biocompatibility testing of biomaterials and medical devices', *Med Device Technol*, vol. 19, no. 2, pp. 30, 2-4.

Nair, L.S. & Laurencin, C.T. 2007, 'Biodegradable polymers as biomaterials', *Progress in Polymer Science*, vol. 32, no. 8–9, pp. 762-98.

Nam, K., Watanabe, J. & Ishihara, K. 2004, 'Modeling of swelling and drug release behavior of spontaneously forming hydrogels composed of phospholipid polymers', *International Journal of Pharmaceutics*, vol. 275, no. 1–2, pp. 259-69.

Navarro, M., Michiardi, A., Castaño, O. & Planell, J.A. 2008, 'Biomaterials in orthopaedics', *Journal of The Royal Society Interface*, vol. 5, no. 27, pp. 1137-58.

Nelson, C.L., Hickmon, S.G. & Skinner, R.A. 1997, 'Treatment of experimental osteomyelitis by surgical debridement and the implantation of bioerodable, polyanhydride-gentamicin beads', *Journal of Orthopaedic Research*, vol. 15, no. 2, pp. 249-55.

Niemelä, T., Niiranen, H., Kellomäki, M. & Törmälä, P. 2005, 'Self-reinforced composites of bioabsorbable polymer and bioactive glass with different bioactive glass contents. Part I: Initial mechanical properties and bioactivity', *Acta Biomaterialia*, vol. 1, no. 2, pp. 235-42.

Niiranen, H. & To¨rma¨la¨, P. 1999, 'Bioabsorbable polymer plates coated with bioactive glass spheres', *Journal of Materials Science: Materials in Medicine*, vol. 10, no. 12, pp. 707-10.

Noh, H. & Vogler, E.A. 2007, 'Volumetric interpretation of protein adsorption: Competition from mixtures and the Vroman effect', *Biomaterials*, vol. 28, no. 3, pp. 405-22.

Norton, M.R. & Wilson, J. 2002, 'Dental implants placed in extraction sites implanted with bioactive glass: human histology and clinical outcome', *Int J Oral Maxillofac Implants*, vol. 17, no. 2, pp. 249-57.

Oktar, F., Tuyel, U., Demirkol, N., Gunduz, O., Samur, R., Kannan, S. & Agathopoulos, S. 2010, 'A new safe method to produce bioceramic nano-powders from

nacre venus verrucosa', paper presented to the *Artificial Organs Conference, FYROM, 2010*.

Orgaz-Orgaz, F. 1988, 'Gel to glass conversion: Densification kinetics and controlling mechanisms', *J. Non-Cryst. Solids*, vol. 100, p. 115.

Palacio, M.L. & Bhushan, B. 2012, 'Bioadhesion: a review of concepts and applications', *Philos Trans A Math Phys Eng Sci*, vol. 370, no. 1967, pp. 2321-47.

Pascaud, P., Bareille, R., Bourget, C., Amedee, J., Rey, C. & Sarda, S. 2012, 'Interaction between a bisphosphonate, tiludronate and nanocrystalline apatite: in vitro viability and proliferation of HOP and HBMSC cells', *Biomed Mater*, vol. 7, no. 5, p. 054108.

Pereira, M.M. & Hench, L.L. 1996, 'Mechanisms of hydroxyapatite formation on porous gel-silica substrates', *Journal of Sol-Gel Science and Technology*, vol. 7, no. 1-2, pp. 59-68.

Pernaa, K., Koski, I., Mattila, K., Gullichsen, E., Heikkila, J., Aho, A. & Lindfors, N. 2011, 'Bioactive Glass S53P4 and Autograft Bone in Treatment of Depressed Tibial Plateau Fractures - A Prospective Randomized 11-year Follow-Up', *J Long Term Eff Med Implants*, vol. 21, no. 2, pp. 139-48.

Pirkey, E.L. & Hurt, J. 1959, 'Roentgen Evaluation of the Soft Tissues in Orthopedics', *American Journal of Roentgenology Radium Therapy and Nuclear Medicine*, vol. 82, no. 2, pp. 271-6.

Pratsinis, S.E. 1998, 'Flame aerosol synthesis of ceramic powders', *Progress in Energy and Combustion Science*, vol. 24, no. 3, pp. 197-219.

Prior, S., Gamazo, C., Irache, J.M., Merkle, H.P. & Gander, B. 2000, 'Gentamicin encapsulation in PLA/PLGA microspheres in view of treating Brucella infections', *Int J Pharm*, vol. 196, no. 1, pp. 115-25.

Qian, J.M., Kang, Y.H., Wei, Z.L. & Zhang, W. 2009, 'Fabrication and characterization of biomorphic 45S5 bioglass scaffold from sugarcane', *Materials Science & Engineering C-Biomimetic and Supramolecular Systems*, vol. 29, no. 4, pp. 1361-4.

Quintero, F., Dieste, O., Pou, J., Lusqui˜nos, F. & Riveiro, A. 2009, 'On the conditions to produce micro- and nanofibres by laser spinning', *J. Phys. D: Appl. Phys.*, vol. 42, pp. 1-10.

Quintero, F., Mann, A.B., Pou, J., Lusquinos, F. & Riveiro, A. 2007, 'Rapid production of ultralong amorphous ceramic nanofibers by laser spinning', *Applied Physics Letters*, vol. 90, no. 15, pp. 153109-3.

Quintero, F., Pou, J., Comesaña, R., Lusquiños, F., Riveiro, A., Mann, A.B., Hill, R.G., Wu, Z.Y. & Jones, J.R. 2009, 'Laser Spinning of Bioactive Glass Nanofibers', *Advanced Functional Materials*, vol. 19, no. 19, pp. 3084-90.

Quintero, F., Pou, J., Lusquiños, F. & Riveiro, A. 2007, 'Experimental analysis of the production of micro- and nanofibres by Laser Spinning', *Applied Surface Science*, vol. 254, no. 4, pp. 1042-7.

Rahman, I.A. & Padavettan, V. 2012, 'Synthesis of Silica Nanoparticles by Sol-Gel: Size-Dependent Properties, Surface Modification, and Applications in Silica-Polymer Nanocomposites—A Review', *Journal of Nanomaterials*, vol. 2012, p. 15.

Ramires, P.A., Wennerberg, A., Johansson, C.B., Cosentino, F., Tundo, S. & Milella, E. 2003, 'Biological behavior of sol-gel coated dental implants', *J Mater Sci Mater Med*, vol. 14, no. 6, pp. 539-45.

Rantakokko, J., Frantzen, J.P., Heinanen, J., Kajander, S., Kotilainen, E., Gullichsen, E. & Lindfors, N.C. 2012, 'Posterolateral Spondylodesis Using Bioactive Glass S53p4 and Autogenous Bone in Instrumented Unstable Lumbar Spine Burst Fractures', *Scandinavian Journal of Surgery*, vol. 101, no. 1, pp. 66-71.

Rao, M.S.a.K.P. 2002, 'Preparation, characterization and in vitro release of gentamicin from coralline hydroxyapatite-gelatin composite microspheres', *Biomaterials*, vol. 23, no. 15, pp. 3175-81.

Rey, C., Marsan, O., Combes, C., Drouet, C., Grossin, D. & Sarda, S. 2014, 'Characterization of Calcium Phosphates Using Vibrational Spectroscopies', in B. Ben-Nissan (ed.), *Advances in Calcium Phosphate Biomaterials*, vol. 2, Springer Berlin Heidelberg, pp. 229-66.

Rothstein, S.N., Federspiel, W.J. & Little, S.R. 2008, 'A simple model framework for the prediction of controlled release from bulk eroding polymer matrices', *Journal of Materials Chemistry*, vol. 18, no. 16, pp. 1873-80.

Rothstein, S.N., Federspiel, W.J. & Little, S.R. 2009, 'A unified mathematical model for the prediction of controlled release from surface and bulk eroding polymer matrices', *Biomaterials*, vol. 30, no. 8, pp. 1657-64.

Ryu, H.-S., Youn, H.-J., Sun Hong, K., Chang, B.-S., Lee, C.-K. & Chung, S.-S. 2002, 'An improvement in sintering property of β-tricalcium phosphate by addition of calcium pyrophosphate', *Biomaterials*, vol. 23, no. 3, pp. 909-14.

Saboori, A., Rabiee, M., Mutarzadeh, F., Sheikhi, M., Tahriri, M. & Karimi, M. 2009, 'Synthesis, characterization and in vitro bioactivity of sol-gel-derived SiO2-CaO-P2O5-MgO bioglass', *Materials Science & Engineering C-Biomimetic and Supramolecular Systems*, vol. 29, no. 1, pp. 335-40.

Sakai, K., Kawano, H., Iwami, A., Nakamura, M. & Moriguchi, M. 2001, 'Isolation of a thermophilic poly-L-lactide degrading bacterium from compost and its enzymatic characterization', *J Biosci Bioeng*, vol. 92, no. 3, pp. 298-300.

Sakka, S. 1985, 'Glasses and glass-ceramics from gels', *J. Non-Cryst. Solids*, vol. 73, p. 651.

Sampath, S.S., Garvin, K. & Robinson, D.H. 1992, 'Preparation and characterization of biodegradable poly(l-lactic acid) gentamicin delivery systems', *International Journal of Pharmaceutics*, vol. 78, no. 1–3, pp. 165-74.

Santos, A., Sinn Aw, M., Bariana, M., Kumeria, T., Wang, Y. & Losic, D. 2014, 'Drug-releasing implants: current progress, challenges and perspectives', *Journal of Materials Chemistry B*, vol. 2, no. 37, pp. 6157-82.

Saska, S., Barud, H.S., Gaspar, A.M., Marchetto, R., Ribeiro, S.J. & Messaddeq, Y. 2011, 'Bacterial cellulose-hydroxyapatite nanocomposites for bone regeneration', *Int J Biomater*, vol. 2011, p. 175362.

Scheele, C.W. 1980, 'Kgl. Vetenskaps-Academiens nya Handlingar', *Stockholm*, vol. 1, pp. 116-24.

Seal, B.L., Otero, T.C. & Panitch, A. 2001, 'Polymeric biomaterials for tissue and organ regeneration', *Materials Science and Engineering: R: Reports*, vol. 34, no. 4–5, pp. 147-230.

Sepulveda, P., Jones, J.R. & Hench, L.L. 2001, 'Characterization of melt-derived 45S5 and sol-gel-derived 58S bioactive glasses', *Journal of Biomedical Materials Research*, vol. 58, no. 6, pp. 734-40.

Severino, P., Santana, M.H.A., Malmonge, S.M. & Souto, E.B. 2011, 'Polymers for Drug Delivery Systems Formulations', *Polimeros-Ciencia E Tecnologia*, vol. 21, no. 5, pp. 361-8.

Shih, C. 1995, 'Chain-end scission in acid catalyzed hydrolysis of poly (d,l-lactide) in solution', *Journal of Controlled Release*, vol. 34, no. 1, pp. 9-15.

Siepmann, J. & Siepmann, F. 2008, 'Mathematical modeling of drug delivery', *International Journal of Pharmaceutics*, vol. 364, no. 2, pp. 328-43.

Singh, R. & Dahotre, N.B. 2007, 'Corrosion degradation and prevention by surface modification of biometallic materials', *J Mater Sci Mater Med*, vol. 18, no. 5, pp. 725-51.

Singh, S., Bhardwaj, P., Singh, V., Aggarwal, S. & Mandal, U.K. 2008, 'Synthesis of nanocrystalline calcium phosphate in microemulsion—effect of nature of surfactants', *Journal of Colloid and Interface Science*, vol. 319, no. 1, pp. 322-9.

Smeaton, W.A. 1986, 'Carl Wilhelm Scheele (1742–1786)', *Endeavour*, vol. 10, no. 1, pp. 28-30.

Södergård, A. & Stolt, M. 2002, 'Properties of lactic acid based polymers and their correlation with composition', *Progress in Polymer Science*, vol. 27, no. 6, pp. 1123-63.

Sokolsky-Papkov, M., Agashi, K., Olaye, A., Shakesheff, K. & Domb, A.J. 2007, 'Polymer carriers for drug delivery in tissue engineering', *Advanced Drug Delivery Reviews*, vol. 59, no. 4–5, pp. 187-206.

Soundrapandian, C., Basu, D., Sa, B. & Datta, S. 2011, 'Local drug delivery system for the treatment of osteomyelitis: In vitro evaluation', *Drug Development and Industrial Pharmacy*, vol. 37, no. 5, pp. 538-46.

Soundrapandian, C., Datta, S., Kundu, B., Basu, D. & Sa, B. 2010, 'Porous Bioactive Glass Scaffolds for Local Drug Delivery in Osteomyelitis: Development and In Vitro Characterization', *Aaps Pharmscitech*, vol. 11, no. 4, pp. 1675-83.

Stark, W.J., Madler, L., Maciejewski, M., Pratsinis, S.E. & Baiker, A. 2003, 'Flame synthesis of nanocrystalline ceria-zirconia: effect of carrier liquid', *Chemical Communications*, no. 5, pp. 588-9.

Stephens, D., Li, L., Robinson, D., Chen, S., Chang, H., Liu, R.M., Tian, Y., Ginsburg, E.J., Gao, X. & Stultz, T. 2000, 'Investigation of the in vitro release of gentamicin from a polyanhydride matrix', *J Control Release*, vol. 63, no. 3, pp. 305-17.

Stewart, P.S. 2002, 'Mechanisms of antibiotic resistance in bacterial biofilms', *International Journal of Medical Microbiology*, vol. 292, no. 2, pp. 107-13.

Suchanek, W. & Yoshimura, M. 1998, 'Processing and properties of hydroxyapatite-based biomaterials for use as hard tissue replacement implants', *Journal of Materials Research*, vol. 13, no. 01, pp. 94-117.

Sun, Y., Guo, G., Tao, D. & Wang, Z. 2007, 'Reverse microemulsion-directed synthesis of hydroxyapatite nanoparticles under hydrothermal conditions', *Journal of Physics and Chemistry of Solids*, vol. 68, no. 3, pp. 373-7.

Tacconelli, E., Smith, G., Hieke, K., Lafuma, A. & Bastide, P. 2009, 'Epidemiology, medical outcomes and costs of catheter-related bloodstream infections in intensive care units of four European countries: literature- and registry-based estimates', *Journal of Hospital Infection*, vol. 72, no. 2, pp. 97-103.

Talal, A., Waheed, N., Al-Masri, M., McKay, I.J., Tanner, K.E. & Hughes, F.J. 2009, 'Absorption and release of protein from hydroxyapatite-polylactic acid (HA-PLA) membranes', *J Dent*, vol. 37, no. 11, pp. 820-6.

Talha, M., Behera, C.K. & Sinha, O.P. 2013, 'A review on nickel-free nitrogen containing austenitic stainless steels for biomedical applications', *Mater Sci Eng C Mater Biol Appl*, vol. 33, no. 7, pp. 3563-75.

Tang, Q.L., Zhu, Y.J., Duan, Y.R., Wang, Q., Wang, K.W., Cao, S.W., Chen, F. & Wu, J. 2010, 'Porous nanocomposites of PEG-PLA/calcium phosphate: room-temperature synthesis and its application in drug delivery', *Dalton Transactions*, vol. 39, no. 18, pp. 4435-9.

Thanh, N.T.K. & Green, L.A.W. 2010, 'Functionalisation of nanoparticles for biomedical applications', *Nano Today*, vol. 5, no. 3, pp. 213-30.

Thevenot, P., Hu, W. & Tang, L. 2008, 'SURFACE CHEMISTRY INFLUENCE IMPLANT BIOCOMPATIBILITY', *Current topics in medicinal chemistry*, vol. 8, no. 4, pp. 270-80.

Thompson, I.D. & Hench, L.L. 1998, 'Mechanical properties of bioactive glasses, glass-ceramics and composites', *Proceedings of the Institution of Mechanical Engineers Part H-Journal of Engineering in Medicine*, vol. 212, no. H2, pp. 127-36.

Tian, H., Tang, Z., Zhuang, X., Chen, X. & Jing, X. 2012, 'Biodegradable synthetic polymers: Preparation, functionalization and biomedical application', *Progress in Polymer Science*, vol. 37, no. 2, pp. 237-80.

Tirrell, M., Kokkoli, E. & Biesalski, M. 2002, 'The role of surface science in bioengineered materials', *Surface Science*, vol. 500, no. 1–3, pp. 61-83.

Tjong, S.C. 2006, 'Structural and mechanical properties of polymer nanocomposites', *Materials Science and Engineering: R: Reports*, vol. 53, no. 3–4, pp. 73-197.

Tokgozoglu, A.M. 1998, 'The use of bioresorbable implants in orthopaedics', *Biomedical Science and Technology*, pp. 197-203.

Törmälä, P. 1992, 'Biodegradable self-reinforced composite materials; Manufacturing structure and mechanical properties', *Clinical Materials*, vol. 10, no. 1–2, pp. 29-34.

Tsigkou, O., Hench, L.L., Boccaccini, A.R., Polak, J.M. & Stevens, M.M. 2007, 'Enhanced differentiation and mineralization of human fetal osteoblasts on PDLLA containing Bioglass® composite films in the absence of osteogenic supplements', *Journal of Biomedical Materials Research Part A*, vol. 80A, no. 4, pp. 837-51.

Tsukrov, I. & Kachanov, M. 1997, 'Stress concentrations and microfracturing patterns in a brittle-elastic solid with interacting pores of diverse shapes', *International Journal of Solids and Structures*, vol. 34, no. 22, pp. 2887-904.

Ustariz-Peyret, C., Coudane, J., Vert, M., Kaltsatos, V. & Boisramene, B. 2000, 'Labile conjugation of a hydrophilic drug to PLA oligomers to modify a drug delivery system: cephradin in a PLAGA matrix', *Journal of Microencapsulation*, vol. 17, no. 5, pp. 615-24.

Valenzuela, F., Covarrubias, C., Martinez, C., Smith, P., Diaz-Dosque, M. & Yazdani-Pedram, M. 2012, 'Preparation and bioactive properties of novel bone-repair bionanocomposites based on hydroxyapatite and bioactive glass nanoparticles', *J Biomed Mater Res B Appl Biomater*, vol. 100, no. 6, pp. 1672-82.

Vedantham, K., Swet, J.H., McKillop, I.H. & El-Ghannam, A. 2012, 'Evaluation of a bioresorbable drug delivery system for the treatment of hepatocellular carcinoma', *Journal of Biomedical Materials Research Part A*, vol. 100A, no. 2, pp. 432-40.

Vendra, V.K., Wu, L. & Krishnan, S. 2015, 'Polymer Thin Films for Biomedical Applications', in C.S.S.R. Kumar (ed.), *Nanotechnologies for the Life Sciences*, vol. 5, WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany, p. 54.

Vermonden, T., Censi, R. & Hennink, W.E. 2012, 'Hydrogels for Protein Delivery', *Chemical Reviews*, vol. 112, no. 5, pp. 2853-88.

Verne, E., Vitale-Brovarone, C., Bui, E., Bianchi, C.L. & Boccaccini, A.R. 2009, 'Surface functionalization of bioactive glasses', *J Biomed Mater Res A*, vol. 90, no. 4, pp. 981-92.

Vert, M., Doi, Y., Hellwich, K.-H., Hess, M., Hodge, P., Kubisa, P., Rinaudo, M. & Schué, F. 2012, 'Terminology for biorelated polymers and applications (IUPAC Recommendations 2012)', *Pure and Applied Chemistry*, vol. 84, no. 2, p. 34.

Vert, M., Li, S. & Garreau, H. 1991, 'More about the degradation of LA/GA-derived matrices in aqueous media', *Journal of Controlled Release*, vol. 16, no. 1–2, pp. 15-26.

Vidal, C.V. & Muñoz, A.I. 2009, 'Effect of thermal treatment and applied potential on the electrochemical behaviour of CoCrMo biomedical alloy', *Electrochimica Acta*, vol. 54, no. 6, pp. 1798-809.

Vollenweider, M., Brunner, T.J., Knecht, S., Grass, R.N., Zehnder, M., Imfeld, T. & Stark, W.J. 2007, 'Remineralization of human dentin using ultrafine bioactive glass particles', *Acta Biomaterialia*, vol. 3, no. 6, pp. 936-43.

von Eiff, C., Jansen, B., Kohnen, W. & Becker, K. 2005, 'Infections associated with medical devices: pathogenesis, management and prophylaxis', *Drugs*, vol. 65, no. 2, pp. 179-214.

Wang, G., Liu, S.-J., Ueng, S.W.-N. & Chan, E.-C. 2004, 'The release of cefazolin and gentamicin from biodegradable PLA/PGA beads', *International Journal of Pharmaceutics*, vol. 273, no. 1–2, pp. 203-12.

Wang, M., Hench, L.L. & Bonfield, W. 1998, 'Bioglass/high density polyethylene composite for soft tissue applications: preparation and evaluation', *J Biomed Mater Res*, vol. 42, no. 4, pp. 577-86.

Wiegand, C. & Hipler, U.-C. 2010, 'Polymer-based Biomaterials as Dressings for Chronic Stagnating Wounds', *Macromolecular Symposia*, vol. 294, no. 2, pp. 1-13.

Willerth, S.M. & Sakiyama-Elbert, S.E. 2007, 'Approaches to neural tissue engineering using scaffolds for drug delivery', *Advanced Drug Delivery Reviews*, vol. 59, no. 4–5, pp. 325-38.

Wren, A.W., Coughlan, A., Hassanzadeh, P. & Towler, M.R. 2012, 'Silver coated bioactive glass particles for wound healing applications', *J Mater Sci Mater Med*, vol. 23, no. 5, pp. 1331-41.

Wu, C., Fan, W., Chang, J. & Xiao, Y. 2013, 'Mesoporous bioactive glass scaffolds for efficient delivery of vascular endothelial growth factor', *J Biomater Appl*, vol. 28, no. 3, pp. 367-74.

Xie, F., Ji, S. & Cheng, Z. 2015, 'In vitro dissolution similarity factor (f2) and in vivo bioequivalence criteria, how and when do they match? Using a BCS class II drug as a simulation example', *European Journal of Pharmaceutical Sciences*, vol. 66, no. 0, pp. 163-72.

Xu, C., Zhang, Y., Cheng, G. & Zhu, W. 2008, 'Pitting corrosion behavior of 316L stainless steel in the media of sulphate-reducing and iron-oxidizing bacteria', *Materials Characterization*, vol. 59, no. 3, pp. 245-55.

Yamamoto, A., Honma, R. & Sumita, M. 1998, 'Cytotoxicity evaluation of 43 metal salts using murine fibroblasts and osteoblastic cells', *Journal of Biomedical Materials Research*, vol. 39, no. 2, pp. 331-40.

Yamamoto, A., Kohyama, Y. & Hanawa, T. 2002, 'Mutagenicity evaluation of forty-one metal salts by the umu test', *Journal of Biomedical Materials Research*, vol. 59, no. 1, pp. 176-83.

Yamamoto, A., Mishima, S., Maruyama, N. & Sumita, M. 1998, 'A new technique for direct measurement of the shear force necessary to detach a cell from a material', *Biomaterials*, vol. 19, no. 7–9, pp. 871-9.

Yang, X. & Wang, Z. 1998, 'Synthesis of biphasic ceramics of hydroxyapatite and [small beta]-tricalcium phosphate with controlled phase content and porosity', *Journal of Materials Chemistry*, vol. 8, no. 10, pp. 2233-7.

Yoshioka, S. & Kitano, Y. 1985, 'Transformation of aragonite to calcite through heating', *GEOCHEMICAL JOURNAL*, vol. 19, no. 4, pp. 245-9.

Zammarano, M., Maupin, P.H., Sung, L.P., Gilman, J.W., McCarthy, E.D., Kim, Y.S. & Fox, D.M. 2011, 'Revealing the interface in polymer nanocomposites', *Acs Nano*, vol. 5, no. 4, pp. 3391-9.

Zhang, J., Wang, M., Cha, J.M. & Mantalaris, A. 2009, 'The incorporation of 70s bioactive glass to the osteogenic differentiation of murine embryonic stem cells in 3D bioreactors', *Journal of Tissue Engineering and Regenerative Medicine*, vol. 3, no. 1, pp. 63-71.

Zhou, Q., Pramoda, K.P., Lee, J.M., Wang, K. & Loo, L.S. 2011, 'Role of interface in dispersion and surface energetics of polymer nanocomposites containing hydrophilic POSS and layered silicates', *J Colloid Interface Sci*, vol. 355, no. 1, pp. 222-30.

Zhou, Y., Li, H., Lin, K., Zhai, W., Gu, W. & Chang, J. 2012, 'Effect of heat treatment on the properties of SiO(2)-CaO-MgO-P (2)O (5) bioactive glasses', *J Mater Sci Mater Med*.

Zhou, Z.H., Ruan, J.M., Zou, J.P., Zhou, Z.C. & Shen, X.J. 2007, 'Bioactivity of bioresorbable composite based on bioactive glass and poly-L-lactide', *Transactions of Nonferrous Metals Society of China*, vol. 17, no. 2, pp. 394-9.

Zhu, G., Mallery, S.R. & Schwendeman, S.P. 2000, 'Stabilization of proteins encapsulated in injectable poly (lactide- co-glycolide)', *Nat Biotech*, vol. 18, no. 1, pp. 52-7.

Zhu, M., Wang, H., Liu, J., He, H., Hua, X., He, Q., Zhang, L., Ye, X. & Shi, J. 2011, 'A mesoporous silica nanoparticulate/beta-TCP/BG composite drug delivery system for osteoarticular tuberculosis therapy', *Biomaterials*, vol. 32, no. 7, pp. 1986-95.

EXPERIMENTAL WORK

CHAPTER 2

PLA THIN FILMS

CHAPTER 2: PLA THIN FILMS

2.1 General introduction

This chapter provides a brief background of PLA. It describes the experimental work performed for the aim of producing and characterizing PLA thin films for biomedical applications. It also entails the use of established and modified synthesis methods and modern characterization techniques. The results obtained are also presented in this chapter with detailed discussions, which include comparison of mechanical properties of thin films with bulk samples. The ability to conform to any shape or size, due to its excellent ease of formability, PLA thin films have advantages over other biopolymers for a wide range of clinical applications.

2.2 PLA

Acid anhydrides, such as poly(2-hydroxypropanoic acid), also known as poly(lactic acid) or PLA, and its co-polymers, are among the group of biodegradable synthetic polymers that have been used either alone or in combination for tissue engineering (Macha, Ben-Nissan & Milthorpe 2014). The history of PLA started when its monomer, Lactic acid was discovered by a German-Swedish experimental chemist in 1780 who isolated the acid of milk from sour whey (Scheele 1980; Smeaton 1986). The first commercial production of synthetic lactic acid started in Japan (Benninga 1990) with the idea of producing acetaldehyde and hydrogen cyanide and then hydrolyze in the second stage to lactic acid. The existence of both a hydroxyl and a carboxyl group in lactic acid enables it to be converted directly into polylactic acid via a polycondensation reaction. For many decades, PLA use was limited especially in the biomedical field due to its high cost and low molecular weight. Cargill Inc. brought a revolution in the early 1990s when they succeeded in polymerizing high molecular weight PLA using a commercially viable ring-opening reaction (Carothers, Dorough & Natta 1932; Gruber et al. 2001).

At present, PLA is one of the most promising polymeric clinical materials and has drawn a lot of attention from scientists and industrialists. Its synthesis methods; physical, mechanical, optical and biological properties have been extensively studied (Drumright, Gruber & Henton 2000). Pure PLA is a semi-crystalline polymer with a glass transition temperature T_g of about 55 ^oC and melting point

(T_m) of about 180 ^oC (Södergård & Stolt 2002). Polymers prepared from meso or rac-lactide are in general amorphous, but by applying a stereo-selective catalyst polymers having tacticity high enough for crystallization to also have been obtained. The crystal structure of PLA was studied and reported to be the left-handed helix conformation for the α-form (De Santis & Kovacs 1968). The solubility of PLA highly depends on the degree of crystallinity, polymer molar mass and other co-monomer units present in the polymer. It has been reported that PLA is soluble in most organic solvents such as acetone, pyridine, ethyl lactate, tetrahydrofuran, xylene, ethyl acetate, dimethylsulfoxide, N-N dimethylformamide and methylethyl ketone (Södergård & Stolt 2002). PLA is insoluble in water, alcohols (e.g. ethanol, propylene glycol) and unsubstituted hydrocarbons (e.g. hexane, heptanes).

Lactic acid (2-hydroxypropionic acid) is a simple chiral molecule that exists as two enantiomers, L- and D-lactic acid, which differs in their effect on polarized light. The optically inactive D, L or meso form is an equimolar (racemic) mixture of D(−) and L(+) isomers (Gupta & Kumar 2007). The stereochemistry and thermal history have direct influence on PLA crystallinity, and therefore, on its properties in general. PLA with PLLA content higher than 90% tends to be crystalline, while the lower optically pure is amorphous. Semi-crystalline PLA has higher mechanical properties than the amorphous. It has been reported to have an approximate tensile modulus of 3 GPa, tensile strength of 50 – 70 MPa, flexural modulus of 5 GPa, flexural strength of 100 MPa, and an elongation at break of about 4% (Grijpma et al. 1992; Törmälä 1992; Fambri et al. 1997; Jacobsen & Fritz 1999).

Due to the presence of ester bond in the polymer back bone, PLA degradation occurs by uptake of water followed by the hydrolytic splitting of the ester bonds in a random way, according to the Flory principles, which postulates that all the linkages have the same reactivity (Shih 1995). PLA is also believed to be degraded by enzymes and bacteria. Several bacteria such as *Brevibacillus, Bacillus smithii, Geobacillus*, and *Bacillus licheniformis* (Chu et al. 1999; Sakai et al. 2001) have been reported to induce PLA-degrading ability. Literature shows that PLA-degrading enzymes such as esterases have been investigated and drawn a lot of attention.

Unlike natural polymers, synthetic polymers like PLA, are hydrolyzed into low molecular weight oligomers and then to monomers by either water or cerum. Agarwal and his co-workers investigated the effect of water, microbes and temperature in the degradation process of lactic acid based polymers (Agarwal, Koelling & Chalmers 1998). They concluded that there was no significant difference in the rate or mechanism of degradation attributable to the presence of microorganisms but the extent of degradation increased at higher process temperatures. Low molecular weight polymers are more susceptible to degradation than high molecular weight polymers and the amorphous materials degrade more easily than the crystalline materials. Degradation kinetics depend on different factors, such as: chemical composition and configurational structure, processing history, molar mass M_w, mass to polydispersity ratio M_w/M_n, environmental conditions, stress and strain, crystallinity, device size, morphology (e.g. porosity) and chain orientation. Moreover, it depends on distribution of chemically reactive compounds within the matrix and overall hydrophilicity (Vert, Li & Garreau 1991; Dunn, Campbell & Marra 2001; Heidemann et al. 2001).

Within biomedical fields, PLA has been widely used for clinical implant materials, drug delivery systems and also as degradable scaffolds. PLA provides excellent clinical properties at relatively low cost, which increases its use in biomedical applications. Different medical devices have been developed using PLA from degradable sutures to membranes for wound dressings. Different properties can be easily tuned from the simple modifications of the physical structural properties of PLA.

It has been shown that blending or co-polymerizing PLA with either degradable or no-degradable biocompatible materials results in new products with the desired behavior without compromising its biocompatibility; which consequently improves the quality and reduces the cost of production. Surface properties play an important role in both biocompatibility and bio-functionability of biomaterials hence its applications. Different surface modification strategies, such as physical, chemical, plasma, and radiation induced methods, have been employed to create desirable surface properties of PLA biomaterials (Lasprilla et al. 2012). Resorbable

fixation can be used for both anterior and middle cranial base surgical approaches. Imola and Schramm in their study reported that bioresorbable fixation systems represent a major advance in pediatric craniomaxillofacial surgery (Imola & Schramm 2002).

PLA microsphere and microcapsules have been used as controlled drug release systems in short or prolonged periods for different active clinical agents like contraceptives, narcotic antagonists, antimetabolic, local anesthetics, vaccines and antibiotics (Kimura et al. 1992; Prior et al. 2000).

2.3 Study Rationale

Biodegradable polymer thin films have unique properties attractive to biomedical field, particularly in tissue engineering and gene/drug delivery systems. They can be coated on biomedical devices for different applications using simple techniques such as spray coating, dip coating, or solvent casting. They also offer great versatility for surface modifications, which enhance their bio-functionalities. There is an increasing interest in preparing thin films using self-assembly monolayer (SAM), layer-by-layer (LBL) assembly or chemical vapour deposition (CVD) because they precisely control the location and orientation of chemical groups and biomolecules on the surface of the coating. In addition, it is also possible to design thin films to have mechanical properties close to soft biological tissues.

Incorporation of a drug into medical devices represents an emerging new era of drug delivery systems. Thin films of biodegradable polymer like PLA could be an ideal implantable therapeutic, due to their propensity to uptake and release clinical active agents, as a consequence of their degradability. Implant associated infection is common to all implanted medical devices from temporal like intravascular and urinary catheters, to more permanent devices like orthopaedic implants.

Bacterial-device adhesion and the ability of many microorganisms to form biofilms, play a major role in implant-associated infections. It results in host patient morbidity and device removal, or mortality. It has been reported that, of

the three million cases of central venous catheter insertions per year in the USA, 30% result in infection-related mortality (Vendra, Wu & Krishnan 2015) with ~US$ 3700 to US$ ~28,000 medical remediation costs, depending on the original central venous catheter placed. Among surgical site infections, those related to the implant of orthopaedic devices are of great relevance for public health due to the increasing number of aged and disabled patients requiring this type of surgical intervention (Francolini & Donell 2010). The increase in use of medical devices in our modern society accompanied by device related infections prompt for the immediate need to redesign medical devices to improve performance, or innovate new and effective methods for prevention of drug related infections. Direct and controlled antibiotic release at the site of implantation is one of the effective methods to mitigate this problem. This could be done by either incorporating the drug directly into/onto the implants or using drug release coatings. Functionalizing the medical devices' surface to render them non-bacterial adhesive or using non-drug non-adhesive coatings could be the second choice. The choice of the methods is normally based on the general cost and intended medical device application.

The main focus in this section of the research was to produce and characterize biodegradable thin films using PLA. It is believed that successful production of these films using a simple and effective method is the key to successful applications in the biomedical field, specifically in slow drug delivery applications.

2.4 Materials and procedures

PLA, 3052D, (Specific gravity = 1.24, Tg = 55 – 60^{o}C, MP = 200^{o}C) with Mw 75,000 (g/mol) was obtained from NatureWorks LLC Australia. Chloroform with ≥ 99% assay was obtained from Sigma Aldrich.

PLA was purified using the method described by (Gu et al. 2010) *Method B: warm water extraction and cold EtOH precipitation*. Briefly, crude PLA 25 g was dissolved in 170 mL of $CHCl_3$ by stirring at room temperature (RT). The solution was poured into 170 mL of warm water (initially at 60 ◦C) in a 1 L beaker and stirred for 0.5 h. The organic layer was separated using a separating funnel and washed with water (initially at 60 ◦C) again. The organic solution was added drop-wise to 520 mL of

EtOH that was being cooled in an acetone/dry ice bath to precipitate the PLA. After removal of the solvent by decantation, the residue obtained was dried under high vacuum for 5 days and yield a colourless waxy solid.

Film samples were prepared using a solvent casting techniques published by (Buzarovska & Grozdanov 2012; Macha, Ben-Nissan & Milthorpe 2014). Briefly, PLA films were prepared by dissolving 0.50 g of purified polymer in 30ml of chloroform (1.12 %wt/v polymer concentration) under magnetic stirrer at room temperature until it was completely dissolved. Then the solution was transferred into petri dish and chloroform was allowed to evaporate under low vacuum (in a desiccator) for 48 h. The films were stored in desiccators for further analyses.

Injection molding of PLA dog-bone samples (bulk) was produced using injection molding machine SP2634TC 966 (The Small Power Machine Co. LTD. Wiltshire, England) at 165^{o}C and 551.6 MPa.

The mechanical characterization of films and bulk samples was performed by means of tensile tests according to the ASTM D882 – 10 and ASTMD638 – 10 standards respectively. Tests were carried out in a universal testing machine Instron 6022 (Instron Pty Ltd. Melbourne, Australia) equipped with a 5 kN load cell. Deformation was followed with Windows based software.

2.5 Results and Discussion

2.5.1 Thin film by solution casting

Polymer films were prepared using both adapted and modified solution casting techniques. With the adapted method (Buzarovska & Grozdanov 2012) film with air bubbles, entrapped during drying in the air were observed. On the other hand, transparent films of an average thickness of 29 ± 1.2 μm were produced by a modified technique (Macha, Ben-Nissan & Milthorpe 2014) where the films were dried under small vacuum of 0.028mm Hg. In general the main advantage of the solution casting technique is the ease of fabrication without the need for specialized equipment. On the other hand, the disadvantages of this technique are the limitation to cast different shapes, the possible retention of toxic solvent within

the polymer and the possible denaturation of proteins and other molecules incorporated into the polymer. However, with the additional vacuum drying method introduced, the possibility of solvent retention in the film is very small. Three different drying methods were experimented: 1) Drying in the air, where the samples after casting were left in fume cabinet to dry, 2) Controlled drying, the samples after casting were put in desiccator without vacuum and; 3) drying under vacuum, samples were put in desiccator with low vacuum.

Figure 3 shows the stereo microscopy pictures of air-dried PLA film and vacuum dried PLA. The pictures revealed that drying in the air creates many air bubbles (black spots) in the film due to air entrapment during the stirring and casting processes.

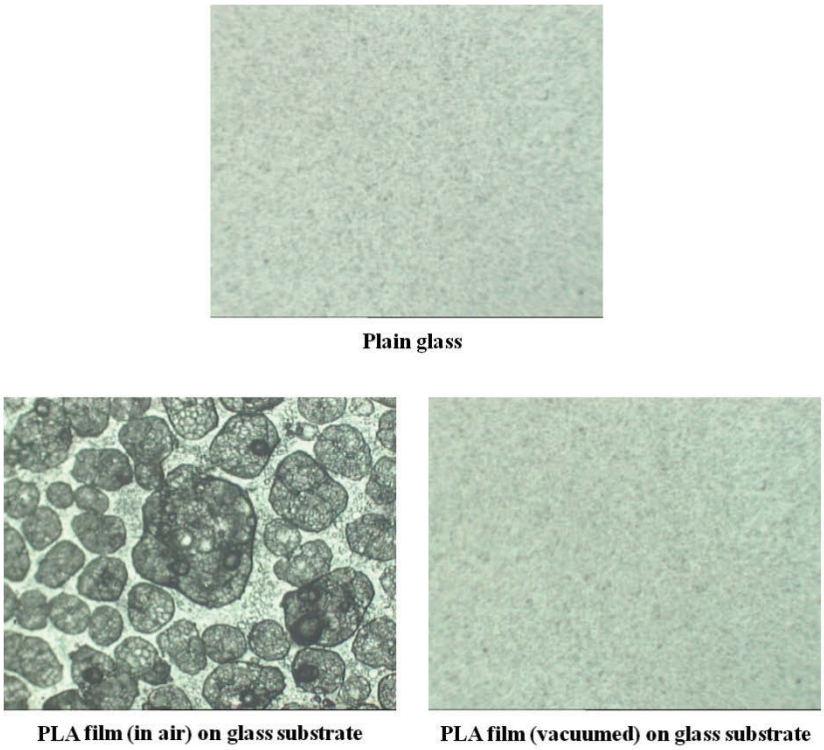

Figure 3: Optical microscopy pictures of PLA films dried in the air and vacuum on glass substrate.

The presence of air bubbles in the films creates stress concentrations, which reduce the mechanical properties of these films. Consequently, it affects the effectiveness of the films in the intended medical applications: specifically its

flexibility and conformability, which are important properties in tissue engineering applications. It was also observed that with the modified method we could produce thin films without compromising mechanical properties.

Figure 4 shows the comparison of tensile strength of films prepared by the two techniques. The results suggest that with the modified technique, tensile strength and elongation to fracture increase significantly. It was also discovered that when we reduce the rate of solvent evaporation from the solution, it results in transparent film with mechanical properties superior to air dried films, as shown in Figure 3.2. It was observed that vacuum drying improved film tensile strength by 300% while in controlled drying (samples were allowed to dry in a desiccator without vacuum) the tensile strength improved by 200%. The improvements widen the biomedical applications of thin films and have significantly addressed the shortcomings of both industrial and clinical production techniques

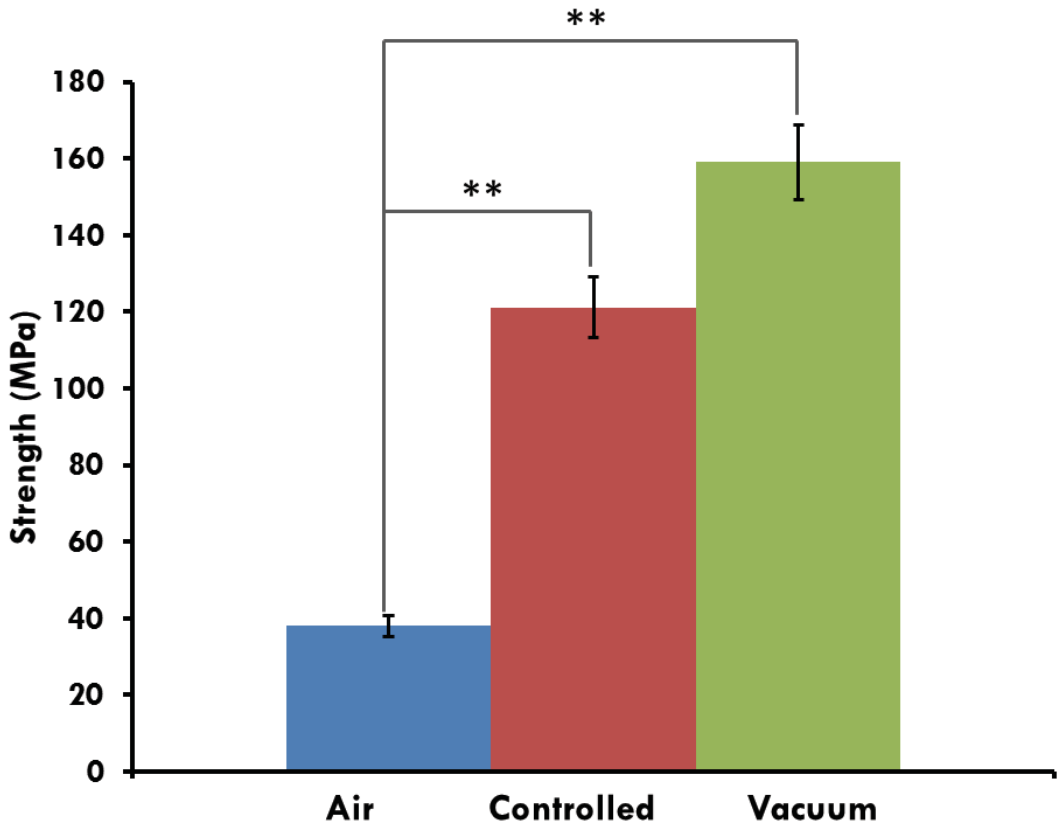

Figure 4: Comparison of mechanical tensile strength for different drying techniques (n=5)

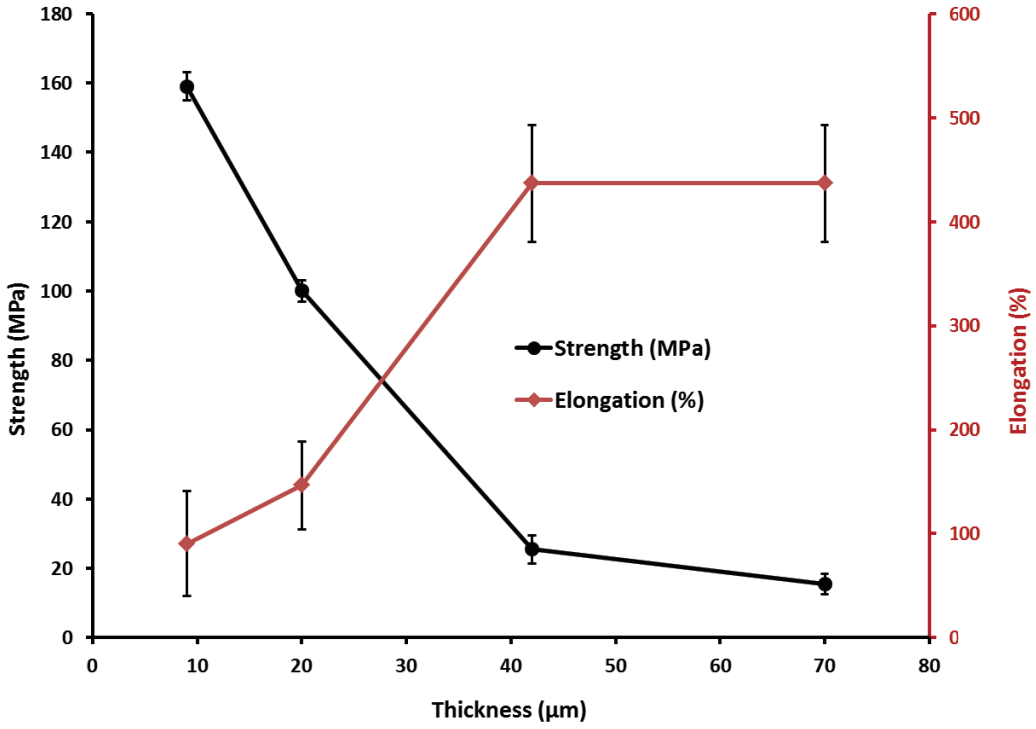

Figure 5: The effect of thickness on films tensile strength and elongation

Figure 5 shows the effect of film thickness on the mechanical properties. The results suggest that by increasing the thickness of the film, tensile strength decreases while the elongation increases. The increase in thickness is likely to increase the number of defects within the material, which act as stress concentrators and lower the material's strength. At early stages of mechanical testing of PLA composites, flexural strength was evaluated on the v-notched samples and the strength, as well as fracture morphologies, were compared with thin film composites.

2.5.3 Comparison of Dog bone tensile samples by injection moulding

Figure 6 shows the tensile strength of thin films compared to dog-bone (bulk) mechanical strength. The results revealed that thin films are stronger than dog-bone samples due to the possibility of an increasing in number of flaws in dog-bone samples. Since dog-bone samples are less flexible compared to thin films, it is expected to affect elongation and in the presence of flaws, the decrease in elongation was significant. Another possibility is possible oxidation of the PLA

during melting and the change of the structure and influence that oxidation does to the mechanical properties (Sungsanit 2011).

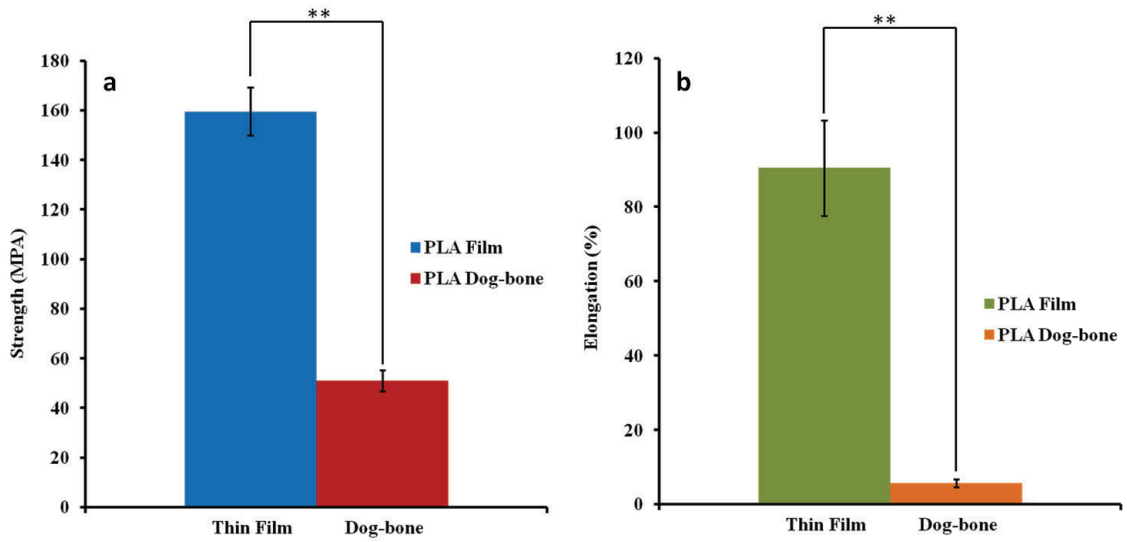

Figure 6: Comparison of thin films and dog-bone samples a) Tensile Strength b) Percentage Elongation

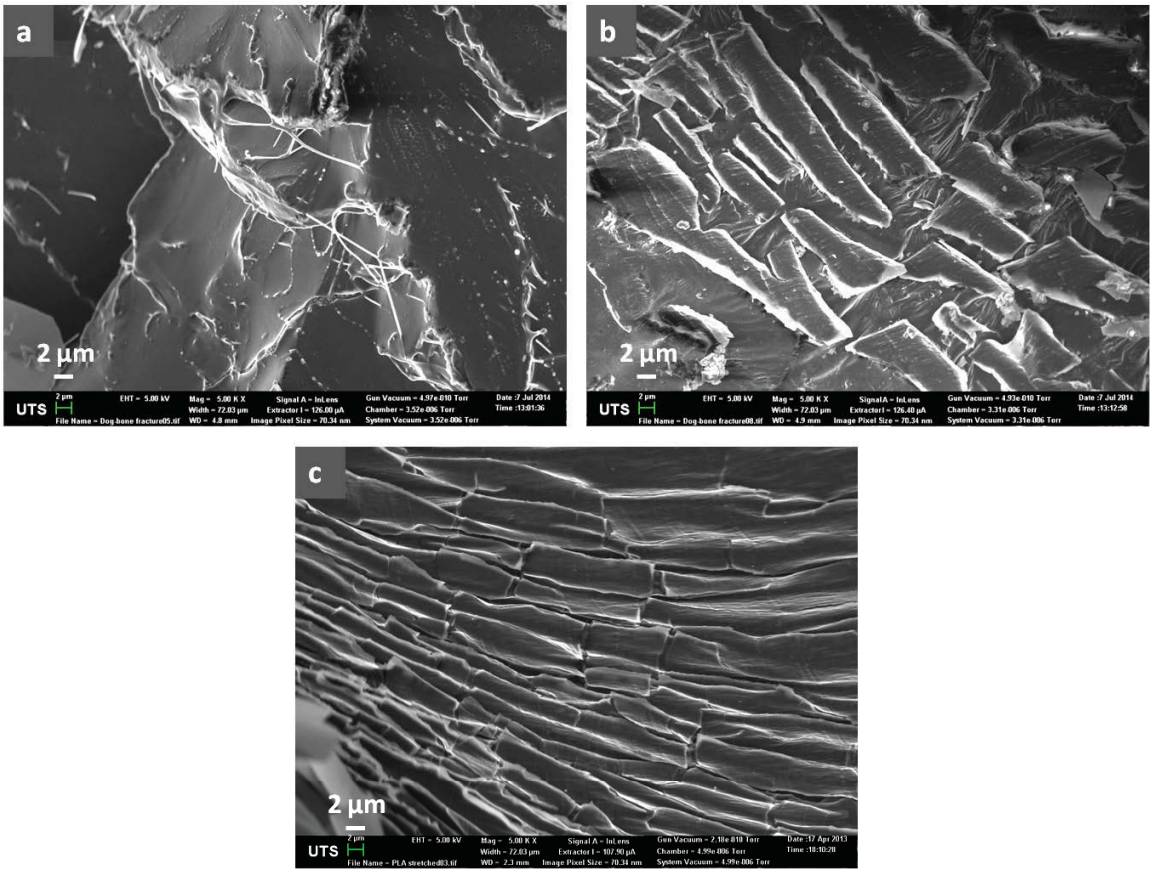

Figure 7: Tensile fracture morphology of a&b) dog-bone b) thin film showing riverlines

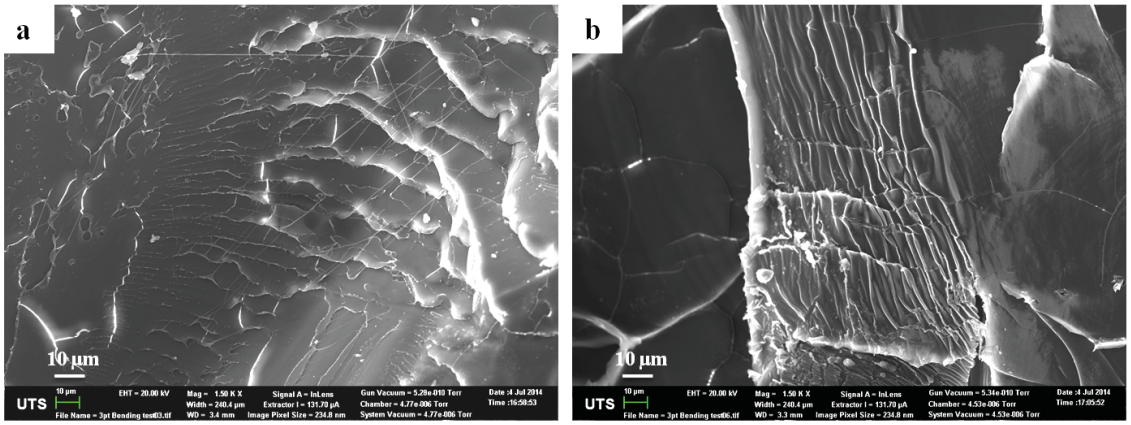

Figure 8: Flexural fracture morphology of a&b) PLA V-notched samples

Figure 7 shows the fracture morphology of PLA dog-bone samples (a and b) and thin films (c). The observed deformation of these samples is consistent with semi-crystalline polymer fracture. Figure 7 a and b images clearly show two distinct zones of fracture associated with dog-bone tensile fracture. Figure 7a shows the fibrils of plastically deformed polymer due to microscopic inhomogeneities in the polymer sample. The riverlines were observed in both dog bone 7a and films 7c which suggest that multiple fractures initiate along the crack front and propagate on several slightly different planes (Macha, Ben-Nissan & Milthorpe 2014). There is a little formation of curps which appears on the fracture surface as raised platelets along riverlines (Lee 1997).

Figure 8 show the morphology of fracture under flexural loading on PLA samples v-notched. 8a shows the delaminations in multidirectional laminates. It is suggested that delaminations start from embedded defects and migrate through different planes (Kenane & Benzeggagh 2009) due to the combination of shear and peel stress at this site. Figure 8b on the other hand reveals the effect of tensile stress, which results into riverlines, and formation of cusps on the fracture site.

2.6 Summary and Conclusions

The use of thin films for biomedical applications has a number of clinical advantages due to their biocompatibility and biodegradability, easy and faster processing times, the ability to conform into different shapes, and good mechanical properties. Further usage includes their adaptability on local drug delivery which

will be discussed in the later chapters. The results in this chapter suggest that novel drug release devices could be made either from thin film alone or in combination with other materials for a wide range of biomedical applications.

2.7 Reference

Agarwal, M., Koelling, K.W. & Chalmers, J.J. 1998, 'Characterization of the degradation of polylactic acid polymer in a solid substrate environment', *Biotechnol Prog*, vol. 14, no. 3, pp. 517-26.

Benninga, H. 1990, 'A History of Lactic Acid Making: A Chapter in the History of Biotechnology', *The British Journal for the History of Science*, vol. 24, pp. 424 - 75.

Buzarovska, A. & Grozdanov, A. 2012, 'Biodegradable Poly(L-lactic acid)/TiO2 Nanocomposites: Thermal Properties and Degradation', *Journal of Applied Polymer Science*, vol. 123, no. 4, pp. 2187-93.

Carothers, W.H., Dorough, G.L. & Natta, F.J.v. 1932, 'STUDIES OF POLYMERIZATION AND RING FORMATION. X. THE REVERSIBLE POLYMERIZATION OF SIX-MEMBERED CYCLIC ESTERS', *Journal of the American Chemical Society*, vol. 54, no. 2, pp. 761-72.

Chu, G.C., Tomita, T., Sonnichsen, F.D., Yoshida, T. & Ikeda-Saito, M. 1999, 'The heme complex of Hmu O, a bacterial heme degradation enzyme from Corynebacterium diphtheriae. Structure of the catalytic site', *J Biol Chem*, vol. 274, no. 35, pp. 24490-6.

De Santis, P. & Kovacs, A.J. 1968, 'Molecular conformation of poly(S-lactic acid)', *Biopolymers*, vol. 6, no. 3, pp. 299-306.

Drumright, R.E., Gruber, P.R. & Henton, D.E. 2000, 'Polylactic Acid Technology', *Advanced Materials* vol. 12, no. 23, pp. 1841–6.

Dunn, A.S., Campbell, P.G. & Marra, K.G. 2001, 'The influence of polymer blend composition on the degradation of polymer/hydroxyapatite biomaterials', *Journal of Materials Science: Materials in Medicine*, vol. 12, no. 8, pp. 673-7.

Fambri, L., Pegoretti, A., Fenner, R., Incardona, S.D. & Migliaresi, C. 1997, 'Biodegradable fibres of poly(l-lactic acid) produced by melt spinning', *Polymer*, vol. 38, no. 1, pp. 79-85.

Francolini, I. & Donell, G. 2010, 'Prevention and control of biofilm-based medical-device-related infections', *FEMS Immunology & Medical Microbiology*, vol. 59, no. 3, p. 12.

Grijpma, D.W., Nijenhuis, A.J., Wijk, P.G.T. & Pennings, A.J. 1992, 'High impact strength as-polymerized PLLA', *Polymer Bulletin*, vol. 29, no. 5, pp. 571-8.

Gruber, P.R., Hall, E.S., Kolstad, J.J., Iwen, M.L., Benson, R.D. & Borchardt, R.L. 2001, *Continuous process for the manufacture of lactide and lactide polymers*, *US Patent*, vol. 6,326,458, USA.

Gu, J.H., Huang, Y.J., Beekman, A. & Goldenberg, M. 2010, 'Simplified preparation of low polydispersity, low molecular weight poly(D,L-lactic acid) by liquid/liquid phase separation for drug delivery systems with discussion of molecular weight characterization', *Polymer International*, vol. 59, no. 12, pp. 1571-7.

Heidemann, W., Jeschkeit, S., Ruffieux, K., Fischer, J.H., Wagner, M., Krüger, G., Wintermantel, E. & Gerlach, K.L. 2001, 'Degradation of poly(d,l)lactide implants with or without addition of calciumphosphates in vivo', *Biomaterials*, vol. 22, no. 17, pp. 2371-81.

Imola, M.J. & Schramm, V.L. 2002, 'Resorbable internal fixation in pediatric cranial base surgery', *Laryngoscope*, vol. 112, no. 10, pp. 1897-901.

Jacobsen, S. & Fritz, H.G. 1999, 'Plasticizing polylactide—the effect of different plasticizers on the mechanical properties', *Polymer Engineering & Science*, vol. 39, no. 7, pp. 1303-10.

Kenane, M. & Benzeggagh, M.L. 2009, 'Characterization of Mixed Mode Delamination Growth and Thresholds', in T. Boukharouba, M. Elboujdaini & G. Pluvinage (eds), *Damage and Fracture Mechanics*, Springer Netherlands, pp. 523-30.

Kimura, H., Ogura, Y., Moritera, T., Honda, Y., Wada, R., Hyon, S.H. & Ikada, Y. 1992, 'Injectable microspheres with controlled drug release for glaucoma filtering surgery', *Invest Ophthalmol Vis Sci*, vol. 33, no. 12, pp. 3436-41.

Lasprilla, A.J.R., Martinez, G.A.R., Lunelli, B.H., Jardini, A.L. & Filho, R.M. 2012, 'Poly-lactic acid synthesis for application in biomedical devices — A review', *Biotechnology Advances*, vol. 30, no. 1, pp. 321-8.

Lee, S. 1997, 'Mode II delamination failure mechanisms of polymer matrix composites', *Journal of Materials Science*, vol. 32, no. 5, pp. 1287-95.

Macha, I.J., Ben-Nissan, B. & Milthorpe, B. 2014, 'Improvement of Elongation in Nanosurface Modified Bioglass/PLA Thin Film Composites', *Current Nanoscience*, vol. 10, no. 2, pp. 200-4.

Prior, S., Gamazo, C., Irache, J.M., Merkle, H.P. & Gander, B. 2000, 'Gentamicin encapsulation in PLA/PLGA microspheres in view of treating Brucella infections', *Int J Pharm*, vol. 196, no. 1, pp. 115-25.

Sakai, K., Kawano, H., Iwami, A., Nakamura, M. & Moriguchi, M. 2001, 'Isolation of a thermophilic poly-L-lactide degrading bacterium from compost and its enzymatic characterization', *J Biosci Bioeng*, vol. 92, no. 3, pp. 298-300.

Scheele, C.W. 1980, 'Kgl. Vetenskaps-Academiens nya Handlingar', *Stockholm*, vol. 1, pp. 116-24.

Shih, C. 1995, 'Chain-end scission in acid catalyzed hydrolysis of poly (d,l-lactide) in solution', *Journal of Controlled Release*, vol. 34, no. 1, pp. 9-15.

Smeaton, W.A. 1986, 'Carl Wilhelm Scheele (1742–1786)', *Endeavour*, vol. 10, no. 1, pp. 28-30.

Södergård, A. & Stolt, M. 2002, 'Properties of lactic acid based polymers and their correlation with composition', *Progress in Polymer Science*, vol. 27, no. 6, pp. 1123-63.

Törmälä, P. 1992, 'Biodegradable self-reinforced composite materials; Manufacturing structure and mechanical properties', *Clinical Materials*, vol. 10, no. 1–2, pp. 29-34.

Vendra, V.K., Wu, L. & Krishnan, S. 2015, 'Polymer Thin Films for Biomedical Applications', in C.S.S.R. Kumar (ed.), *Nanotechnologies for the Life Sciences*, vol. 5, WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany, p. 54.

Vert, M., Li, S. & Garreau, H. 1991, 'More about the degradation of LA/GA-derived matrices in aqueous media', *Journal of Controlled Release*, vol. 16, no. 1–2, pp. 15-26.

CHAPTER 3

BIOGLASS

CHAPTER 3: BIOGLASS

To improve the mechanical properties and allow additional particulate matter to facilitate drug delivery, bioglass microspheres were utilized to reinforce a PLA matrix. This chapter covers Bioglass composition, development and its use as PLA composite reinforced particles.

3.1 Introduction

After its invention by Professor Larry Hench in 1969, silicate bioactive glasses commercially known as Bioglass© provided for the first time an alternative: second generation, interfacial bonding of an implant with host tissues (Hench 2006) and has been widely researched for biomedical applications. Bioglasses undergo a rapid sequence of chemical reactions on the surface of the material when inserted into the body, leading to the formation of an hydroxy carbonate apatite (HCA) layer. Because this HCA layer is similar to the mineral constituent of bone, it bonds firmly with both hard and soft tissues. The formation of this layer on the materials when inserted in simulated body fluid (SBF) is often considered as an indication of its bioactivity (Kokubo & Takadama 2006).

Low toughness of bioactive glasses limits their use in load bearing applications. Currently, composites of polymers and ceramics are being developed with the aim of increasing the mechanical stability and improving tissue interaction; and have been reported to be a promising choice, in particular for bone tissue engineering (Boccaccini & Maquet 2003). Since the 1980s, Bioactive glass/biodegradable polymer composite materials have been reported to be a new family of bioactive materials with applications ranging from structural implants to tissue engineering scaffolds (Ducheyne 1985). These materials are advantageous since they exploit the flexibility of polymers, with the stiffness, strength and bioactive character of the bioactive glass fillers.

One of the clinically successful bioactive composites is that developed by Professor William Bonfield and colleagues at the Interdisciplinary Research Centre in Biomedical Materials, University of London. It is composed of bioactive HA

particles uniformly dispensed in a dense polyethylene matrix (Bonfield et al. 1981). The composite is used clinically as "HAPEX" for middle ear reconstruction.

Although bioactive glasses are mechanically weak, they have excellent biological properties and have been used in tissue engineering and regeneration strategies since their inception. It has also been discovered that Bioglass®, when heated to above (950 ^{o}C), crystallize partially to a mechanically strong phase that can transform into a biodegradable, amorphous calcium phosphate at body temperature and in a biological environment (Chen, Thompson & Boccaccini 2006; Boccaccini et al. 2007).

There are a number of potential applications for bioactive glasses and related bioactive composites materials in the biomedical field, from tissue engineering to drug and growth factor delivery. As promising materials, more research efforts are focused on the production and functionalization, in terms of chemistry and local topography, which have a pronounced effect on *in-vitro* and *in-vivo* bone regeneration. In this part of the research the aim was to produce, characterize and use bioglass in PLA polymer composites.

3.2 Production

In this section, apart from the sol-gel method, other different processing methods used to fabricate nanoscale bioactive glasses are presented.

3.2.1 Micro-emulsion techniques

Microemulsions are thermodynamically stable dispersions of oil and water stabilized by a surfactant and, in many cases a co-surfactant. The microemulsions can be of the droplet type, either with spherical oil droplets dispersed in a continuous medium of water (oil-in-water microemulsions, O/W), or with spherical water droplets dispersed in a continuous medium of oil (water-in-oil microemulsions, W/O). Studies showed that adjusting microemulsion and/or operation variables provides a key to controlling nanoparticle size and polydispersity (Arriagada & Osseo-Asare 1999; Singh et al. 2008). This method has been known as a suitable technique able to obtain inorganic particles with particle

size in the range of nanometers with minimum agglomeration (Karagiozov & Momchilova 2005; Sun et al. 2007). However, the main disadvantages of this technique are the low production yield and the usage of a large amount of oil and surfactant phases (Boccaccini et al. 2010). Despite of the fact that micro emulsion techniques provide an alternative way to other production methods for synthesis of several types of inorganic and organic nano-sized particles (Lim et al. 1996, 1999) only few reports are available on the synthesis of nano-sized bioactive particles by this method.

3.2.2 Laser spinning techniques

In the past few years extensive experimental works have been conducted to develop laser spinning techniques with definite control of the results to produce tailored products (Quintero, Mann, et al. 2007; Quintero, Pou, et al. 2007; Quintero, Dieste, et al. 2009). Recently, a team of researchers from the University of Vigo, Rutgers University in the United States and Imperial College London, in the United Kingdom, has developed "laser spinning", a novel method of producing bioglass nanofibres (Quintero, Pou, et al. 2009). This technique involves using a high-energy laser that melts a small amount of precursor material and creates a super-fine filament that is lengthened and cooled by a powerful gas current. The process is very fast; nanofibres are produced in several microseconds. Laser spinning makes it possible to produce glass nanofibres of compositions that would be impossible to obtain using other methods. The diameters of the fibres obtained range from hundreds down to tenths of microns, as well as the types of products vary from disordered maths to continuous filaments (Quintero, Pou, et al. 2007).

The capability of the laser-spinning technique to produce nanofibers with a wide range of compositions makes evident its potential to produce nanofibers with different rates of bioresorption, to control the release of active ions that have the potential to stimulate the gene expression and cellular response necessary for tissue regeneration (Quintero, Pou, et al. 2009). The disadvantage of this technique is the use of high energy in the production process, which consequently increases the production cost.

3.2.3 Gas phase synthesis (Flame spray synthesis)

Possibly the flame spray technology has been used since prehistoric times as depicted with paintings on cave walls and in Chinese ink artwork (Pratsinis 1998). This technology uses metal–organic precursor compounds to produce nanoparticles at temperatures above 1000 ^{o}C. The basic principle of all gas phase synthesis methods is the formation of molecular nuclei, which is followed by condensation and coalescence inducing the subsequent growth of nanoparticles in high temperature regions during the process (Stark et al. 2003; Boccaccini et al. 2010). This is important since flame technology has high potential for inexpensive manufacture of ceramics, especially nanoparticles that can be used for synthesis of a wide spectrum of new materials (Pratsinis 1998).

Several investigations have been carried out related to the flame spray process dynamics and the understanding of the key variables involved and how they can be controlled to obtain nanoparticles of given size range and chemical composition (Stark et al. 2003; Athanassiou, Grass & Stark 2010). It has been shown that the metal carboxylate system is a very convenient precursor because it allows the synthesis of oxide nanoparticles of almost any composition (Athanassiou, Grass & Stark 2010). In addition, metal–organic salts are highly stable in air, tolerate humidity and most importantly they are fully miscible among each other. Consequently, the process allows the production of any kind of nanoparticulate mixed-oxides with high chemical homogeneity. By using flame spray synthesis, therefore, the preparation of nanoparticles of different bioactive glass compositions has become possible. Bioactive glass nanoparticles in the range 20 – 50 nm were successful synthesized by flame spray techniques and used in remineralization of human dentin. A pronounced increase of mineral content of the dentin samples suggested rapid remineralization (Vollenweider et al. 2007). The technique also demonstrated the ability to produce radio-opaque nanosized bioactive glass for potential root canal application (Mohn et al. 2010). In spite of the fact that this is an energy intensive technique, the only advantage in comparison to other gas phase techniques is precursors do not require an addition of energy.

3.2.4 Sol-gel Bioglass

The sol-gel processing of ceramic and glass materials started more than one and a half centuries ago on silica gel (Ebelmen 1846; Graham 1864). Early investigation on sol-gel suggested that the hydrolysis of tetraethyl orthosilicate (TEOS), $Si(OC_2H_5)_4$, under acidic conditions yielded SiO_2 in the form of a glass-like material (Ebelmen 1846) which could be drawn into fibres or formed composites. In order to avoid the silica gels fracturing into a fine powder the gels were dried for one year or more, and as result the whole process lost technological interest. The formation of Liesegang Rings (Liesegang 1896) from gels attracted many researchers to investigate the problem of the periodic precipitation phenomena that lead to the formation of Liesegang rings and the growth of crystals from gels.

A variety of coatings and films have also been developed by using sol-gel methods. Of particular importance are the anti-reflection coatings of indium tin oxide (ITO) and related compositions applied to glass window panes to improve insulation characterization (Hench & West 1990).

Bioactive glasses have been produced using conventional glass technology. The glass components in the form of grains of oxides or carbonates are mixed and then melted and homogenized at high temperatures, 1250-1400°C (Li, Clark & Hench 1991). The molten glass is then cast into steel or graphite moulds to make bulk implants. A final grind and polish is often necessary to achieve required tolerances. The motivation for sol-gel processing is primarily the potentially higher purity and homogeneity and the lower processing temperatures associated with sol-gels, compared with traditional glass melting or ceramic powder methods (Hench & West 1990).

The bioglass produced by the sol-gel process has become an interesting research field for the past two decades (Hench & West 1990; Greenspan et al. 1997; Jie et al. 2004; Saboori et al. 2009; Chen & Thouas 2011; Cacciotti et al. 2012). The sol gel process involves the synthesis of an inorganic network by mixing the metal alkoxides in solution, followed by hydrolysis, gelation, and low-temperature firing to produce a dense and stable glass powder. The network structure of the gel can

be modified by controlling hydrolysis and polycondensation reactions during production. Therefore, structural variation can be produced without compositional changes. Bioactive glasses can be prepared from gels by sintering at relatively low temperatures (600 – 700 ^{o}C), which nullify most of the disadvantages of high-temperature processing with much higher control over purity.

In addition, sol-gel processing offers potential advantages of ease of powder production, a broader range of bioactivity, and a better control of bioactivity by changing either the composition or the microstructure through processing parameters (Sakka 1985). Li and his co-workers (Li, Clark & Hench 1991) showed that SiO_2-CaO-P_2O_5 powders produced by this technique are more bioactive than the melt-derived glasses of the same composition. Moreover, Sepulveda and his co-workers studied the dissolution rates and rates of surface layer formations on melt derived and sol gel bioglass and the results suggested that 45S5 melt derived bioglass exhibited lower rates than 58S sol gel bioglass powder (Sepulveda, Jones & Hench 2001). The high bioactivity of the sol-gel derived materials is related to the textural features of the gels, i.e., pore size and pore volume associated with the large surface area, higher rate of dissolution, and the negative surface charge (Pereira & Hench 1996). In addition, bioactive sol-gel glasses have been proposed as alternatives to glasses produced by melt and quenching methods, because they exhibit higher rates of apatite-layer formation, more rapid bone bonding, improved homogeneity and purity and excellent degradation/resorption properties (Sepulveda, Jones & Hench 2001).

3.3 Composites

Bioactive glasses are a category of biomaterials that bond to living tissues both soft and hard through the formation of a hydroxyl carbonate apatite (HCA) layer on their surfaces (Hench et al. 1972; Hench & Wilson 1984; Gross et al. 1988). However, it has been shown that the key phenomenon for bioactivity or bond formation is controlled rates of release of ionic dissolution products from the bioglass surface, especially critical concentrations of soluble silica and calcia ions (Hench et al. 2000). Reactions occurring on the surface of the glass lead to the formation of a silica gel layer and subsequent crystallization of HCA.

Bioactive glasses have been used successfully as bone-filling materials in orthopaedic and dental surgery, but as stated earlier their poor mechanical strength limits their applications in load-bearing positions (Merwin 1990; Cai & Zhou 2005; Hench 2006). This motivates researchers to combine the excellent mechanical properties of metals or polymers with a bioactive phase of either particles or fibres to produce a bioactive composite with optimized properties. The purpose of these composite materials is to impart strength and bioactivity by inorganic bioactive fillers while keeping the positive properties of the polymer such as flexibility and capacity to deform under loads. Although there are only a few polymer/ceramic composite clinical devices on the market, it should be taken as a challenge to continue researching in this area, with the aim to improving the available data or developing an alternative approach.

A number of bioglass composites have been developed and tested for biomedical applications such as bone regeneration matrix and scaffolds (Chen & Boccaccini 2006; Kim, Song & Kim 2006; Hajiali et al. 2010; Valenzuela et al. 2012), dental implants (Mehdikhani-Nahrkhalaji et al. 2012), and drug delivery systems (Arcos, Ragel & Vallet-Regi 2001; Soundrapandian et al. 2010; Zhu et al. 2011). It has been reported that the mechanical properties, bioactivity, degradation kinetics and osteoblast responses were improved in the presence of bioactive glasses in the composites with respect to pure polymer or bioglass (Chen & Boccaccini 2006; Kim, Lee & Chun 2008; Lee et al. 2008).

The improvement of mechanical properties of the composite materials is solely dependent on the number of things such as dispersion of bioglass particles in the polymeric matrix and the bioglass contents. Although some researchers have reported to have successfully developed uniform dispersion of bioglass particles in polymer matrix (Kim, Lee & Chun 2008; Lee et al. 2008), it is difficult without treating the bioglass surfaces if they are in nanoscale. The mechanical properties of the composites increase as the amount of bioactive glass particles increase and start to decrease above 30%. However, optimal bioglass content in the composites depends on the application of the composite materials (Niemelä et al. 2005).

Generally, the properties of composite materials depend on the nature and properties of the polymer and filler materials. The interface between the polymer and filler materials plays a defining role in the material's properties. It has been reported that, in polymer composite, the properties of the polymer near the nanoparticles are different from those of the bulk (Zammarano et al. 2011). Zhou and his co-workers reported that interfacial interactions in the form of hydrogen bonding played an important role in affecting the dispersion state of particles in the composite materials (Zhou et al. 2011).

Depending on the application, composite materials for biomedical applications face a number of challenges. The price of biodegradable polymers as one of the raw materials is one of the challenges. There are several issues that need to be addressed for the production of these polymers and the lowering of the costs of raw materials. In addition, the control of accuracy and reproducibility of the manufactured nanocomposite is very important for future biomedical applications. Moreover, lack of well-known structure-property relationships between polymer and nanoparticle hampers the design of complex biomedical useful materials. Currently, the available database for these materials does not give a well-established theory to predict the properties resulting from the combination of nanoparticles and polymeric biomaterials (Dou, Lin & Chang 2011; Keledi, Hari & Pukanszky 2012).

This study is focused on using sol-gel bioglass to make biodegradable polymer composites for biomedical applications. Polymer, bioglass powder and their composites with different bioglass contents was characterized in terms of their mechanical, physic-chemical and bioactive properties with the aim of obtaining the optimum composite properties for hard tissue regeneration. More of this will be discussed in the independent chapter of sol-gel bioglass polymer nanocomposites.

3.4 Applications

Bioactive glasses have gained several clinical applications after their invention in the late 1960s due to their bioactivity and biocompatibility properties. It has been proved to be an effective implant material for tissue repair, tissue regeneration

and drug delivery in the human body. In this section, several clinical applications of bioglass will be discussed. The regulatory aspect of bioactive glass materials will also be presented.

3.4.1 Maxillofacial and dental repair

The ability of bioactive glasses to bond with host tissue has led its applications as bone substitute in dental, oral and maxillofacial bone augmentation (Bosetti et al. 2003). Chen and his co-workers showed that machinable bioactive glass ceramics (MBGC) could be used in maxillofacial augmentation as a substitute for bone grafts. The results suggested that the implant does not show any inflammatory reaction or rejection by the host tissue thus proving clinical reliability of this material (Chen, Li & Huang 1995; Hill et al. 2004).

The need to repair and augment dento-alveolar defects necessitates the use of autogenous bone or a substitute that may be seen to avoid the additional morbidity of a donor site procedure and without risk of cross infection. The use of bioactive glass has been proposed as a viable bone substitute (Norton & Wilson 2002). In addition, biological behaviour of dental implants has been improved by coating them with bioglass (Kontonasaki et al. 2003; Ramires et al. 2003). It was reported that bioglass PerioGlas® granules could be used in the treatment of dental extraction sites to effect bone regeneration and to give early fixation to the implant (Gatti et al. 2006).

3.4.2 Bone substitutes in orthopaedics and traumatology

Bioactive glass S53P4 is an osteoconductive bone substitute with antibacterial and bone bonding properties. It has been shown that bioglass S53P4 is a good and well-tolerated bone substitute, and can be used in the treatment of osteomyelitis with good primary results (Lindfors et al. 2010). Recently, a synthetic osteoconductive bone graft material composed of bioactive glass has been described, with high effectiveness in animal models. This graft material was compared with gold standard iliac crest autograft in the treatment of thoracic adolescent idiopathic scoliosis (AIS) and it was shown that bioactive glass can be proposed in the treatment of AIS, with the advantage of avoiding the morbidity of

iliac crest harvesting (Ilharreborde et al. 2008). Pernaa and his co-workers reported that bioactive glass S53P4 can be used as a bone substitute in depressed lateral tibial plateau fractures with good functional and radiological long-term results (Pernaa et al. 2011). They compared S53P4 bioglass with autograft bone used as bone-graft substitute for prospective 11 years and the results suggested that there was no significant difference in the tibial-femoral angle or deviation of mechanical axes observed between the two groups. In addition, studies have shown bioglass S54P4 is safe to be used as a bone graft extender in spine surgery (Rantakokko et al. 2012).

Bioglass coating has been used to enhance bioactivity of bone substitute in orthopaedics. It was shown that a bioglass coating on the PET artificial ligament and on a porous Ti-based implant surface has a positive effect in the induction of artificial ligament osseointegration within the bone (Drnovsek et al. 2012; Li et al. 2012). Biomorphic silicon carbide ceramics coated with a bioactive glass layer with excellent mechanical properties and low density was shown to be a base material for bone substitutions with a good biological response. The results show that this material can be used as an alternative dental and orthopaedic implant with enhanced mechanical and biochemical properties that ensure optimum fixation to living tissue (Gonzalez et al. 2003).

3.4.3 Treatment of bone infections

Bioactive glass scaffolds can effectively be used in the treatment of bone infections. Research findings indicate that bioactive borate glass could provide a promising biodegradable and bioactive material for use as drug delivery systems as well as scaffolds for bone repair (Liu, Xie, et al. 2010). Local antimicrobial delivery is a potential area of research conceptualized to provide alternative and better methods of treatment for cases such as osteomyelitis where avascular zones prevent the delivery of drugs from conventional routes of administration. Soundrapandian and his colleagues showed that porous bioactive glass scaffolds could be suitable for local delivery of the drugs in cases of osteomyelitis (Soundrapandian et al. 2010). In their further investigations they suggest that bioactive glass SSS2 porous scaffolds with pores predominantly in the range of 10-

60 micron, released the drugs gatifloxacin and fluconazole effectively for 6 weeks and are deemed suitable for local delivery of drugs to treat osteomyelitis (Soundrapandian et al. 2011).

A recurrent aneurysmal bone cyst of the proximal phalanx of the index finger has been reported to be treated with bioglass and the results were positive suggesting both the growth in length of the phalanx and remodelling to an almost normal shape (Lindfors 2009). Recently, magnetic bioactive glass ceramic (MG) in the system CaO-SiO_2-P_2O_5-MgO-CaF_2-MnO_2-Fe_2O_3 for hyperthermia treatment of bone tumor was synthesized. In vitro results suggested that cells could successfully attach and proliferate well on MG (Li, Feng & Zhou 2011). The excellent bioactivity, osteoconductive and drug release properties of bioactive glass materials open the possibility of developing effective new devices in the treatment of bone diseases.

3.4.4 Bioactive glass and biodegradable polymer composites

The biomedical applications of bioresorbable polymer nanocomposites have addressed several limitations of non-resorbable or metal medical devices in orthopaedics (Agrawal, Athanasiou & Heckman 1997; Tokgozoglu 1998; Covani et al. 2007; Brown & Farrar 2008; Calandrelli et al. 2010; Lee et al. 2011; Saska et al. 2011; Lei et al. 2012), soft tissue regeneration (Pirkey & Hurt 1959; de Tayrac et al. 2007), and drug delivery systems (Ustariz-Peyret et al. 2000) (Asandei et al. 2004; Habraken, Wolke & Jansen 2007; Willerth & Sakiyama-Elbert 2007; Liu & Webster 2010a; Liu & Webster 2010b; Tang et al. 2010) and can perfectly replace them if more effort will be focused on investigating bioresorbable polymers. The main purpose of composite technology is to impart strength and bioactivity by the use of an inorganic bioactive filler, while keeping the positive properties of the polymer such as flexibility and capacity to deform under loads. Nevertheless, it is important to optimize their performances, especially in load-bearing and implantable medical devices.

Bioglass biodegradable polymer nanocomposites devices have shown to be useful in supporting periodontal tissue regeneration. The nanocomposite membrane of chitosan and bioactive glass nanoparticles was suggested to potentially be used as

a temporary guided tissue regeneration membrane in periodontal regeneration, with the possibility of inducing bone regeneration (Mota et al. 2012). Moreover, nanocomposite scaffolds of degradable polymers and bioglass have been widely used in tissue engineering. The ability to manipulate morphology and size of bioglass polymer nanocomposites to tailor better material properties for specific applications has been a key to widen their applications and motivation for research in the biomedical field.

Hajiali and his colleagues prepared a novel biodegradable nanocomposite scaffold based on poly (3-hydroxybutyrate)/bioglass nanoparticles with acceptable porosity and morphologic character for bone tissue engineering (Hajiali et al. 2010). Other properties of polymer bioglass nanocomposites like osteoblas responses, mineralization properties, water absorption ability, *in-vitro* cytotoxicity and bioactivity have been extensively studied (Hong, Reis & Mano 2008; Erdemli et al. 2010). It was reported that a nanocomposite of bioactive glass nanofibre and degradable polymer proved to have excellent bioactivity and osteoblas responses and can be considered as a promising bone regeneration matrix (Kim, Lee & Chun 2008). The excellent properties of bioglass and biodegradable polymers pave a new way to synthesize their composites for a wide range of biomedical applications.

3.4.5 Bioactive glasses for wound healing

Several studies revealed that bioactive glass can bond well with soft tissue and can promote regeneration of soft tissue such as skin (Gatti, Valdre & Andersson 1994; Wang, Hench & Bonfield 1998). Bioglass has demonstrated the ability to stimulate the release of angiogenic growth factors and to promote angiogenesis that is critical for stimulating neovascularization of tissue-engineered constructs (Day 2005; Day et al. 2005). A method has been developed for treating wounds including contacting a wound with an effective wound healing amount of bioactive glass and topical antibiotic and composition, for the accelerated healing of wounds and burns, including particulates of bioactive glass and at least one topical antibiotic. The study on the healing effect of bioactive glass by Cai and his colleagues revealed that bioglass can accelerate the recovery of skin wounds and

has great potential for use in wound repair in the future (Lin et al. 2012). Although controversial, an attempt to coat silver particles with bioactive glass for wound healing application has been carried out (Blaker, Nazhat & Boccaccini 2004; Wren et al. 2012). The effects of a bioactive glass on healing of closed skin wounds were studied by Gillete and his co-workers. The results suggested that bioactive glass has potential for increasing tissue strength and could be beneficial in treating wounds in which early healing strength is needed (Gillette et al. 2001).

3.3 Experimental Work

In this research, bioactive glass nanoparticles were synthesized using the sol-gel method. The method was selected based on the reasons mentioned in the previous sections of this chapter. The main aim was to develop bioglass PLA composites that are capable of delivering clinical active substances locally and to improve and measure the properties to ascertain their suitability for orthopaedic and maxillofacial applications.

3.3.1 Materials

Tetraethyl orthosilicate (TEOS, $Si(OC_2H_5)_4$, 99.90%), Citric acid monohydrate (99-100%), Ethanol absolute, Ammonium hydroxide solution (NH_4OH, 33%) Ammonium phosphate dibasic ($(NH_4)_2HPO_4$), Calcium nitrate tetrahydrate ($Ca(NO_3)_2.4H_2O$, 99.60%) and Polyvinylpyrrolidone (PVP) were purchased from Sigma Aldrich Australia Pty Ltd.

3.3.2 Methods

3.3.2.1 Bioglass Synthesis Sol-gel method

The procedure for preparing the bioglass nanoparticles (SiO_2:CaO:P_2O_5 (mol) 55:40:5) (55S5) was based on the method reported by (Li, Clark & Hench 1991) and modified by (Hong, Reis & Mano 2009b) was described as follows: (1) In an well-washed beaker equipped with a magnetic stirrer, 7.639 g calcium nitrate was dissolved in 120 mL of deionized water at room temperature. The TEOS-ethanol solution was created by diluting 9.167 g of TEOS in 60 mL of ethanol and added to the calcium nitrate solution. Then, citric acid was added into the solution to adjust the pH value to 1–2. The reaction mixture was kept stirring until a homogeneous

and transparent solution is obtained. (2) Under vigorous stirring, the homogenous solution was slowly dropped into 1500 mL of ammoniated demonized water, in which 1.078 g of ammonium dibasic phosphate was dissolved in advance. During the dripping process, the pH value of solution was kept at around 11 using ammonia water. (3) After stirred for 48 h and aged for 24 h at 50 ^{o}C, the precipitate was separated from the reaction solution by centrifugation at 3500 rpm, washed three times with deionized water, and finally separated in 200 mL of 2% (w/v) PEG-water solution and kept still. (4) The precipitation was then freeze-dried followed by calcination at 700 ^{o}C in a muffle furnace for 3 h, after which the white BG nanoparticle was obtained and kept in desiccator until characterized. 55S5 was chosen due to its balanced bioactivity where bioactive glasses bond to both bone and soft tissues and are gene activating, Figure 1.

3.3.2.2 Microstructure evolution and determination of calcination temperature

Simultaneous thermogravimetry and differential thermal analysis of bioglass was conducted at different temperatures after freeze-drying to determine the proper calcinations temperature. Then the freeze-dried sol-gel bioactive glass was thermally treated in air at different temperatures, 700 ^{o}C, 750 ^{o}C, 800 ^{o}C, 850 ^{o}C, 900 ^{o}C, 1000 ^{o}C and 1050 ^{o}C for three hours.

The results of different characterization techniques are presented, such as Fourier transform Infrared (FT-IR) spectroscopy, thermogravimetry and differential thermal analysis (TG–DTA), X-ray diffraction (XRD), Scanning electron microscopy (SEM) coupled to energy-dispersion spectroscopy (EDS) analysis.

3.3.2.3 Fourier Transform Infrared Spectroscopy, (FT-IR)

Structural analysis of the bioactive glass was characterized by FT-IR. The synthesized Bioglass powders was ground in an agate mortar and thoroughly mixed with dried KBr (FTIR Grade). Three milligrams of powder sample was mixed with 300 mg of KBr powder (1% w/w). Pellets were prepared in a stainless steel die by applying a uniaxial load of 6.89 GPa pressure (Carver press). The FTIR spectra were collected using a Nicolet, Magna-IR 6700 Spectrometer FTIR in the range 4000–400 cm^{-1}. KBr pellet was used to collect the background.

3.3.2.4 Simultaneous thermogravimetry and differential thermal analysis TG-DTA

Thermal behavior of sol-gel bioglass was investigated by use of a simultaneous thermogravimetry and differential analysis TG-DTA, SDT 2960 SDT V3.0F TA Instrument. A sample weight of 15 – 20 mg bioglass was used during analysis under air condition with a heating rate of 10 ^{o}C/min from room temperature to 1,100 ^{o}C.

3.3.2.5 X-ray diffraction, XRD analysis

X-ray diffraction (XRD) analyses were performed in a Seimens D5000 X-ray Diffractometer, (Cu Kα radiation λ = 1.5405600 Å, 2θ from 3–70^{o}, step size 0.010^{o}, time per step 8 s, scan speed 0.005^{o}/s) at 45Kv and 40mA on the sol-gel bioglass powders.

3.3.2.6 Particle size measurements

Particle size of sol-gel bioglass was measured using a Mastersizer (Malvern Instrument Ltd, Marven UK) 2000 laser diffraction particle size analyzer. The bioglass was dispersed in water and sonicated for 2 hrs to break down agglomeration before measurements.

3.4 Results and Discussions

3.4.1 Microstructural evolution and calcinations temperature

It is believed that the sol-gel process technique produces more a purer product compared to conventional methods. However, the method seemed to be limited in terms of the compositions that can be produced. The remaining organic reactants and water may pose some complications to the final biomedical application of the final products. To remove retained organic material impurities, high temperature treatments are normally employed. This temperature should be less than the melting temperature of the final products. Calcination of freeze-dried bioglass at different temperatures were carried out from 700 to 1050 ^{o}C in order to establish the proper calcination temperature condition. Figure 9 shows XRD spectra of 55S5 calcined at different temperatures for 3hrs. Thermal analysis showed crystalline phases appeared from 750 ^{o}C. The XRD spectra of 55S5 treated at 750 ^{o}C mainly showed the presence of a small amount of Tricalcium disilicate (Rankinite)

($Ca_3Si_2O_7$) crystalline phase (JCPDS Card No. 22-0539). This is a complex silicate compound formed due to the presence of non-bridging oxygen in bioglass. At an elevated temperature above 750 °C, Rankinite was not observed. The evolution of calcium phosphate oxide $Ca_{10}(PO_4)_6O$ (JCPDS Card No. 89-6495) and calcium silicate $Ca_2(SiO_4)$ (JCPDS Card No. 87-1257) phases appeared from 800 °C and stayed stable to 1050 °C. In the powder treated at 1000 and 1050 °C low quartz SiO_2 (JCPDS Card No. 65-0466) was identified as the main phase. Other peaks were assigned to calcium phosphate oxide and calcium silicate formed from 800 °C. The powder treated at 700 °C did not show any diffraction maxima and is believed to be amorphous.

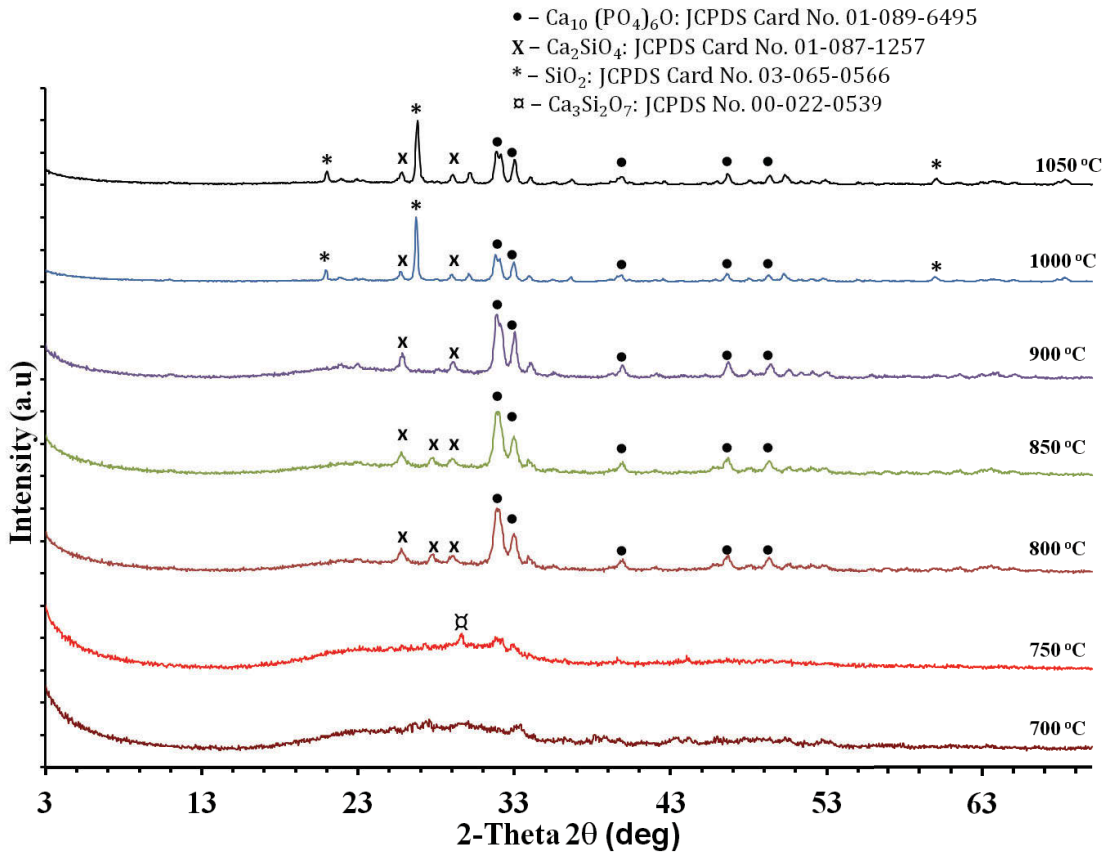

Figure 9: XRD patterns of 55S5 thermally treated at 700, 750, 800, 850, 900, 1000 and 1050 °C.

Thermal treated powders were analysed by FT-IR as shown in Figure 10. Samples treated at 700 and 750 showed the characteristic pattern consistent with amorphous calcium silicate materials. The spectral peak at 871 cm^{-1} is assigned to the Si – O – Si asymmetric stretching mode (Ma et al. 2010). The vibration band at

462 cm^{-1} is attributed to Si–O–Si bending mode. The peak at 1066 is the stretching vibration of phosphate groups that is masked by the broad silicate band (Hong, Reis & Mano 2009a). However, the diffraction pattern of 750 ^{o}C samples (Figure 9) showed the presence of a small amount of tricalcium disilicate phase. The broad bands around 1639 and 1477 ^{o}C are associated with C-O asymmetric stretching and a small peak around 876 cm^{-1} corresponds to out of plane C-O bending vibration of polyethylene glycol (PEG) used in during production.

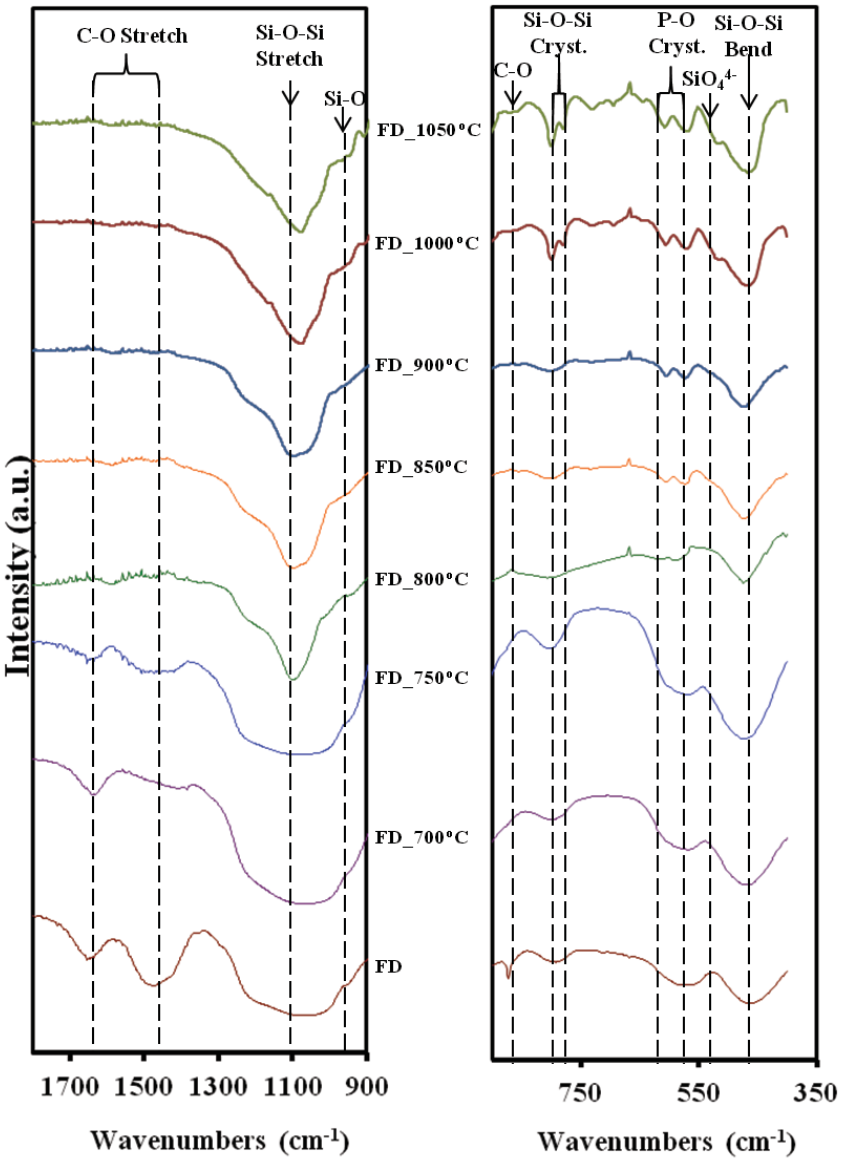

Figure 10: FT-IR spectra of bioglass calcined at 700, 750, 800, 850, 900, 1000, and 1050 ^{o}C for 3 hrs.

3.4.2 Thermal analysis

Thermogravimetric analysis was conducted on freeze dried bioglass before and after calcination. Figure 11a shows the TG-DTA curve of freeze dried bioglass and its comparison with heat treated at 700 °C and 900 °C in 11b.

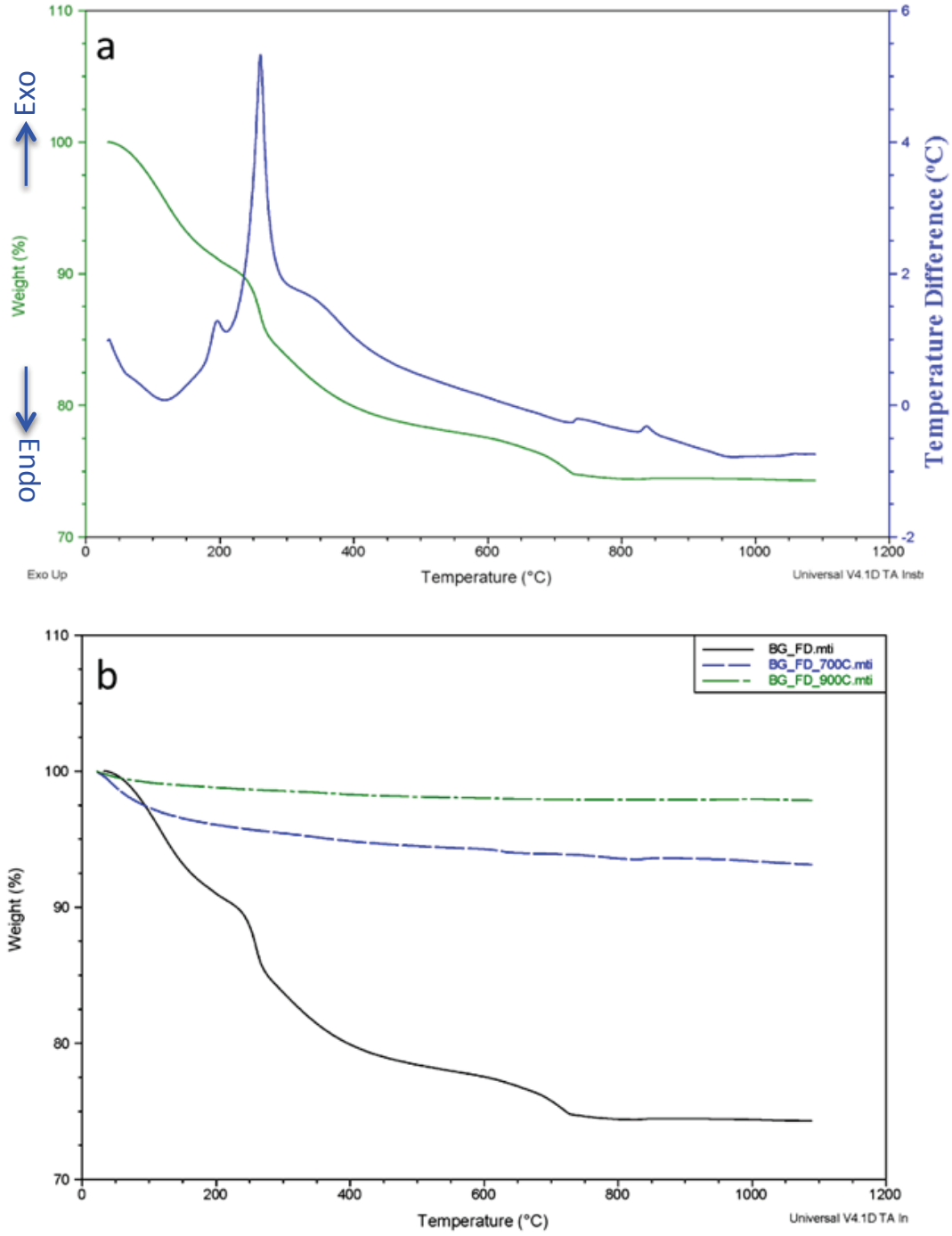

Figure 11: a) Thermogram of freeze dried bioglass 11b) Comparison of thermograms of freeze dried bioglass, freeze dried and calcined at 700 °C and 900 °C bioglass. After calcination in 2b, thermograms show a fairly stable bioglass with a small weight loss for the 700 °C sample.

In Figure 11a, it was observed that the weight loss from TG-DTA occurred in three stages. The first mass loss occurred between 50°C and 140°C, corresponding to an endothermic curve in the DT around 116 ^{o}C. This is associated with the removal of physically adsorbed water and little liquor. The second stage of weight loss commenced from the end of the first weight loss (135°C) until about 275°C and might be correlated to an exothermic peak in the DT curve at 260 ^{o}C, corresponding to the pyrolysis reaction of free organic species and/or the release of the resulting water from the further condensation of silanol and P–OH groups (Qian et al. 2009). The third drop in mass occurred from the end of the second weight loss (275°C) until around 650°C. This is due to the departure of nitrate groups that are usually removed during the thermal stabilization process. The elimination of the species at these temperatures is reflected in the weight loss from the TG curve.

3.4.3 Bioglass morphology, particle size and surface area

The sol-gel process is well known for the production of nanoparticle bioglass with spherical morphology. Using spherical nanoparticles for nanocomposites production has attracted many researchers due to the increased demand for new materials with improved thermal, mechanical, physical, and chemical properties (Moncada, Quijada & Retuert 2007). However, a major problem associated with the use of nanoparticles is that they are highly agglomerated. In many cases if used in bulk composites, they tend to lose their high-surface area and introduce voids or defects within the composites, which weakens the mechanical properties.

The bioglass powder produced showed a similar behaviour of agglomeration. Particle size analysis performed in a Marven Instrument, mastersize 200, with water as a solvent, suggests that bioglass powders are in the size of between 1 to 100 μm as shown in Figure 12a. This was also confirmed by SEM pictures (Figure 12b) which showed the agglomeration of these particles to be in the range of 1 to 50 μm. At higher magnifications (Figure 12c), it was revealed that these big particles are made of agglomerated spherical bioglass nanoparticles of a size in the range of 50 – 100nm. The BET surface area of produced bioglass was determined in Micromeritics ASAP 2010, to be 76.03 ± 0.31 m^2/g.

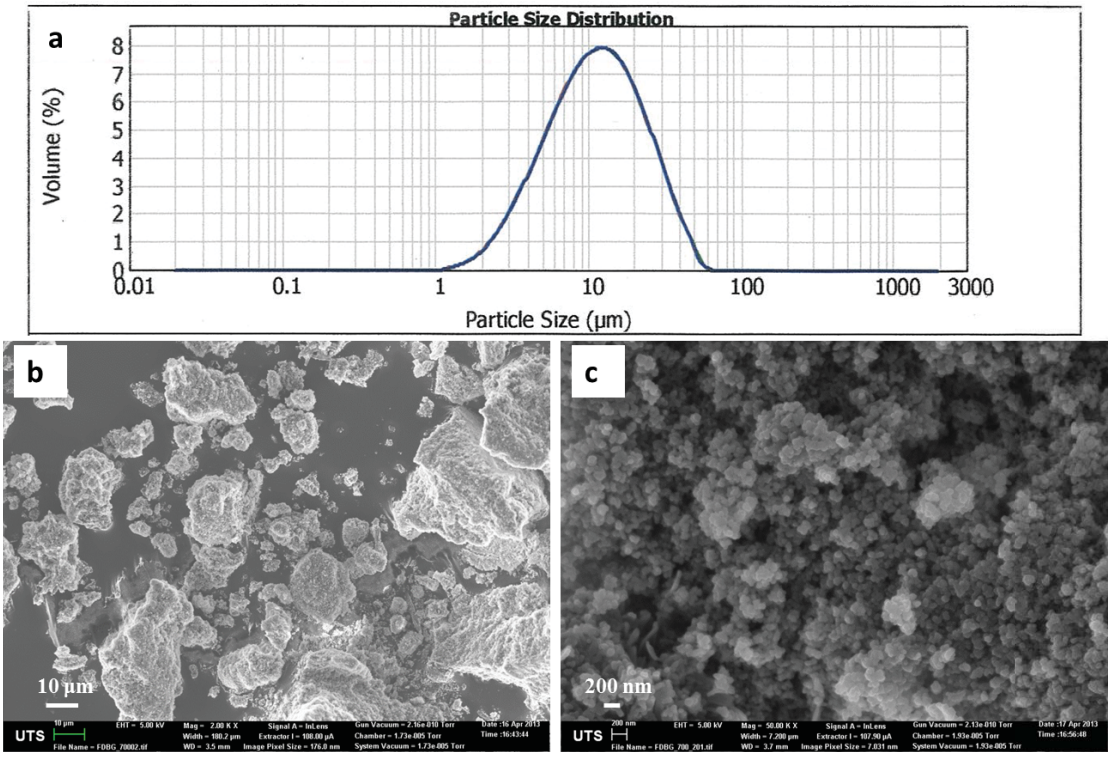

Figure 12: a) Particle size analysis b) SEM picture of sol-gel bioglass 55S5 C) High magnification SEM of 55S5 revealing nanoparticles

3.4.4 Bioglass –polymer composites

One of the applications in advanced clinical devices of bioglass particles is to be used as fillers or reinforcement in advanced composites. The performance of Bioglass-polymer composites depends on the bioglass distribution or dispersion in the polymer matrix. The attaining of a homogenous bioglass nanoparticle distribution in bioglass-polymer composites has been reported to be one of the major challenges because hydrophobic polymers like PLA form a mixture with sol-gel bioglass that is macroscopically phase separated.

To mitigate this problem, effective mixing techniques and surface treatment of bioglass particles can be employed to lead to homogenous distribution of the particles in the polymer matrix. On effective mixing, three techniques are commonly used to enhance uniform dispersion of nanoparticles in nanocomposite production: solution mixing, the in-situ polymerization process and the melt mixing process (Kim et al. 2007). It was also reported that solution mixing and in-situ polymerization usually produce higher levels of nanoparticle dispersion.

On the other hand, the melt mixing process is the favourite due to its compatibility with current industrial compounding facilities (Rahman & Padavettan 2012). The absence of solvents makes the process environmentally friendly and economically viable. In the melt mixing process, due to thermal energy and mechanical mixing of fillers, polymer molecules gain increasingly mobility - a key to attaining uniform filler distribution. Solution mixing using magnetic stirrer and ultra-sonication were employed in making bioglass-PLA composites. Characterizations of the resulted composites are presented in **Chapter 4.**

3.4.4.1 Surface treatments of sol-gel bioglass

Chemical modification of the bioglass surface to enhance uniform distribution in the polymer matrix has been one of the important steps in preparation of silica based particles-polymer nanocomposites. It has been reported that surface functionalization improves adhesion between inorganic particles and the organic polymeric materials and enhances particle distribution with the matrix (Huadong, Xiaohong** & Zhijun 2008).

We have previously reported that chemical modification of the bioglass nanosurface by using silane such as 3-Aminopropyl)triethoxysilane (APTES), affects the chemical and physical properties of the surface layer and improves the elastic properties of PLA/bioglass thin film composites (Macha, Ben-Nissan & Milthorpe 2014). They suggested that surface treated bioglass with APTES provides a better bonding between the amine part of APTES and the carbonyl group in the PLA matrix that can contribute to a better interfacial adhesion than untreated ones especially at the nanoparticle level. Kickelbick suggested that due to the interactions between the polymer and the silanol groups generated during the sol–gel process, a macrophase separation is avoided, and the resulting materials have a high degree of homogeneity (Kickelbick 2003). The treatment results of these experiments are also reported in **Chapter 4.**

3.5 Summary and Conclusions

Production, characterization and surface treatment of bioglass 55S5 was carried out in this chapter. The use of bioactive materials and biodegradable polymer in

developing medical devices would be ideal for a wide range of biomedical applications. However, the big challenge to prepare composite is to achieve homogeneity in the mixing between the bioglass particles and the polymer. The surface-functionalized bioglasses are ready to fabricate bioglass-polymer composites with improved physical-chemical and mechanical properties. Drug loading and delivering requires materials to be porous, bioglass powders do not possess this property. However, several people have reported using mesoporous bioglass scaffolds prepared by sol-gel, using polymer templates for drug and growth factor delivery (Chung et al. 2011; Li, Liu, et al. 2013; Wu et al. 2013). However the non-porous nature of the bioglass produced did not allow us to use them as a drug delivery vehicle but to fabricate bioglass-polymer composites with improved mechanical properties that can be modified to deliver drugs with other means.

3.6 References

Agrawal, C.M., Athanasiou, K.A. & Heckman, J.D. 1997, 'Biodegradable PLA-PGA polymers for tissue engineering in orthopaedics', *Porous Materials for Tissue Engineering*, vol. 250, pp. 115-28.

Arcos, D., Ragel, C.V. & Vallet-Regi, M. 2001, 'Bioactivity in glass/PMMA composites used as drug delivery system', *Biomaterials*, vol. 22, no. 7, pp. 701-8.

Arriagada, F.J. & Osseo-Asare, K. 1999, 'Synthesis of Nanosize Silica in a Nonionic Water-in-Oil Microemulsion: Effects of the Water/Surfactant Molar Ratio and Ammonia Concentration', *Journal of Colloid and Interface Science*, vol. 211, no. 2, pp. 210-20.

Asandei, A.D., Erkey, C., Burgess, D.J., Saquing, C., Saha, G. & Zolnik, B.S. 2004, 'Preparation of drug delivery biodegradable nanocomposites by rapid expansion from supercritical solutions.', *Abstracts of Papers of the American Chemical Society*, vol. 228, pp. U345-U.

Athanassiou, E.K., Grass, R.N. & Stark, W.J. 2010, 'Chemical Aerosol Engineering as a Novel Tool for Material Science: From Oxides to Salt and Metal Nanoparticles', *Aerosol Science & Technology*, vol. 44, no. 2, pp. 161-72.

Blaker, J.J., Nazhat, S.N. & Boccaccini, A.R. 2004, 'Development and characterisation of silver-doped bioactive glass-coated sutures for tissue engineering and wound healing applications', *Biomaterials*, vol. 25, no. 7-8, pp. 1319-29.

Boccaccini, A.R., Chen, Q., Lefebvre, L., Gremillard, L. & Chevalier, J. 2007, 'Sintering, crystallisation and biodegradation behaviour of Bioglass-derived glass-ceramics', *Faraday Discuss*, vol. 136, pp. 27-44; discussion 107-23.

Boccaccini, A.R., Erol, M., Stark, W.J., Mohn, D., Hong, Z.K. & Mano, J.F. 2010, 'Polymer/bioactive glass nanocomposites for biomedical applications: A review', *Composites Science and Technology*, vol. 70, no. 13, pp. 1764-76.

Boccaccini, A.R. & Maquet, V. 2003, 'Bioresorbable and bioactive polymer/Bioglass® composites with tailored pore structure for tissue engineering applications', *Composites Science and Technology*, vol. 63, no. 16, pp. 2417-29.

Bonfield, W., Grynpas, M.D., Tully, A.E., Bowman, J. & Abram, J. 1981, 'Hydroxyapatite reinforced polyethylene--a mechanically compatible implant material for bone replacement', *Biomaterials*, vol. 2, no. 3, pp. 185-6.

Bosetti, M., Zanardi, L., Hench, L. & Cannas, M. 2003, 'Type I collagen production by osteoblast-like cells cultured in contact with different bioactive glasses', *Journal of Biomedical Materials Research Part A*, vol. 64A, no. 1, pp. 189-95.

Brown, M. & Farrar, D. 2008, 'Development of bioresorbable polymers in orthopaedics', *Plastics Rubber and Composites*, vol. 37, no. 2-4, pp. 46-9.

Cacciotti, I., Lombardi, M., Bianco, A., Ravaglioli, A. & Montanaro, L. 2012, 'Sol-gel derived 45S5 bioglass: synthesis, microstructural evolution and thermal behaviour', *J Mater Sci Mater Med*.

Cai, Y.R. & Zhou, L. 2005, 'Effect of thermal treatment on the microstructure and mechanical properties of gel-derived bioglasses', *Materials Chemistry and Physics*, vol. 94, no. 2-3, pp. 283-7.

Calandrelli, L., Annunziata, M., Della Ragione, F., Laurienzo, P., Malinconico, M. & Oliva, A. 2010, 'Development and performance analysis of PCL/silica nanocomposites for bone regeneration', *J Mater Sci Mater Med*, vol. 21, no. 11, pp. 2923-36.

Chen, Q.Z. & Boccaccini, A.R. 2006, 'Poly(D,L-lactic acid) coated 45S5 Bioglass-based scaffolds: processing and characterization', *J Biomed Mater Res A*, vol. 77, no. 3, pp. 445-57.

Chen, Q.Z., Thompson, I.D. & Boccaccini, A.R. 2006, '45S5 Bioglass-derived glass-ceramic scaffolds for bone tissue engineering', *Biomaterials*, vol. 27, no. 11, pp. 2414-25.

Chen, Q.Z. & Thouas, G.A. 2011, 'Fabrication and characterization of sol-gel derived 45S5 Bioglass (R)-ceramic scaffolds', *Acta Biomaterialia*, vol. 7, no. 10, pp. 3616-26.

Chen, X., Li, S. & Huang, Z. 1995, '[The applications of machinable bioactive glass ceramics in maxillofacial augmentation]', *Zhonghua Zheng Xing Shao Shang Wai Ke Za Zhi*, vol. 11, no. 6, pp. 419-20.

Chung, E.J., Kodali, P., Laskin, W., Koh, J.L. & Ameer, G.A. 2011, 'Long-term in vivo response to citric acid-based nanocomposites for orthopaedic tissue engineering', *J Mater Sci Mater Med*, vol. 22, no. 9, pp. 2131-8.

Covani, U., Giacomelli, L., Krajewski, A., Ravaglioli, A., Spotorno, L., Loria, P., Das, S. & Nicolinil, C. 2007, 'Biomaterials for orthopedics: A roughness analysis by atomic force microscopy', *Journal of Biomedical Materials Research Part A*, vol. 82A, no. 3, pp. 723-30.

Day, R.M. 2005, 'Bioactive glass stimulates the secretion of angiogenic growth factors and angiogenesis in vitro', *Tissue Engineering*, vol. 11, no. 5-6, pp. 768-77.

Day, R.M., Maquet, V., Boccaccini, A.R., Jerome, R. & Forbes, A. 2005, 'In vitro and in vivo analysis of macroporous biodegradable poly(D,L-lactide-co-glycolide) scaffolds containing bioactive glass', *Journal of Biomedical Materials Research Part A*, vol. 75A, no. 4, pp. 778-87.

de Tayrac, R., Oliva-Lauraire, M.C., Guiraud, I., Henry, L., Vert, M. & Mares, P. 2007, 'Long-lasting bioresorbable poly(lactic acid) (PLA(94)) mesh: A new approach for soft tissue reinforcement based on an experimental pilot study', *International Urogynecology Journal*, vol. 18, no. 9, pp. 1007-14.

Dou, Y., Lin, K. & Chang, J. 2011, 'Polymer nanocomposites with controllable distribution and arrangement of inorganic nanocomponents', *Nanoscale*, vol. 3, no. 4, pp. 1508-11.

Drnovsek, N., Novak, S., Dragin, U., Ceh, M., Gorensek, M. & Gradisar, M. 2012, 'Bioactive glass enhances bone ingrowth into the porous titanium coating on orthopaedic implants', *International Orthopaedics*.

Ducheyne, P. 1985, 'Bioglass coatings and bioglass composites as implant materials', *Journal of Biomedical Materials Research*, vol. 19, no. 3, pp. 273-91.

Ebelmen, M. 1846, 'On the synthesis of silica gels from alkoxides ', *Annales de chimie et de physique* vol. 16, p. 129.

Erdemli, O., Captug, O., Bilgili, H., Orhan, D., Tezcaner, A. & Keskin, D. 2010, 'In vitro and in vivo evaluation of the effects of demineralized bone matrix or calcium sulfate addition to polycaprolactone-bioglass composites', *J Mater Sci Mater Med*, vol. 21, no. 1, pp. 295-308.

Gatti, A.M., Simonetti, L.A., Monari, E., Guidi, S. & Greenspan, D. 2006, 'Bone augmentation with bioactive glass in three cases of dental implant placement', *Journal of Biomaterials Applications*, vol. 20, no. 4, pp. 325-39.

Gatti, A.M., Valdre, G. & Andersson, O.H. 1994, 'Analysis of the in vivo reactions of a bioactive glass in soft and hard tissue', *Biomaterials*, vol. 15, no. 3, pp. 208-12.

Gillette, R.L., Swaim, S.F., Sartin, E.A., Bradley, D.M. & Coolman, S.L. 2001, 'Effects of a bioactive glass on healing of closed skin wounds in dogs', *Am J Vet Res*, vol. 62, no. 7, pp. 1149-53.

Gonzalez, P., Serra, J., Liste, S., Chiussi, S., Leon, B., Perez-Amor, M., Martinez-Fernandez, J., de Arellano-Lopez, A.R. & Varela-Feria, F.M. 2003, 'New biomorphic SiC ceramics coated with bioactive glass for biomedical applications', *Biomaterials*, vol. 24, no. 26, pp. 4827-32.

Graham, T. 1864, 'XXXV.-On the properties of silicic acid and other analogous colloidal substances', *Journal of the Chemical Society*, vol. 17, pp. 318-27.

Greenspan, D.C., Zhong, J.P., Chen, X.F. & LaTorre, G.P. 1997, 'The evaluation of degradability of melt and sol-gel derived Bioglass (R) in-vitro', *Bioceramics, Vol 10*, pp. 391-4.

Gross, U., Kinne, R., Schmitz, H.J. & Strunz, V. 1988, 'The response of bone to surface active glassceramics', *CRC Crit. Rev. Biocomput.*, vol. 4, pp. 2-15.

Habraken, W.J.E.M., Wolke, J.G.C. & Jansen, J.A. 2007, 'Ceramic composites as matrices and scaffolds for drug delivery in tissue engineering', *Advanced Drug Delivery Reviews*, vol. 59, no. 4–5, pp. 234-48.

Hajiali, H., Karbasi, S., Hosseinalipour, M. & Rezaie, H.R. 2010, 'Preparation of a novel biodegradable nanocomposite scaffold based on poly (3-hydroxybutyrate)/bioglass nanoparticles for bone tissue engineering', *J Mater Sci Mater Med*, vol. 21, no. 7, pp. 2125-32.

Hench, L.L. 2006, 'The story of Bioglass', *J Mater Sci Mater Med*, vol. 17, no. 11, pp. 967-78.

Hench, L.L., Polak, J.M., Xynos, I.D. & Buttery, L.D.K. 2000, 'Bioactive materials to control cell cycle', *Materials Research Innovations*, vol. 3, no. 6, pp. 313-23.

Hench, L.L., Splinter, R.J., Allen, W.C. & Greenlee, T.K. 1972, 'Bonding mechanisms at the interface of ceramic prosthetic materials', *J. Biomed. Muter. Res*, vol. 2.

Hench, L.L. & West, J.K. 1990, 'The sol-gel process', *Chemical Reviews*, vol. 90, no. 1, pp. 33-72.

Hench, L.L. & Wilson, J. 1984, 'Surface-Active Biomaterials', *Science*, vol. 226, no. 4675, pp. 630-6.

Hong, Z., Reis, R.L. & Mano, J.F. 2008, 'Preparation and in vitro characterization of scaffolds of poly(L-lactic acid) containing bioactive glass ceramic nanoparticles', *Acta Biomater*, vol. 4, no. 5, pp. 1297-306.

Hong, Z., Reis, R.L. & Mano, J.F. 2009, 'Preparation and in vitro characterization of novel bioactive glass ceramic nanoparticles', *J Biomed Mater Res A*, vol. 88, no. 2, pp. 304-13.

Huadong, S., Xiaohong, L. & Zhijun, Z. 2008, 'Surface Modified Nano-Silica and Its Action on Polymer', *Progress in Chemistry*, vol. 20, no. 10, pp. 1509-14.

Ilharreborde, B., Morel, E., Fitoussi, F., Presedo, A., Souchet, P., Pennecot, G.F. & Mazda, K. 2008, 'Bioactive Glass as a Bone Substitute for Spinal Fusion in Adolescent Idiopathic Scoliosis A Comparative Study With Iliac Crest Autograft', *Journal of Pediatric Orthopaedics*, vol. 28, no. 3, pp. 347-51.

Jie, Q., Lin, K.L., Zhong, J.P., Shi, Y.F., Li, Q., Chang, J.A. & Wang, R.D. 2004, 'Preparation of macroporous sol-gel bioglass using PVA particles as pore former', *Journal of Sol-Gel Science and Technology*, vol. 30, no. 1, pp. 49-61.

Karagiozov, C. & Momchilova, D. 2005, 'Synthesis of nano-sized particles from metal carbonates by the method of reversed mycelles', *Chemical Engineering and Processing: Process Intensification*, vol. 44, no. 1, pp. 115-9.

Keledi, G., Hari, J. & Pukanszky, B. 2012, 'Polymer nanocomposites: structure, interaction, and functionality', *Nanoscale*, vol. 4, no. 6, pp. 1919-38.

Kickelbick, G. 2003, 'Concepts for the incorporation of inorganic building blocks into organic polymers on a nanoscale', *Progress in Polymer Science*, vol. 28, no. 1, pp. 83-114.

Kim, D., Lee, J.S., Barry, C.M.F. & Mead, J.L. 2007, 'Effect of fill factor and validation of characterizing the degree of mixing in polymer nanocomposites', *Polymer Engineering & Science*, vol. 47, no. 12, pp. 2049-56.

Kim, H.W., Lee, H.H. & Chun, G.S. 2008, 'Bioactivity and osteoblast responses of novel biomedical nanocomposites of bioactive glass nanofiber filled poly(lactic acid)', *J Biomed Mater Res A*, vol. 85, no. 3, pp. 651-63.

Kim, H.W., Song, J.H. & Kim, H.E. 2006, 'Bioactive glass nanofiber-collagen nanocomposite as a novel bone regeneration matrix', *J Biomed Mater Res A*, vol. 79, no. 3, pp. 698-705.

Kontonasaki, E., Papadopoulou, L., Zorba, T., Pavlidou, E., Paraskevopoulos, K. & Koidis, P. 2003, 'Apatite formation on dental ceramics modified by a bioactive glass', *J Oral Rehabil*, vol. 30, no. 9, pp. 893-902.

Lee, H.H., Sang Shin, U., Lee, J.H. & Kim, H.W. 2011, 'Biomedical nanocomposites of poly(lactic acid) and calcium phosphate hybridized with modified carbon nanotubes for hard tissue implants', *J Biomed Mater Res B Appl Biomater*, vol. 98B, no. 2, pp. 246-54.

Lee, H.H., Yu, H.S., Jang, J.H. & Kim, H.W. 2008, 'Bioactivity improvement of poly(epsilon-caprolactone) membrane with the addition of nanofibrous bioactive glass', *Acta Biomater*, vol. 4, no. 3, pp. 622-9.

Lei, B., Shin, K.H., Noh, D.Y., Koh, Y.H., Choi, W.Y. & Kim, H.E. 2012, 'Bioactive glass microspheres as reinforcement for improving the mechanical properties and biological performance of poly(e-caprolactone) polymer for bone tissue regeneration', *Journal of Biomedical Materials Research Part B-Applied Biomaterials*, vol. 100B, no. 4, pp. 967-75.

Li, G., Feng, S. & Zhou, D. 2011, 'Magnetic bioactive glass ceramic in the system CaO-P2O5-SiO2-MgO-CaF2-MnO2-Fe2O3 for hyperthermia treatment of bone tumor', *J Mater Sci Mater Med*, vol. 22, no. 10, pp. 2197-206.

Li, H., Chen, S., Wu, Y., Jiang, J., Ge, Y., Gao, K., Zhang, P. & Wu, L. 2012, 'Enhancement of the osseointegration of a polyethylene terephthalate artificial ligament graft in a bone tunnel using 58S bioglass', *International Orthopaedics*, vol. 36, no. 1, pp. 191-7.

Li, R., Clark, A.E. & Hench, L.L. 1991, 'An investigation of bioactive glass powders by sol-gel processing', *Journal of Applied Biomaterials*, vol. 2, no. 4, pp. 231-9.

Li, Y., Liu, Y.Z., Long, T., Yu, X.B., Tang, T.T., Dai, K.R., Tian, B., Guo, Y.P. & Zhu, Z.A. 2013, 'Mesoporous bioactive glass as a drug delivery system: fabrication, bactericidal properties and biocompatibility', *J Mater Sci Mater Med*, vol. 24, no. 8, pp. 1951-61.

Liesegang, R.E. 1896, 'Ueber einige Eigenschaften von Gallerten', *Naturwissenschaftliche Wochenschrift,*, vol. 11, no. 30, pp. 353 - 62.

Lim, G.K., Wang, J., Ng, S.C. & Gan, L.M. 1996, 'Processing of fine hydroxyapatite powders via an inverse microemulsion route', *Materials Letters*, vol. 28, no. 4–6, pp. 431-6.

Lim, G.K., Wang, J., Ng, S.C. & Gan, L.M. 1999, 'Formation of Nanocrystalline Hydroxyapatite in Nonionic Surfactant Emulsions', *Langmuir*, vol. 15, no. 22, pp. 7472-7.

Lin, C., Mao, C., Zhang, J., Li, Y. & Chen, X. 2012, 'Healing effect of bioactive glass ointment on full-thickness skin wounds', *Biomedical Materials*, vol. 7, no. 4, p. 045017.

Lindfors, N.C. 2009, 'Treatment of a recurrent aneurysmal bone cyst with bioactive glass in a child allows for good bone remodelling and growth', *Bone*, vol. 45, no. 2, pp. 398-400.

Lindfors, N.C., Hyvonen, P., Nyyssonen, M., Kirjavainen, M., Kankare, J., Gullichsen, E. & Salo, J. 2010, 'Bioactive glass S53P4 as bone graft substitute in treatment of osteomyelitis', *Bone*, vol. 47, no. 2, pp. 212-8.

Liu, H. & Webster, T.J. 2010, 'Ceramic/polymer nanocomposites with tunable drug delivery capability at specific disease sites', *Journal of Biomedical Materials Research Part A*, vol. 93A, no. 3, pp. 1180-92.

Liu, X., Xie, Z.P., Zhang, C.Q., Pan, H.B., Rahaman, M.N., Zhang, X., Fu, Q. & Huang, W.H. 2010, 'Bioactive borate glass scaffolds: in vitro and in vivo evaluation for use as a drug delivery system in the treatment of bone infection', *Journal of Materials Science-Materials in Medicine*, vol. 21, no. 2, pp. 575-82.

Ma, J., Chen, C., Wang, D., Meng, X. & Shi, J. 2010, 'In vitro degradability and bioactivity of mesoporous CaO-MgO-P_2O_5 glasses synthesized by sol–gel method', *Journal of Sol-Gel Science and Technology*, vol. 54, no. 1, pp. 69-76.

Macha, I.J., Ben-Nissan, B. & Milthorpe, B. 2014, 'Improvement of Elongation in Nanosurface Modified Bioglass/PLA Thin Film Composites', *Current Nanoscience*, vol. 10, no. 2, pp. 200-4.

Mehdikhani-Nahrkhalaji, M., Fathi, M.H., Mortazavi, V., Mousavi, S.B., Hashemi-Beni, B. & Razavi, S.M. 2012, 'Novel nanocomposite coating for dental implant applications in vitro and in vivo evaluation', *J Mater Sci Mater Med*, vol. 23, no. 2, pp. 485-95.

Merwin, G.E. 1990, 'Review of Bioactive Materials for Otologic and Maxillofacial Applications', in T. Yamamuro, L.L. Hench & J.Wilson (eds), *Handbook of Bioactive Ceramics*, vol. 1, CRC Press, Boca Raton, Florida, p. 323.

Mohn, D., Zehnder, M., Imfeld, T. & Stark, W.J. 2010, 'Radio-opaque nanosized bioactive glass for potential root canal application: evaluation of radiopacity, bioactivity and alkaline capacity', *International Endodontic Journal*, vol. 43, no. 3, pp. 210-7.

Moncada, E., Quijada, R. & Retuert, J. 2007, 'Nanoparticles prepared by the sol–gel method and their use in the formation of nanocomposites with polypropylene', *Nanotechnology*, vol. 18, no. 33, p. 335606.

Mota, J., Yu, N., Caridade, S.G., Luz, G., Gomes, M.E., Reis, R.L., Jansen, J.A., Walboomers, X.F. & Mano, J.F. 2012, 'Chitosan/bioactive glass nanoparticle composite membranes for periodontal regeneration', *Acta Biomater*.

Niemelä, T., Niiranen, H., Kellomäki, M. & Törmälä, P. 2005, 'Self-reinforced composites of bioabsorbable polymer and bioactive glass with different bioactive glass contents. Part I: Initial mechanical properties and bioactivity', *Acta Biomaterialia*, vol. 1, no. 2, pp. 235-42.

Norton, M.R. & Wilson, J. 2002, 'Dental implants placed in extraction sites implanted with bioactive glass: human histology and clinical outcome', *Int J Oral Maxillofac Implants*, vol. 17, no. 2, pp. 249-57.

Orgaz-Orgaz, F. 1988, 'Gel to glass conversion: Densification kinetics and controlling mechanisms', *J. Non-Cryst. Solids*, vol. 100, p. 115.

Pereira, M.M. & Hench, L.L. 1996, 'Mechanisms of hydroxyapatite formation on porous gel-silica substrates', *Journal of Sol-Gel Science and Technology*, vol. 7, no. 1-2, pp. 59-68.

Pernaa, K., Koski, I., Mattila, K., Gullichsen, E., Heikkila, J., Aho, A. & Lindfors, N. 2011, 'Bioactive Glass S53P4 and Autograft Bone in Treatment of Depressed Tibial Plateau Fractures - A Prospective Randomized 11-year Follow-Up', *J Long Term Eff Med Implants*, vol. 21, no. 2, pp. 139-48.

Pirkey, E.L. & Hurt, J. 1959, 'Roentgen Evaluation of the Soft Tissues in Orthopedics', *American Journal of Roentgenology Radium Therapy and Nuclear Medicine*, vol. 82, no. 2, pp. 271-6.

Pratsinis, S.E. 1998, 'Flame aerosol synthesis of ceramic powders', *Progress in Energy and Combustion Science*, vol. 24, no. 3, pp. 197-219.

Qian, J.M., Kang, Y.H., Wei, Z.L. & Zhang, W. 2009, 'Fabrication and characterization of biomorphic 45S5 bioglass scaffold from sugarcane', *Materials Science & Engineering C-Biomimetic and Supramolecular Systems*, vol. 29, no. 4, pp. 1361-4.

Quintero, F., Dieste, O., Pou, J., Lusqui˜nos, F. & Riveiro, A. 2009a, 'On the conditions to produce micro- and nanofibres by laser spinning', *J. Phys. D: Appl. Phys.*, vol. 42, pp. 1-10.

Quintero, F., Mann, A.B., Pou, J., Lusquinos, F. & Riveiro, A. 2007a, 'Rapid production of ultralong amorphous ceramic nanofibers by laser spinning', *Applied Physics Letters*, vol. 90, no. 15, pp. 153109-3.

Quintero, F., Pou, J., Comesaña, R., Lusquiños, F., Riveiro, A., Mann, A.B., Hill, R.G., Wu, Z.Y. & Jones, J.R. 2009b, 'Laser Spinning of Bioactive Glass Nanofibers', *Advanced Functional Materials*, vol. 19, no. 19, pp. 3084-90.

Quintero, F., Pou, J., Lusquiños, F. & Riveiro, A. 2007b, 'Experimental analysis of the production of micro- and nanofibres by Laser Spinning', *Applied Surface Science*, vol. 254, no. 4, pp. 1042-7.

Rahman, I.A. & Padavettan, V. 2012, 'Synthesis of Silica Nanoparticles by Sol-Gel: Size-Dependent Properties, Surface Modification, and Applications in Silica-Polymer Nanocomposites—A Review', *Journal of Nanomaterials*, vol. 2012, p. 15.

Ramires, P.A., Wennerberg, A., Johansson, C.B., Cosentino, F., Tundo, S. & Milella, E. 2003, 'Biological behavior of sol-gel coated dental implants', *J Mater Sci Mater Med*, vol. 14, no. 6, pp. 539-45.

Rantakokko, J., Frantzen, J.P., Heinanen, J., Kajander, S., Kotilainen, E., Gullichsen, E. & Lindfors, N.C. 2012, 'Posterolateral Spondylodesis Using Bioactive Glass S53p4 and Autogenous Bone in Instrumented Unstable Lumbar Spine Burst Fractures', *Scandinavian Journal of Surgery*, vol. 101, no. 1, pp. 66-71.

Saboori, A., Rabiee, M., Mutarzadeh, F., Sheikhi, M., Tahriri, M. & Karimi, M. 2009, 'Synthesis, characterization and in vitro bioactivity of sol-gel-derived SiO2-CaO-P2O5-MgO bioglass', *Materials Science & Engineering C-Biomimetic and Supramolecular Systems*, vol. 29, no. 1, pp. 335-40.

Sakka, S. 1985, 'Glasses and glass-ceramics from gels', *J. Non-Cryst. Solids*, vol. 73, p. 651.

Saska, S., Barud, H.S., Gaspar, A.M., Marchetto, R., Ribeiro, S.J. & Messaddeq, Y. 2011, 'Bacterial cellulose-hydroxyapatite nanocomposites for bone regeneration', *Int J Biomater*, vol. 2011, p. 175362.

Sepulveda, P., Jones, J.R. & Hench, L.L. 2001, 'Characterization of melt-derived 45S5 and sol-gel-derived 58S bioactive glasses', *Journal of Biomedical Materials Research*, vol. 58, no. 6, pp. 734-40.

Singh, S., Bhardwaj, P., Singh, V., Aggarwal, S. & Mandal, U.K. 2008, 'Synthesis of nanocrystalline calcium phosphate in microemulsion—effect of nature of surfactants', *Journal of Colloid and Interface Science*, vol. 319, no. 1, pp. 322-9.

Soundrapandian, C., Basu, D., Sa, B. & Datta, S. 2011, 'Local drug delivery system for the treatment of osteomyelitis: In vitro evaluation', *Drug Development and Industrial Pharmacy*, vol. 37, no. 5, pp. 538-46.

Soundrapandian, C., Datta, S., Kundu, B., Basu, D. & Sa, B. 2010, 'Porous Bioactive Glass Scaffolds for Local Drug Delivery in Osteomyelitis: Development and In Vitro Characterization', *Aaps Pharmscitech*, vol. 11, no. 4, pp. 1675-83.

Stark, W.J., Madler, L., Maciejewski, M., Pratsinis, S.E. & Baiker, A. 2003, 'Flame synthesis of nanocrystalline ceria-zirconia: effect of carrier liquid', *Chemical Communications*, no. 5, pp. 588-9.

Sun, Y., Guo, G., Tao, D. & Wang, Z. 2007, 'Reverse microemulsion-directed synthesis of hydroxyapatite nanoparticles under hydrothermal conditions', *Journal of Physics and Chemistry of Solids*, vol. 68, no. 3, pp. 373-7.

Tang, Q.L., Zhu, Y.J., Duan, Y.R., Wang, Q., Wang, K.W., Cao, S.W., Chen, F. & Wu, J. 2010, 'Porous nanocomposites of PEG-PLA/calcium phosphate: room-temperature synthesis and its application in drug delivery', *Dalton Transactions*, vol. 39, no. 18, pp. 4435-9.

Tokgozoglu, A.M. 1998, 'The use of bioresorbable implants in orthopaedics', *Biomedical Science and Technology*, pp. 197-203.

Ustariz-Peyret, C., Coudane, J., Vert, M., Kaltsatos, V. & Boisramene, B. 2000, 'Labile conjugation of a hydrophilic drug to PLA oligomers to modify a drug delivery system: cephradin in a PLAGA matrix', *Journal of Microencapsulation*, vol. 17, no. 5, pp. 615-24.

Valenzuela, F., Covarrubias, C., Martinez, C., Smith, P., Diaz-Dosque, M. & Yazdani-Pedram, M. 2012, 'Preparation and bioactive properties of novel bone-repair bionanocomposites based on hydroxyapatite and bioactive glass nanoparticles', *J Biomed Mater Res B Appl Biomater*, vol. 100, no. 6, pp. 1672-82.

Vollenweider, M., Brunner, T.J., Knecht, S., Grass, R.N., Zehnder, M., Imfeld, T. & Stark, W.J. 2007, 'Remineralization of human dentin using ultrafine bioactive glass particles', *Acta Biomaterialia*, vol. 3, no. 6, pp. 936-43.

Wang, M., Hench, L.L. & Bonfield, W. 1998, 'Bioglass/high density polyethylene composite for soft tissue applications: preparation and evaluation', *J Biomed Mater Res*, vol. 42, no. 4, pp. 577-86.

Willerth, S.M. & Sakiyama-Elbert, S.E. 2007, 'Approaches to neural tissue engineering using scaffolds for drug delivery', *Advanced Drug Delivery Reviews*, vol. 59, no. 4–5, pp. 325-38.

Wren, A.W., Coughlan, A., Hassanzadeh, P. & Towler, M.R. 2012, 'Silver coated bioactive glass particles for wound healing applications', *J Mater Sci Mater Med*, vol. 23, no. 5, pp. 1331-41.

Wu, C., Fan, W., Chang, J. & Xiao, Y. 2013, 'Mesoporous bioactive glass scaffolds for efficient delivery of vascular endothelial growth factor', *J Biomater Appl*, vol. 28, no. 3, pp. 367-74.

Zammarano, M., Maupin, P.H., Sung, L.P., Gilman, J.W., McCarthy, E.D., Kim, Y.S. & Fox, D.M. 2011, 'Revealing the interface in polymer nanocomposites', *Acs Nano*, vol. 5, no. 4, pp. 3391-9.

Zhou, Q., Pramoda, K.P., Lee, J.M., Wang, K. & Loo, L.S. 2011, 'Role of interface in dispersion and surface energetics of polymer nanocomposites containing hydrophilic POSS and layered silicates', *J Colloid Interface Sci*, vol. 355, no. 1, pp. 222-30.

Zhu, M., Wang, H., Liu, J., He, H., Hua, X., He, Q., Zhang, L., Ye, X. & Shi, J. 2011, 'A mesoporous silica nanoparticulate/beta-TCP/BG composite drug delivery system for osteoarticular tuberculosis therapy', *Biomaterials*, vol. 32, no. 7, pp. 1986-95.

CHAPTER 4

PLA-BIOGLASS COMPOSITES

CHAPTER 4: PLA-BIOGLASS COMPOSITES

In this chapter, PLA with the sol-gel bioglass biomaterials were used to form biocomposites and their properties are hereby discussed.

4.1 Introduction

Biomaterials have been designed for medical and related applications. It is a reasonable prediction that in the near future, the use of biodegradable biomaterials in many of the permanent prosthetic devices used for temporary therapeutic applications, will be replaced by bioresorbable devices (Nair & Laurencin 2007). The chief advantage of using bioresorbable devices begins with a simple desire: to have a device that can be used as an implant yet will not necessitate a second surgical event for its removal (Middleton & Tipton 2000). In addition, the long term biocompatibility of some existing implants has shown to have longevity problems. Moreover, novel biomedical technologies available today like tissue engineering, regenerative medicine, gene therapy, controlled drug delivery and bio nanotechnology require biodegradable platform materials to build on (Nair & Laurencin 2007).

Polylactic acid (PLA) has been extensively described in **Chapter 2** and has been shown to have suitable abilities for medical applications. PLA, and its copolymers, received FDA approval for use in medical applications and have been used as surgical sutures or drug delivery systems for many years (Howard et al. 2002). However, lactic acid-based synthetic polymers are not inherently bioactive and will not bond directly to bone. They are also initially highly hydrophobic and lack the mechanical strength required to meet the demands of orthopaedic surgery (Cohen et al. 1993; Liu and Ma 2004). The combination of such polymers with a bioactive component such as hydroxyapatite and bioglass therefore takes advantage of both the osteoconductive properties (bioactivity) and their possible strengthening effect on polymer matrices. Moreover, it has been stated that bioactive glass in polyester controls the hydrolytic degradation characteristics of the polymer thus improving the structural integrity of the polymer composites (Niiranen & To¨rma¨la¨ 1999). With respect to their excellent osteoconductivity

and high bioactivity of bioglass as described in **Chapter 3**, its composites with PLA have a good potential for wide medical applications.

Excessive agglomeration of bioactive particles in polymer matrices has been one of the main challenges in making homogeneous particle distributed composites. Effort has been devoted into functionalizing the surface of these particles in order to improve adhesion, and their physicochemical and mechanical properties (Macha, Ben-Nissan & Milthorpe 2014). Surface modification of bioactive glasses to improve mechanical and biological properties of polylactic acid composites has been investigated using different grafting materials (Liu et al. 2008; Gao & Chang 2009; Verne et al. 2009). Past studies on improvement of mechanical properties have been focused on improving tensile strength, tensile modulus and impact energy of the bulk PLA composites (Gerhardt & Boccaccini 2010). Improvement of mechanical properties of sol-gel bioglass-PLA composites to adapt the complex elastic nature and elongation of human tissues during biomechanical functional loading would be beneficial in tissue engineering.

In this chapter functionalization of bioactive bioglass to improve elastic properties of PLA thin film composites are covered.

4.2 Experimental Work

4.2.1 Materials

PLA was obtained from NatureWorks LLC, 3052D Australia. Materials for bioglass production are mentioned in Chapter 3. Tetraethyl orthosilicate (TEOS, $Si(OC_2H_9)_4$, 99.90%), Citric acid monohydrate (99%), Ethanol absolute (99.8%), Ammonium hydroxide solution (NH_4OH, 33%), Ammonium phosphate dibasic ($(NH_4)_2HPO_4$, 98%), Calcium nitrate tetrahydrate ($Ca(NO_3)_2.4H_2O$, 99.60%), PEG (Mw 20,000), acetic acid (99.7%) and (3-Aminopropyl)triethoxysilane (APTES) (99%) were obtained from Sigma Aldrich Australia.

4.2.2 Methods

4.2.2.1 Sol-gel Bioglass

Bioglass powders (SiO_2:CaO:P_2O_5 at mole ratio 55:40:5) were based on the method describe in previous chapter (Chapter 3)

4.2.2.2 Bioglass Surface Functionalization

The deposition method from aqueous alcohol solutions was used to obtain silanated bioglass surfaces. A 95% ethanol-5% water solution was adjusted to pH 4.5-5.5 with 0.1M acetic acid. Silane (3-aminopropyltriethoxysilane, APTES) was added with stirring to yield a 1% final concentration and then left for five minutes to allow for hydrolysis and silanol formation. Bioglass powders were added, suspended in the same solution and stirred for 2-3 minutes and then the solution was decanted. The particles were briefly rinsed twice with ethanol and cured for 5-10 minutes at 110°C in an oven. The obtained silanised bioglass powders were stored in desiccators for further analyses.

4.2.2.3 Polymer Films and Composites

All the composites were prepared using a solvent casting technique described and modified by (Macha, Ben-Nissan & Milthorpe 2014). PLA films were prepared by dissolving 0.50 g of polymer in 30ml of chloroform. The solution was transferred into a 9 cm diameter petri dish for 24h, and chloroform was allowed to evaporate under a low vacuum (in a desiccator). The films were stored in desiccators for further analyses. The same procedure with 15 minutes ultrasonication was employed to prepare PLA/Bioglass composites with loadings of 0.1, 0.5 and 1% weight percentage bioglass.

4.2.2.4 Characterization

Calcination of bioglass was performed using simultaneous thermo-gravimetry and differential analysis (TG-DTA, SDT 2960, TA Instruments, New Castle, DE, USA). A sample weight of 30-40 mg was used during analysis under a circulating air environment with a heating rate of 10 ^oC/min from room temperature to 700 ^oC and maintained at 700 ^oC for three hours.

The morphological analysis of bioglass, polymer films and composite samples was performed in a Scanning Electron Microscopy (ZEISS Supra55VP, Zeiss, Sydney, Australia). Samples were fixed by mutual conductive adhesive tape on to aluminium stubs and covered with carbon using a sputter coater. Images were taken at various magnifications at acceleration voltages of 5 kV to avoid beam damage to the polymer.

Phase analysis of the synthesized powders of Bioglass was conducted using primarily X-ray diffraction, using a Siemens D5000 X-ray Diffractometer (Siemens, Bayswater, Australia) employing CuKα radiation (λ =0.15418 nm) with detector (X'celerator). The diffractometer was operated at 45 kV and 40 mA at a 2θ range of 20–70° employing a step size of 0.01 and a 4 s exposure.

The synthesized and surface functionalized bioglass were ground in an agate mortar and thoroughly mixed with KBr (FTIR Grade, 1% w/w). The FTIR spectra were collected using a Nicolet, Magna-IR 6700 Spectrometer FTIR (Thermo Fisher Scientific, Madison USA) in the range 4000–400 cm-1. The mechanical characterizations were performed by means of tensile and tear tests according to the ASTM D882 – 10 and ASTM D1004 – 13 standards respectively. Tests were carried out in a universal testing machine - an Instron 6022 equipped with a 5 kN load cell. Deformation was followed with Windows based software.

4.3 Results and Discussions

4.3.1 APTES functionalized bioglass

Sol-gel bioglass characterized in **Chapter 3** with FTIR showed the characteristic peaks of amorphous bioglass. After the modification with APTES, some notable changes are observed in the spectrum of Bioglass-APTES, Figure 13.

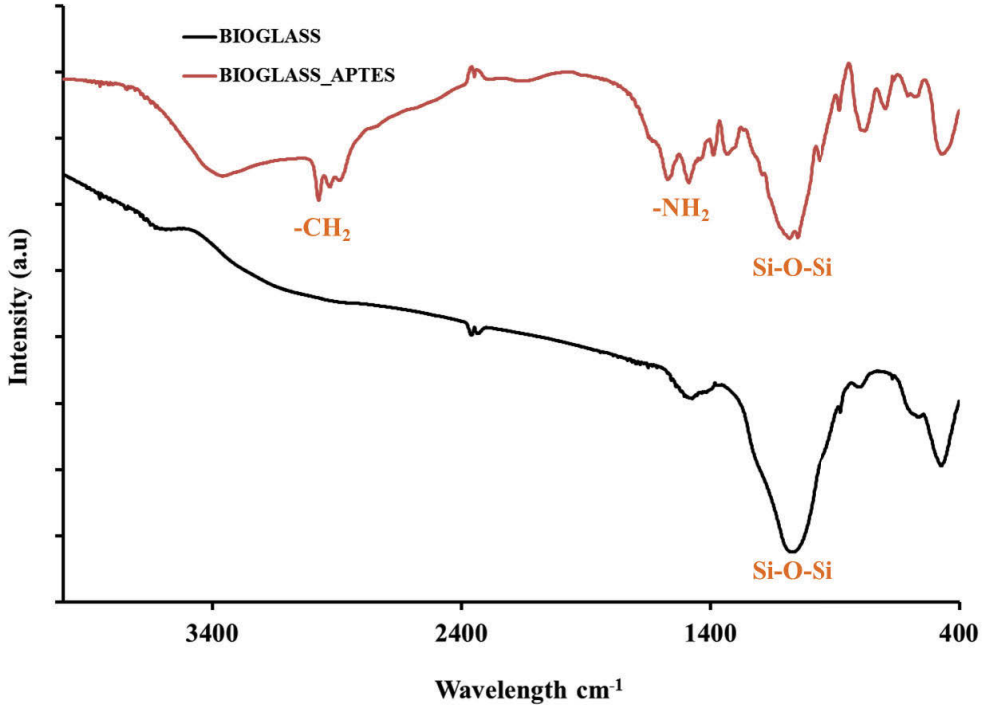

Figure 13: FTIR spectrum of bioglass and bioglass treated with APTES.

The strong bands at 2970 cm^{-1} and 2897 cm^{-1} are assigned to stretching mode of $–CH_2$ (Bianco et al. 2009). Deformation mode of $–NH_2$ was observed around 1558 cm^{-1} and 1473 cm^{-1} absorption band (Li, Ma, et al. 2013). The FTIR showed peaks at 1050 cm^{-1} and 457 cm^{-1}, corresponding to the Si-O-Si stretching and bending mode. These results suggest that APTES is successfully bonded on the bioglass surface.

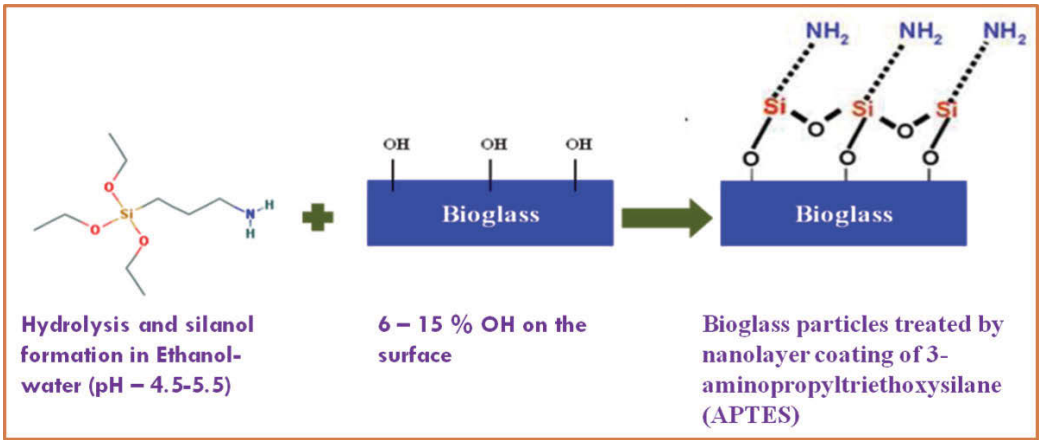

Figure 14: Schematic diagram showing the process of attaching APTES on bioglass surfaces.

It can be suggested that surface treated bioglass with APTES provides better bonding between the amine part of APTES and the carbonyl group in the PLA matrix that could contribute to a better interfacial adhesion than untreated ones, especially at the nanoparticle level. Figure 14 shows the schematic diagram of bioglass functionalization.

4.3.2 Particle size and morphology of composites

As was mentioned in **Chapter 3,** section **3.4.3**, bioglass highly agglomerates and the results suggested having an agglomeration size of between 1 to 100 μm. Due to the hydrophobicity of polylactic acid and the agglomeration nature of bioglass, their composite was shown to have poor particle distributions within the matrix and possess poor mechanical properties.

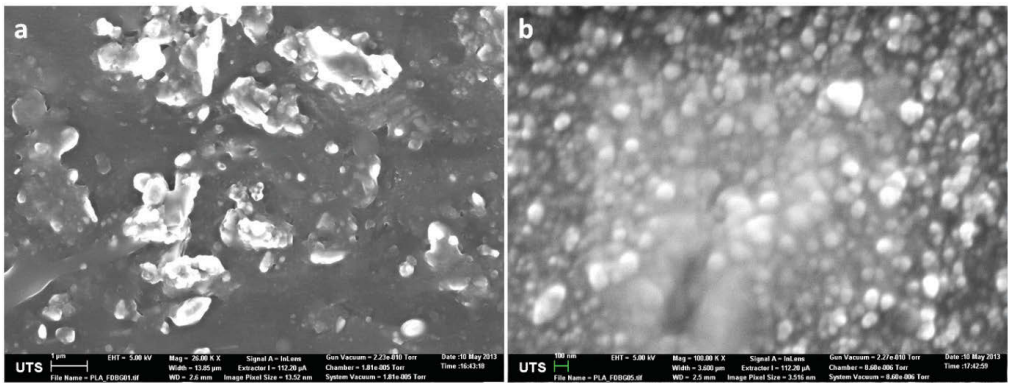

Figure 15: SEM image of PLA-Bioglass composites revealing agglomerated bioglass in PLA matrix (a) 26KX (b) 100KX.

Figure 15 shows that bioglass particles in the PLA matrix protruded cleanly from the matrix surface, indicating a weak interfacial bonding with the compact agglomerated bioglass powders, which are in some parts partially bonded to the PLA matrix. After treating the bioglass with APTES, the morphology of composites look different from untreated bioglass. SEM analysis of the treated bioglass composites showed both agglomerated and well dispersed powders, with particle sizes ranging from 50-100 nm; and some large agglomeration of particles were found within the matrix (Fig. 16a and 16b). These big particles seemed to be made of agglomerated spherical nanoparticles of the size in the range 50 – 100 nm.

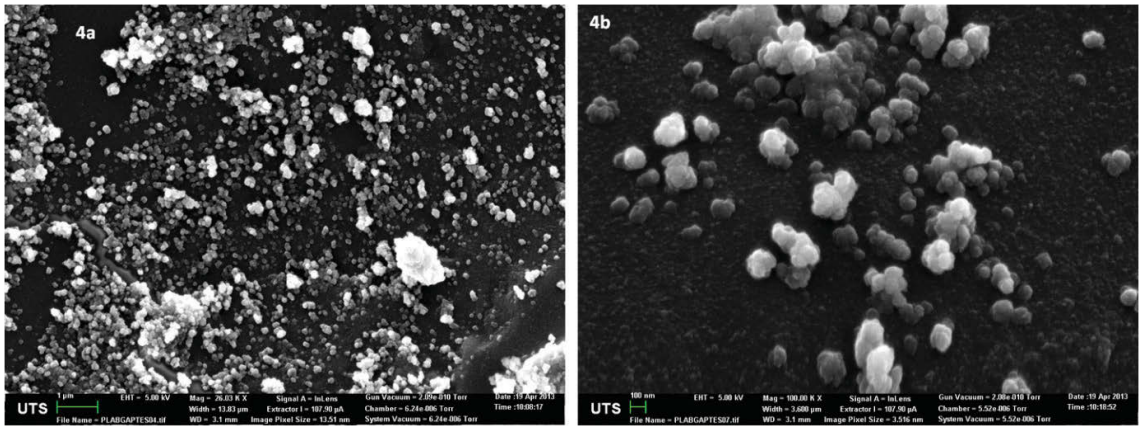

Figure 16: SEM micrograph of PLA-1% APTES treated bioglass composite at different magnifications a) 26KX and b) 100KX.

4.3.3 Mechanical properties of thin film composites

Tensile and tear tests were performed on both treated and untreated bioglass-PLA thin film composites. Elongation to fracture, and fracture behaviour of these films were studied and compared.

4.3.3.1 Tensile testing - Elongation at fracture of the composites

In Figure (17a), the effect of APTES treated bioglass on elongation at break of PLA composites is presented. The prepared PLA composite with treated bioglass shows improved mechanical properties. The APTES treated bioglass-PLA composites have a very high percentage elongation at break (an increase of 92 %) compared to pure PLA films and improvement of about 55% compared with untreated composites. Figure (17b) shows the tear resistance of PLA-untreated bioglass composites with 1, 3 and 5% bioglass loadings. It was observed that the tear resistant force and percentage elongation of PLA composites increases with an increase with the bioglass content from 1 to 3%.

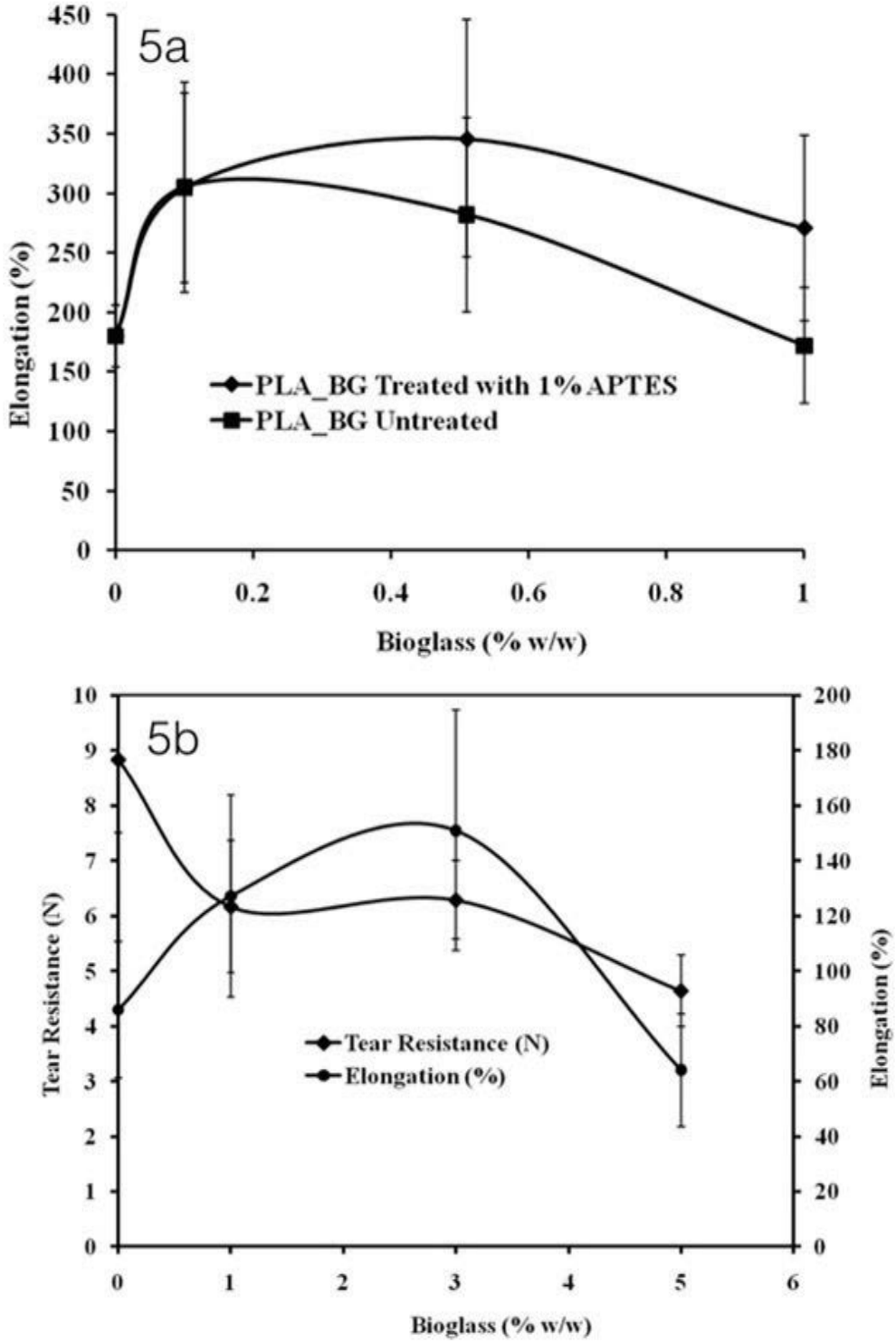

Figure 17: a) Effect of treated and untreated bioglass on elongation at break of PLA composites under tensile testing b) Tear test showing tear resistance and percent elongation for untreated bioglass/PLA composites. Error bars represent standard deviation (SD).

The tensile results showed that pure PLA film has yield and failure strength of 140 MPa and 81 MPa respectively with elongation at failure of 180 %. The maximum elongation at break for untreated bioglass composites occurred at 0.1 % bioglass content with a yield and failure strength and elongation at break of 6.5 MPa, 4.8 MPa and 305%, respectively. On the other hand, treated bioglass composites showed the maximum elongation at break of 346% with yield strength and failure strength of 5.2 MPa and 10.1 MPa respectively at 0.5% bioglass content. Tear results (Figure 17b) for untreated bioglass composites showed that pure PLA film has a tear resistant load of 6.6 N while its tear failure resistance was 2.2 N with elongation at break of 86%. The maximum elongation at break occurred at 3% bioglass loading with tear yield resistance of 2.9 N, tear failure resistance of 2.1 N and elongation at break of 151%.

4.3.3.2 Fracture Morphology of Pure PLA and Composites

The morphology of pure PLA film after tensile testing is shown in Fig. 18. It was observed that the deformation behaviour is slightly different between the outer and inner surfaces of the films, which may be due to variations in the drying rates during fabrication. One of the fundamental features observed in PLA thin films after fracture is the “riverlines”, which indicate clearly the crack growth direction. It is suggested that riverlines observed in multiple fractures initiated along the crack front during deformation and then began to propagate on several slightly different planes to then subsequently converge onto one plane. Similar observations in other polymers were also reported (Greenhalgh 2009). Another feature observed on the fracture surface is the formation of “cusps”, which appear on the fractured surface as raised platelets along riverlines, which was described earlier by Lee (Lee 1997).

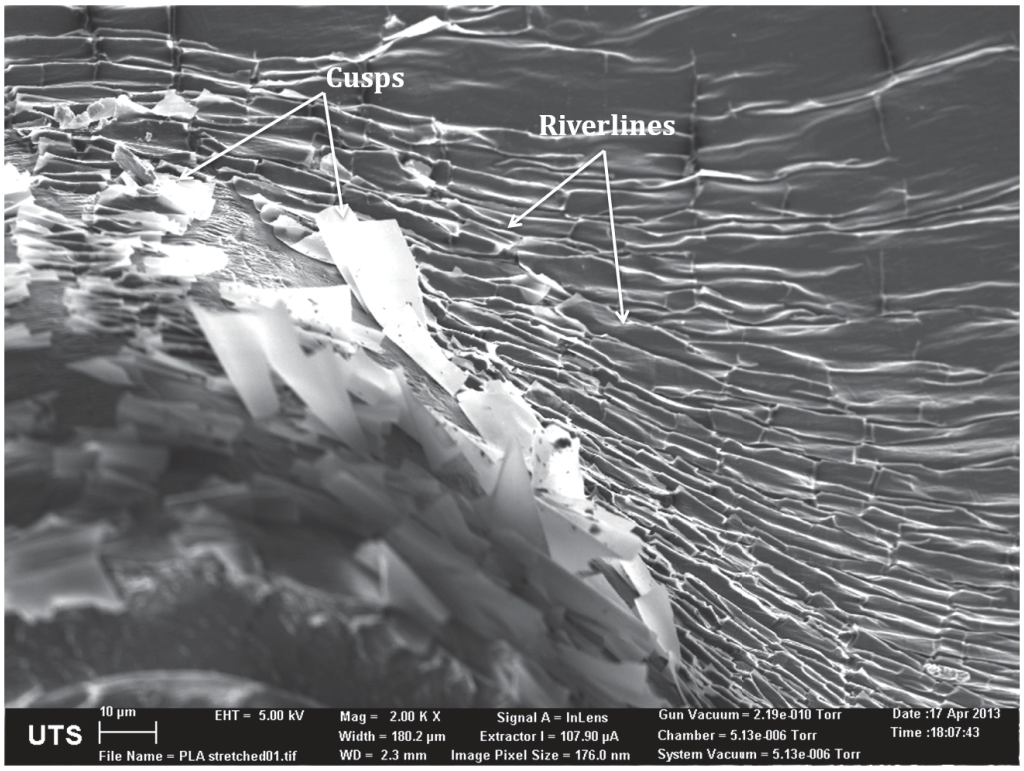

Figure 18: SEM micrograph of tensile fractured pure PLA film showing the riverlines at the edge and the surface of the thin film.

In Figure 19, the morphology of fractured PLA-untreated bioglass composite is shown. The bioglass particles in the PLA matrix protruded cleanly from the matrix surface, indicating a weak interfacial bonding with the compact agglomerated bioglass powders, which are in some parts partially bonded to the PLA matrix. The fracture seems to be initiated within the bioglass agglomerated particle and at the particle/PLA interface and spreads outwards to the surface. In this particular juncture, agglomerated bioglass particles create voids within the matrix (Fig. 19). These pores generate weak interfaces between the particles and the matrix. Furthermore, the particles are weakly adhered within the agglomerate itself due to weak adhesion of the individual particles; and dissociate to smaller particles under load. The morphology of fracture is quite similar to pure PLA films with riverlines and cusps at the fracture point.

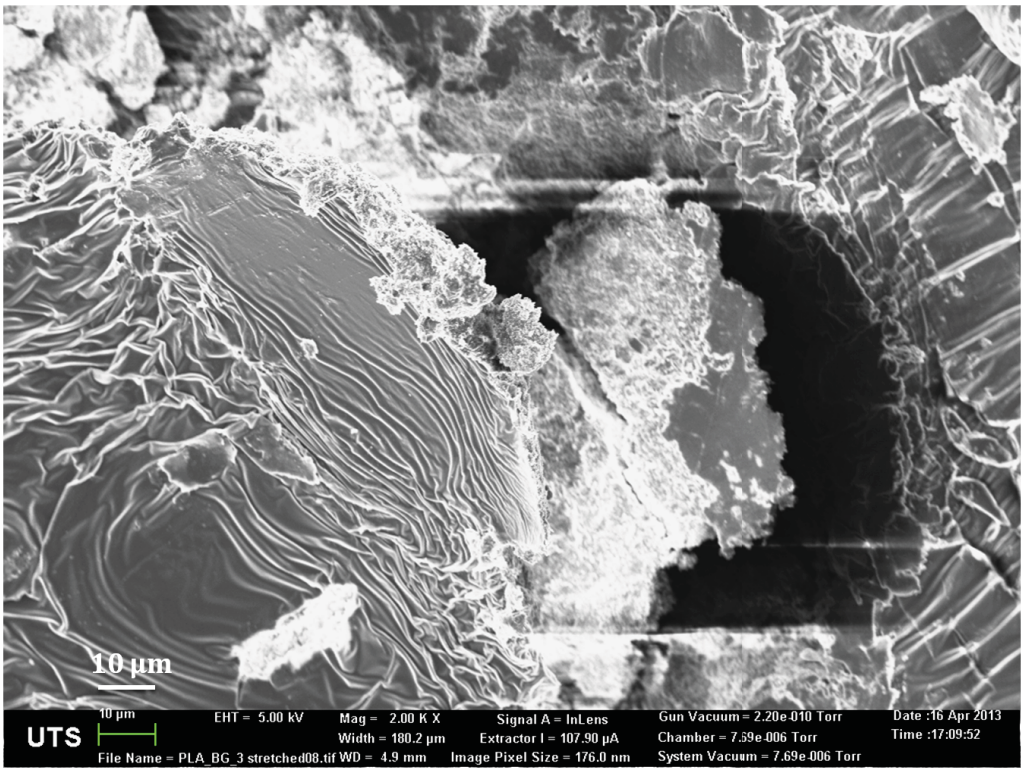

Figure 19: SEM micrograph of fractured PLA-untreated bioglass composite showing agglomerated particles and related pore within PLA matrix (2KX).

The presence of voids or pores within the polymer matrix could explain the difference in increased elongation of the PLA/bioglass composites compared to pure PLA films. The effect of voids on the elastic modulus of the composites could also be easily calculated theoretically and compared to the experimental results. Pores act as stress concentrators in adjacent materials and give rise to anfractuous material networks that result in an uneven distribution of strains on loading (Tsukrov & Kachanov 1997).

Predictions of elastic properties of porous polymer composites using mixtures model developed by Alam (Alam 2010) (Equation 1) are more accurate than classical mixture rules which do not incorporate sufficient details on how microstructural features such as pores affect the overall resistance to loading.

Table 2 shows the comparison of elastic modulus estimation using both rules of mixtures given in equation 1, which incorporates pores effect and the experimentally measured values. As the results suggested, poor adhesion between

bioglass and PLA introduces voids within the matrix, which consequently affect the Elastic modulus of composite, E_c.

$$E_c = \left(E_r F_r \left(\frac{\overline{L_r}}{d_r}.\frac{1}{S_t}\right) + E_b F_{c,eff}\right).\frac{1}{\bar{A}}.\left(1 - \frac{w_{p,max}}{w}\right) \quad (1)$$

Where,

E - elastic modulus, $F_{c,\ eff}$ - effective area, F_r - particle fraction, $\overline{L_r}$ - mean reinforcement length, $\overline{D_r}$ - mean diameter, c - composites, b - binder (PLA), r - reinforcement (bioglass), S_t - stress transfer aspect ratio, W - sample width, $W_{p,\ max}$ - maximum pore length and $\overline{A}$- mean anfractuosity.

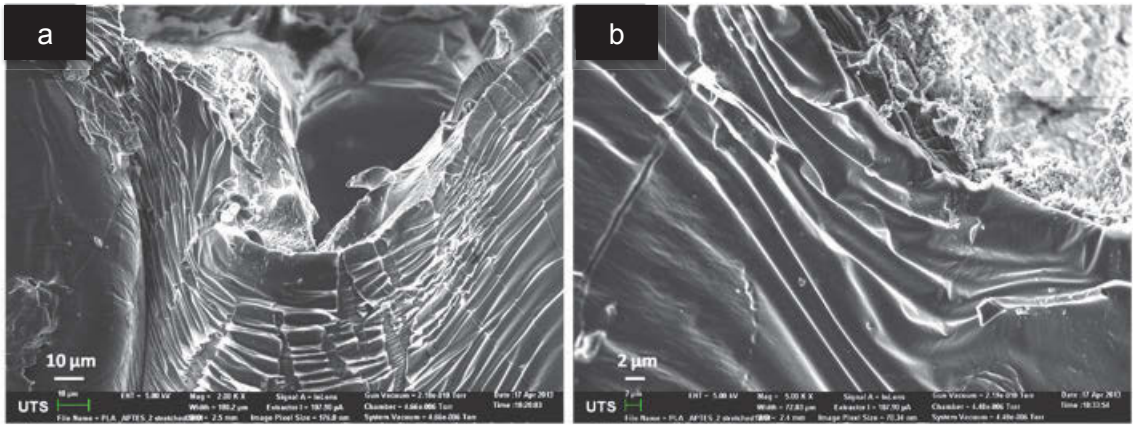

Figure 20: SEM micrograph fractured treated bioglass PLA composite at different magnifications: a) 2 KX b) 5KX.

The morphological changes of fractured PLA-nanosurface treated bioglass with 1% APTES composites is shown in Figure 20a and 20b. As explained earlier, untreated bioglass particles agglomerated within the matrix with an average particle size of 1 to 100 µm. After surface treatment with 1% APTES, bioglass easily dispersed in all levels within matrix. In SEM observations, particle size distributions of 5-50 nm were observed. A close examination of the fractured surface of the PLA-untreated bioglass and PLA-treated bioglass composite revealed a distinct difference in the fracture mode around the pores and at the bioglass-PLA matrix interface.

Table 2. Summary of the experimental and calculated elastic modulus of samples

Parameters	**Calculated (Eq. 1) or published values**	**Experimental values**	
		Treated	**Untreated**
Pure PLA Elongation %	180.0	NA	NA
Bioglass (0.1%) composite Elongation %		304.5	305.1
Bioglass (0.5%) composite Elongation %		345.9	282.1
Bioglass (1%) composite Elongation %		270.9	171.9
E_r (GPa)	4.1(Garlotta 2001)		
$E_{b,}$ (GPa)	35 (Thompson & Hench 1998)		
$E_{c\ (0.1\%\ Bioglass)}$ (GPa)±SD	3.8±0.2	3.2±1.0	2.8±1.0
$E_{c\ (0.5\%\ Bioglass)}$ (GPa) ±SD	3.6±0.1	3.1±1.2	3.2±1.2
$E_{c\ (1.0\ bioglass)}$ (GPa) ±SD	3.5±0.4	2.4±0.6	3.2±0.7

4.4 Discussion

The fractured surface of treated bioglass PLA composites shows thinner riverlines around the particle-matrix interface, indicating that the fracture did not start from the particle and spread outwards like in untreated bioglass composites. It was observed that most of the bioglass agglomerated particles were protruded from the fracture surface indicating a weak interfacial adhesion. Higher magnification observations shows well distributed nano particles within the matrix.

It can be suggested that surface treated bioglass with APTES provides better bonding between the amine part of APTES and the carbonyl group in the PLA matrix, that can contribute to a better interfacial adhesion than untreated ones, especially at the nanoparticle level. In addition it can be postulated that a better

dispersion of treated bioglass in the PLA matrix is observed due to the charge created on the outer part of the particle giving way to coulombic repulsion between the particles as shown in other functionalized nanoparticles (Thanh & Green 2010).

Mechanical properties of pure PLA and composites

Figure 17a shows the effect of APTES treated bioglass on the elongation at break of PLA-bioglass composites. SEM analysis of the treated bioglass composites (Figure 16 and 20) showed both agglomerated and well dispersed powders within the polymer matrix. The improvement of elongation at break of PLA composites compared with pure PLA films suggests that the introduction of voids within the PLA matrix due to poor interfacial adhesion between bioglass and PLA might be the cause of increase in composite flexibility. The effect of voids within the PLA matrix on the elastic modulus was evaluated by comparing experimental values to values calculated using a mixtures' model for porous-polymer composites. The results do not show any significant differences between treated and untreated bioglass (55% difference) and the experimental elastic modulus correlate well with the calculated values.

In addition, nanosurface treated bioglass with 1% APTES composites depicted a higher elongation at break, possibly due to the coupling effect of APTES. APTES having bonded one side to the bioglass surface, other sides are able to interpose between the linear chains of PLA and interfere with attractive hydrogen bonding and Van der Waals' forces between the polymer chains, making it more flexible and hence enhancing interfacial adhesion between PLA and bioglass powders. Similar behaviour was observed in other composites using APTES (Chen, Liang & Thouas 2013). All these microscopic features reinforce the reasoning why bioglass treated composites shown in Figure 8a and 8b have improved flexibility with narrower riverlines at the fracture point.

Comparison of elongation at break in treated bioglass composite and pure PLA thin films showed an increase of 92%. In comparison to the untreated bioglass, an improvement of 55% was observed. It is envisaged that the improvement obtained

could allow these materials to be used in wider tissue engineering applications where porosity and elongation is required.

In addition to tensile loading, tear tests were carried out according to the ASTM standards (ASTM D1004 - 13). Figure 17b shows the effect of bioglass contents on PLA bioglass composite elongation during tear test. It was observed that elongation at break during tear tests increases around 152% at 3% bioglass addition and then decreases afterwards. The different loading mode than tensile testing and poor interfacial adhesion during tear between bioglass and PLA matrix could explain the relatively lower elongation observed at break and at only higher bioglass contents than 3%.

4.5 Summary and Conclusions

PLA/ APTES nanosurface modified bioglass composites were prepared by the solution casting method using different loadings of 0.1, 0.5 and 1% bioglass and showed improved elongation at break under tensile testing conditions. Bioglass treated with a nanolayer of 1% APTES is suggested to be effective in improving tensile elongation at break of PLA/bioglass composites by enhancing dispersion and adhesion of bioglass particles in the polymer bioglass interfaces. The maximum tensile elongation at break for PLA/untreated bioglass was 305% obtained at 0.1% bioglass which was an increase of 69.5% and decreased by -4.5% at 1% bioglass content compared with values for pure PLA films. For PLA/treated bioglass composites, the maximum tensile elongation at break was 346%, which occurred at 0.5% bioglass loading with an increase of 92%, then decreased to 50% at 1% bioglass compared to pure PLA film. PLA/treated bioglass composites show an improvement on tensile elongation at break of 55% at 1% bioglass compared to untreated bioglass composites. The morphology of the fracture surfaces showed that treated bioglass composites had stronger interfacial bonding at the PLA interface than untreated bioglass composites. The results suggest that by nano-surface treating the bioglass with 1% APTES significantly influences the percentage elongation of the PLA/bioglass composite at fracture. SEM shows more agglomeration of untreated bioglass within the composite. In the treated samples, a better distribution of nanosized bioglass within the PLA matrix was observed.

Although non porous nanobioglass is difficult to be used as an efficient drug delivery vehicle, the modified bioglass/PLA thin film composites may have a wide range of other biomedical applications in tissue engineering with improved elastic properties.

4.6 References

Alam, P. 2010, 'A mixtures' model for porous particle–polymer composites', *Mechanics Research Communications*, vol. 37, no. 4, pp. 389-93.

Bianco, A., Cacciotti, I., Lombardi, M. & Montanaro, L. 2009, 'Si-substituted hydroxyapatite nanopowders: Synthesis, thermal stability and sinterability', *Materials Research Bulletin*, vol. 44, no. 2, pp. 345-54.

Chen, Q., Liang, S. & Thouas, G.A. 2013, 'Elastomeric biomaterials for tissue engineering', *Progress in Polymer Science*, vol. 38, no. 3–4, pp. 584-671.

Gao, Y. & Chang, J. 2009, 'Surface modification of bioactive glasses and preparation of PDLLA/bioactive glass composite films', *J Biomater Appl*, vol. 24, no. 2, pp. 119-38.

Garlotta, D. 2001, 'A Literature Review of Poly(Lactic Acid)', *Journal of Polymers and the Environment*, vol. 9, no. 2, pp. 63-84.

Gerhardt, L.-C. & Boccaccini, A.R. 2010, 'Bioactive Glass and Glass-Ceramic Scaffolds for Bone Tissue Engineering', *Materials*, vol. 3, no. 7, pp. 3867-910.

Greenhalgh, E.S. 2009, *Failure analysis and fractographiy of polymer composites*, Woodhead Publishing Limited, Cambridge, UK.

Hong, Z., Reis, R.L. & Mano, J.F. 2009, 'Preparation and in vitro characterization of novel bioactive glass ceramic nanoparticles', *J Biomed Mater Res A*, vol. 88, no. 2, pp. 304-13.

Howard, D., Partridge, K., Yang, X., Clarke, N.M.P., Okubo, Y., Bessho, K., Howdle, S.M., Shakesheff, K.M. & Oreffo, R.O.C. 2002, 'Immunoselection and adenoviral genetic modulation of human osteoprogenitors: in vivo bone formation on PLA scaffold', *Biochemical and Biophysical Research Communications*, vol. 299, no. 2, pp. 208-15.

Lee, S. 1997, 'Mode II delamination failure mechanisms of polymer matrix composites', *Journal of Materials Science*, vol. 32, no. 5, pp. 1287-95.

Li, R., Clark, A.E. & Hench, L.L. 1991, 'An investigation of bioactive glass powders by sol-gel processing', *Journal of Applied Biomaterials*, vol. 2, no. 4, pp. 231-9.

Li, S., Ma, W., Zhou, Y., Chen, X., Ma, M., Xu, Y., Ding, Z. & Wu, X. 2013, '3-aminopropyltriethoxysilanes Modified Porous Silicon as a Voltammetric Sensor for Determination of Silver Ion', *International Journal of Electrochemical Science*, vol. 8, no. 2, p. 11.

Liu, A., Hong, Z., Zhuang, X., Chen, X., Cui, Y., Liu, Y. & Jing, X. 2008, 'Surface modification of bioactive glass nanoparticles and the mechanical and biological properties of poly(L-lactide) composites', *Acta Biomater*, vol. 4, no. 4, pp. 1005-15.

Macha, I.J., Ben-Nissan, B. & Milthorpe, B. 2014, 'Improvement of Elongation in Nanosurface Modified Bioglass/PLA Thin Film Composites', *Current Nanoscience*, vol. 10, no. 2, pp. 200-4.

Middleton, J.C. & Tipton, A.J. 2000, 'Synthetic biodegradable polymers as orthopedic devices', *Biomaterials*, vol. 21, no. 23, pp. 2335-46.

Nair, L.S. & Laurencin, C.T. 2007, 'Biodegradable polymers as biomaterials', *Progress in Polymer Science*, vol. 32, no. 8–9, pp. 762-98.

Niiranen, H. & To¨rma¨la¨, P. 1999, 'Bioabsorbable polymer plates coated with bioactive glass spheres', *Journal of Materials Science: Materials in Medicine*, vol. 10, no. 12, pp. 707-10.

Thanh, N.T.K. & Green, L.A.W. 2010, 'Functionalisation of nanoparticles for biomedical applications', *Nano Today*, vol. 5, no. 3, pp. 213-30.

Thompson, I.D. & Hench, L.L. 1998, 'Mechanical properties of bioactive glasses, glass-ceramics and composites', *Proceedings of the Institution of Mechanical Engineers Part H-Journal of Engineering in Medicine*, vol. 212, no. H2, pp. 127-36.

Tsukrov, I. & Kachanov, M. 1997, 'Stress concentrations and microfracturing patterns in a brittle-elastic solid with interacting pores of diverse shapes', *International Journal of Solids and Structures*, vol. 34, no. 22, pp. 2887-904.

Verne, E., Vitale-Brovarone, C., Bui, E., Bianchi, C.L. & Boccaccini, A.R. 2009, 'Surface functionalization of bioactive glasses', *J Biomed Mater Res A*, vol. 90, no. 4, pp. 981-92.

CHAPTER 5

CORAL AND CONVERSIONS TO BIOCERAMICS

CHAPTER 5: CORAL AND CONVERSION TO BIOCERAMICS

To introduce porous particles to allow PLA to carry a drug as a biocomposite, a number of calcium carbonate materials, both natural and synthetic, were converted to calcium phosphates. The following chapter covers the conversion of porous coral to hydroxyapatite and their characterisation to be used within the PLA matrix as biodegradable polymer for slow drug delivery.

5.1 Introduction

Calcium phosphate (CaP) materials have gained clinical acceptance for the past 40 years (LeGeros 2008b). Hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$, HAp] and β-tricalcium phosphate [$Ca_3(PO_4)_2$, β-TCP] are one of the most widely used synthetic (CaPs) in the areas of orthopaedic and dentistry for augmentation, bone substitution and repair due to their similarity with the mineral phase of bone. It is known that bone formation involves a series of complex events leading to mineralization of extracellular matrix proteins by cells with specific functions for maintaining the integrity of the bone (LeGeros 2002). The scientific and clinical communities agreed that bone apatite can be better described as carbonate hydroxyapatite (CHA) and approximated by the formula: $(Ca,X)_{10}(PO_4,CO_3)_6(OH,Y)_2$ where X are cations (magnesium, sodium, strontium ions) that can substitute for the calcium ions, and Y are anions (chloride or fluoride ions) that can substitute for the hydroxyl group (LeGeros 1988). Theoretical composition of HAp is 39.68 wt% Ca, 18.45 wt% P; with Ca/P wt ratio of 2.151 and Ca/P molar ratio of 1.667. HAp is stable in a wide range of pH 4.4 – 8.0 and has higher stability in aqueous media than other calcium phosphate ceramics (Best et al. 2008).

Tricalcium phosphate (TCP) is a biodegradable calcium phosphate, it dissolves at a much faster rate than HAp in the physiological environment and can be replaced by bone *in-vitro* (Calafiori et al. 2007). The Ca/P molar ratio for TCP is 1.5. Other calcium phosphate compounds for bioceramic interest are presented in Chapter 1: Table 1.

Table 3: Calcium phosphate materials

Chemical Name	Abbr	Chemical Formula	Phase	Ca/P
Amorphous calcium phosphate	ACP	-	-	-
Dicalcium Phosphate	DCP	$CaHPO_4$	Monetite	1.00
Tricalcium Phosphate	α-TCP	$Ca_3(PO_4)_2$		1.50
Tricalcium Phosphate	β-TCP	$Ca_3(PO_4)_2$		1.50
Pentacalcium Hydroxyl Apatite	HAp	$Ca_{10}(PO_4)_6(OH)_2$	Hydroxyapatite	1.67
Tetracalcium Phosphate Monoxide	TTCP	$Ca_4O(PO_4)_2$	Hilgenstockite	2.00

The use of synthetic materials in the biomedical field has been greatly successful for many years. Natural materials have superior biological and structural properties compared to synthetic materials and they provide an abundant source of novel biomedical applications (Ben-Nissan 2003b). Calcium phosphates, specifically HAp and TCP, can be prepared from natural materials composed of calcium carbonate with a unique architecture such as sea coral (Ben-Nissan 2003b), mussel (Macha et al. 2013), egg shells (Macha, Ozyegin, et al. 2015) and nacre venus verrucosa (Oktar et al. 2010) for biomedical applications. The high price of bioceramics in the market reflects the significant costs of raw materials that can easily be replaced by natural biogenic materials.

The potential applications of natural biogenic materials such as marine structures can be easily overlooked due to the environmental concerns. While it is true that a wide range of marine structures are limited and protected, similarly there are also a variety of materials that are abundantly available and are yet to be exploited for their possible use (Green & Ben-Nissan 2010). Previous work has shown that corals can be artificially grown as synthetic corals in specific areas and containers (Ben-Nissan, Milev & Vago 2004). Among marine structures, coral mineral, which mainly consists of calcium carbonate in the forms of aragonite or calcite with trace

elements of strontium, magnesium and sodium, has considerable success as the apatite precursors and bone graft materials (Ben-Nissan, 2004). Corals have a porous structure with pore size ranges from 150 to 500 μm, similar to cancellous bone and form chemical bonds with bone and soft tissue in vivo (Ben-Nissan 2003a). Kühne and his colleagues analysed osseous reactions in the rabbit femoral condyle to coralline hydroxyapatite bone substitutes of various pore sizes by radiology and histology (Kuhne et al. 1994). Their results suggest that there was a substantial production of bone within the 500-micron pore size. In addition they concluded that the pore size of the coralline hydroxyapatite influenced the development of bone in the implants. It was further reported that the interaction of the primary osteons between the pores via the interconnections allows propagation of osteoblasts (Heness & Ben-Nissan 2004).

A number of synthesis routes for calcium phosphates have been reported in the literature. The main two are the wet chemical and solid-state reaction methods. Other alternative methods like mechano-chemical, electrospray, hydrothermal, and microwave heating to mention just a few, have been reported previously (Chen et al. 2004; Meejoo, Maneeprakorn & Winotai 2006).

This chapter presents the production and characterizations of calcium phosphate bioceramic materials, HAp, from Australian coral materials. Different biogenic materials were converted to CaPs using similar techniques for comparison.

5.2 Materials and Methods

5.2.1 Materials

Corals skeleton samples were obtained from the Great Barrier Reef, QLD. Ammonium dihydrogen phosphate dibasic ($NH_4H_2PO_4$, 98%), hydrophosphoric acid (H_3PO_4, 85%) and sodium hypochlorite (NaClO) were obtained from Sigma Aldrich Australia.

5.2.2 Methods

5.2.2.1 Coral conversion by hydrothermal and mechano-chemical techniques

The coral samples were crushed then cleaned with 2% (v/v) NaClO, and then ground within an aluminium oxide ball mill (46 rpm, 2 h), sieved with 100 µm sieve, cleaned with 2% (v/v) NaClO and then dried at 100°C for 2 hours before use. Hydrothermal conversion was carried out following the Ben-Nissan procedures (Ben-Nissan 2003b; Ben-Nissan & Green 2014). Briefly as shown in Figure 21, coral powder was mixed with the required amount of diammonium hydrogen phosphate $(NH_4)_2HPO_4$ to obtain HAp and then converted in a Parr reactor at 250°C and 8.0MPa pressure for three hours. Mechano-chemical conversion was carried out as described in (Cegla et al. 2014a).

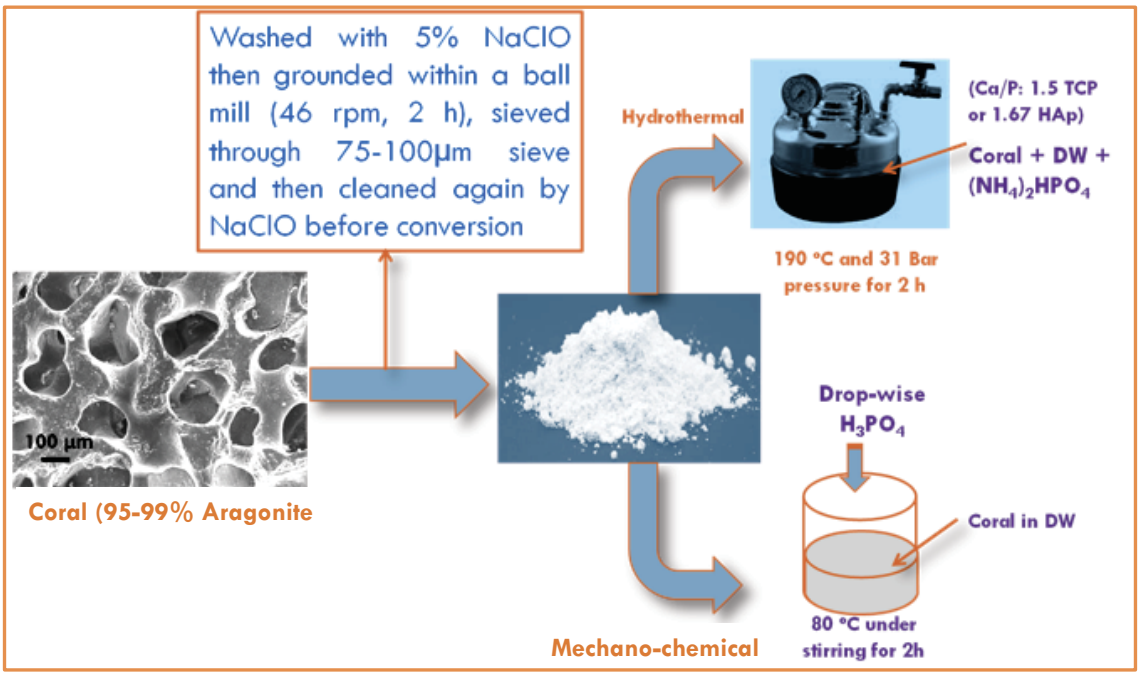

Figure 21: Coral conversion to calcium phosphate materials through hydrothermal and mechano-chemical techniques

Briefly, the required amount of H_3PO_4 or $NH_3H_2PO_4$, to obtain HAp or TCP, was dissolved in 25 ml of distilled water. Then it was added, drop-by-drop, to 3 g of coral powder suspended in a cleaned flask with 150 ml distilled water at 80 °C on a temperature control hot plate with a magnetic stirrer. The stirring rate was 200 rpm and the temperature was kept at 80 °C for 24 hrs. After conversion, all samples were calcined at 700 °C for three hours.

5.2.2.2 Specific surface area, pore size and pore size distribution

Specific surface areas were measured in a Tristar II apparatus from Micromeritics. Samples were initially degassed under vacuum (16 MPa) at 60°C for 15h before analysis. Analysis was performed using the static method at -196.15 ^{o}C with nitrogen as the adsorbent. The Brunauer–Emmett–Teller (BET) method was applied to calculate the total surface area. Porosity measurements were carried out with a mercury intrusion porosimeter (Autopore III, Micromeritics Instruments Inc., Norcross, GA, USA) with a 5-cm^3 powder penetrometer.

5.2.2.3 Morphology of coral from solid piece to HAp powder

The morphology of coral samples were analysed by a Scanning Electron Microscope (SEM) (ZEISS Supra55VP, Zeiss, Germany). All samples were fixed by mutual conductive adhesive tape on aluminium stubs and coated with thin layer of carbon using a sputter coater. Images were taken at various magnifications at acceleration voltages of 20 kV.

5.2.2.4 Fourier transform infrared spectroscopy (FTIR)

FTIR spectra were collected using a Nicolet, Magna-IR 6700 Spectrometer FTIR (Thermo Fisher Scientific, Madison USA) in the range 4000–400 cm^{-1}. Samples were ground in an agate mortar and thoroughly mixed with dried KBr (FTIR Grade). For the analyses, 3 mg was ground with 300 mg of KBr and pressed into a pellet (Carver press).

5.2.2.5 X-ray Powder Diffraction (XRD)

The structural analysis was carried out using X-ray diffraction analysis (Seimens D5000, Germany) employing CuKα radiation (λ=1.5418 Å). The diffractometer was operated at 45 kV and 40 mA at a 2θ range of 20-70° employing a step size of 0.01°/s and a 4 sec exposure. Matching the XRD patterns with the Joint Committee on Powder Diffraction Standards (JCPDS) database identified the crystalline phases of the powders.

5.2.2.6 Inductively coupled plasma-mass spectroscopy (ICP-MS)

Powders were digested to quantify trace elements in coral such as magnesium and strontium ions by inductively coupled plasma-mass spectroscopy (ICP-MS). An Agilent Technologies 7500ce series ICP-MS was used with sample introduction via a micromist concentric nebuliser (Glass expansion). The ICP operating parameters and the lens conditions were selected to maximize the sensitivity of a 1% HNO_3: HCl solution containing 1ng/ml of Li, Co, Y, Ce and Tl. Calibration standards were prepared in 1% nitric acid. Approximately 0.005g of sample was digested with 0.25 mL of HNO_3. The samples underwent a further 1:100 dilution with a 1% nitric acid solution before ICP-MS analysis.

5.2.2.7 Thermal analysis (DTA/TGA)

The thermal analysis of coral was determined by differential thermal and thermogravimetric analysis (TG-DTA, SDT 2960, TA Instruments, New Castle, DE, USA). A sample weight of 30 - 40 mg was used during analysis under a circulating air environment with a heating rate of 10 ^{o}C/min from room temperature to 1100 ^{o}C.

5.3 Results

5.3.1 Morphology of coral from solid piece to HAp powder

The morphology of the coral before conversion is presented in Figure 22. Figure 22a shows the porous structure of a coral piece with its unique architecture of interconnected pores before being ball milled. Figure 22b&c show the coral particles with platelets morphology which is consistent with the morphology of aragonite present in coralline materials. Figure 23 shows the morphology of coralline materials hydrothermally converted to hydroxyapatite in phosphate solution. Figure 23a suggests that HAp retained the morphology of the original coral after conversion. Figure 23d also shows coral after conversion, revealing the platelets morphology which is consistent with hydroxyapatite morphology.

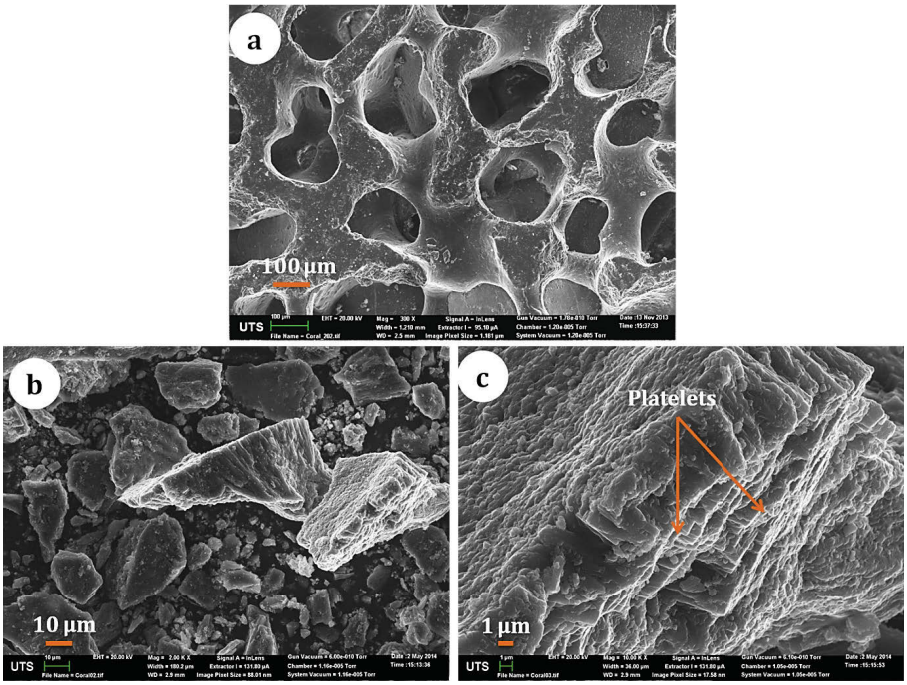

Figure 22: SEM pictures showing the morphology of coral a) before ball mill showing pores and interconnected pores b) after ball mill showing different particle sizes c) higher magnifications of (b) revealing platelets morphology of singer particle

Figure 24 shows the morphology of coral powder converted to HAp by mechano-chemical technique in two different phosphate solutions. Figure 24a revealed platelets morphology of HAp converted coral in ammonium dihydrogen phosphate dibasic similar to hydrothermally HAp converted coral, while Figure 24b shows the rod-like morphology of HAp converted coral in orthophosphoric acid. The observed morphology indicates the evolution of rod-like morphology perpendicular to the platelets. Both hydrothermal and mechano-chemical techniques with ammonia phosphate solution produced HAp with morphologies of platelets similar to the original coral suggesting the solid state topotactic ion-exchange reaction mechanism (Macha, Boonyang, et al. 2015b). On the other hand, with orthophosphoric phosphate solution, the reaction mechanism is suggested to be dissolution-recrystallization.

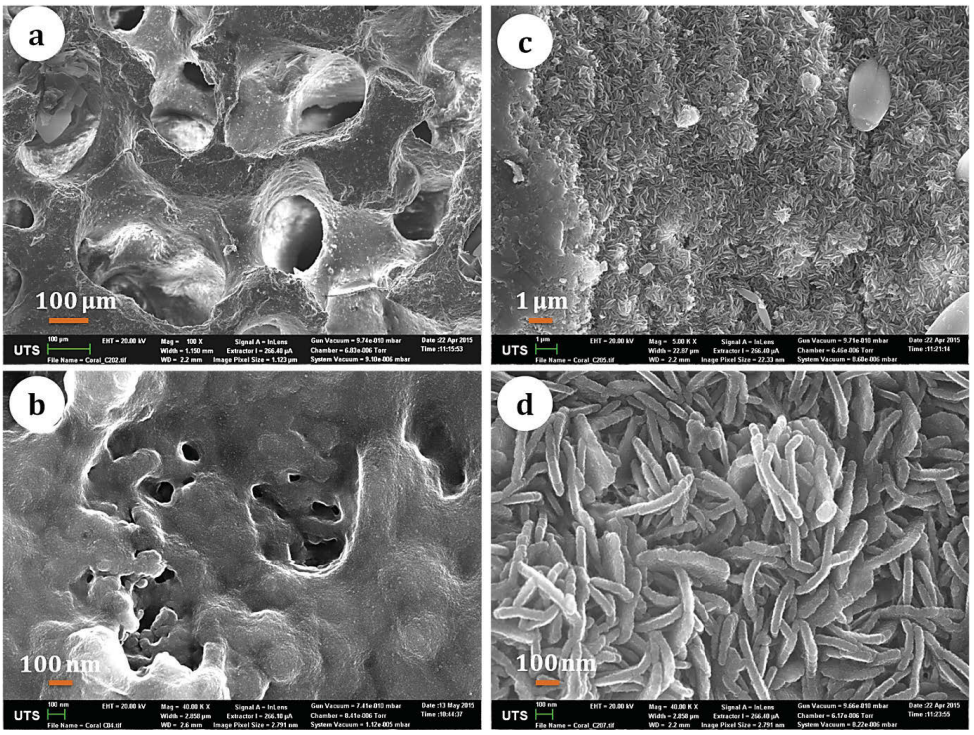

Figure 23: SEM picture of coral after conversion of a) coral solid piece showing the retained porous morphology b) higher magnification of coral solid piece before conversion for comparison, showing nano-pores, c) higher magnification of converted coral piece showing platelets morphology of hydroxyapatite

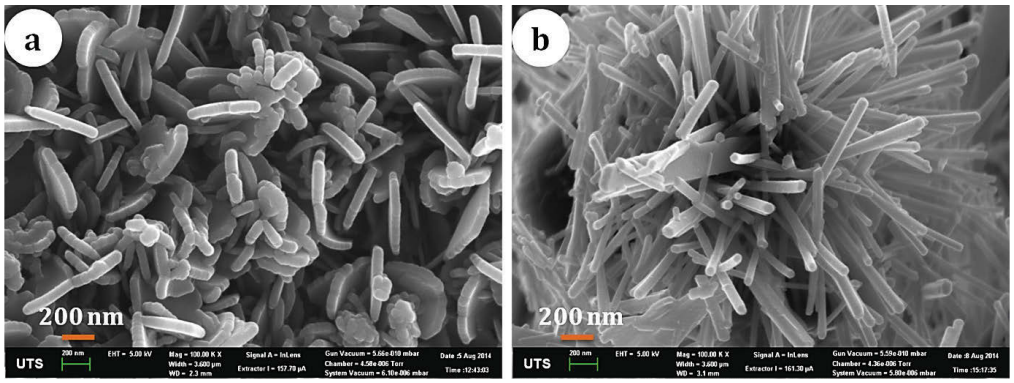

Figure 24: SEM pictures showing the morphology of HAp mechanochemical converted coral by a) Ammonium phosphate solution, platelets morphology and b) orthophosphoric phosphate solution, rod-like morphology.

5.3.2 X-ray Powder Diffraction (XRD)

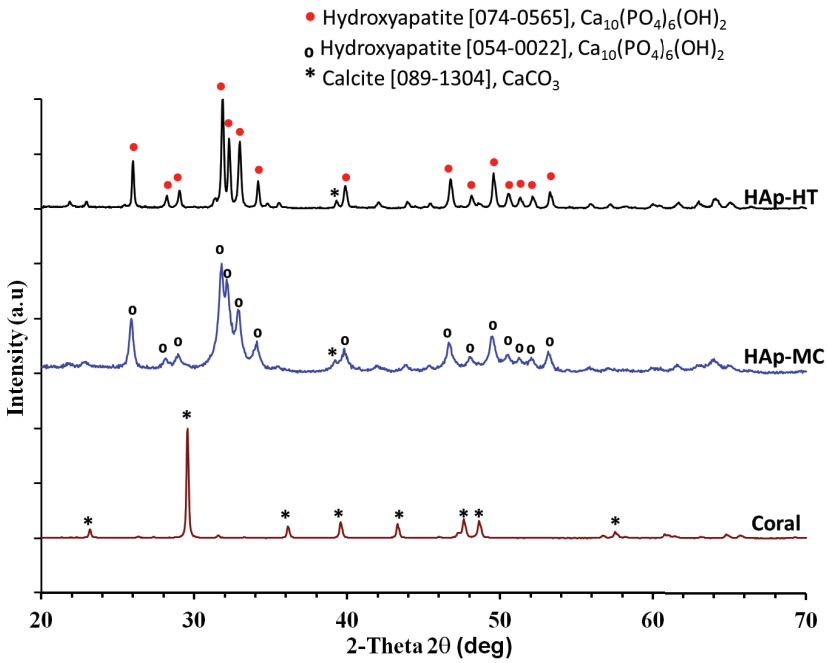

Figure 25: XRD patterns of HAp derived coral by hydrothermal (HAp-HT) and mechano-chemical (HAp-MC) techniques compared to coral before conversion.

Figure 25 presents the identified phases in the products obtained from hydrothermal and mechano-chemical conversion techniques. Both methods successfully converted coralline materials to calcium phosphate compounds with significant degree of crystallinity. The HT method gives HAp with sharp and well defined peaks, especially in the region (2θ = 30 – 35) indicating that the HAp crystallinity is more well developed in this technique than MC. XRD spectra registered that a small amount of other phases remained unconverted in the final products.

5.3.3 Fourier transform infrared spectroscopy (FTIR)

Figure 26 displays IR shifts of HAp derived coral from the two techniques. The results suggest that HAp obtained from the two techniques have FTIR shifts consistent with the ones reported in literature (Rey et al. 2014). Although XRD did not show other minor phases in the final products. The characteristic bands of the functional groups of Hap are clearly seen in Figure 26. Characteristic vibrations of PO_4 tetrahedral such as v_1, v_2 and v_3 occurred at 962 cm^{-1}, 472 cm^{-1} and 1040 cm^{-1} respectively. Absorption maximum of CO_3 group at 876 cm^{-1} can also suggest AB-

type PO_4 and OH group's substitution in the structure of HAp. The increasing peak resolution for HAp-HT is in conjunction with the increase of peak intensity in the XRD spectra (Figure 25) of HAp-HT compared to HAp-MC.

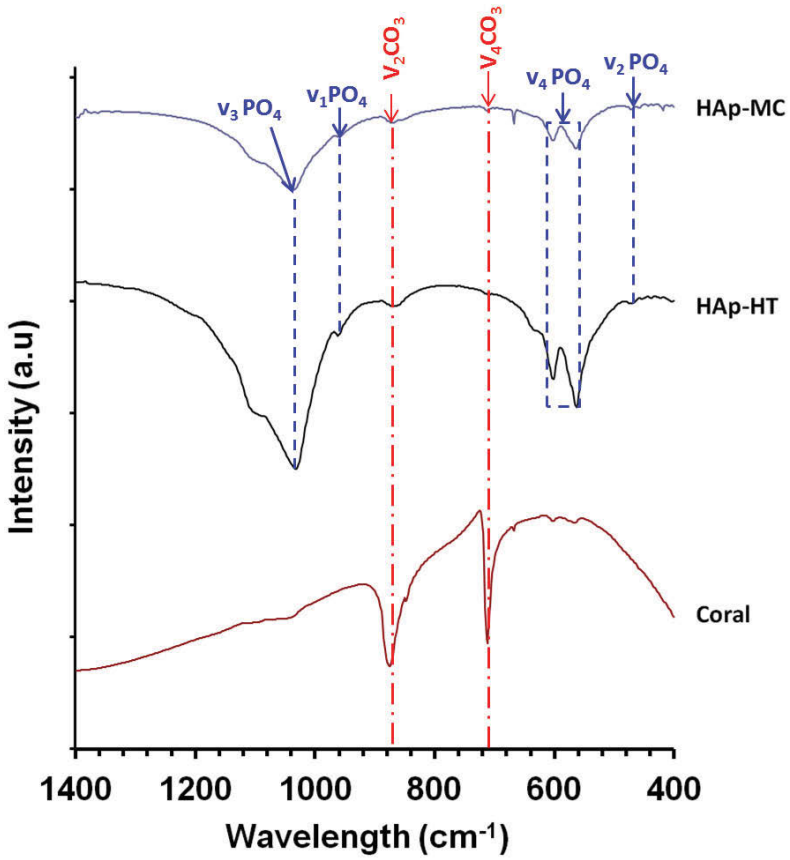

Figure 26: FTIR spectra of coral and HAp derived coral from hydrothermal (HAp-HT) and mechano-chemical (HAp-MC) conversion techniques.

5.3.4 Specific surface area, pore size and pore size distribution

Figure 27 shows the porosity analysis of coral powder. Figure 6a shows the pore size distribution of coral powder while Figure 27b suggests the existence of nanopores in coral by SEM. Pore sizes are suggested to be in the range of 1nm - ~100 µm. It was also indicated that most of the crushed and ground particles have pore sizes around 4.5 µm with BET surface area determined to be 3.23 m^2/g. The porosity of coral particles was determined to be 58.15%. The results obtained are consistent with the results obtained previously using Nuclear Magnetic Resonance (NMR) (McCutcheon et al. 2004).

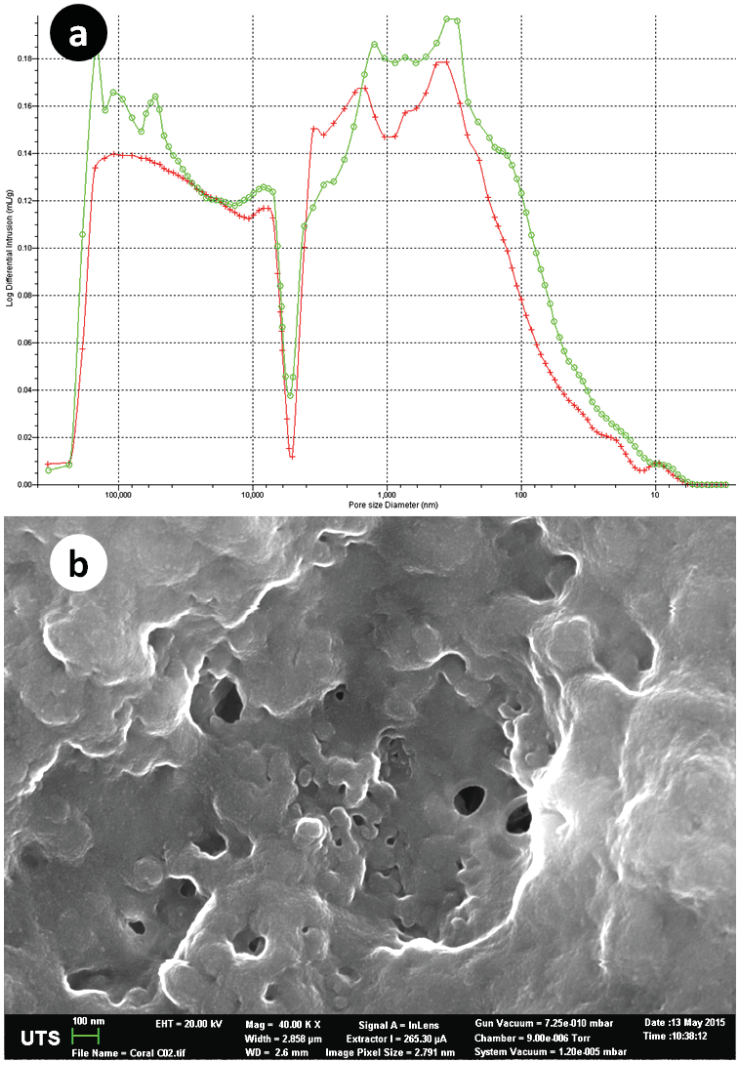

Figure 27: Porosity within coral and coral powder a) pore size and pore size distribution b) evidence of pre-existing nanopores in solid piece coral

5.3.5 Inductively coupled plasma-mass spectroscopy (ICP-MS)

ICP-MS performed on coral and HAp derived coral samples at the end of reaction for trace elements are presented in Table 4. It is suggested that coral doesn't contain impurities that could be harmful to humans. The results showed no presence of any heavy metals. Previous analysis of coral on an imaging LA-ICP (Chou, Austin, et al. 2014) showed similar results on the presence of Magnesium and Strontium in HAp derived coral, which are beneficial to the human body.

Table 4: Trace elements by ICP-MS of coral and HAp derived coral

Sample	**23 Na (mg/g)**	**24 Mg (mg/g)**	**88 Sr (mg/g)**
Coral	7.07E-03	9.06E-04	7.60E-03
HAp-HT	3.32E-03	3.84E-03	4.72E-03

5.3.6 Thermal analysis (DTA/TGA)

Thermal analysis of coral and sample products from the two methods were studied using SDT. Coral showed thermal decomposition from 642.6 ^{o}C to 803.5 ^{o}C with a maximum peak at around 785.1 ^{o}C, which corresponds to the decomposition of calcite/aragonite ($CaCO_3$) present in coralline materials. The weight loss between these temperatures is around 44%, indicating that the inorganic matter of coral materials consists of $CaCO_3$ (calcite/Aragonite), which decomposes according to Eq. 1. Figure 28a represents the coral thermal decomposition, while 28b compares HAp derived coral from hydrothermal and mechanochemical techniques and 26c compares them with coral. There is not much weight loss before 600 ^{o}C because most of the organic materials in coral were removed during cleaning.

$$CaCO_3 \Longrightarrow CaO + CO_2 \qquad (1)$$

After conversion, thermal analysis suggests that the produced HAp is thermally stable with a small weight loss before 600 ^{o}C. Weight loss before 400 ^{o}C (Figure 28b) could be any moisture or unreacted reactants present in the final products. The small amount of calcite remained decomposed between 640.7^{o}C and 770.7^{o}C with a maximum at 752.4^{o}C, indicating a weight loss of 1.4%.

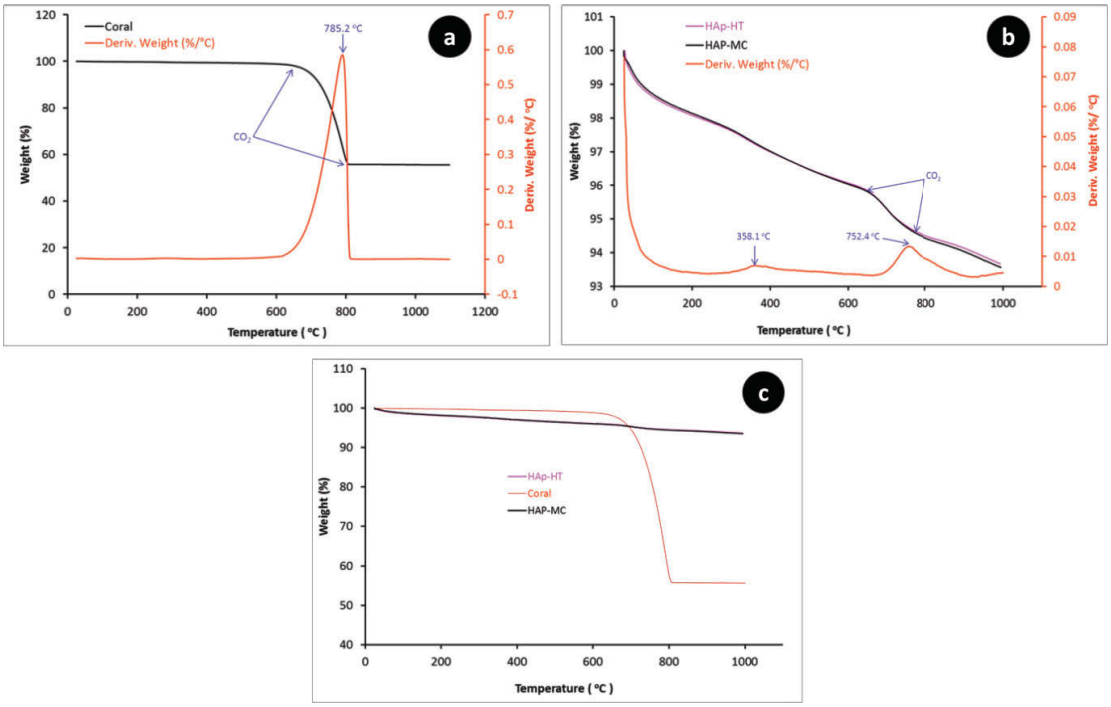

Figure 28: Thermal analysis of coral and HAp derived coral

5.3.7 Microstructural evolution during mechano-chemical conversion

Previous study (Cegla et al. 2014b) on comparison of orthophosphoric and ammonium phosphate solution conversion of coral to HAp suggested a number of factors that influenced the conversion process. Among them, including ratio of Ca/P, reaction time and pH seemed to have more effects on crystal size, reaction mechanism and the structure of the final products. Experiments were set up to explore the evolution of crystalline calcium phosphate and their morphology with respect to the pH of the environment and reaction time (Macha, Boonyang, et al. 2015a). Crystallography analysis and morphological results showed the evolution of calcium phosphate crystalline from two different mechanisms as shown in Table 5&6 and Figure 29.

Table 5: Quantification for HAp derived coral by orthophosphoric phosphate solution experiment showing the amount of transformed phases and crystal growth of HAp

Time (h)	Aragonite (%)	Calcite (%)	Monetite (%)	HAp (%)	Size 002 Scherer (nm)	pH
0.5	4.4±0.4	24.4±0.3	29.0 ±0.3	41.4±0.5	42	4.40
1.0	2.8±0.3	19.4±0.2	38.1±0.3	38.8±0.4	61	4.80
1.5	2.6±0.2	16.8±0.2	42.8±0.3	36.6±0.3	58	5.48
2.0	2.5±0.3	14.8±0.2	48.8±0.3	32.4±0.3	53	6.04
3.0	2.5±0.2	16.0±0.2	43.7±0.3	35.5±0.3	61	6.24
4.0	1.5±0.2	13.3±0.2	40.7±0.3	41.3±0.3	62	6.25
5.0	1.3±0.2	12.5±0.2	31.8±0.2	49.9±0.3	72	5.97
7.0	0.9±0.2	9.6±0.2	24.5±0.2	59.2±0.3	82	5.75
24.0	0.3±0.1	7.2±0.1	0.6±0.1	84.8±0.4	77	7.90

Table 6: Quantification for HAp derived coral by ammonium phosphate solution experiment showing the amount of transformed phases and crystal growth of HAp

Time (h)	Aragonite (%)	Calcite (%)	HAp (%)	Size 002 Scherer (nm)	pH
0.5	13.6±0.6	48.4±0.6	38.0±0.5	53	7.41
1.0	13.0±0.5	49.2±0.6	37.8±0.6	61	7.42
1.5	12.3±0.5	48.0±0.6	39.7±0.6	54	7.58
2.0	12.2±0.5	48.6±0.5	39.2±0.6	51	7.59
3.0	11.5±0.5	46.5±0.5	42.0±0.6	53	7.62
4.0	11.5±0.4	47.4±0.5	41.0±0.6	48	7.63
5.0	11.4±0.4	44.4±0.5	44.2±0.5	56	7.66
7.0	10.9±0.3	44.6±0.5	44.5±0.5	52	7.66
24.0	7.5±0.3	33.5±0.3	58.9±0.4	53	7.69

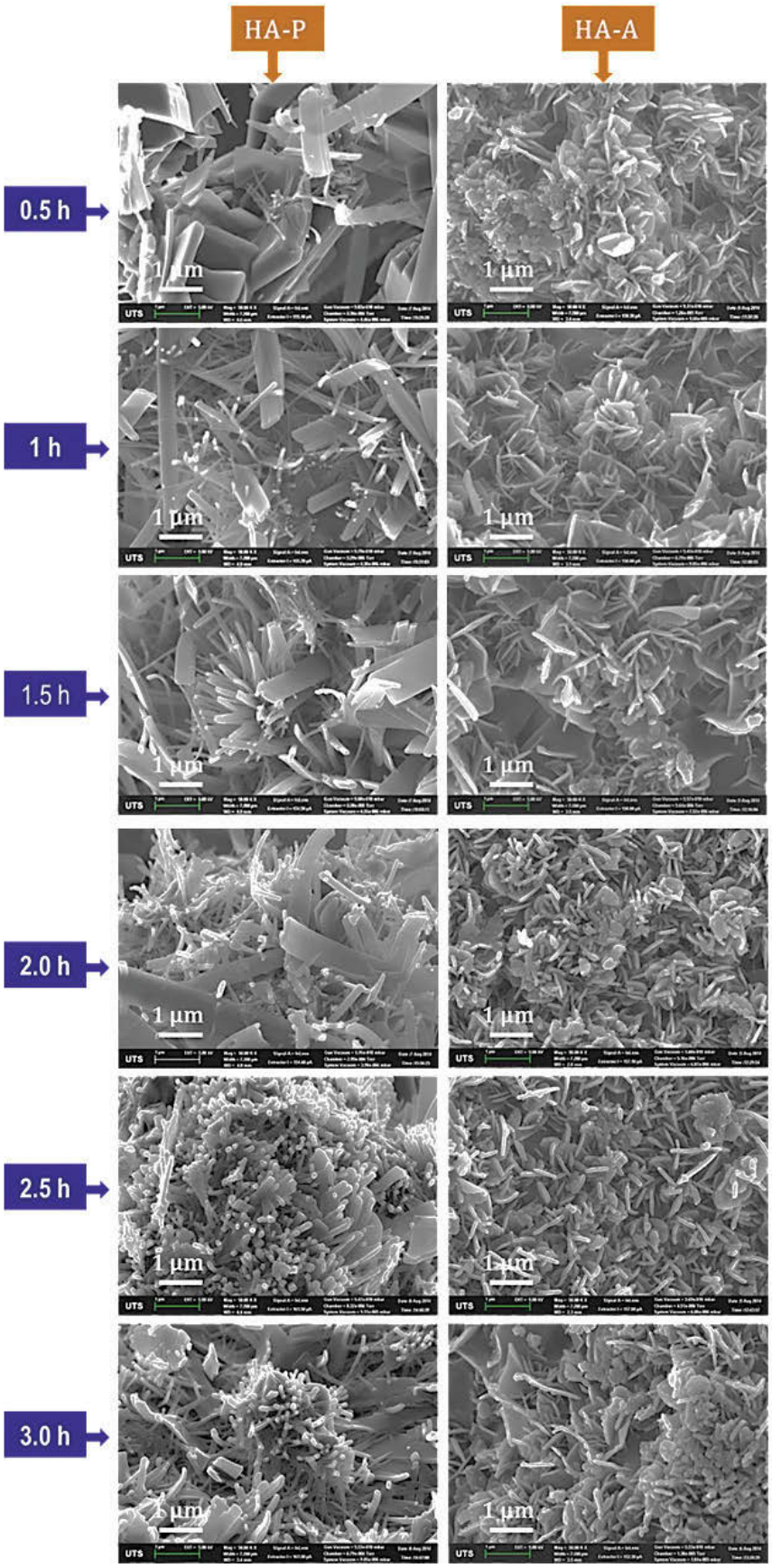

Figure 29: SEM images showing morphology of microstructural evolution in the first 3 hours.

5.4 Discussions

This chapter demonstrated that $CaCO_3$ of coralline materials can be converted both hydrothermally and mechano-chemically to HAp using two different phosphate sources. With ammonium phosphate solution there was a retention of sample form and internal microstructure suggesting the solid state topotactic ion-exchange reaction mechanism (Macha, Boonyang, et al. 2015b). With orthophosphoric phosphate solution, the results indicate the evolution of rod-like morphology of HAp perpendicular to the platelets suggesting the dissolution-recrystallization mechanism (Figure 4a&b). These results may be the consequences of the unique architecture of coralline materials, which imparts a kinetic and orientational effect on the conversion. The retention of porous morphology and interconnectivities between pores after conversion (Figure 23a) has the advantage of supporting biological activities such as bone tissue growth and vascularization (Ben-Nissan 2003a).

Thermal analysis of coral complements XRD and FTIR results on the presence of calcium carbonate ($CaCO_3$) in the coral, which is consistent with many published data. The loss of around 43% weight indicating that cleaned coral contains more than 98% calcium carbonate.

The presence of meso and nanopores in coral powder (Figure 27a&b) signifies the potential to uptake biologically active substances and release them in a controlled manner. The drug solution can easily be encapsulated within the pores by different techniques for slow drug deliveries in the clinical setup. It has been recently reported that mesoporous surfaces loaded with magnesium significantly increase the viability of human adipose-derived stromal cells (ADScs) at a longer cell culture time (Cecchinato et al. 2015). In addition, they reported that specific osteogenic markers could be incorporated into interconnected nanopores that helps (ADScs) differentiation into osteoblast faster (Green et al. 2012).

ICP results (Table 4) indicated the presence of magnesium and strontium in coral materials before and after conversions. Though they are in small quantity their role in bone formation and general intercellular functions are important. Using

coralline materials as implants has the advantage of releasing these important elements into the human body. Magnesium fulfils various intercellular physiological functions as a cofactor in numerous enzymatic reactions (Jahnen-Dechent & Ketteler 2012). On the other hand it has been reported that strontium may play a role in bone formation, and preliminary evidence suggests that women with osteoporosis may have reduced absorption of strontium.

The evolution of crystalline calcium phosphate materials from wet chemical conversion of coral structure with respect to time and pH was studied. The observed morphology of products from conversion of coral in ammonium phosphate solution suggests that the method could help to retain the original coral structure, specifically the micro pores that are pertinent in bone graft or scaffolding applications and nano and mesopores for slow drug delivery applications. For ammonium phosphate solution, HAp crystalline evolution took place on the surface of platelets morphology of aragonite/calcite without changing the original morphology. While with orthophosphoric acid the observed morphology indicates the evolution (and growth) of rod-like morphology. The crystal growth results for the orthophosphoric phosphate solution experiment, suggest that the HAp crystal size grew at the expense of monetite crystallise (Table 5), which is consistent with the morphology observed in Figure 29. The results suggest that the transformation of monetite to hydroxyapatite or whitlockite via monetite route, involves a dissolution-recrystallization mechanism.

The reaction mechanism and final products are influenced by pH. The effect of pH on the reaction mechanisms and products can be further explained using classic solubility isotherms (Figure 30).

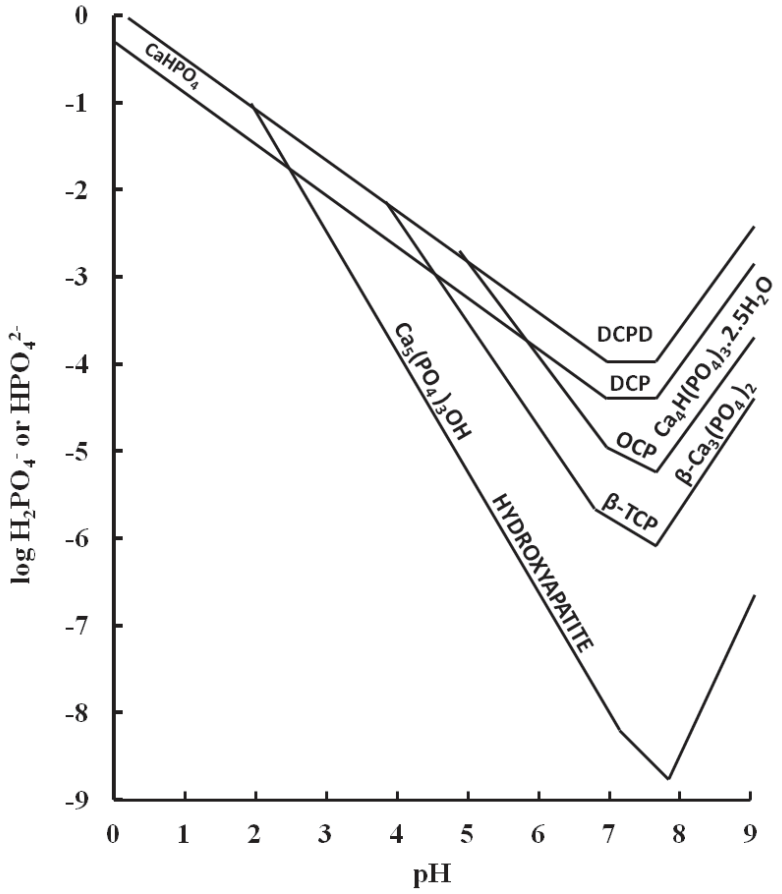

Figure 30: Solubility isotherms for differing calcium phosphate forms versus pH (Modified using our data from (Wang & Nancollas 2008)).

Orthophosphoric acid phosphate solution

For orthophosphoric acid phosphate solution reactions, pH seems to change in the beginning from 7.0 to 4.0 immediately after the addition of the acid and then it starts to continuously increase to 6.5 during the first 2 hour period as shown in Table 3. As stated above, during that period of the addition of the acid, the pH drops immediately to around 3.8- 4.0. At this point part of a "defect HAp" initially formed (the HAp formed is calcium deficient or "defect HAp") and further coral dissolves in the acidic condition and introduces further Ca^{2+} into the environment to form monetite ($CaHPO_4$) (which is more stable at this point as shown in Figure 30). $CaHPO_4$ is one of the mildly acidic calcium phosphates and its amount increases within the solution (Elmore & Farr 1940). The release of PO_4 from dissolution of some of the initial "defect" HAp under increased acidity and phosphate ions generated from the acid and Ca^{2+} from dissolution of $CaCO_3$

induces the precipitation of further monetite. During the following period, some of the $CaCO_3$ continues to dissolve to counterbalance the acidic solution and this will increase the pH observed in Table 5 to 6.5 during the first 2 hour period.

After 2 hours, the pH is around 6.2 and according to Figure 30, HAp is more stable and the monetite formed earlier transforms to Hap; and HAp starts to increase in the amounts as shown in Table 5, from 50% to approximately 80%. Crystal sizes were measured using the (002) reflections and Scherrer-Topaz method. Dissolution products from monetite participate in the formation of additional Hap, in which CO_2 and H_2O formation counter balance the decrease in pH due to HAp formation. This process is confirmed by the increase of HAp crystalline sizes shown in Table 3 and the change of morphology from platelet to rod like structures.

Ammonium phosphate solution

Under ammonium phosphate solution environment, pH starts to increase from around 7 before dropping marginally (Table 6). The formation of HAp starts immediately as the first drop is added into the solution. Since the formation of HAp results in a decrease in pH due to the formation of HCO_3^-, a sudden drop and then increase of pH is observed. After this initial position, pH is well balanced and an increase is observed with the addition of ammonium solution which is basic. The pH condition in this transformation favours formation of HAp which is more stable at this pH compared to precursors such as monetite (Figure 30). Similar behaviour in plate-like hydroxyapatite formation and related morphological changes were observed and it was suggested that the reaction mechanism in these conditions is based on the "solid state topotactic ion-exchange reaction mechanism" (Milev, Kannangara & Ben-Nissan 2003).

5.5 Summary and Conclusions

In this chapter coralline materials were characterized and then successfully converted to HAp in two different techniques. Hydrothermally, coral converts into HAp in ammonium phosphate solutions with preservation of sample form and morphology indicating topotactic iron exchange reaction mechanisms. Mechano-

chemical conversion of coral to HAp follows the topotactic reaction mechanism under ammonium phosphate solution and dissolution-recrystallization using orthophosphoric acid phosphate solution.

The results suggest the possibility of producing single phase HAp with defined morphology. Utilization of natural biogenic materials such as coral in the production of HAp will address the significant cost of synthetic raw materials and the cost of HAp generally. HAp derived coral has the wide potential in the medical field, especially in orthopaedic and tissue engineering.

Drug delivery is an ideal application for these materials and we are planning to use the converted coral to deliver clinical active substances as presented in the next chapters. Since using ammonium phosphate solution results in single phase HAp, all HAp derived corals that will be used in the next chapters for drug delivery will be produced from using the ammonium phosphate solution.

5.6 References

Ben-Nissan, B. 2003. Natural bioceramics: from coral to bone and beyond. *Current Opinion in Solid State and Materials Science* 7:283-288.

Ben-Nissan, B. and Green, D. 2014. Marine Structures as Templates for Biomaterials. *In* Advances in Calcium Phosphate Biomaterials, vol. 2 (B. Ben-Nissan, ed.) pp. 391-414. Springer Berlin Heidelberg.

Best, S.M., Porter, A.E., Thian, E.S., and Huang, J. 2008. Bioceramics: Past, present and for the future. *Journal of the European Ceramic Society* 28:1319-1327.

Calafiori, A.R., Di Marco, G., Martino, G., and Marotta, M. 2007. Preparation and characterization of calcium phosphate biomaterials. *Journal of materials science. Materials in medicine* 18:2331-2338.

Cecchinato, F., Karlsson, J., Ferroni, L., Gardin, C., Galli, S., Wennerberg, A., Zavan, B., Andersson, M., and Jimbo, R. 2015. Osteogenic potential of human adipose-derived stromal cells on 3-dimensional mesoporous TiO2 coating with magnesium impregnation. *Materials Science and Engineering: C* 52:225-234.

Cegla, R.-N.R., Macha, I.J., Ben-Nissan, B., Grossin, D., Heness, G., and Chung, R.-J. 2014. Comparative Study of Conversion of Coral with Ammonium

Dihydrogen Phosphate and Orthophosphoric Acid to Produce Calcium Phosphates. *Journal of the Australian Ceramics Society* 50:154-161.

Chen, C.-W., Riman, R.E., TenHuisen, K.S., and Brown, K. 2004. Mechanochemical–hydrothermal synthesis of hydroxyapatite from nonionic surfactant emulsion precursors. *Journal of Crystal Growth* 270:615-623.

Chou, J., Austin, C., Doble, P., Ben-Nissan, B., and Milthorpe, B. 2014. Trace elemental imaging of coralline hydroxyapatite by laser-ablation inductively coupled plasma-mass spectroscopy. *Journal of tissue engineering and regenerative medicine* 8:515-520.

Elmore, K.L. and Farr, T.D. 1940. Equilibrium in the System Calcium Oxide–Phosphorus Pentoxide–Water. *Industrial & Engineering Chemistry* 32:580-586.

Green, D.W. and Ben-Nissan, B. 2010. Biomimetic Applications in Regenerative Medicine: Scaffolds, Transplantation Modules, Tissue Homing Device and Stem Cells. *In* Handbook of Materials for Nanomedicine (V. Tochilin and M. Amiji, eds.) pp. 821-850. Pan Stanford Publishing Pte Ltd, Singapore.

Heness, G. and Ben-Nissan, B. 2004. Innovative Bioceramics. *Materials Forum* 27:104-114.

Jahnen-Dechent, W. and Ketteler, M. 2012. Magnesium basics. *Clinical Kidney Journal* 5:i3-i14.

Kuhne, J.H., Bartl, R., Frisch, B., Hammer, C., Jansson, V., and Zimmer, M. 1994. Bone formation in coralline hydroxyapatite. Effects of pore size studied in rabbits. *Acta Orthop Scand* 65:246-252.

LeGeros, R.Z. 1988. Calcium phosphate materials in restorative dentistry: a review. *Advances in dental research* 2:164-180.

LeGeros, R.Z. 2002. Properties of osteoconductive biomaterials: calcium phosphates. *Clin Orthop Relat Res* 81-98.

Liu, C., Huang, Y., Shen, W., and Cui, J. 2001. Kinetics of hydroxyapatite precipitation at pH 10 to 11. *Biomaterials* 22:301-306.

Macha, I.J., Boonyang, U., Cazalbou, S., Ben-Nissan, B., Charvillat, C., Oktar, F.N., and Grossin, D. 2015. Comparative study of Coral Conversion, Part 2: Microstructural evolution of calcium phosphate. *Journal of the Australian Ceramic Society* 52.

Macha, I.J., Ozyegin, L.S., Chou, J., Samur, R., Oktar, F.N., and Ben-Nissan, B. 2013. An Alternative Synthesis Method for Di Calcium Phosphate (Monetite) Powders from Mediterranean Mussel (Mytilus galloprovincialis) Shells. *Journal of The Australian Ceramic Society* 49:122-128.

Macha, I.J., Ozyegin, L.S., Oktar, F.N., and Ben-Nissan, B. 2015b. Conversion of Ostrich Eggshells (Struthio camelus) to Calcium Phosphates. *J Aust Ceram Soc* 51:125-133.

McCutcheon, A.L., Kannangara, G.S.K., Wilson, M.A., and Ben-Nissan, B. 2004. Preliminary analysis of pore distributions using NMR in natural coral and hydrothermally prepared hydroxyapatite. *J Mater Sci* 39:5711-5717.

Meejoo, S., Maneeprakorn, W., and Winotai, P. 2006. Phase and thermal stability of nanocrystalline hydroxyapatite prepared via microwave heating. *Thermochimica Acta* 447:115-120.

Milev, A., Kannangara, G.S.K., and Ben-Nissan, B. 2003. Morphological Stability of Plate-Like Hydroxyapatite. *Key Engineering Materials* 240-242:481-484.

Oktar, F., Tuyel, U., Demirkol, N., Gunduz, O., Samur, R., Kannan, S., and Agathopoulos, S. 2010. A new safe method to produce bioceramic nano-powders from nacre venus verrucosa. Artificial Organs Conference, FYROM, 2010, pp. 467-467.

Rey, C., Marsan, O., Combes, C., Drouet, C., Grossin, D., and Sarda, S. 2014. Characterization of Calcium Phosphates Using Vibrational Spectroscopies. *In* Advances in Calcium Phosphate Biomaterials, vol. 2 (B. Ben-Nissan, ed.) pp. 229-266. Springer Berlin Heidelberg.

Yang, X. and Wang, Z. 1998. Synthesis of biphasic ceramics of hydroxyapatite and [small beta]-tricalcium phosphate with controlled phase content and porosity. *Journal of Materials Chemistry* 8:2233-2237.

Yoshioka, S. and Kitano, Y. 1985. Transformation of aragonite to calcite through heating. *GEOCHEMICAL JOURNAL* 19:245-249.

CHAPTER 6

DRUG LOADING AND RELEASE STUDY

CHAPTER 6: DRUG LOADING AND RELEASE STUDY

In this chapter loading and in vitro release of a number of pharmaceuticals into the hydroxyapatite particles and to the PLA matrix are covered. A strong, widely used antibiotic Gentamicin and a drug used for osteoporosis Bisphosphonate (Clodronate) were loaded into PLA and PLAHAp biocomposites under SINK conditions (in the volume of dissolution medium enough to provide complete dissolution of the expected amount of drug in the samples) with the following objectives: (i) to analyze the experimental release profiles and their fit to available kinetic models, and (ii) to study their kinetic release behaviour.

6.1 Introduction

Slow drug release has been an important research subject in the field of drug delivery for decades. Drug release systems have been proved to provide an outstanding alternative to conventional clinical therapies. With the advancement in both science and material design and engineering, more sophisticated therapeutic agent release systems have been developed with improved capabilities and performances for the treatment of resilient diseases such as musculoskeletal disorders and bone diseases. Drug delivery technology presents an interesting interdisciplinary challenge for pharmaceutical, chemical engineering, biomaterials and medical communities (Rao 2002). In general, a biomaterial that will act as a drug carrier must have the ability to incorporate a drug, to retain it in a specific site, and to deliver it progressively over time to the surrounding tissues.

In order to allow greater potency and less toxicity to healthy tissue, therapeutic agent release systems are required to control the release rate of the drugs locally, in order to maintain a desired drug concentration level without reaching a toxic level or dropping below a minimum effective level capability which cannot be attained by conventional systemic administrations. The major challenges though are centred on how to regulate the drug releasing rate in order to keep its concentration within the therapeutic window, how to personalise the dosage regime for different people and the effective way to target affected tissues while keeping healthy ones spared. Using biodegradable materials in designing drug release devices addresses these challenges by providing the outstanding capability

of performing localized and controlled delivery of drugs to different parts of the host body.

There has been an enormous effort directed to the development of biodegradable materials that are capable of releasing drugs by reproducible and predictable kinetics (Ginebra MP 2006; Habibe et al. 2009) to meet these demands.

It has been reported that calcium phosphate bone substitutes derived from mixed hydroxyapatite and β tricalcium phosphate (β-TCP) are one of the most promising materials for bone drug delivery systems. During the last decade there have been several studies on both commercial and experimental calcium phosphate drug carriers. Major attention has been focused on the delivery of antibiotics, due to their wide areas of application as prevention against infection during surgical interventions or in general in the treatment of bone related infections.

Ceramics and other materials such as polymers and biocomposites have been proposed in the past, but it is difficult to form an appropriate shape with adequate micro porosity in order to be fitted into any type and size of bone defect. The treatment of bone infection remains difficult because of problems with the local penetration of systemically administered antibiotic. Furthermore, bacteria adhere to bone matrix and orthopaedic implants, eluding host defences by developing a biofilm or acquiring a very slow metabolic rate (Baro et al. 2002). Effective treatment against infection may be possible by killing the bacteria during the early stages of colonization, followed by the continuous long term steady state delivery of appropriate amounts of antibiotics. Recently it has been demonstrated by Ben-Nissan and co-workers that marine shells with specific microspherical design offer desired functions for the delivery of Bisphosphonate (paminodrate) and antibiotic (Gentamicin) (Chou, Valenzuela, et al. 2014). This has been possible by virtue of its unique structure and architecture of the foraminifera shells which are extraordinarily difficult to manufacture with the current know-how (Chou et al. 2011).

As stated earlier, biodegradable PLA and polyD,L-lactic-co-glycolic acid polymer films loaded with gentamicin have been developed to serve as "coatings" for possible fracture fixation devices and prevent implant-associated infections (Aviv, Berdicevsky & Zilberman 2007). The use of biodegradable polymer films is advantageous due to their propensity to uptake and release antibiotics, as a consequence of their degradability. Although their drug release rates are high, they could be tailored to form biocomposites with different biodegradability rates by incorporating other materials.

Biodegradable polymer-bioceramic composites would be ideal in this endeavour because of the bioactive nature of ceramic materials, which promote tissue growth. Incorporation of bioceramics derived from converted coral in the polymer will improve not only controlled drug release but also bioactivity and tissue regeneration, especially in orthopaedic and maxillofacial applications. As explained in **Chapter 4**, coral derived bioceramic has clinical benefits due to its unique architecture and the presence of essential elements such as Calcium, Magnesium and Strontium. Sampath *et al.* (Sampath, Garvin & Robinson 1992) demonstrated that PLA microcapsules could release more than 80% of loaded gentamicin sulphate within 3 weeks for the treatment of osteomyelitis. Due to the production process, the pore size and interconnectivity of these pores generated problems in drug release rates. Bioerodable polyanhydride-gentamicin beads were used *in vivo* study for the treatment of Osteomyelitis by surgical debridement by Nelson *et al.* (Nelson, Hickmon & Skinner 1997). They reported to have reduced osteomyelitis by 93% after 4 weeks of implantation for 20% gentamicin loaded beads.

However, most of these past published investigations have shown that antibiotics have been ototoxic and nephrotoxic at high dosages. For most controlled release systems, the loaded dosages are usually high, and therefore the systemic exposure of antibiotic in blood and urine is the major safety concern. Moreover none of these reported systems contain the key minerals like Ca^{2+} to support bone repair and regeneration.

6.1.1 Release kinetics

Drug release from PLA and PLA-HAp biocomposite is not only due to PLA degradation but also the diffusion of the drug entrapped into the polymer matrix, which plays an important role depending on the extension of the experiments. The assessment of kinetic release from drug delivery devices provides the confidence to predict the release behaviour before the release systems are realized. There are three classified categories to analyse kinetics of drug release from controlled release systems:

- Statistical methods (exploratory data analysis method, repeated measures design, multivariate approach (MANOVA) (Mauger, Chilko & Howard 1986; Dash et al. 2010).
- Mathematical model dependent method such as zero order, first order, Higuchi, Korsmeyer-Peppas, Hixson Crowell, Baker-Lonsdale and Weibull models just to mention few (Liu, Luo, et al. 2010; Adrover & Nobili 2015)
- Model independent methods (difference factor (f_1), similarity factor (f_2) Model independent methods [difference factor (Costa 2001; Xie, Ji & Cheng 2015)

At the end of this chapter the release kinetics based on these theories will be discussed.

6.2 Materials and Methods

6.2.1 Materials

Corals skeleton samples were obtained from the Great Barrier Reef, QLD Australia by Prof B. Ben-Nissan, Gentamicin sulfate, Clodronate (Dichloromethylenediphosphonic acid disodium salt), Chloroform diammonium hydrogen phosphate ($(NH_4)_2HPO_4$, 98%), and sodium hypochlorite (NaClO) were obtained from Sigma Aldrich, Castle Hill, Australia.

6.2.2 Methods

Coral materials were converted to HAp and characterized as described in **Chapter 5** section **5.2.2** and used in this study without any further modifications.

Drug loading and film composites

Figure 31 shows schematic procedures to load drugs in to HAp derived from corals. These hydrothermally converted coralline-hydroxyapatite were loaded with 25% w/w or 10% of clodronate or gentamicin by dissolving the required amount of drug in polished 18 MΩ (MilliQ, Millipore, Victoria, Australia) water, mixed with HAp, sonicated and then allowed to dry in a rotavapor (Rotavapor R-210) coupled with a vacuum controller (V-850, BUCHI, In vitro Technologies, Australia) at 60 ^{o}C. Clodronate and gentamicin individually loaded to HAp microspheres were then dried in vacuumed desiccators and then used to produce PLA-thin film composites. Drug loading to the PLA-films was performed by adding the required amount of clodronate/gentamicin or clodronate/gentamicin loaded HAp into PLA-Chloroform solution to give 10% drug in the composites under magnetic stirring then followed by sonication to break down the agglomerations of particles, cast in petri-dishes and then dried in desiccator under vacuum.

Experimental samples used were designated as follows, Polylactic acid film as PLA, PLA film loaded with Bisphosphonate (throughout this chapter Clodronate is designated as BP) or gentamicin (GM) as PLABP or PLAGM, PLA-hydroxyapatite composite film as PLAHAp and composite films of Bisphosphonate/gentamicin loaded hydroxyapatite with PLA as PLAHApBP or PLAHApGM. The confirmation of drug purity after loading into PLA matrix was evaluated by running an FTIR of drugs and drug loaded PLA and PLAHAp films. The films were transparent enough. A spectrum in the range 400 – 4000 cm^{-1} was obtained with FTIR (Nicolet Magna 6700 FTIR spectrometer) for the product samples. BP/GM were ground in an agate mortar and thoroughly mixed with KBr (FTIR Grade). For the analyses, 3 mg was ground with 300 mg of KBr and pressed into a pellet (Carver press).

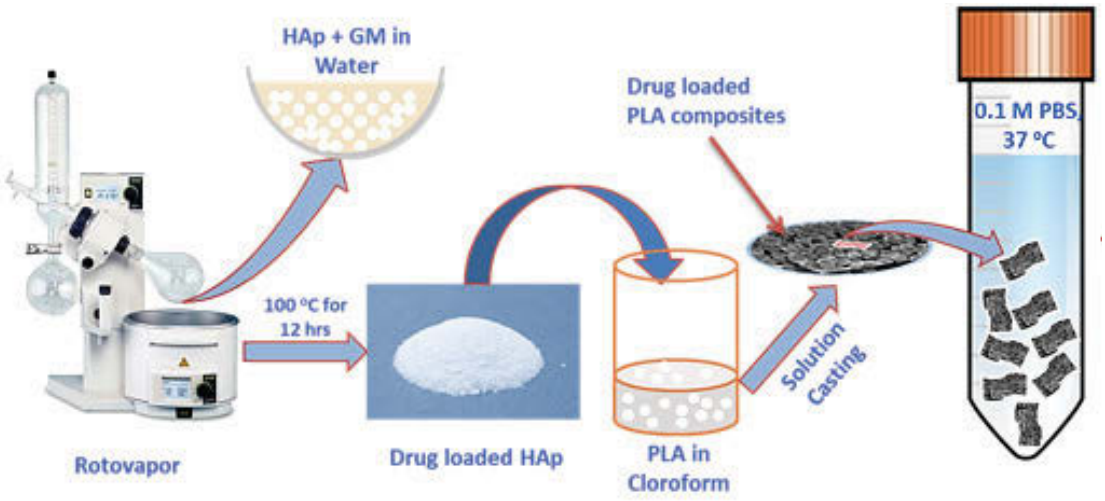

Figure 31: Drug loading and release study of gentamicin and bisphosphonate

In vitro release experiment

Drug release study from PLA thin film composites was conducted under SINK conditions in phosphate buffered saline (PBS) ((0.1 M, Na_3N 0.1%, pH 7.4) at 37 ± 0.1 °C) for gentamicin and in Tris-HCl buffer ((0.1 M, pH 7.4) at 37 ± 0.1 °C) for clodronate in a temperature controlled water bath shaker running at a constant speed of 100 rpm. Each sampling time had its own independent samples under the same conditions and experiments were respectively terminated after sampling. Gentamicin concentrations in the solution were determined by using a Cary 100 UV-Vis spectrophotometer (Agilent Technologies, Victoria, Australia, Cary Series UV-Vis Spectrophotometer) at the maximum absorbance of gentamicin-*o*-phthaldialdehyde complex, λ_{max} = 332 nm, using procedures described in (Aviv, Berdicevsky & Zilberman 2007; Chou, Valenzuela, et al. 2014). Ophthaldialdehyde reagent was prepared by dissolving 2.5 g ophthaldialdehyde in 62.5 mL methanol and adding with 3 mL 2-hydroxyethylmercaptan to 560 mL 0.04 M sodium borate in distilled water. 2 mL gentamicin solution, 2 mL *o*-phthaldialdehyde reagent were reacted for 45 minutes at room temperature. The absorbance, which corresponds to the gentamicin concentration, was then measured at 332 nm.

The quantification of released clodronate concentration was measured using a ^{31}P Nuclear Magnetic Resonance (NMR). ^{31}P NMR spectra were recorded on Agilent 500 MHz NMR magnet apparatus (Agilent Technologies, Australia) integrated with

Varian NMR systems with broadband probe, ø 5 mm, equipped with manual tuning and matching. All spectra were recorded with the standard phosphorus parameters: Decoupling with 90◦ pulses, 1024 number of scans, and an acquisition time of 0.668 s with a relaxation delay of 1 s, no rotation, and the temperature was 24 ◦C. The measuring time for one spectrum was 28 minutes. Tuning, lock and shimming on aqueous dispersions were performed on Triphenylphosphate in deuterochloroform $CDCl_3$. Data were processed using VnmrJ software version 4.0 A and a calibration curve was prepared for each set of measurements. Each prescribed sampling time had its own independent sample, therefore after sampling the respective experiment was terminated.

Minimal Inhibitory Concentration, Antimicrobial Efficacy and Drug Encapsulation Efficiency (DEE)

According to CLSI, the Minimal Inhibitory Concentration (MIC) is the lowest concentration of an antimicrobial agent that prevents visible growth of a microorganism in an agar or broth dilution susceptibility test (Nelson et al. 2002; Buzarovska & Grozdanov 2012) Gentamicin MICs were determined using 96-well microtitre trays and broth microdilution techniques. Inoculum concentration of 5×10^6 CFU/mL was used in each well. MICs were determined in accordance with CLSI (CLSI 2012) guidelines. *S. aureus* ATCC 25923 strain was also used as the control *S. aureus* strain.

An antimicrobial efficacy test was conducted in Tryptone Soya Broth (TSB). Inoculum density equivalent to 0.5 McFarland standards (1×10^8 CFU/mL) from overnight culture, with a 50 mL TSB sub-culture was used. PLA film composites loaded with gentamicin were either introduced in the beginning or after 90 min of bacterial growth in a dry incubator at 37 ± 0.1 °C, with shaking at 220 rpm. The optical density of the culture was monitored by spectrophotometer at 595 nm from the experiment at time 0, which was immediately after introduction of gentamicin loaded PLA films, and every half an hour for the first 6 to 6.5 h. When OD reaches about 1, samples were diluted to 1:10 before measurement. An additional time-point was obtained after 24 h to check for recovery. Replicates of all experiments were done in different days.

The Minimum Inhibitory Concentration of Gentamicin against *S. aureus* (SH1000) was found to be 1 μg/mL. DEE was determined to be 94.2% and 98.9% for PLAGM and PLAHApGM films respectively.

Drug encapsulation efficiency (DEE) was determined by soaking 2 mg of samples in 2 mL of distilled water at 37 °C for 5 min and then measured the drug concentration of the solution using UV-Vis. This process dissolves drugs that are on the outer surface of the films. The efficiency was determined by the formula Equation (1).

$$DEE = \frac{W_{df} - W_{ds}}{W_{df}} \quad (1)$$

where, W_{df} = Weight of drug in the film, W_{ds} = Weight of drug dissolved in solution.

The Minimum Inhibitory Concentration of Gentamicin against *S. aureus* (SH1000) was found to be 1 μg/mL. DEE was determined to be 94.2% and 98.9% for PLAGM and PLAHApGM films respectively.

6.2.3 *In vitro* drug release: Theoretical Mechanism

It is worth mentioning that several factors influence the release mechanism, including the type of drug, environmental conditions during drug release as well as the geometry and dimensions of the drug delivery system, and preparation technique, to mention just a few (Siepmann & Siepmann 2008). The assumption taken into consideration here is that, the drug particles or particles loaded with drugs are dispersed randomly throughout the uniform polymer matrix with known geometry, such that the probability of finding drug at any point in the polymer matrix is constant at all positions within the matrix itself (Rothstein, Federspiel & Little 2008). Based on previous studies, it was shown that release behaviour of a clinical active substance from degradable material follows the common pattern consisting of three stages (Stephens et al. 2000; Rothstein, Federspiel & Little 2009). However, a number of other studies have indicated that the behaviour of drug release from biodegradable materials can follow two or four stages

(Siepmann & Siepmann 2008). The common three stages of drug release could briefly be elucidated as follows:

i. Stage I: At time zero, water or buffer begins to hydrate the matrix, a process that happens quickly for the bulk eroding polymer matrices. As the matrix hydrates, encapsulated drug adjacent to the matrix surface or on the surface diffuses into the water or buffer in a phase reservoir typically known as ‘‘the initial burst’’. It’s magnitude depends on the amount of drugs present adjacent to the matrix and the drug solubility.

ii. Stage II: As the initial burst release takes place, degradation of the polymer chains begins, producing soluble oligomers and increasing chain mobility effectively leading to the formation of pores in the polymer matrix. It is believed that heterogeneous degradation of the polymer matrix starts with an amorphous region of polymer matrix leaving behind pores, creating a secondary surface porosity favourable for fluid transport phenomena and a further pathway for dissociation inside the composite. These pores appear to be essential for subsequent release of drugs (Fredenberg, Reslow & Axelsson 2005). The drug release by diffusion through the narrow or tortuous pores controls the mass transfer process. Graphical observation of the release profile indicates changes in the slope of the curve.

iii. Stage III: Cumulative growth and coalescence of these pores as degradation of the polymer matrix progresses, as the results of water or PBS penetrates into the polymer network and hydrolyzes the polymer into more soluble oligomers, providing the channels for the drug to be able to diffuse towards the surface of a polymer matrix that would otherwise be too dense to allow their passage (Fredenberg, Reslow & Axelsson 2005). Drug is released progressively until complete polymer degradation, a process that depends on the polymer degradation behavior. In our case, the duration of the experiments for gentamicin and bisphosphonate loaded polymers were 15 (Macha, Cazalbou, Ben-Nissan, et al. 2015) and 11 weeks (Macha, Cazalbou, Shimmon, et al. 2015a) respectively, insufficient time to achieve the complete full degradation.

For the fourth stage drug release behavior has been proposed to have a secondary bust before the final stages of drug release from the biodegradable matrix. These stages are for the pure polymeric matrix and addition of the particulate matter as drug carrier introduces additional stages to the process, which will be discussed in the following section.

6.3 Results and Discussions

6.3.1 Drug loading to HAp

Scanning electron microscopy (SEM) was used to confirm the physical presence of loaded drugs in HAp particles and to study the degradation of a polymer matrix loaded with drugs. FTIR was used to evaluate any alteration of drugs loaded in a PLA matrix. Figure 31 shows images of HAp before and after gentamicin (Fig. 32b) and bisphosphonate (Fig.32c) loading. It is evident that converted coral particles are coated with drug on the surface and into the micro, meso and nanopores as the drug solution could easily penetrate into these pores.

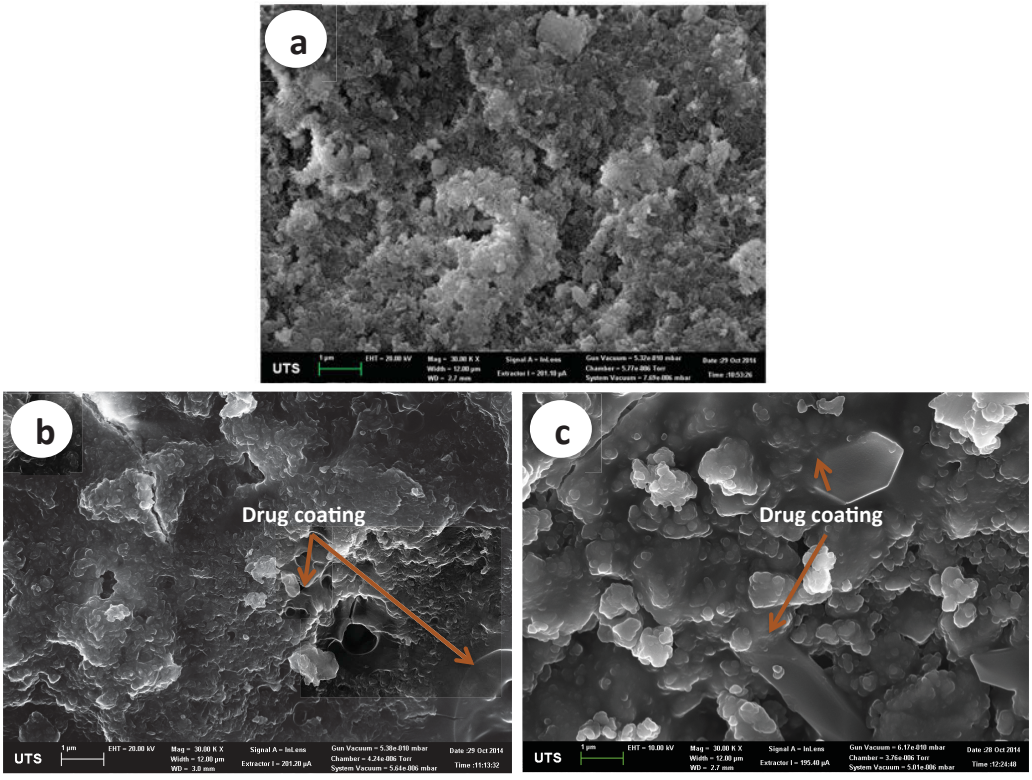

Figure 32: SEM picture showing coral (a) before loading (b) after loading with gentamicin and (c) after loading with Bisphosphonate

On the other hand, the surface of HAp before loading reveals thin platelets of HAp crystals, while after loading the surface looks relatively smooth due to the drug coating.

Figure 33 shows the IR shifts of gentamicin (Fig.33a) and bisphosphonate (Fig.33b) in PLA matrix consistence with IR shifts of these drugs before imbedded into the matrix. It suggests that there is no evidence of chemical interaction between the drugs and the polymer.

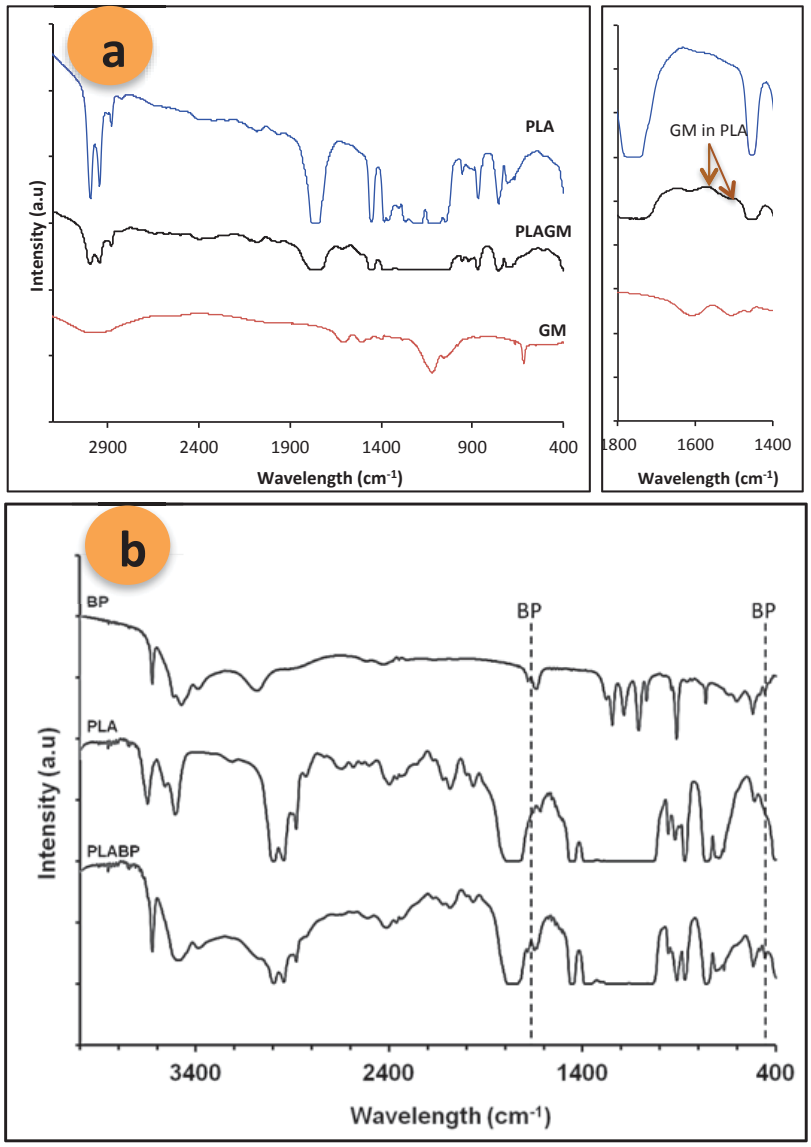

Figure 33: FTIR spectra of drug loaded PLA confirming non-denaturation of drugs in the PLA matrix after loading (a) Gentamicin and (b) Bisphosphonate

The drugs are not regenerated or consumed in any reaction within the polymer matrix. Thus, drug release is mainly due to the diffusion phenomena of fluids within the PLA matrix, which are limited by the hydrolysis rate and by the barrier effect (due to its hydrophobicity) of PLA.

6.3.2 Antibacterial Efficacy Test

Drug loaded PLA composite films were introduced to a 50 mL subculture of *S. aureus* in the beginning and also in time-delay of 90 min where the bacteria growth is at the middle of log phase. In both cases, the released gentamicin concentration was enough to control the growth of bacterial as shown in Figure 34a–c. It was also revealed that after 24 h there was no bacterial recovery seen. Most of the drug released in this period of time is surface associated drug molecules. Furthermore, samples from release study, after four weeks of release were also collected and subjected to efficacy testing. This was important specifically when it is intended to be used as drug delivery system during prolonged periods.

6.3.3 Morphological study of gentamicin and bisphosphonate release

The morphological changes of the PLA film composites during the release study were carried out by using SEM. For extended drug controlled release systems, degradation of the polymeric matrix and the addition of particulate matter (HAp, GM or BP) control the release of drug. The drug release rate and release time are mainly influenced by the degradation kinetics of the polymer, especially in stage two and three of the release behaviour, which means the slower the degradation the longer the release time. Figure 35 represents the morphology comparison of films loaded with gentamicin in the release study after the 1st and 3rd weeks to zero time, while Figure 35 represents the morphology of samples loaded with bisphosphonate after seven weks of release compared to zero time samples.

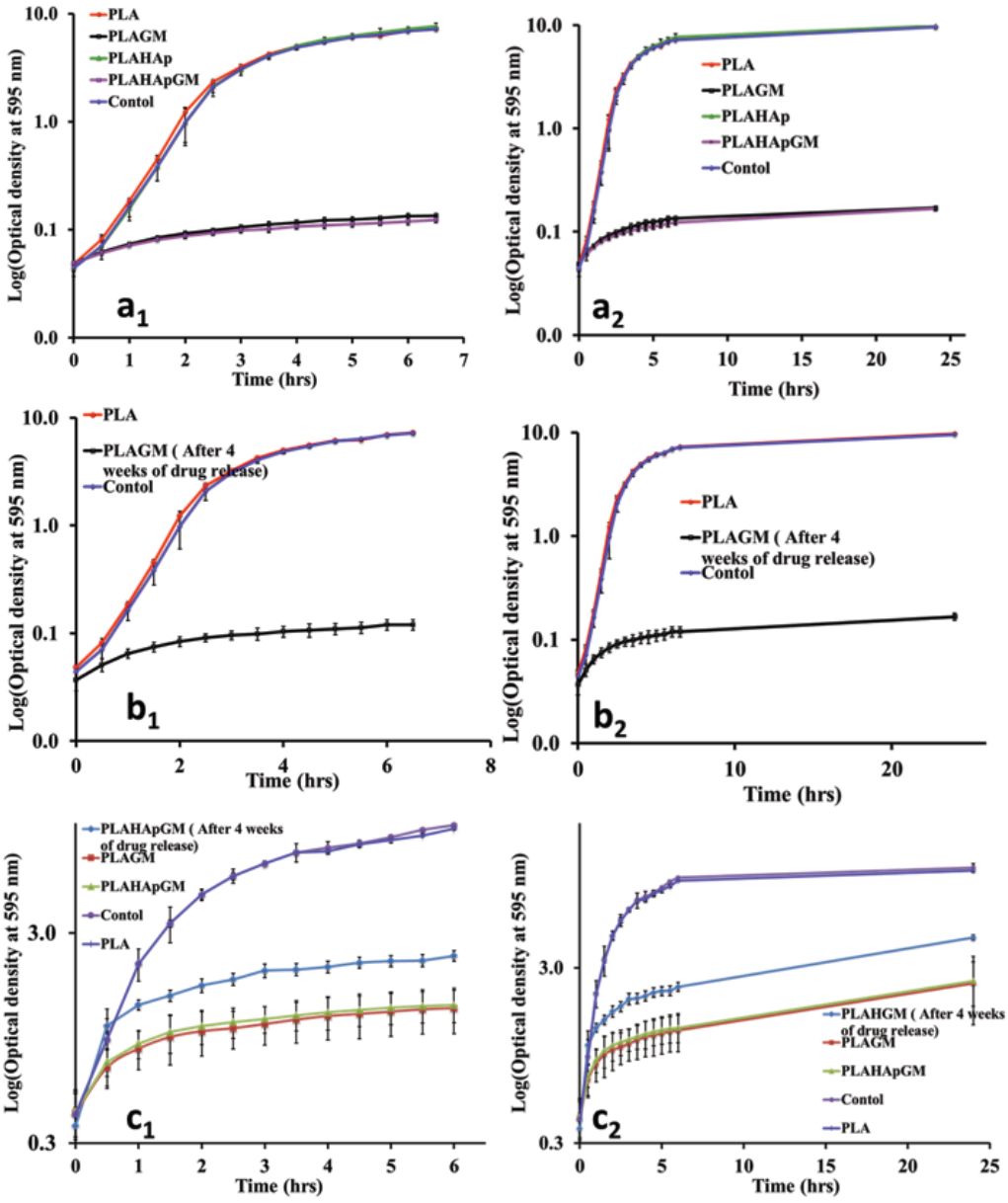

Figure 34: Antibacterial efficacy (a_1) Controls compared with films loaded with gentamicin. All films were introduced immediately after inoculating media with bacteria; (b_1) PLA films loaded with gentamicin after releasing gentamicin for four weeks in PBS (pH 7.4, 37 °C and 100 rpm). Plain PLA films and media were used as positive and negative control, all films were introduced immediately after bacteria inoculation; (c_1) Films loaded with gentamicin and PLAHApGM film after releasing gentamicin for four weeks. Plain PLA films and media were used as positive and negative control; all films were introduced immediately after bacteria inoculation. a_2, b_2, and c_2 are post-bacterial growth level up to 24 h of respective experiment. Error bars are mean standard deviation (SD) of two biological and technical replicates of the experiments conducted in different days.

The general structures of PLAHAp, gentamicin loaded PLA and PLAHAp thin film composites (Figure 35), shows the loaded particles existed in agglomerate forms within the PLA matrix with an average size of 100 nm to 1.5 µm for PLAGM and PLAHAp and 200 nm to 1 µm for PLAHApGM (Figure 35-Week 0). The distribution of drug and HAp drug loaded particles within the PLA matrix is good. The micrographs also suggests that the PLAGM sample has more gentamicin protruding close to the polymer surfaces compared to PLAHApGM which is consistent with drug encapsulation efficiency data (Macha, Cazalbou, Ben-Nissan, et al. 2015). This also explains the affinity between gentamicin and hydroxyapatite, the factor that influences the physical interaction between them and the PLA matrix.

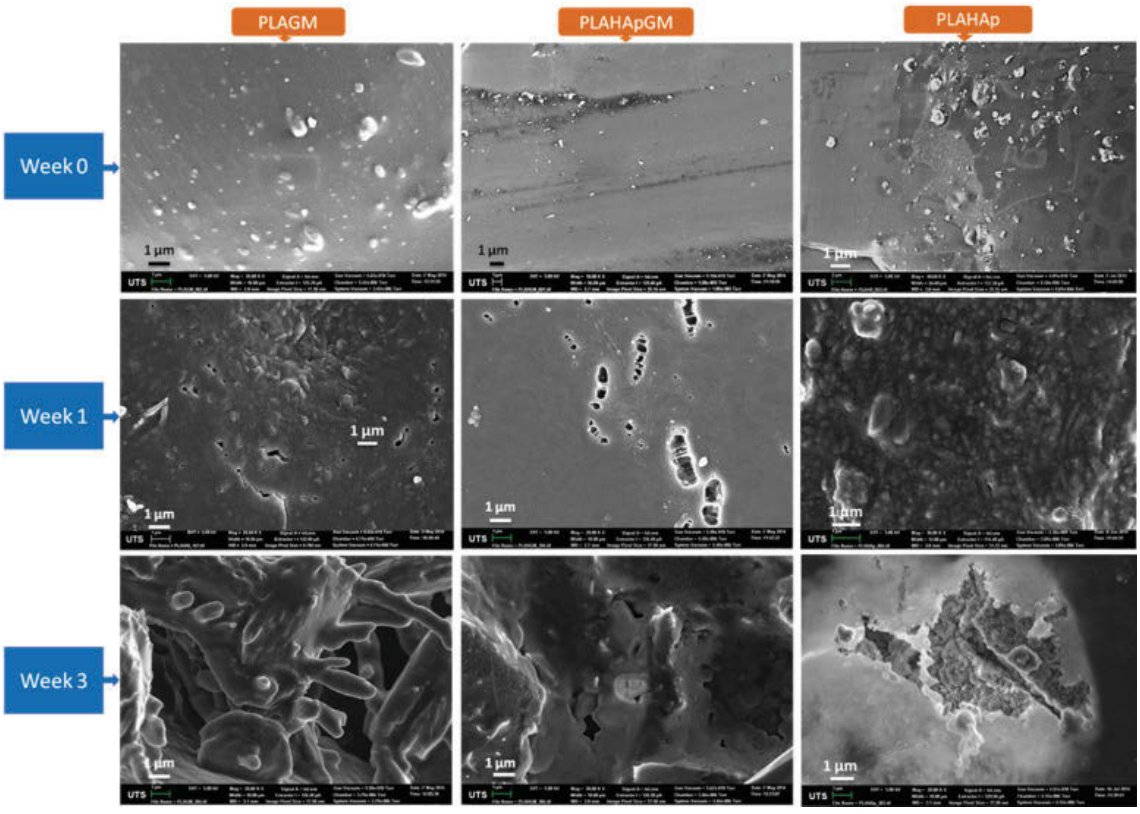

Figure 35: SEM pictures of PLAGM, PLAHApGM and PLAHAp composites revealing the degraded morphology after 1st and 3rd weeks in PBS solution.

Microstructures of samples after seven weeks of release were compared with the samples before release as shown in Figure 36. It is shown that for both drug-PLA and drug loaded HAp-PLA matrices an agglomeration of the drug particles exists as

100 nm to 2 µm sizes at time 0. The results show the evidence of the PLA matrix degradation as a function of time.

The presence of clodronate (BP) in the films seems to affect the degradation behaviour of the matrix. Normally, degradation depends on the rate of buffer or water absorption into the polymeric matrix. Clodronate has extensive ionization, which makes PLABP more hydrophilic than PLAHApBP and PLAHAp. Buffer or water diffused into the polymeric matrix affects both polymer degradation and the particles, as it is simultaneously consumed through hydrolysis of the matrix, and drug release rates as it dissolves the drug within the matrix.

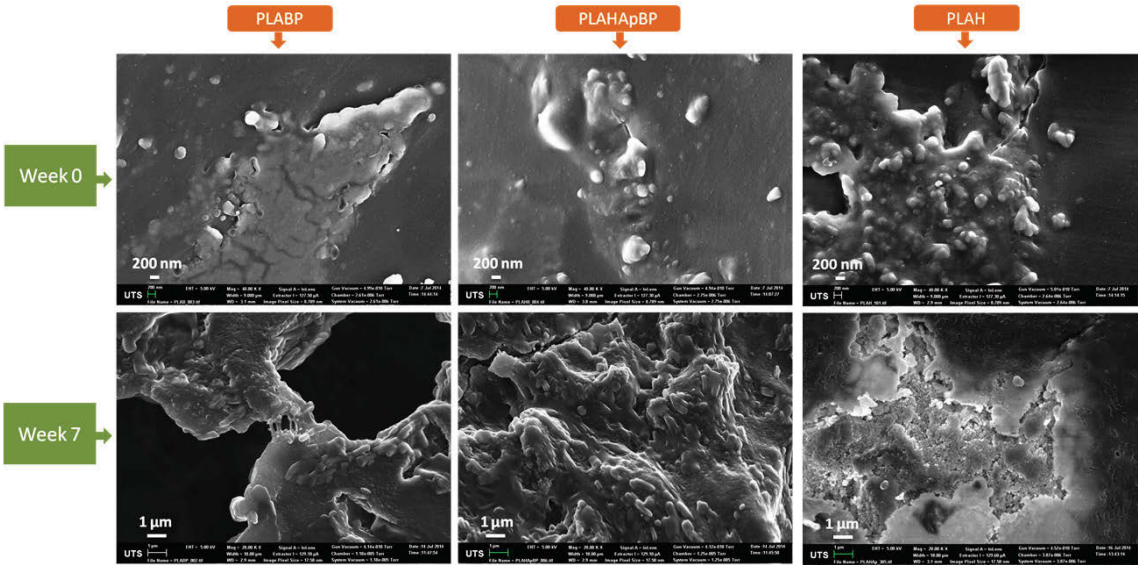

Figure 36: Structure of PLA drug release devices in sink conditions comparing before and after 7 weeks of drug release.

Clodronate loaded in HAp is located on the surface, and also in nano and mesopores of the particles. As measured by McCutcheon et al. (McCutcheon et al. 2004) coral structure contains in addition to micro pores a large number of nano and mesopores. There is also some clodronate dispersed in the matrix alone which degrades as in PLABP. The PLAHApBP is relatively less hydrophilic and is observed to degrade the least. Based on these results it is observed that clodronate release will be faster in PLABP than PLAHApBP composite.

6.3.4 *in-vitro* gentamicin and bisphosphonate release in PBS and Tris-Cl buffer respectively

Figure 37 shows drug release profiles composed of three stages for Bisphosphonate (Figure 37a) and five stages for gentamicin (Figure 37b) loaded PLA films and PLAHAp composites. It is possible that the first stage consists only of the surface bound drug release that it is simple dissolution from the surfaces by diffusion that includes both polymer and particle surfaces exposed to the environment. The process is fast and the "burst effect" is observed. The second stage includes a number of sub-stages dependent on the drugs, particles and the environment but can be summarized as "progressive cumulative dissolution"(PCD). In this stage the polymer matrix starts to degrade and allows the liquid medium to penetrate into the HAp particles where surface bound drug on the surfaces of the particles starts to dissolve and diffuse out. The rate of dissolution is slower due to the strong ceramic nature of the particulate matter (HAp).

In the third or last stage in addition to PCD the particulate matter starts to break down, which is a relatively slow process and the dissolution rate is further reduced but it includes the polymeric matrix dissolution, particle degradation and further drug release at steady state rate.

It is possible to appreciate different shapes of the release curves depending on the type of drug and release medium. The release time was also enough to appreciate significant degradation of the polymer matrix as shown in Figure 35 and 36.

A mathematical model proposed to fit the experimental data comprises three different equations addressing full physical meaning according to the release mechanism described previously. In the release profiles of drugs from degradable polymer matrix the most sustained release part is by degradation of the polymer matrix. Table 7 shows the time range of each release stage for bisphosphonate and gentamicin (these values are an approximation considering the shape of the release profiles, the final time of one stage occurs with the initial time of the next one). Each stage time goes with the assumption that the drug is homogeneously

distributed within the matrix and therefore there is uniform steady state release of the drugs from the matrix.

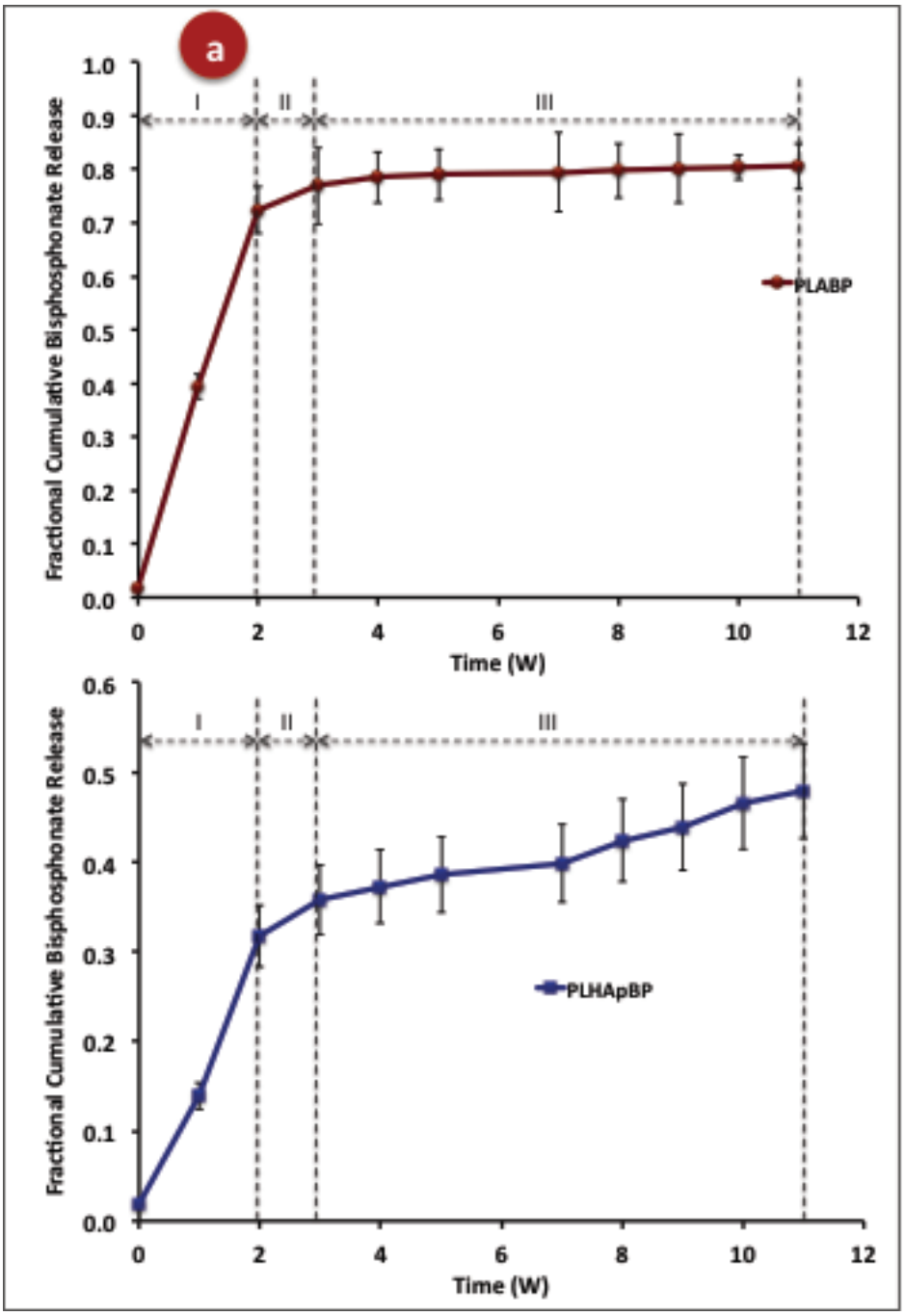

Figure 37: In vitro fractional cumulative release of (a) clodronate (Bisphosphonate, BP) from PLA thin film composite in Tris-HCl buffer solution (pH 7.4, 37^{o}C and 100 rpm) for eleven weeks and

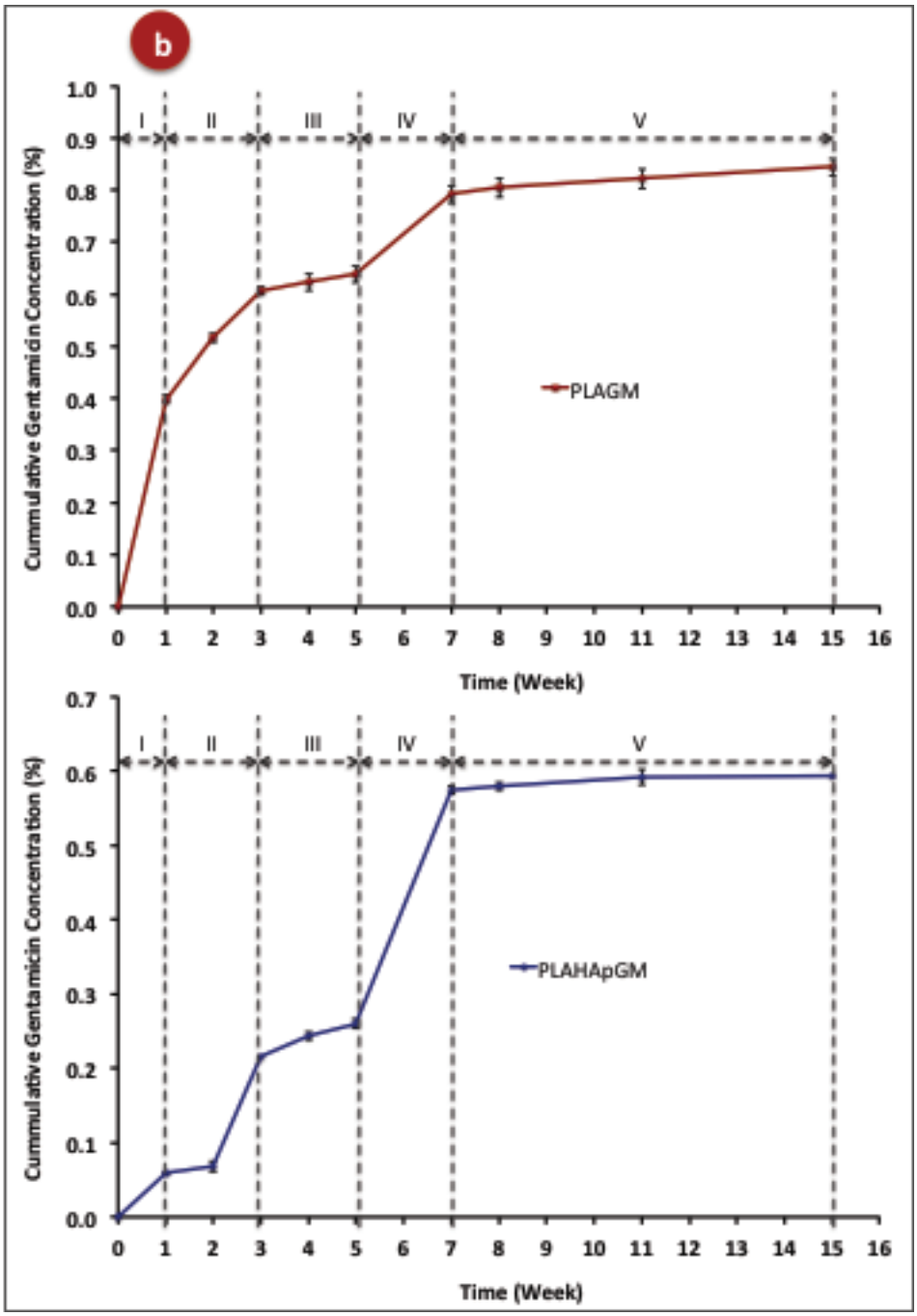

Figure 37: (b) gentamicin from PLA thin film composite in phosphate buffered saline (PBS) solution (pH 7.4, 37 °C and 100 rpm) for fifteen weeks showing different stages of drug release.

6.3.4.1 Stage I

The initial very quick release (burst) of gentamicin or bisphosphonate can be assimilated as the direct dissolution of drugs in water. It is shown in Figure 37a that the initial burst took two weeks for bisphosphonate and only one week for

gentamicin. At this stage it can be assumed that the release is purely governed by diffusion of drugs from polymer surfaces (Liechty et al. 2010b; Severino et al. 2011).

6.3.4.2 Stage II

For both BP and GM this step is driven by the internal diffusion of drugs impregnated within the matrix possibly in the porous part of the matrix generated during preparation. For gentamicin release from PLAHApGM samples, this stage is preceded by a "lag phase" which occurs between 1 and 2 hours of release. The presence of HAp loaded with gentamicin could in many ways hinder or slow down the release of gentamicin through these micropores. This stage is a bit slower release compared to the previous one due to the drug transporting through these small and narrow pores.

Table 7: Specific time frames for different release stages and their numerical values for bisphosphonate (Three stages) and gentamicin (Four stages)

Type of drug released	**Stages**	**t_o (w)**	**t_f (w)**
Bisphosphonate	Stage I (burst ext. release)	0	2
	Stage II (internal release)	2	3
	Stage III (degradation)	3	∞
Gentamicin	Stage I (burst ext. release)	0	1
	Stage II (Internal release)	1	3
	Stage II (lag phase)	3	5
	Stage III (degradation)	5	∞

6.3.4.3 Sub Stage III

Bisphosphonate

This is the terminal release phase or stage for bisphosphonate loaded devices (Figure 37). At this stage there is pore growth due to both mass loss by polymer degradation and pore coalescence (micropores coalescing (or joining) to form

mesopores). For the device containing HAp (PLAHApBP) the release amount in all stages is lower than PLABP because drug diffusion is delayed by an induction time sufficient to allow nano and micropores of HAp to coalesce and permit the passage of the macromolecular drug out from the occlusion through the microporated matrix (Batycky et al. 1997). Moreover, for PLAHApBP, drug (BP) previously adsorbed on mineral particles is reported to have strong affinity to the nanocrystalline apatites with adsorption phenomena occurring at the surface of apatite crystals (Pascaud et al. 2012).

Gentamicin

This is a 'lag stage or phase' for devices loaded with gentamicin. The lagging to release a significant amount of drug from the devices could be attributed to the pH change of entrapped, released acidic degradation products from the polymer matrix. The acidic environment would trigger unfolding of the encapsulated gentamicin until the degradation products dissolve and diffuse to the outer surface of the matrix providing a way for the drugs to be released (Zhu, Mallery & Schwendeman 2000).

6.3.4.4 Sub Stage IV

Gentamicin

This is the second burst stage for gentamicin loaded PLA and PLAHAp devices. It is more vivid in the PLAHAp device due to the fact that at this stage we have more drugs retained in this device compared to PLA for the reasons described previously. After the retardation of drug release in the previous stage, drugs saturated close to the surface and released immediately after the degradation products dissolve and pH increases.

6.3.4.5 Stage III

Gentamicin

This is the terminal release stage for gentamicin-loaded devices. After a greater fraction of the drug is released, the remaining drug release is directly associated with polymer degradation (Cabezas et al. 2014). There is the possibility of drug release by conventional diffusion at this stage, but it is considered to be negligible

compared to that released by polymer degradation. In this study, it was observed that this stage was the slowest and steady stage drug release phase.

6.3.5 Release kinetics-(Gentamicin)

The release kinetic study was assessed by a model dependent method. Based on number of kinetic models available in literature, which described the overall release of drug from the dosage forms, the models were carefully selected and used to fit the release data (Costa & Sousa Lobo 2001; Liechty et al. 2010a; Mohammadi et al. 2010; Ford Versypt, Pack & Braatz 2013). Finally, data were fitted to seven different models: Zero order, 1st Order, Higuchi, Hixson-Crowell, Korsmeyer-Peppas and Reciprocal powered time.

Table 8. Modelled dissolution characteristics of the mean dissolution profile.

Model	**Mathematical expression**		**PLAGM**	**PLAHApGM**
Zero order	$F = Q_0 + k_0 t$	r^2	0.440	0.937
		k_0	0.125	0.070
First order	$Ln(1-F) = -kt$	r^2	0.866	0.874
		k	0.226	0.098
Higuchi	$F = at^{1/2} + b$	r^2	0.959	0.803
		a	0.273	0.214
Hixson- Crowell	$1 - (1-F)^{1/3} = kt$	r^2	0.758	0.898
		k	0.060	0.029
Korsmeyer-Peppas	$F = kt^n$	r^2	0.992	0.962
		n	0.282	1.315
Baker Lonsdale	$\frac{3}{2}\left[1-(1-F)^{2/3}\right] \times F = kt$	r^2	0.945	0.727
		k	0.107	0.038
Reciprocal powered time	$\left(\frac{1}{F} - 1\right) = \frac{m}{t^b}$	r^2	0.971	0.835
		b	0.744	1.037
		m	1.524	17.190

F = fraction of drug released up to time t, r^2 = Square correlation coefficient, Q_o, k_0, k, a, b, n, m and b are parameters of the models.

The correlation coefficients (r^2) indicate that drug release kinetic of PLAGM and PLAHApGM fitted with the power law model described by Korsmeyer-Peppas (Table 8). If this semi-empirical equation does not allow for the determination of all of the mechanisms involved in the release, it is still possible to determine the mechanisms of transport by considering two borderline cases, which correspond to distinct physical realities (when $n = 0.5$ and $n = 1$). The n coefficient obtained for PLAGM ($n < 0.5$) indicates that the release mechanism was mainly controlled by diffusion while the value obtained for PLAHApGM ($n > 1$) is characteristic of a number of mixed transport mechanisms including diffusion, possibly super case II kinetics as well as a release due to damage to the composite surface through dissolution.

6.3.5.1 Release Kinetics—Comparison of Drug Release Profiles

PLAGM and PLAHApGM dissolution profiles were compared using statistical difference factors, difference factor (f1), and similarity factor (f2) (Table 9). The test indicates that there is a significant statistical difference between the two kinetic profiles. This difference is confirmed by the release half-life ($t_{50\%}$) obtained for each material loaded with gentamicin. Thus, the release half-life of PLAGM is obtained around 14 days while 124 days are necessary for PLAHApGM.

Table 9. Modelled dissolution characteristics and difference and similarity factors of PLA film and PLAHAp composites loaded with gentamicin.

	PLAGM	**PLAHApGM**
$t_{50\%}$ (weeks)	1.76	15.53
f1 difference factor	54.4	
f2 similarity factor	24.2	

The release of gentamicin from these devices seems to follow the semi-empirical equation described by the Korsmeyer-Peppas model. Nevertheless, the 'n' coefficient obtained for PLAGM and PLAHApGM indicate that somehow a number of different mechanisms might control release. Thus, the release of gentamicin contained from the PLA matrix seems to be mainly controlled by diffusion whereas

for PLAHApGM it is possibly a mixture of diffusion, the super case II mechanism and other mechanisms of transport which control the drug release.

It should be added that the analysis shows that the diffusion mechanism is observed for the PLAGM model (regression coefficients of models which use diffusion are good). However it is certain that at the end of the initial release, degradation of the polymeric network will “favour” the dissolution of the remainder of the drug. So, this phenomenon (diffusion and degradation) should occur at the end of the surface drug release (when dissolution of the drug has created a secondary porosity inside the polymeric network and the resultant “fragility” of it) and/or depends to the dissolution rate, surface area, of the PLA film used. For this reason, we have to consider only the early part of the dissolution kinetic (just below 60% drug released) and hence, in the case of PLAGM, the release can be assumed to proceed and be mainly controlled by the diffusional process (Macha, Cazalbou, Ben-Nissan, et al. 2015).

6.3.6 Release kinetics - Bisphosphonate

The release study of BP from thin films shows kinetic profiles characteristic of a sustained release. However, the profiles show a substantial difference, since the whole quantity of BP was totally dissolved from the PLA film in 4 weeks, whereas only 44% of the drug was released in the same time period when BP was loaded onto HA particles before being introduced into a PLA matrix. This difference was confirmed by the (f1) and similarity (f2) factors obtained, 49 and 18 respectively, indicating that adsorbing BP onto the surface of HA before introduction to the PLA film induces a modification of the drug-release behaviour. Thus, when BP is loaded onto HA particles, it allows a significant decrease of the initial burst effect and a decrease of the release rate, therefore prolonging the release period and permitting the extension of the therapeutic efficacy of the material.

To enable a better understanding of the phenomena occurring during drug release, we used mathematical models to allow a quantitative interpretation of the dissolution profiles and thus describe the mechanisms that govern the dissolution. In the case of PLABP, we chose some mathematical models adapted to film forms

(zero-order, first-order, Higuchi, Hixson–Crowell and Korsmeyer–Peppas) to describe the drug dissolution. Only the first weeks were considered, as the rest of the curve corresponded to the plateau. Dissolution data closely fitted to the zero-order equation (r2 = 0.997), which is usually used to describe the drug dissolution from transdermal systems and corresponds to a kinetic with the same amount of drug dissolved/unit of time (in this case, 24.3 μg BP/day). In the case of PLAHApBP, the drug is previously adsorbed on mineral particles embedded in the PLA thin matrix film. Pascaud et al. (2012) have highlighted the strong affinity of BP molecules for the nanocrystalline apatites and have described the adsorption phenomena that occur at the surfaces of apatite crystals. Thus, BP molecules in contact with apatite crystals are exchanged with phosphate ions located in the reactive labile apatitic layer, and cannot be spontaneously released without another anionic exchange or without the dissolution of the crystals. This is unlike the kinetic release of BP dispersed into PLA (PLABP), which is constituted by two clearly defined portions (the first constituting zero-order drug release and the other corresponding to the plateau, i.e. when the drug is totally dissolved).

On the other hand, the release kinetics of BP contained in the composite film (PLAHApBP) seems to present three separate linear parts. Considering the fact that each portion is linear, we were able to determine the daily dose released for each part of the kinetics. The first part corresponds to the first 2 weeks; during this period, the daily drug released is quite important and is equal to 10.59 μg/day (r2 = 0.995). The second part of the curve corresponds to the release that occurs during weeks 3–5; the slope of the curve is greatly reduced and the daily dose then released is 0.91 μg/day (r2 = 0.999). The third part of the release kinetics occurs during weeks 7–11; there was a noticeable increase of the release rate, with the daily dose released equal to 1.33/μg/day (r2 = 0.990). Finally, 60% of the drug contained in the composite is released after 11 weeks, which allows considering a release, and therefore a local therapeutic action, over several months (Macha, Cazalbou, Shimmon, et al. 2015b).

6.4 Summary and Conclusions

The study of PLA–calcium phosphate thin film composites reveals possible potential applications of these devices in the biomedical field as drug delivery systems. The flexibility they provide allows them to conform to any desired clinical shape and size. Incorporation of hydroxyapatite in the matrix, has the added advantages of controlled release, improved encapsulation efficiency, increases drug stability and maintenance of bioactivity and the continuous supply of calcium Ca^{2+} and phosphate PO_4^{2-} ions which can assist in bone regeneration and repair. Gentamicin release profiles, exhibited a steady state release rate, with significant antimicrobial activity even at high concentration of bacteria (Macha, Cazalbou, Ben-Nissan, et al. 2015). The systems also showed the potential for prolonged release of antibiotic. The loading of drug onto HAp particles induces a significant decrease of the release period, for both gentamicin and bisphosphonate, permitting the therapeutic efficacy of composite biomaterial locally to be extended. The bisphosphonate release study showed the ability of these devices to control clodronate release for a prolonged period of time. Depending on the degree of clinical dosage required, the devices developed could be tuned to release the drug in both short and prolonged periods of time.

6.5 References

Adrover, A. & Nobili, M. 2015, 'Release kinetics from oral thin films: Theory and experiments', *Chemical Engineering Research and Design*, vol. 98, no. 0, pp. 188-201.

Aviv, M., Berdicevsky, I. & Zilberman, M. 2007, 'Gentamicin-loaded bioresorbable films for prevention of bacterial infections associated with orthopedic implants', *J Biomed Mater Res A*, vol. 83, no. 1, pp. 10-9.

Baro, M., Sánchez, E., Delgado, A., Perera, A. & Evora, C. 2002, 'In vitro- in vivo characterization of gentamicin bone implants', *J Control Release.*, vol. 83, no. 3, pp. 353-64.

Batycky, R.P., Hanes, J., Langer, R. & Edwards, D.A. 1997, 'A theoretical model of erosion and macromolecular drug release from biodegrading microspheres', *J Pharm Sci*, vol. 86, no. 12, pp. 1464-77.

Ben-Nissan, B. 2003, 'Natural bioceramics: from coral to bone and beyond', *Current Opinion in Solid State and Materials Science*, vol. 7, no. 4–5, pp. 283-8.

Cabezas, L.I., Gracia, I., de Lucas, A. & Rodríguez, J.F. 2014, 'Novel Model for the Description of the Controlled Release of 5-Fluorouracil from PLGA and PLA Foamed Scaffolds Impregnated in Supercritical CO2', *Industrial & Engineering Chemistry Research*, vol. 53, no. 40, pp. 15374-82.

Chou, J., Ben-Nissan, B., Green, D.W., Valenzuela, S.M. & Kohan, L. 2011, 'Targeting and Dissolution Characteristics of Bone Forming and Antibacterial Drugs by Harnessing the Structure of Microspherical Shells from Coral Beach Sand', *Advanced Engineering Materials*, vol. 13, no. 1-2, pp. 93-9.

Chou, J., Valenzuela, S., Green, D.W., Kohan, L., Milthorpe, B., Otsuka, M. & Ben-Nissan, B. 2014, 'Antibiotic delivery potential of nano- and micro-porous marine structure-derived beta-tricalcium phosphate spheres for medical applications', *Nanomedicine (Lond)*, vol. 9, no. 8, pp. 1131-9.

Costa, P. 2001, 'An alternative method to the evaluation of similarity factor in dissolution testing', *International Journal of Pharmaceutics*, vol. 220, no. 1–2, pp. 77-83.

Costa, P. & Sousa Lobo, J.M. 2001, 'Modeling and comparison of dissolution profiles', *Eur J Pharm Sci*, vol. 13, no. 2, pp. 123-33.

Dash, S., Murthy, P.N., Nath, L. & Chowdhury, P. 2010, 'Kinetic modeling on drug release from controlled drug delivery systems', *Acta Pol Pharm*, vol. 67, no. 3, pp. 217-23.

Ford Versypt, A.N., Pack, D.W. & Braatz, R.D. 2013, 'Mathematical modeling of drug delivery from autocatalytically degradable PLGA microspheres--a review', *J Control Release*, vol. 165, no. 1, pp. 29-37.

Fredenberg, S., Reslow, M. & Axelsson, A. 2005, 'Measurement of Protein Diffusion Through Poly(d,l-Lactide-Co-Glycolide)', *Pharmaceutical Development and Technology*, vol. 10, no. 2, pp. 299-307.

Ginebra MP, T.T., Planell JA. 2006, 'Calcium phosphate cements as bone drug delivery systems: A review', *J Control Release.* , vol. 113, no. 2, pp. 102-10.

Habibe, A.F., Maeda, L.D., Souza, R.C., Barboza, M.J.R., Daguano, J.K.M.F., Rogero, S.O. & Santos, C. 2009, 'Effect of bioglass additions on the sintering of Y-TZP

bioceramics', *Materials Science and Engineering: C*, vol. 29, no. 6, pp. 1959-64.

Langer, R. 1990, 'New methods of drug delivery', *Science*, vol. 249, no. 4976, pp. 1527-33.

Liechty, W.B., Kryscio, D.R., Slaughter, B.V. & Peppas, N.A. 2010, 'Polymers for drug delivery systems', *Annu Rev Chem Biomol Eng*, vol. 1, pp. 149-73.

Liu, P., Luo, Q., Guan, Y. & Zhang, Y. 2010, 'Drug release kinetics from monolayer films of glucose-sensitive microgel', *Polymer*, vol. 51, no. 12, pp. 2668-75.

Macha, I.J., Cazalbou, S., Ben-Nissan, B., Harvey, K.L. & Milthorpe, B. 2015, 'Marine structure derived calcium phosphate-polymer biocomposites for local antibiotic delivery', *Mar Drugs*, vol. 13, no. 1, pp. 666-80.

Macha, I.J., Cazalbou, S., Shimmon, R., Ben-Nissan, B. & Milthorpe, B. 2015, 'Development and dissolution studies of Bisphosphonate (Clodronate) containing hydroxyapatite/PLA biocomposites for slow drug delivery ', *Journal of Tissue Engineering and Regenerative Medicine*.

Mauger, J.W., Chilko, D. & Howard, S. 1986, 'On the Analysis of Dissolution Data', *Drug Development and Industrial Pharmacy*, vol. 12, no. 7, pp. 969-92.

McCutcheon, A.L., Kannangara, G.S.K., Wilson, M.A. & Ben-Nissan, B. 2004, 'Preliminary analysis of pore distributions using NMR in natural coral and hydrothermally prepared hydroxyapatite', *Journal of Materials Science*, vol. 39, no. 18, pp. 5711-7.

Mohammadi, G., Barzegar-Jalali, M., Valizadeh, H., Nazemiyeh, H., Barzegar-Jalali, A., Siahi Shadbad, M.R., Adibkia, K. & Zare, M. 2010, 'Reciprocal powered time model for release kinetic analysis of ibuprofen solid dispersions in oleaster powder, microcrystalline cellulose and crospovidone', *J Pharm Pharm Sci*, vol. 13, no. 2, pp. 152-61.

Nelson, C.L., Hickmon, S.G. & Skinner, R.A. 1997, 'Treatment of experimental osteomyelitis by surgical debridement and the implantation of bioerodable, polyanhydride-gentamicin beads', *Journal of Orthopaedic Research*, vol. 15, no. 2, pp. 249-55.

Pascaud, P., Bareille, R., Bourget, C., Amedee, J., Rey, C. & Sarda, S. 2012, 'Interaction between a bisphosphonate, tiludronate and nanocrystalline apatite: in vitro

viability and proliferation of HOP and HBMSC cells', *Biomed Mater*, vol. 7, no. 5, p. 054108.

Rao, M.S.a.K.P. 2002, 'Preparation, characterization and in vitro release of gentamicin from coralline hydroxyapatite-gelatin composite microspheres', *Biomaterials*, vol. 23, no. 15, pp. 3175-81.

Rothstein, S.N., Federspiel, W.J. & Little, S.R. 2008, 'A simple model framework for the prediction of controlled release from bulk eroding polymer matrices', *Journal of Materials Chemistry*, vol. 18, no. 16, pp. 1873-80.

Rothstein, S.N., Federspiel, W.J. & Little, S.R. 2009, 'A unified mathematical model for the prediction of controlled release from surface and bulk eroding polymer matrices', *Biomaterials*, vol. 30, no. 8, pp. 1657-64.

Sampath, S.S., Garvin, K. & Robinson, D.H. 1992, 'Preparation and characterization of biodegradable poly(l-lactic acid) gentamicin delivery systems', *International Journal of Pharmaceutics*, vol. 78, no. 1–3, pp. 165-74.

Santos, A., Sinn Aw, M., Bariana, M., Kumeria, T., Wang, Y. & Losic, D. 2014, 'Drug-releasing implants: current progress, challenges and perspectives', *Journal of Materials Chemistry B*, vol. 2, no. 37, pp. 6157-82.

Severino, P., Santana, M.H.A., Malmonge, S.M. & Souto, E.B. 2011, 'Polymers for Drug Delivery Systems Formulations', *Polimeros-Ciencia E Tecnologia*, vol. 21, no. 5, pp. 361-8.

Siepmann, J. & Siepmann, F. 2008, 'Mathematical modeling of drug delivery', *International Journal of Pharmaceutics*, vol. 364, no. 2, pp. 328-43.

Stephens, D., Li, L., Robinson, D., Chen, S., Chang, H., Liu, R.M., Tian, Y., Ginsburg, E.J., Gao, X. & Stultz, T. 2000, 'Investigation of the in vitro release of gentamicin from a polyanhydride matrix', *J Control Release*, vol. 63, no. 3, pp. 305-17.

Xie, F., Ji, S. & Cheng, Z. 2015, 'In vitro dissolution similarity factor (f2) and in vivo bioequivalence criteria, how and when do they match? Using a BCS class II drug as a simulation example', *European Journal of Pharmaceutical Sciences*, vol. 66, no. 0, pp. 163-72.

Zhu, G., Mallery, S.R. & Schwendeman, S.P. 2000, 'Stabilization of proteins encapsulated in injectable poly (lactide- co-glycolide)', *Nat Biotech*, vol. 18, no. 1, pp. 52-7.

CHAPTER 7

STEM CELLS AND BIOFILM STUDY

CHAPTER 7: STEM CELLS AND BIOLFIM STUDY

The in vivo efficiency of the thin film biocomposites were investigated with both biofilm formation and stem cell culture studies. In this chapter, biological testing of stem cell culture human adipose derived stem cells (hADSC) and biofilm study of *S. aureus* and *P. aeruginas* on PLA-hydrothermally converted coral composites loaded with gentamicin are covered.

7.1 Introduction

Different types of cells have been used in tissue engineering and in therapeutic strategies in cell therapy including stem cell transplantation. Stem cells are primarily used to understand the mechanisms by which natural or synthetic biomaterials are able to elicit a cellular response when implanted in vivo. In the early days, adult stem cells were used in this process but proved difficult to grow in culture because of being full developed. The attention was then focused onto the use of fetal cells, which are not stem cells but behaved similarly and better than adult cells when tested in conjunction with biomaterials (Dvir et al. 2006; Tsigkou et al. 2007).

7.1.1 Stem cells

Stem cells are different from the other types of cells in the human body. They are capable of dividing and renewing themselves for an extended period of time. They are also unspecialized and can be differentiated into specialized cells. It was previously believed that embryonic stem cells (ES) derived from inner cell mass of preplantation embryos and adult stem cells were found in tissue or organs. However, recent research indicates otherwise. ES can be found at all stages during development (Lovell-Badge 2007). Bone marrow stem cells that are tough reside in all tissues as a repair mechanism against injury have been extensively investigated.

Currently, haemapoetic stem cell transplant (sometimes known as bone marrow transplant) is most often recommended as a treatment option for people with leukemia, multiple myeloma, and some types of lymphoma. It may also be used to treat some genetic diseases that involve the blood. A patient undergoing either

autologous or allogeneic transplantation can be treated by replacing diseased bone marrow with highly specialized stem cells that develop into healthy bone marrow.

There has been an increase in the number of researches on implantable biomaterials and their application in regenerative medicine. This has also created necessary testing procedures to be undertaken using in-vitro laboratory tests, according to ISO 10993 to avoid dangers for patients and unnecessary animal experiments.

Cell compatibility of biomaterials involves three important stages, which are adhesion of cells on the surface, proliferation and finally differentiation. When biomaterial is implanted into a living body, rapidly the biomaterial is coated with a protein layer before the cells' attachment. Serum protein and various extracellular matrices (ECM) are involved such as fibronectin, fibrinogen, albumin and vitronectin (Palacio & Bhushan 2012). The protein adsorption and conformation is partly influenced by the morphology of the biomaterial surface, which also influences the cell adhesion and proliferation process (Noh & Vogler 2007). It has been reported that micro/nano surface topography has a direct impact on cell adhesion and proliferation with a micro-textured surface favouring the adhesion.

Apart from morphology, the chemical composition of the biomaterial surface has been suggested to play a significant role in the adhesion and proliferation of cells. Keselowsky and his co-workers (Keselowsky, Collard & Garcia 2003) showed that the cell adhesion through integrin group of cell surface receptors depend on the conformation of adsorbed fibronectin. In their demonstration, they used a self assembly-monolayer of different functional groups such as OH, COOH, NH_2 and CH_3 termini, to create surfaces with different chemistry that are hydrophilic and neutrally charged, hydrophilic and acidic, hydrophilic and basic, and hydrophobic, respectively. Their findings suggested that the adhesion strength of cell binding (as determined by a centrifugation assay) followed the trend: OH>COOH>NH_2>CH_3. They also found that specific gene expression of cells such as osteoblast, alkaline phosphatase enzyme activity and matrix mineralization, showed the dependence on surface chemistry in which OH- and NH_2- terminated surfaces were more

advantageous compared with COOH and CH_3 SAMs. Hydrophobic surfaces seem to cause denaturation of proteins and prevent the surface exposure to cell-binding groups responsible to cell adhesion. On the other hand, hydrophilic and neutrally charged surfaces induce the least extent of unfolding or denaturation, leading to a good cell adhesion on the fibronectin (E et al. 2003).

7.1.2 Bacteria and biofilm

The most frequent complications related to the use of implantable medical devices such as vascular and urinary catheters, orthopaedic or cardiac prostheses and endotracheal tubes are bacterial infections. Medical device-related infections caused by biofilm are the public health concern and country's economic burden resulting in host patient morbidity and device removal, or mortality (Desrousseaux, Sautou, Descamps & Traore 2013). It was reported by the European Center for Disease Prevention and Control [http://ecdc.europa.eu/en/healthtopics/Healthcare-associated_infections/Pages/index. aspx] that approximately 4,100,000 patients are estimated to acquire a healthcare-associated infection (HAI) in the European Union each year. The number of deaths occurring as a direct consequence of these infections is estimated to be at least 0.9% (37,000) and these infections are thought to contribute to an additional 110,000 deaths (2.7%) each year. In the United States, it was estimated that 1,737,125 patients acquired HAIs for the year 2002, including 417,946 in intensive care units (ICUs) and 1,266,851 outside ICUs. The number of associated deaths was 98,987; of these, 30,665 were from bloodstream infections (BSIs), 13,088 from urinary tract infections (UTIs) and 8,205 from surgical site infections including those associated with orthopaedic implants (Klevens et al. 2007). Efforts have been made by governments to prevent HAIs and commit to provide the best and safest care. The Centre for Disease Control and Prevention (CDC) has recently reported the decrease in HAI cases ((CDC) 2015) for central line-associated bloodstream infections (CLABSI), a 46 percent decrease between 2008 and 2013, catheter-associated urinary tract infections (CAUTI), a 6 percent increase between 2009 and 2013; although initial data from 2014 seem to indicate that these infections have started to decrease, select surgical site infections (SSI), a 19 percent decrease between 2008 and 2013,

and hospital-onset methicillin-resistant *Staphylococcus aureus* (MRSA) bacteremia (bloodstream infections), an 8 percent decrease between 2011 and 2013.

Surgical site infection (SSI) is the most common HAI among surgical patients, with a mortality rate of 3% and 77% of patient deaths reported to be related to infection (Cataife et al. 2014). SSI related to the orthopaedic implants are of great relevance for public health due to the increasing number of aged and disabled patients requiring this type of surgical intervention. It has been estimated that 5% of patients undergoing clean surgical procedures and up to 20% of patients having intra-abdominal surgical procedures develop a surgical site infection. Such infections result in 3.7 million excess hospital days and more than US $1.6-3 billion in excess hospital costs per year.

Because of the large number of patients suffering from biofilm-based device-related infections, a number of strategies for their prevention have been developed in the last two decades. Different strategies have been proposed for either preventing or controlling biofilm development. Modification or development of devices with surface properties that have an effect against microbial adhesion or viability seems to be a promising approach for the prevention of device-related infections. The designing of medical devices with an antimicrobial agent is one of the strategies. Antibiotics can be incorporated into the materials, coated, covalently bonded or loaded into the thin film that can be used to cover the implants resulting into either slow release of antibiotic or in contact killing without release of antibiotic (Cerca et al. 2012; Desrousseaux, Sautou, Descamps & Traoré 2013). Another strategy is to modify the surface of the medical device chemically or physically to render the surface microbial-adhesion free. Chemical surface modifications have been mostly targeted on the hydrophobicity properties of the materials. (Garrett, Bhakoo & Zhang 2008; Kargupta et al. 2014).

Bacteria adhesion is a complex phenomenon affected by many factors, including the properties of surface materials, some characteristics of bacteria itself and the environment where the adhesion takes place such as the presence of serum protein or bactericidal substances (An & Friedman 1998). According to

thermodynamic theory, bacteria attachment is interpreted as a spontaneous decrease in the free energy in a system. Some of the proposed theory and models of adhesion seem to be limited because they consider physical interaction between the surface and bacteria and neglect biological aspects of adhesion in which specific bacterial structures responsible for adhesive activities called adhesins that control cell to cell or cell to abiotic surface adhesion. Bacteria may have different adhesives for different surfaces (different acceptor). The ionic strength and pH of the medium in which the adhesion takes place influence the charge of the cell wall and of the substrate (in terms of surface chemistry, charge and hydrophobicity) and therefore affects their interaction.

Bacterial biofilms are protected from antibiotic killing. Poor diffusion and penetration of antibiotic through the biofilm, contribute to the persistence of biofilm infections especially those associated with implanted devices (Stewart 2002). A variety of reasons on microbial resistance to antimicrobial agents have been postulated. An increase in the depletion of oxygen and nutrients resulting in slow growth of bacteria, adaptive stress responses and formation of persistant cells are hypothesized to constitute a multilayered defense. The focus is directed towards disabling biofilm resistance, which may enhance the ability of existing antibiotics to treat infections involving biofilms (Hoiby et al. 2010). It has been reported that in most cases, biofilm can be prevented aggressively by antibiotic in their early stages and can also be treated by chronic suppressive therapy. Mah and his co-workers (Mah et al. 2003) suggested that the use of traditional antibiotics combined with a drug that interferes with biofilm-specific resistance would be the right approach to render biofilms more susceptible to treatment

The major challenge associated with the use of antibiotics is ensuring retention of antibiotic release and activity for a prolonged period of time after post-operation (Kargupta et al. 2014). In **Chapter 6** we have shown that the use of biodegradable polymer – ceramic composite for prevention of bacterial infections associated with orthopaedic implants would be an ideal approach. It was reported that the release of antimicrobial agent from PLA thin film composites sustained for more that 8 weeks (Macha, Cazalbou, Ben-Nissan, et al. 2015).

P. aeruginosa is regarded as an opportunistic pathogen causing indwelling device-related infections especially in catheters. *P. aeruginosa* infection is a leading cause of morbidity and mortality in cystic fibrosis (CF) patients. It was reported that the median survival age of a patient with CF in 2011 was predicted to be 36.8 years, a slight rise compared to 2010 (Marshall & Hazl 2011). *S. aureus* infection on the other hand causes serious infectious complications such as severe sepsis, septic-thrombosis and/or severe deep-seated infections (endocarditis, osteomyelitis and other metastatic infections) (von Eiff et al. 2005).

7.2 Materials and Methods

7.2.1 Materials

Samples PLA, PLAGM, PLAHAp and PLAHApGM were prepared using materials and chemicals stated in **Chapter 2** in section **2.4** and **Chapter 6** Section **6.2.**

7.2.2 Methods

8.2.2.1 Sample preparations for Biofilm and Stem cells experiments

PLA films and film composites loaded with gentamicin were prepared as described in **Chapter 6** Section **6.2.2**. Samples for biofilm experiments were cut into a circular shape of approximately 1 cm diameter and glued on the sterilized cylindrical coupons. Then the glued samples were sterilized in UV for 40 minutes. For stem cell experiments, samples were casted into the sterilized glass petri dishes 60 x 15 mm and these dishes were used in the experimental work.

7.2.2.2 Cell culture experiment

Cell attachment and morphology on the PLA thin film composite samples were evaluated *in vitro* using cultures of human adipose derived stem cells (hADSC) at passage 5. Approximately 5 x 10^4 cells/ ml were seeded on glass petri dishes with the cast samples in DMEM/F12 Glutmax (Gibco) 10 % fetal bovine serum (Gibco) and incubated at 37°C, 5% CO_2 for 7 days. Briefly, the PLA and PLAGM samples were coated with Poly-L-lysine at room temperature for 30 minutes and rinsed twice with phosphate buffered saline (pH 7.4) before cell culture. Samples were washed with PBS (pH 7.4), fixed in 4 % formaldehyde (Sigma) and then dehydrated in an ethanol gradient from 10%-100% at 10% increments at 10

minutes each before the cells were observed in SEM (ZEISS Supra55VP, Zeiss, Germany) operated at 5kV accelerated voltage, using back scatter electron detector.

7.2.2.3 Preparation of media

Trytpic Soy Broth (TSB) for *Staphylococcus aureus (S. aureus)* and Mueller Hinton II BR cation adjusted media (MHB) for *Pseudonomas aeruginosa (P. aerugonas)* were prepared by thoroughly dissolving 30 g of TSB powder (Bacto™ TSB (BD) and 22 g of MHB powder in 1 L of polished 18 MΩ (MilliQ, Millipore, Victoria, Australia) water respectively. Then the solutions were autoclaved to sterilize (121 ^{o}C, liquid cycle) and stored at room temperature.

7.2.2.4 Bacteria strains and static biofilm formation assay

*S. aureus (*ATCC 25923*)* and *P. aeruginosa* (ATCC 15692) were used for this study and were cultured in a shaking incubator, 250 rpm at 37 ◦C under anaerobic and aerobic conditions respectively. For static biofilm formation, cells were grown in broth medium overnight, and diluted 1:100 into TSB and MHB media respectively. These cell suspensions were inoculated into a 12-well plate containing a triplicate of samples glued on coupons. The plate was sealed with sterile breathable film (Aeraseal; Excel Scientific, Victorville, CA, USA) and statically grown at 37°C, 5% CO_2 for 24 h. Biofilm samples were washed with PBS, stained using SYTO9 Green fluorescent Nucleic Acid Stain (Life Technologies Corp, Carlsbad, CA, USA) and fixed with 4% paraformaldehyde.

7.2.2.5 Confocal Laser Scanning Fluorescence Microscopy (CLSM)

The morphologies of the biofilm were analyzed using confocal laser scanning microscopy (CLSM) (Nikon A1, Tokyo, Japan), using an oil-immersion lens (60 Objective lens and numerical aperture of 1.4 with Z-series images taken in 1.0 μm slices) with NIS Elements Confocal software. A total of eight images were acquired randomly from each specimen.

7.2.2.6 Images Analysis with COMSTAT

Recorded CLSM images were reconstructed by IMARIS (Bitplane AG, Zurich, Switzerland) for biofilm structural quantification in computer statistics software COMSTAT (Heydorn et al. 2000) and presented as three-dimensional structures. The reconstructed Tagged Image Format File (.TIFF) images were converted from gray-scale into a black-and-white picture that could be analyzed by the COMSTAT program. The threshold settings were achieved by comparing the original gray-scale picture with the black-and-white picture, and the best value was chosen to give the most accurate conversion of the gray-scale to the black-and-white picture. This threshold value is fixed and then used for all image stacks (Kreth et al. 2004).

7.2.2.7 Scanning Electron Microscopy (SEM)

The morphological analyses of PLA films and composites were performed in a Scanning Electron Microscope (SEM) (ZEISS Supra55VP, Zeiss, Germany). Samples were fixed by conductive adhesive tape on aluminium stubs and covered with carbon using a sputter coater. Images were taken at various magnifications at acceleration voltages of 5 kV.

7.3 Results and Discussions

7.3.1 Biofilm

In the biofilm study, four biofilm image features calculated by COMSTAT were chosen to characterize biofilm development by *S. aureus* and *P. aerugino*sa on PLA thin film composites. These variables, biomass, average thickness, roughness coefficient and surface to biovolume ratio were selected for interpretation of biological and physical characteristics of biofilm (Heydorn et al. 2000) on these surfaces. Biomass represents the overall volume of the biofilm, and also provides an estimate of the biomass in the biofilm, average thickness provides a measure of the spatial size of the biofilm, roughness represents a measure of biofilm heterogeneity and surface to biovolume ratio tells us how large a portion of the biofilm is exposed to the nutrient flow. Tables 10 and 11 represent the results in summary of these four parameters of biofilm calculated by COMSTAT.

P. aeroginosa showed a stronger tendency to form micro-colonies on the surface of PLA films than *S. aureus*, which is indicated by higher roughness coefficients (Table 10). The higher surface to biovolume ratio of *P. aeroginosa* is an indication of its flat growth on the surface compared to *S. aureus*. This is also consistent with the lower average thickness of the biofilm it forms on the surface. It is also suggested that *S. aureus* grows faster than *P. aeroginosa* on PLA surfaces indicated by lower surface to volume ratio, higher average thickness and hence higher biomass in the biofilm (Figure 38).

Table 10: Biomass, average thickness, roughness coefficient and surface to biovolume ratio of biofilm of *S. aureus* and *P. aeroginosa* on PLA thin film composites after 24 hours.

	Samples	**Biomass ($\mu m^3/\mu m^2$)**	**Average Thickness (μm)**	**Roughness Coefficient**	**Surface to biovolume ratio ($\mu m^2/\mu m^3$)**
P. aeruginosa	**PLA**	1.50±0.70	2.09±0.87	0.56±0.18	9.68±2.64
	PLAGM	1.34±0.71	2.16±0.99	0.65±0.36	7.13±2.01
	PLAHAp	2.20±0.40	3.19±0.45	0.77±0.31	14.80±5.59
	PLAHApGM	2.36±0.48	2.59±0.84	0.72±0.28	15.43±6.42
S. aureus	**PLA**	1.10±0.57	2.31±0.72	0.90±0.08	4.33±0.58
	PLAGM	1.04±0.52	2.66±0.70	0.48±0.21	4.02±0.42
	PLAHAp	2.75±0.43	4.51±0.50	0.48±0.21	3.56±0.31
	PLAHApGM	2.03±0.44	2.90±0.35	0.41±0.20	3.50±0.32

Values are means of data from 30 image stacks (five images from each of 3 specimens in biological replicates and technical triplicate experiments)

The effect of antibiotic in the films (PLAGM and PLAHApGM) against biofilm after 24 hours seemed to be minimal for *S. aureus* and none at all for *P. aeroginosa*. This is possibly due to the fact that static conditions may influence the low release rate of drugs from the device and also 24 hours is not enough time to realize polymer degradation and significant release of antibiotic. Both bacteria displayed more growth of biofilm (highest biomass and highest average thickness) on PLAHAp due to the improvement of bioactivity by HAp in the samples.

Table 11: Biomass, average thickness, roughness coefficient and surface to biovolume ratio of biofilm of *S. aureus* and *P. aeroginosa* on PLA thin film composites after 5 days.

	Samples	Biomass ($\mu m^3/\mu m^2$)	Average Thickness (μm)	Roughness Coefficient	Surface to biovolume ratio ($\mu m^2/\mu m^3$)
P. aeruginosa	**PLA**	3.68±0.67	4.99±0.48	0.24±0.23	2.85±0.42
	PLAGM	3.08±0.64	3.68±0.69	0.57±0.37	1.14±0.39
	PLAHAp	3.67±0.83	4.03±0.60	0.30±0.08	2.12±0.59
	PLAHApGM	3.79±0.70	4.40±1.10	0.32±0.35	1.28±0.28
S. aureus	**PLA**	2.51±0.82	3.52±1.27	0.34±0.22	2.85±0.74
	PLAGM	3.61±0.64	3.70±0.50	0.37±0.16	1.79±0.16
	PLAHAp	4.89±0.74	5.14±0.97	0.28±0.17	1.74±0.07
	PLAHApGM	2.38±0.41	4.17±1.16	0.21±0.02	1.81±0.19

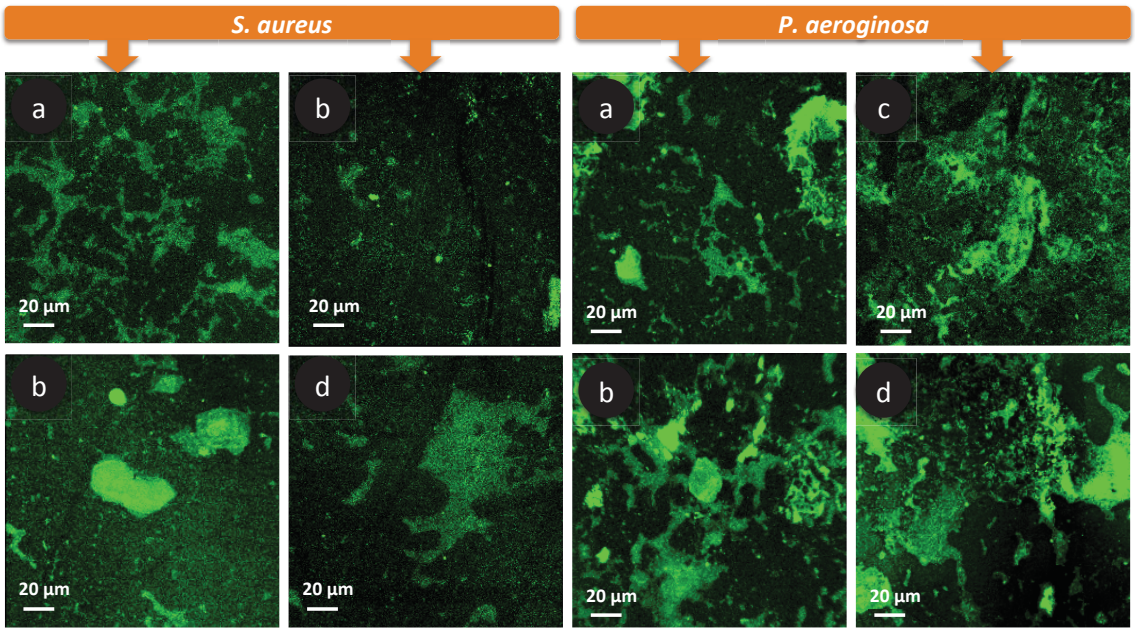

Figure 38: CLSM of 1-day old static grown biofilm of *S. aureus* and *P. aeroginosa* on a) PLA b) PLAGM c) PLAHAp and d) PLAHApGM showing their distributions. a, b and d for *S. aureus* reveals large and high micro-colonies while b shows single cells and small cell clusters cover the surface on of the film. *P. aeroginosa* shows more growth and wider coverage of the surface than *S. aureus*.

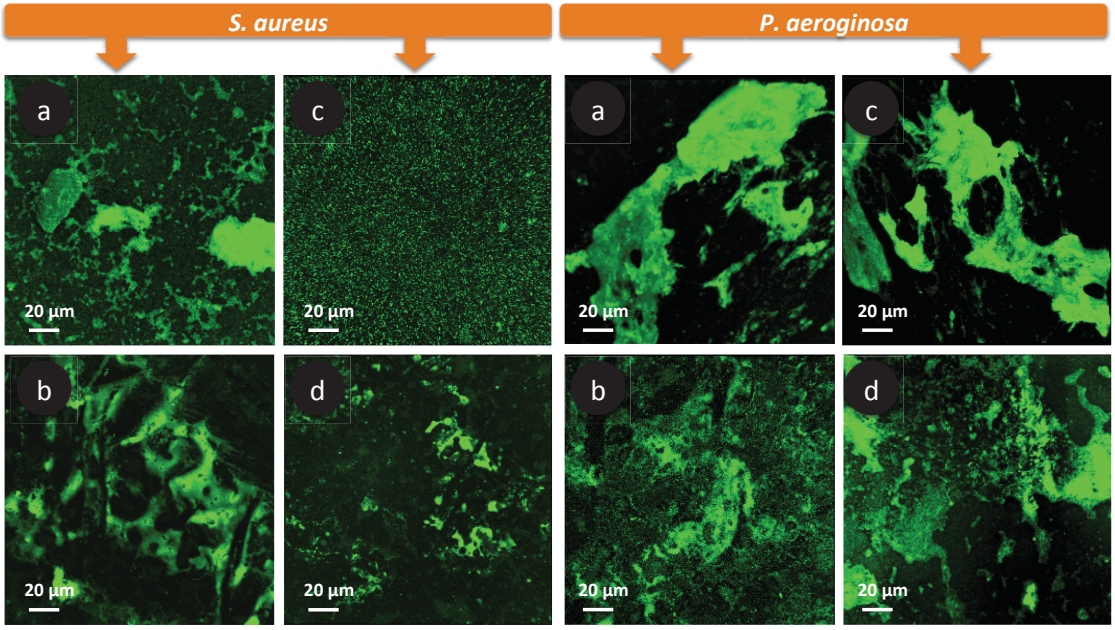

Figure 39: CLSM of 5-day old static grown biofilm of *S. aureus* and *P. aeroginosa* on a) PLA b) PLAGM c) PLAHAp and d) PLAHApGM showing their distributions. a, b and d for *S. aureus* reveals large and high micro-colonies while b shows single cells and small cell clusters cover the surface on of the film. *P. aeroginosa* b and d shows more coverage of the surface by biofilm compared to a and b.

The preliminary results obtained from five day's experiments for *S. aureus* and *P. aeroginosa* on PLA thin film samples are presented in Table 11. Both strains at day 5 had grown into micro colonies, reflected by their low surface to volume ratio compared to biofilm at day 1. On the other hand, microscopy images of *S. aureus* on the films show large and high micro-colonies on PLA, PLAHAp and PLAHApGM samples while on PLAGM high number of single cells and small cell clusters were observed (Figure 38 and 39). This could be due to the effect of antibiotic presence in the film. It is suggested that cells were able to attach onto the surface of PLAGM samples but the drug released from the surface suppresses the ability of bacteria to make a biofilm.

It could also be envisaged that there is a possibility under flow-biofilm-growth conditions that more drugs would be released from the surface and may suppress the attachments of bacteria on the surfaces. Being high-level antimicrobial

resistant in Gram-negative, *P. aeroginosa* displays similar structure characteristics on the surface of PLA thin films.

7.3.2 Cell attachment and morphology

Synthetic polymeric biomaterials can only support cell adhesion and proliferation to a limited extent due to the lack of functional groups necessary for cell interaction (Alvarez-Barreto et al. 2011). Figure 40 presents SEM pictures showing the morphology and attachment of hADSC seeded on PLA thin film composites. The results show abundant cell attachment on PLAHAp and PLAHApGM samples and none on PLA and PLAGM. PLA has an alkyl pendant group (CH_3-) in its backbone, which makes the polymer more hydrophobic, and tends to denaturalize protein responsible for cell binding and adhesion. Gentamicin has NH_2- group, which with CH_3- on the polymer backbone reduces any chance for protein binding on the surface. This was evident because these samples (PLA and PLGM) do not show any cells on their surfaces. The addition of hydroxyapatite (HAP) in the matrix improves the bioactivity of the materials by changing the surface chemistry from hydrophobic to hydrophilic and neutrally charged with the presence of OH group, which favours binding of adhesive protein (vitronectin and fibronectin) and subsequently cellular interaction (Thevenot, Hu & Tang 2008). There are several techniques currently used to treat biomaterial surfaces in order to improve their biocompatibility, including physical, chemical and mechanical techniques. Surface treatments such as exposure to plasma, coatings, corona discharge, ions and ultraviolet (UV)-ozone can enhance the cell attachments. Specific functional groups can be covalently attached to biomaterial surfaces using chemical synthesis techniques to attach biomolecules (Palacio & Bhushan 2012).

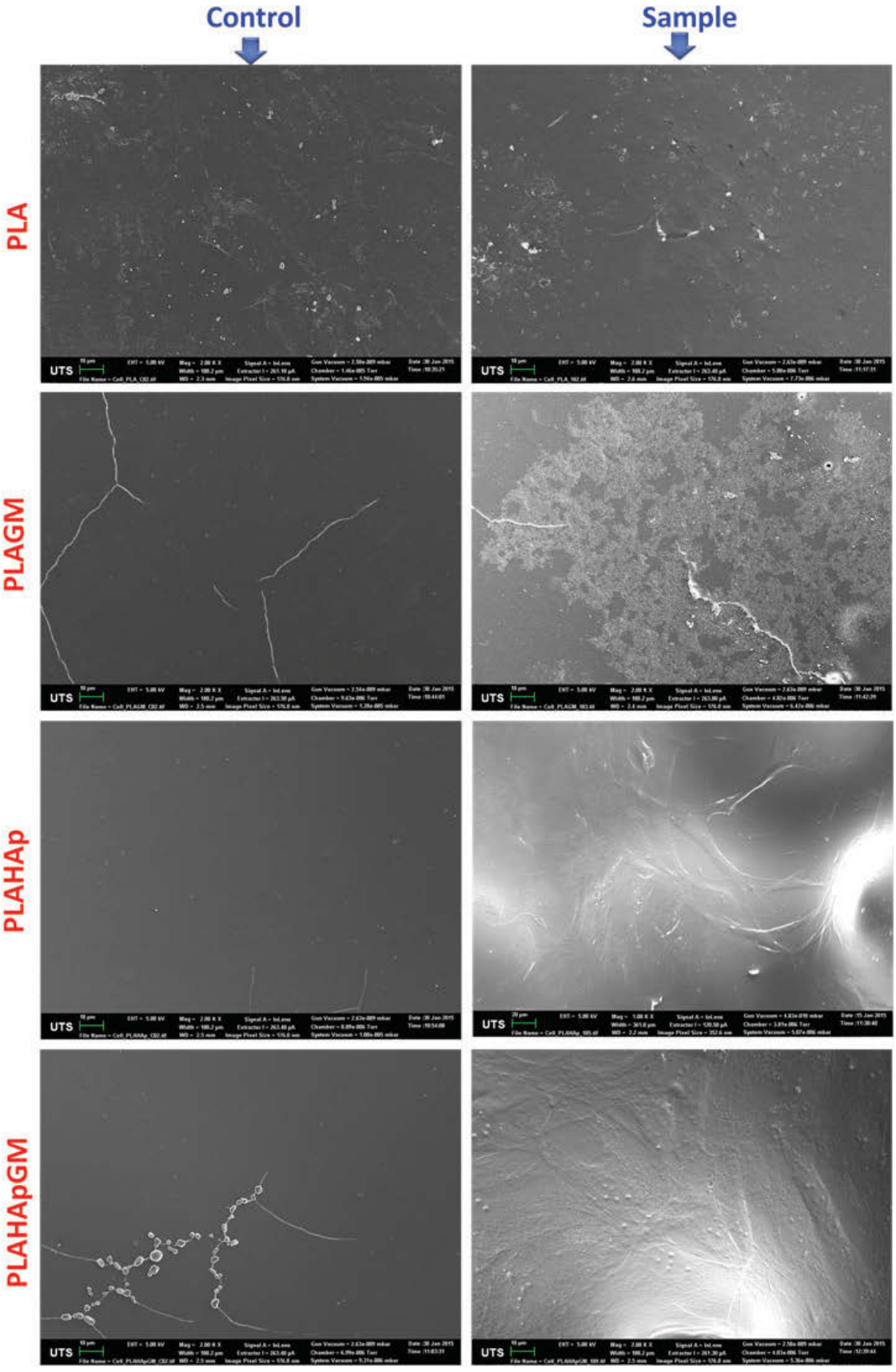

Figure 40: SEM picture of stem cell cultured PLA thin film composites for 10 days, showing attachment and morphology of cells.

In order to confirm the lack of protein adsorption on the surface of PLA and PLGM samples, we coated them with polylysine as an attachment factor to enhance the electrostatic interaction between negatively charged ions of the cell membrane and positively-charged ions of the culture surface by increasing the number of positively-charged sites available for cell binding. The results suggest that cells were able to attach on the PLA and PLAGM surfaces after coating in less than 24 hours (Figure 41).

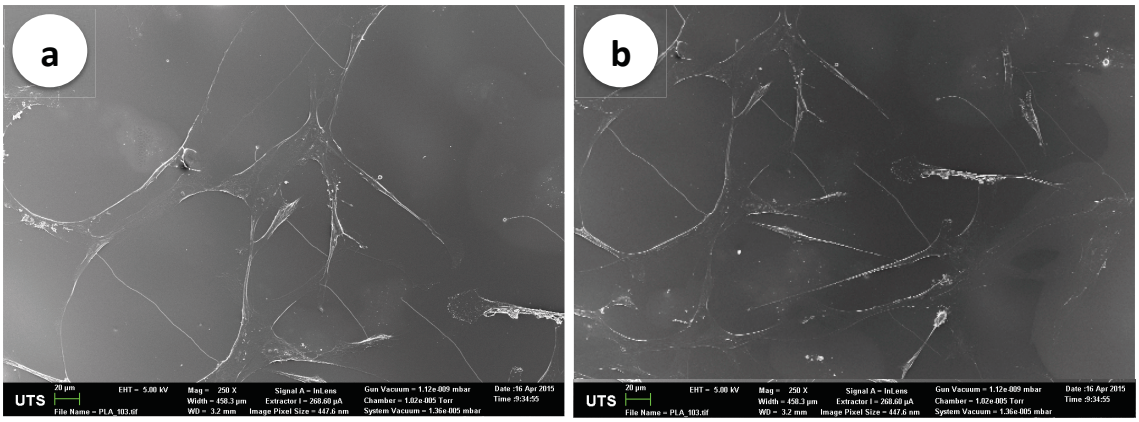

Figure 41: SEM picture of cell cultured samples coated with polylysine a) PLA b) PLAGM

7.4 Summary and Conclusions

Cell attachment and proliferation on synthetic biomaterials is an important step for the material's biocompatibility. PLAHAp and PLAHApGM display strong affinity to hADSC due to the presence of HAp in a polymer matrix. Lack of cells attachment on PLA and PLAGM is not a problem since PLA is biodegradable and is meant in many applications to degrade over time and be replaced with tissue. Polylysine coating on PLA and PLAGM reveals the possibility of surface treatments in case the attachment is of medical importance. Other surface methods like plasma treatments could also be employed to render the surface suitable for protein binding. The use of PLA-HAp composites as drug release systems could give us the possibility of avoiding any additional revision surgery as currently used in PMMA antibiotic delivery systems (Chou, Valenzuela, et al. 2014).

The bacterial strains used in this study play a significant role in the medical context. They are responsible for most of the medical implant-related infections. The activities of these organisms have resulted in a growing interest in their physiology and molecular biology performance especially in biofilm formations. It appears that biofilm development occurs differently on the same surface under different strains. An alternative model for biofilm development (flow chamber biofilms) will give the other side of biofilm development, a phenomenon that is close to in-vitro settings. Reduction of the medical device-related infections lie on the ability to develop medical devices with modified materials. However, further investigations are still needed to widen our current understanding of the biofilms and their prevention mechanisms in order to achieve the minimum number of medical device related infection incidents.

7.5 References

(CDC), C.f.D.C.a.P. 2015, *The National and State Healthcare-Associated Infections Progress Report (HAI Progress Report)*, Centers for Disease Control and Prevention (CDC), USA.

Adrover, A. & Nobili, M. 2015, 'Release kinetics from oral thin films: Theory and experiments', *Chemical Engineering Research and Design*, vol. 98, no. 0, pp. 188-201.

Agarwal, M., Koelling, K.W. & Chalmers, J.J. 1998, 'Characterization of the degradation of polylactic acid polymer in a solid substrate environment', *Biotechnol Prog*, vol. 14, no. 3, pp. 517-26.

Agrawal, C.M., Athanasiou, K.A. & Heckman, J.D. 1997, 'Biodegradable PLA-PGA polymers for tissue engineering in orthopaedics', *Porous Materials for Tissue Engineering*, vol. 250, pp. 115-28.

Agrawal, P., Soni, S., Mittal, G. & Bhatnagar, A. 2014, 'Role of polymeric biomaterials as wound healing agents', *Int J Low Extrem Wounds*, vol. 13, no. 3, pp. 180-90.

Alam, P. 2010, 'A mixtures' model for porous particle–polymer composites', *Mechanics Research Communications*, vol. 37, no. 4, pp. 389-93.

Albee, F.H. & Morrison, H.F. 1920, 'Studies in bone growth: Triple CaP as a stimulus to osteogenesis', *Annals of Surgery*, vol. 71, no. 1, pp. 32-9.

Alvarez-Barreto, J.F., Landy, B., VanGordon, S., Place, L., DeAngelis, P.L. & Sikavitsas, V.I. 2011, 'Enhanced osteoblastic differentiation of mesenchymal stem cells seeded in RGD-functionalized PLLA scaffolds and cultured in a flow perfusion bioreactor', *J Tissue Eng Regen Med*, vol. 5, no. 6, pp. 464-75.

An, Y.H. & Friedman, R.J. 1998, 'Concise review of mechanisms of bacterial adhesion to biomaterial surfaces', *Journal of Biomedical Materials Research*, vol. 43, no. 3, pp. 338-48.

Arciola, C.R., Campoccia, D., Speziale, P., Montanaro, L. & Costerton, J.W. 2012, 'Biofilm formation in Staphylococcus implant infections. A review of molecular mechanisms and implications for biofilm-resistant materials', *Biomaterials*, vol. 33, no. 26, pp. 5967-82.

Arcos, D., Ragel, C.V. & Vallet-Regi, M. 2001, 'Bioactivity in glass/PMMA composites used as drug delivery system', *Biomaterials*, vol. 22, no. 7, pp. 701-8.

Arriagada, F.J. & Osseo-Asare, K. 1999, 'Synthesis of Nanosize Silica in a Nonionic Water-in-Oil Microemulsion: Effects of the Water/Surfactant Molar Ratio and Ammonia Concentration', *Journal of Colloid and Interface Science*, vol. 211, no. 2, pp. 210-20.

Asandei, A.D., Erkey, C., Burgess, D.J., Saquing, C., Saha, G. & Zolnik, B.S. 2004, 'Preparation of drug delivery biodegradable nanocomposites by rapid expansion from supercritical solutions.', *Abstracts of Papers of the American Chemical Society*, vol. 228, pp. U345-U.

Athanassiou, E.K., Grass, R.N. & Stark, W.J. 2010, 'Chemical Aerosol Engineering as a Novel Tool for Material Science: From Oxides to Salt and Metal Nanoparticles', *Aerosol Science & Technology*, vol. 44, no. 2, pp. 161-72.

Aviv, M., Berdicevsky, I. & Zilberman, M. 2007, 'Gentamicin-loaded bioresorbable films for prevention of bacterial infections associated with orthopedic implants', *J Biomed Mater Res A*, vol. 83, no. 1, pp. 10-9.

Baradari, H., Damia, C., Dutreih-Colas, M., Champion, E., Chulia, D. & Viana, M. 2011, 'beta-TCP porous pellets as an orthopaedic drug delivery system: ibuprofen/carrier physicochemical interactions', *Science and Technology of Advanced Materials*, vol. 12, no. 5.

Baro, M., Sánchez, E., Delgado, A., Perera, A. & Evora, C. 2002, 'In vitro- in vivo characterization of gentamicin bone implants', *J Control Release.*, vol. 83, no. 3, pp. 353-64.

Batycky, R.P., Hanes, J., Langer, R. & Edwards, D.A. 1997, 'A theoretical model of erosion and macromolecular drug release from biodegrading microspheres', *J Pharm Sci*, vol. 86, no. 12, pp. 1464-77.

Ben-Nissan, B. 2003a, 'Natural bioceramics: from coral to bone and beyond', *Current Opinion in Solid State & Materials Science*, vol. 7, no. 4-5, pp. 283-8

Ben-Nissan, B. 2003b, 'Natural bioceramics: from coral to bone and beyond', *Current Opinion in Solid State and Materials Science*, vol. 7, no. 4–5, pp. 283-8.

Ben-Nissan, B. 2004, 'Nanoceramics in Biomedical Applications', *MRS Bulletin*, vol. 29, no. 1, pp. 28-32.

Ben-Nissan, B. & Choi, A.H. 2006, 'Sol-gel production of bioactive nanocoatings for medical applications. Part 1: an introduction', *Nanomedicine (Lond)*, vol. 1, no. 3, pp. 311-9.

Ben-Nissan, B. & Green, D. 2014, 'Marine Structures as Templates for Biomaterials', in B. Ben-Nissan (ed.), *Advances in Calcium Phosphate Biomaterials*, vol. 2, Springer Berlin Heidelberg, pp. 391-414.

Ben-Nissan, B., Milev, A., Green, D., Conway, R.M., Kannangara, G.S.K., Russel, J., Hu, J., Gillott, G. & Trefry, C. 2003, *Sol-gel coating*.

Ben-Nissan, B., Milev, A. & Vago, R. 2004, 'Morphology of sol-gel derived nano-coated coralline hydroxyapatite', *Biomaterials*, vol. 25, no. 20, pp. 4971-5.

Ben-Nissan, B. & Pezzotti, G. 2002, 'Bioceramics: Processing Routes and Mechanical Evaluations', *Journal of the Ceramic Society of Japan*, vol. 110, no. 7, pp. 601 - 8

Benninga, H. 1990, 'A History of Lactic Acid Making: A Chapter in the History of Biotechnology', *The British Journal for the History of Science*, vol. 24, pp. 424 - 75.

Bergsma, E.J., Rozema, F.R., Bos, R.R.M. & Bruijn, W.C.D. 1993, 'Foreign body reactions to resorbable poly(l-lactide) bone plates and screws used for the fixation of unstable zygomatic fractures', *Journal of Oral and Maxillofacial Surgery*, vol. 51, no. 6, pp. 666-70.

Best, S.M., Porter, A.E., Thian, E.S. & Huang, J. 2008, 'Bioceramics: Past, present and for the future', *Journal of the European Ceramic Society*, vol. 28, no. 7, pp. 1319-27.

Bianco, A., Cacciotti, I., Lombardi, M. & Montanaro, L. 2009, 'Si-substituted hydroxyapatite nanopowders: Synthesis, thermal stability and sinterability', *Materials Research Bulletin*, vol. 44, no. 2, pp. 345-54.

Blaker, J.J., Nazhat, S.N. & Boccaccini, A.R. 2004, 'Development and characterisation of silver-doped bioactive glass-coated sutures for tissue engineering and wound healing applications', *Biomaterials*, vol. 25, no. 7-8, pp. 1319-29.

Boccaccini, A.R., Chen, Q., Lefebvre, L., Gremillard, L. & Chevalier, J. 2007, 'Sintering, crystallisation and biodegradation behaviour of Bioglass-derived glass-ceramics', *Faraday Discuss*, vol. 136, pp. 27-44; discussion 107-23.

Boccaccini, A.R., Erol, M., Stark, W.J., Mohn, D., Hong, Z.K. & Mano, J.F. 2010, 'Polymer/bioactive glass nanocomposites for biomedical applications: A review', *Composites Science and Technology*, vol. 70, no. 13, pp. 1764-76.

Boccaccini, A.R. & Maquet, V. 2003, 'Bioresorbable and bioactive polymer/Bioglass® composites with tailored pore structure for tissue engineering applications', *Composites Science and Technology*, vol. 63, no. 16, pp. 2417-29.

Bonfield, W., Grynpas, M.D., Tully, A.E., Bowman, J. & Abram, J. 1981, 'Hydroxyapatite reinforced polyethylene--a mechanically compatible implant material for bone replacement', *Biomaterials*, vol. 2, no. 3, pp. 185-6.

Bosetti, M., Zanardi, L., Hench, L. & Cannas, M. 2003, 'Type I collagen production by osteoblast-like cells cultured in contact with different bioactive glasses', *Journal of Biomedical Materials Research Part A*, vol. 64A, no. 1, pp. 189-95.

Brown, M. & Farrar, D. 2008, 'Development of bioresorbable polymers in orthopaedics', *Plastics Rubber and Composites*, vol. 37, no. 2-4, pp. 46-9.

Buzarovska, A. & Grozdanov, A. 2012, 'Biodegradable Poly(L-lactic acid)/TiO2 Nanocomposites: Thermal Properties and Degradation', *Journal of Applied Polymer Science*, vol. 123, no. 4, pp. 2187-93.

Cabezas, L.I., Gracia, I., de Lucas, A. & Rodríguez, J.F. 2014, 'Novel Model for the Description of the Controlled Release of 5-Fluorouracil from PLGA and PLA

Foamed Scaffolds Impregnated in Supercritical CO2', *Industrial & Engineering Chemistry Research*, vol. 53, no. 40, pp. 15374-82.

Cacciotti, I., Lombardi, M., Bianco, A., Ravaglioli, A. & Montanaro, L. 2012, 'Sol-gel derived 45S5 bioglass: synthesis, microstructural evolution and thermal behaviour', *J Mater Sci Mater Med*.

Cai, Y.R. & Zhou, L. 2005, 'Effect of thermal treatment on the microstructure and mechanical properties of gel-derived bioglasses', *Materials Chemistry and Physics*, vol. 94, no. 2-3, pp. 283-7.

Calafiori, A.R., Di Marco, G., Martino, G. & Marotta, M. 2007, 'Preparation and characterization of calcium phosphate biomaterials', *J Mater Sci Mater Med*, vol. 18, no. 12, pp. 2331-8.

Calandrelli, L., Annunziata, M., Della Ragione, F., Laurienzo, P., Malinconico, M. & Oliva, A. 2010, 'Development and performance analysis of PCL/silica nanocomposites for bone regeneration', *J Mater Sci Mater Med*, vol. 21, no. 11, pp. 2923-36.

Campbell, A.A. 2003, 'Bioceramics for implant coatings', *Materials Today*, vol. 6, no. 11, pp. 26-30.

Carothers, W.H., Dorough, G.L. & Natta, F.J.v. 1932, 'STUDIES OF POLYMERIZATION AND RING FORMATION. X. THE REVERSIBLE POLYMERIZATION OF SIX-MEMBERED CYCLIC ESTERS', *Journal of the American Chemical Society*, vol. 54, no. 2, pp. 761-72.

Cataife, G., Weinberg, D.A., Wong, H.H. & Kahn, K.L. 2014, 'The effect of Surgical Care Improvement Project (SCIP) compliance on surgical site infections (SSI)', *Med Care*, vol. 52, no. 2 Suppl 1, pp. S66-73.

Cecchinato, F., Karlsson, J., Ferroni, L., Gardin, C., Galli, S., Wennerberg, A., Zavan, B., Andersson, M. & Jimbo, R. 2015, 'Osteogenic potential of human adipose-derived stromal cells on 3-dimensional mesoporous TiO2 coating with magnesium impregnation', *Materials Science and Engineering: C*, vol. 52, no. 0, pp. 225-34.

Cegla, R.-N.R., Macha, I.J., Ben-Nissan, B., Grossin, D., Heness, G. & Chung, R.-J. 2014a, 'Comparative Study of Conversion of Coral with Ammonium Dihydrogen Phosphate and Orthophosphoric Acid to Produce Calcium Phosphates ', *Journal of the Australian Ceramics Society*, vol. 50, no. 2, p. 8.

Cegla, R.-N.R., Macha, I.J., Ben-Nissan, B., Grossin, D., Heness, G. & Chung, R.-J. 2014b, 'Comparative Study of Conversion of Coral with Ammonium Dihydrogen Phosphate and Orthophosphoric Acid to Produce Calcium Phosphates', *Journal of the Australian Ceramics Society*, vol. 50, no. 2, pp. 154-61.

Cerca, N., Gomes, F., Pereira, S., Teixeira, P. & Oliveira, R. 2012, 'Confocal laser scanning microscopy analysis of S. epidermidis biofilms exposed to farnesol, vancomycin and rifampicin', *BMC Res Notes*, vol. 5, p. 244.

Chai, C., Ben-Nissan, B., Pyke, S. & Evans, L. 1995, 'Sol-Gel Derived Hydroxylapatite Coatings for Biomedical Applications', *Materials and Manufacturing Processes*, vol. 10, no. 2, pp. 205-16.

Chen, C.-W., Riman, R.E., TenHuisen, K.S. & Brown, K. 2004, 'Mechanochemical–hydrothermal synthesis of hydroxyapatite from nonionic surfactant emulsion precursors', *Journal of Crystal Growth*, vol. 270, no. 3–4, pp. 615-23.

Chen, Q., Liang, S. & Thouas, G.A. 2013, 'Elastomeric biomaterials for tissue engineering', *Progress in Polymer Science*, vol. 38, no. 3–4, pp. 584-671.

Chen, Q., Roether, J.A. & Boccaccini, A.R. 2008, 'Tissue Engineering Scaffolds from Bioactive Glass and Composite Materials', in N. Ashammakhi, R. Reis & F. Chiellini (eds), *Topics in Tissue Engineering*, Oulu University, Oulu, Finland.

Chen, Q.Z. & Boccaccini, A.R. 2006, 'Poly(D,L-lactic acid) coated 45S5 Bioglass-based scaffolds: processing and characterization', *J Biomed Mater Res A*, vol. 77, no. 3, pp. 445-57.

Chen, Q.Z., Thompson, I.D. & Boccaccini, A.R. 2006, '45S5 Bioglass-derived glass-ceramic scaffolds for bone tissue engineering', *Biomaterials*, vol. 27, no. 11, pp. 2414-25.

Chen, Q.Z. & Thouas, G.A. 2011, 'Fabrication and characterization of sol-gel derived 45S5 Bioglass (R)-ceramic scaffolds', *Acta Biomaterialia*, vol. 7, no. 10, pp. 3616-26.

Chen, X., Li, S. & Huang, Z. 1995, '[The applications of machinable bioactive glass ceramics in maxillofacial augmentation]', *Zhonghua Zheng Xing Shao Shang Wai Ke Za Zhi*, vol. 11, no. 6, pp. 419-20.

Choi, A.H., Cazalbou, S. & Ben-Nissan, B. 2015, 'Nanobiomaterial Coatings in Dentistry', *Front Oral Biol*, vol. 17, pp. 49-61.

Chou, J., Austin, C., Doble, P., Ben-Nissan, B. & Milthorpe, B. 2014, 'Trace elemental imaging of coralline hydroxyapatite by laser-ablation inductively coupled plasma-mass spectroscopy', *J Tissue Eng Regen Med*, vol. 8, no. 7, pp. 515-20.

Chou, J., Ben-Nissan, B., Choi, A., Green, D. & Wuhrer, R. 2007, 'Conversion of coral sand to calcium phosphate for biomedical applications', *Journal of the Australian Ceramic Society* vol. 43, no. 1, p. 5.

Chou, J., Ben-Nissan, B., Green, D.W., Valenzuela, S.M. & Kohan, L. 2011, 'Targeting and Dissolution Characteristics of Bone Forming and Antibacterial Drugs by Harnessing the Structure of Microspherical Shells from Coral Beach Sand', *Advanced Engineering Materials*, vol. 13, no. 1-2, pp. 93-9.

Chou, J., Valenzuela, S., Green, D.W., Kohan, L., Milthorpe, B., Otsuka, M. & Ben-Nissan, B. 2014, 'Antibiotic delivery potential of nano- and micro-porous marine structure-derived beta-tricalcium phosphate spheres for medical applications', *Nanomedicine (Lond)*, vol. 9, no. 8, pp. 1131-9.

Choudhuri, A., Mohanty, P.S. & Karthikeyan, J. 2009, 'Bio-ceramic Composite Coatings by Cold Spray Technology ', *International Thermal Spray Conference*, ASM International, pp. 391-6.

Chu, G.C., Tomita, T., Sonnichsen, F.D., Yoshida, T. & Ikeda-Saito, M. 1999, 'The heme complex of Hmu O, a bacterial heme degradation enzyme from Corynebacterium diphtheriae. Structure of the catalytic site', *J Biol Chem*, vol. 274, no. 35, pp. 24490-6.

Chung, E.J., Kodali, P., Laskin, W., Koh, J.L. & Ameer, G.A. 2011, 'Long-term in vivo response to citric acid-based nanocomposites for orthopaedic tissue engineering', *J Mater Sci Mater Med*, vol. 22, no. 9, pp. 2131-8.

CLSI 2012, *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically* Clinical and Laboratory Standards Institute, Wayne, PA, USA.

Cook, S.D., Thomas, K.A., Kay, J.F. & Jarcho, M. 1988, 'Hydroxyapatite-coated titanium for orthopedic implant applications', *Clin Orthop Relat Res*, no. 232, pp. 225-43.

Costa, P. 2001, 'An alternative method to the evaluation of similarity factor in dissolution testing', *International Journal of Pharmaceutics*, vol. 220, no. 1–2, pp. 77-83.

Costa, P. & Sousa Lobo, J.M. 2001, 'Modeling and comparison of dissolution profiles', *Eur J Pharm Sci*, vol. 13, no. 2, pp. 123-33.

Costerton, J.W., Montanaro, L. & Arciola, C.R. 2005, 'Biofilm in implant infections: its production and regulation', *Int J Artif Organs*, vol. 28, no. 11, pp. 1062-8.

Covani, U., Giacomelli, L., Krajewski, A., Ravaglioli, A., Spotorno, L., Loria, P., Das, S. & Nicolinil, C. 2007, 'Biomaterials for orthopedics: A roughness analysis by atomic force microscopy', *Journal of Biomedical Materials Research Part A*, vol. 82A, no. 3, pp. 723-30.

Dash, S., Murthy, P.N., Nath, L. & Chowdhury, P. 2010, 'Kinetic modeling on drug release from controlled drug delivery systems', *Acta Pol Pharm*, vol. 67, no. 3, pp. 217-23.

Davis, J.R. 2003, 'Metallic Materials', in J.R. Davis (ed.), *Handbook of Materials for Medical Devices*, ASM International, US, pp. 21-50.

Day, R.M. 2005, 'Bioactive glass stimulates the secretion of angiogenic growth factors and angiogenesis in vitro', *Tissue Engineering*, vol. 11, no. 5-6, pp. 768-77.

Day, R.M., Maquet, V., Boccaccini, A.R., Jerome, R. & Forbes, A. 2005, 'In vitro and in vivo analysis of macroporous biodegradable poly(D,L-lactide-co-glycolide) scaffolds containing bioactive glass', *Journal of Biomedical Materials Research Part A*, vol. 75A, no. 4, pp. 778-87.

De Santis, P. & Kovacs, A.J. 1968, 'Molecular conformation of poly(S-lactic acid)', *Biopolymers*, vol. 6, no. 3, pp. 299-306.

de Tayrac, R., Oliva-Lauraire, M.C., Guiraud, I., Henry, L., Vert, M. & Mares, P. 2007, 'Long-lasting bioresorbable poly(lactic acid) (PLA(94)) mesh: A new approach for soft tissue reinforcement based on an experimental pilot study', *International Urogynecology Journal*, vol. 18, no. 9, pp. 1007-14.

Desrousseaux, C., Sautou, V., Descamps, S. & Traore, O. 2013, 'Modification of the surfaces of medical devices to prevent microbial adhesion and biofilm formation', *J Hosp Infect*, vol. 85, no. 2, pp. 87-93.

Desrousseaux, C., Sautou, V., Descamps, S. & Traoré, O. 2013, 'Modification of the surfaces of medical devices to prevent microbial adhesion and biofilm formation', *Journal of Hospital Infection*, vol. 85, no. 2, pp. 87-93.

Doi, Y., Kitamura, S. & Abe, H. 1995, 'Microbial Synthesis and Characterization of Poly(3-hydroxybutyrate-co-3-hydroxyhexanoate)', *Macromolecules*, vol. 28, no. 14, pp. 4822-8.

Dou, Y., Lin, K. & Chang, J. 2011, 'Polymer nanocomposites with controllable distribution and arrangement of inorganic nanocomponents', *Nanoscale*, vol. 3, no. 4, pp. 1508-11.

Drnovsek, N., Novak, S., Dragin, U., Ceh, M., Gorensek, M. & Gradisar, M. 2012, 'Bioactive glass enhances bone ingrowth into the porous titanium coating on orthopaedic implants', *International Orthopaedics*.

Drumright, R.E., Gruber, P.R. & Henton, D.E. 2000, 'Polylactic Acid Technology', *Advanced Materials* vol. 12, no. 23, pp. 1841–6.

Ducheyne, P. 1985, 'Bioglass coatings and bioglass composites as implant materials', *Journal of Biomedical Materials Research*, vol. 19, no. 3, pp. 273-91.

Dunn, A.S., Campbell, P.G. & Marra, K.G. 2001, 'The influence of polymer blend composition on the degradation of polymer/hydroxyapatite biomaterials', *Journal of Materials Science: Materials in Medicine*, vol. 12, no. 8, pp. 673-7.

Dvir, T., Benishti, N., Shachar, M. & Cohen, S. 2006, 'A novel perfusion bioreactor providing a homogenous milieu for tissue regeneration', *Tissue Eng*, vol. 12, no. 10, pp. 2843-52.

E, M.K., N, V.V., G, K.B., Carson, M.J., A, L.R. & J, G.A. 2003, ' Adsorption-induced conformational changes in fibronectin due to interactions with well-defined surface chemistries ', *Langmuir*, vol. 19, no. 19, pp. 8033-40.

Ebelmen, M. 1846, 'On the synthesis of silica gels from alkoxides ', *Annales de chimie et de physique* vol. 16, p. 129.

Elbert, D.L. & Hubbell, J.A. 1996, 'Surface Treatments of Polymers for Biocompatibility', *Annual Review of Materials Science*, vol. 26, no. 1, pp. 365-94.

Elliott, J.C. 1994, 'Structure and Chemistry of the Apatites and Other Calcium Orthophosphates', *Elsevier*.

Elmore, K.L. & Farr, T.D. 1940, 'Equilibrium in the System Calcium Oxide–Phosphorus Pentoxide–Water', *Industrial & Engineering Chemistry*, vol. 32, no. 4, pp. 580-6.

Erdemli, O., Captug, O., Bilgili, H., Orhan, D., Tezcaner, A. & Keskin, D. 2010, 'In vitro and in vivo evaluation of the effects of demineralized bone matrix or calcium sulfate addition to polycaprolactone-bioglass composites', *J Mater Sci Mater Med*, vol. 21, no. 1, pp. 295-308.

Fambri, L., Pegoretti, A., Fenner, R., Incardona, S.D. & Migliaresi, C. 1997, 'Biodegradable fibres of poly(l-lactic acid) produced by melt spinning', *Polymer*, vol. 38, no. 1, pp. 79-85.

FDA 2013, '888-INFO-FDA, New medical device treats urinary symptoms related to enlarged prostate', in M. Liscinsky (ed.).

Ford Versypt, A.N., Pack, D.W. & Braatz, R.D. 2013, 'Mathematical modeling of drug delivery from autocatalytically degradable PLGA microspheres--a review', *J Control Release*, vol. 165, no. 1, pp. 29-37.

Francolini, I. & Donell, G. 2010, 'Prevention and control of biofilm-based medical-device-related infections', *FEMS Immunology & Medical Microbiology*, vol. 59, no. 3, p. 12.

Fredenberg, S., Reslow, M. & Axelsson, A. 2005, 'Measurement of Protein Diffusion Through Poly(d,l-Lactide-Co-Glycolide)', *Pharmaceutical Development and Technology*, vol. 10, no. 2, pp. 299-307.

Gao, P., Nie, X., Zou, M., Shi, Y. & Cheng, G. 2011, 'Recent advances in materials for extended-release antibiotic delivery system', *J Antibiot (Tokyo)*, vol. 64, no. 9, pp. 625-34.

Gao, Y. & Chang, J. 2009, 'Surface modification of bioactive glasses and preparation of PDLLA/bioactive glass composite films', *J Biomater Appl*, vol. 24, no. 2, pp. 119-38.

Garlotta, D. 2001, 'A Literature Review of Poly(Lactic Acid)', *Journal of Polymers and the Environment*, vol. 9, no. 2, pp. 63-84.

Garrett, T.R., Bhakoo, M. & Zhang, Z. 2008, 'Bacterial adhesion and biofilms on surfaces', *Progress in Natural Science*, vol. 18, no. 9, pp. 1049-56.

Gatti, A.M., Simonetti, L.A., Monari, E., Guidi, S. & Greenspan, D. 2006, 'Bone augmentation with bioactive glass in three cases of dental implant placement', *Journal of Biomaterials Applications*, vol. 20, no. 4, pp. 325-39.

Gatti, A.M., Valdre, G. & Andersson, O.H. 1994, 'Analysis of the in vivo reactions of a bioactive glass in soft and hard tissue', *Biomaterials*, vol. 15, no. 3, pp. 208-12.

Gerhardt, L.-C. & Boccaccini, A.R. 2010, 'Bioactive Glass and Glass-Ceramic Scaffolds for Bone Tissue Engineering', *Materials*, vol. 3, no. 7, pp. 3867-910.

Gillette, R.L., Swaim, S.F., Sartin, E.A., Bradley, D.M. & Coolman, S.L. 2001, 'Effects of a bioactive glass on healing of closed skin wounds in dogs', *Am J Vet Res*, vol. 62, no. 7, pp. 1149-53.

Ginebra, M.-P., Canal, C., Espanol, M., Pastorino, D. & Montufar, E.B. 2012, 'Calcium phosphate cements as drug delivery materials', *Advanced Drug Delivery Reviews*, vol. 64, no. 12, pp. 1090-110.

Ginebra MP, T.T., Planell JA. 2006, 'Calcium phosphate cements as bone drug delivery systems: A review', *J Control Release.* , vol. 113, no. 2, pp. 102-10.

Gomes, M.E. & Reis, R.L. 2004, 'Biodegradable Polymers and Composites in Biomedical Applications: From Catgut to Tissue Engineering: Part 1 - Available Systems and Their Properties', *International Materials Reviews*, vol. 49, no. 5, p. 13.

Gomes, M.E., Reis, R.L., Cunha, A.M., Blitterswijk, C.A. & de Bruijn, J.D. 2001, 'Cytocompatibility and response of osteoblastic-like cells to starch-based polymers: effect of several additives and processing conditions', *Biomaterials*, vol. 22, no. 13, pp. 1911-7.

Gonzalez, P., Serra, J., Liste, S., Chiussi, S., Leon, B., Perez-Amor, M., Martinez-Fernandez, J., de Arellano-Lopez, A.R. & Varela-Feria, F.M. 2003, 'New biomorphic SiC ceramics coated with bioactive glass for biomedical applications', *Biomaterials*, vol. 24, no. 26, pp. 4827-32.

Graham, T. 1864, 'XXXV.-On the properties of silicic acid and other analogous colloidal substances', *Journal of the Chemical Society*, vol. 17, pp. 318-27.

Green, D.W. & Ben-Nissan, B. 2010, 'Biomimetic Applications in Regenerative Medicine: Scaffolds, Transplantation Modules, Tissue Homing Device and

Stem Cells', in V. Tochilin & M. Amiji (eds), *Handbook of Materials for Nanomedicine*, Pan Stanford Publishing Pte Ltd, Singapore, pp. 821-50.

Green, D.W., Li, G., Milthorpe, B. & Ben-Nissan, B. 2012, 'Adult stem cell coatings for regenerative medicine', *Materials Today*, vol. 15, no. 1–2, pp. 60-6.

Greenhalgh, E.S. 2009, *Failure analysis and fractographiy of polymer composites*, Woodhead Publishing Limited, Cambridge, UK.

Greenspan, D.C. & Hench, L.L. 1976, 'Chemical and mechanical behavior of bioglass-coated alumina', *J Biomed Mater Res*, vol. 10, no. 4, pp. 503-9.

Greenspan, D.C., Zhong, J.P., Chen, X.F. & LaTorre, G.P. 1997, 'The evaluation of degradability of melt and sol-gel derived Bioglass (R) in-vitro', *Bioceramics, Vol 10*, pp. 391-4.

Griffith, L.G. 2000, 'Polymeric biomaterials', *Acta Materialia*, vol. 48, no. 1, pp. 263-77.

Grijpma, D.W., Nijenhuis, A.J., Wijk, P.G.T. & Pennings, A.J. 1992, 'High impact strength as-polymerized PLLA', *Polymer Bulletin*, vol. 29, no. 5, pp. 571-8.

Gross, U., Kinne, R., Schmitz, H.J. & Strunz, V. 1988, 'The response of bone to surface active glasdglassceramics', *CRC Crit. Rev. Biocomput.*, vol. 4, pp. 2-15.

Gruber, P.R., Hall, E.S., Kolstad, J.J., Iwen, M.L., Benson, R.D. & Borchardt, R.L. 2001, *Continuous process for the manufacture of lactide and lactide*

polymers, 6,326,458.

Gu, J.H., Huang, Y.J., Beekman, A. & Goldenberg, M. 2010, 'Simplified preparation of low polydispersity, low molecular weight poly(D,L-lactic acid) by liquid/liquid phase separation for drug delivery systems with discussion of molecular weight characterization', *Polymer International*, vol. 59, no. 12, pp. 1571-7.

Guo, W., Shi, Z., Guo, R. & Sun, R. 2007, '[Preparation of gentamicin sulfate-polyanhydride sustained-release beads and in vitro bacteriostatic activity studies]', *Sheng Wu Yi Xue Gong Cheng Xue Za Zhi*, vol. 24, no. 2, pp. 360-2, 84.

Gupta, A.P. & Kumar, V. 2007, 'New emerging trends in synthetic biodegradable polymers – Polylactide: A critique', *European Polymer Journal*, vol. 43, no. 10, pp. 4053-74.

Gustavsson, J., Ginebra, M.P., Engel, E. & Planell, J. 2011, 'Ion reactivity of calcium-deficient hydroxyapatite in standard cell culture media', *Acta Biomaterialia*, vol. 7, no. 12, pp. 4242-52.

Ha, C.S. & Gardella, J.A., Jr. 2005, 'Surface chemistry of biodegradable polymers for drug delivery systems', *Chem Rev*, vol. 105, no. 11, pp. 4205-32.

Habibe, A.F., Maeda, L.D., Souza, R.C., Barboza, M.J.R., Daguano, J.K.M.F., Rogero, S.O. & Santos, C. 2009, 'Effect of bioglass additions on the sintering of Y-TZP bioceramics', *Materials Science and Engineering: C*, vol. 29, no. 6, pp. 1959-64.

Habraken, W.J.E.M., Wolke, J.G.C. & Jansen, J.A. 2007, 'Ceramic composites as matrices and scaffolds for drug delivery in tissue engineering', *Advanced Drug Delivery Reviews*, vol. 59, no. 4–5, pp. 234-48.

Hajiali, H., Karbasi, S., Hosseinalipour, M. & Rezaie, H.R. 2010, 'Preparation of a novel biodegradable nanocomposite scaffold based on poly (3-hydroxybutyrate)/bioglass nanoparticles for bone tissue engineering', *J Mater Sci Mater Med*, vol. 21, no. 7, pp. 2125-32.

Heidemann, W., Jeschkeit, S., Ruffieux, K., Fischer, J.H., Wagner, M., Krüger, G., Wintermantel, E. & Gerlach, K.L. 2001, 'Degradation of poly(d,l)lactide implants with or without addition of calciumphosphates in vivo', *Biomaterials*, vol. 22, no. 17, pp. 2371-81.

Hench, L.L. 1980, 'Biomaterials', *Science*, vol. 208, no. 4446, pp. 826-31.

Hench, L.L. 1991, 'Bioceramics: From Concept to Clinic', *Journal of the American Ceramic Society*, vol. 74, no. 7, pp. 1487-510.

Hench, L.L. 1998, 'Biomaterials: a forecast for the future', *Biomaterials*, vol. 19, no. 16, pp. 1419-23.

Hench, L.L. 2006, 'The story of Bioglass', *J Mater Sci Mater Med*, vol. 17, no. 11, pp. 967-78.

Hench, L.L. & Andersson, A. 1993, 'Bioactive Glasses', in L.L. Hench & J. Wilson (eds), *An Introduction of Bioceramics*, vol. 1, World Scientific Publishing Co. Pty. Ltd, Singapore, pp. 41-62.

Hench, L.L., Boccaccini, A.R., Day, R.M. & Gabe, S.M. 2003, 'Third-generation gene-activating biomaterials', *Thermec'2003, Pts 1-5*, vol. 426-4, pp. 179-84.

Hench, L.L. & Polak, J.M. 2002, 'Third-generation biomedical materials', *Science*, vol. 295, no. 5557, pp. 1014-+.

Hench, L.L., Polak, J.M., Xynos, I.D. & Buttery, L.D.K. 2000, 'Bioactive materials to control cell cycle', *Materials Research Innovations*, vol. 3, no. 6, pp. 313-23.

Hench, L.L., Splinter, R.J., Allen, W.C. & Greenlee, T.K. 1972, 'Bonding mechanisms at the interface of ceramic prosthetic materials', *J. Biomed. Muter. Res*, vol. 2.

Hench, L.L., Splinter, R.J., Allen, W.C. & T. K. Greenlee, J. 1971, 'Bonding Mechanisms at the Interface of Ceramic Prosthetic Materials', *J. Bi-omed. Maters. Res.*, vol. 2, no. 1, pp. 117-41.

Hench, L.L. & Thompson, I. 2010, 'Twenty-first century challenges for biomaterials', *Journal of the Royal Society Interface*, vol. 7, pp. S379-S91.

Hench, L.L. & West, J.K. 1990, 'The sol-gel process', *Chemical Reviews*, vol. 90, no. 1, pp. 33-72.

Hench, L.L. & Wilson, J. 1984, 'Surface-Active Biomaterials', *Science*, vol. 226, no. 4675, pp. 630-6.

Heness, G. & Ben-Nissan, B. 2004, 'Innovative Bioceramics', *Materials Forum* vol. 27, pp. 104-14.

Heydorn, A., Nielsen, A.T., Hentzer, M., Sternberg, C., Givskov, M., Ersboll, B.K. & Molin, S. 2000, 'Quantification of biofilm structures by the novel computer program COMSTAT', *Microbiology*, vol. 146 (Pt 10), pp. 2395-407.

Hill, R.G., Stamboulis, A., Law, R.V., Clifford, A., Towler, M.R. & Crowley, C. 2004, 'The influence of strontium substitution in fluorapatite glasses and glass-ceramics', *Journal of Non-Crystalline Solids*, vol. 336, no. 3, pp. 223-9.

Hoiby, N., Bjarnsholt, T., Givskov, M., Molin, S. & Ciofu, O. 2010, 'Antibiotic resistance of bacterial biofilms', *Int J Antimicrob Agents*, vol. 35, no. 4, pp. 322-32.

Hong, Z., Reis, R.L. & Mano, J.F. 2008, 'Preparation and in vitro characterization of scaffolds of poly(L-lactic acid) containing bioactive glass ceramic nanoparticles', *Acta Biomater*, vol. 4, no. 5, pp. 1297-306.

Hong, Z., Reis, R.L. & Mano, J.F. 2009a, 'Preparation and in vitro characterization of novel bioactive glass ceramic nanoparticles', *J Biomed Mater Res A*, vol. 88, no. 2, pp. 304-13.

Hong, Z., Reis, R.L. & Mano, J.F. 2009b, 'Preparation and in vitro characterization of novel bioactive glass ceramic nanoparticles', *Journal of Biomedical Materials Research Part A*, vol. 88A, no. 2, pp. 304-13.

Howard, D., Partridge, K., Yang, X., Clarke, N.M.P., Okubo, Y., Bessho, K., Howdle, S.M., Shakesheff, K.M. & Oreffo, R.O.C. 2002, 'Immunoselection and adenoviral genetic modulation of human osteoprogenitors: in vivo bone formation on PLA scaffold', *Biochemical and Biophysical Research Communications*, vol. 299, no. 2, pp. 208-15.

Hryniewicz, T., Rokicki, R. & Rokosz, K. 2008, 'Corrosion Characteristics of Medical-Grade AISI Type 316L Stainless Steel Surface After Electropolishing in a Magnetic Field', *Corrosion*, vol. 64, no. 8, pp. 660-5.

Huadong, S., Xiaohong**, L. & Zhijun, Z. 2008, 'Surface Modified Nano-Silica and Its Action on Polymer', *Progress in Chemistry*, vol. 20, no. 10, pp. 1509-14.

Ilharreborde, B., Morel, E., Fitoussi, F., Presedo, A., Souchet, P., Pennecot, G.F. & Mazda, K. 2008, 'Bioactive Glass as a Bone Substitute for Spinal Fusion in Adolescent Idiopathic Scoliosis A Comparative Study With Iliac Crest Autograft', *Journal of Pediatric Orthopaedics*, vol. 28, no. 3, pp. 347-51.

Imola, M.J. & Schramm, V.L. 2002, 'Resorbable internal fixation in pediatric cranial base surgery', *Laryngoscope*, vol. 112, no. 10, pp. 1897-901.

Ingraham, F.D., Alexander, E., Jr. & Matson, D.D. 1947, 'Polyethylene, a new synthetic plastic for use in surgery; experimental applications in neurosurgery', *J Am Med Assoc*, vol. 135, no. 2, pp. 82-7.

Jacobsen, S. & Fritz, H.G. 1999, 'Plasticizing polylactide—the effect of different plasticizers on the mechanical properties', *Polymer Engineering & Science*, vol. 39, no. 7, pp. 1303-10.

Jahnen-Dechent, W. & Ketteler, M. 2012, 'Magnesium basics', *Clinical Kidney Journal*, vol. 5, no. Suppl 1, pp. i3-i14.

Jarcho, M. 1981, 'Calcium phosphate ceramics as hard tissue prosthetics', *Clinical orthopaedics and related research*, vol. 157, pp. 259-78.

Jie, Q., Lin, K.L., Zhong, J.P., Shi, Y.F., Li, Q., Chang, J.A. & Wang, R.D. 2004, 'Preparation of macroporous sol-gel bioglass using PVA particles as pore former', *Journal of Sol-Gel Science and Technology*, vol. 30, no. 1, pp. 49-61.

Kai, D. & Loh, X.J. 2013, 'Polyhydroxyalkanoates: Chemical Modifications Toward Biomedical Applications', *ACS Sustainable Chemistry & Engineering*, vol. 2, no. 2, pp. 106-19.

Karagiozov, C. & Momchilova, D. 2005, 'Synthesis of nano-sized particles from metal carbonates by the method of reversed mycelles', *Chemical Engineering and Processing: Process Intensification*, vol. 44, no. 1, pp. 115-9.

Kargupta, R., Bok, S., Darr, C.M., Crist, B.D., Gangopadhyay, K., Gangopadhyay, S. & Sengupta, S. 2014, 'Coatings and surface modifications imparting antimicrobial activity to orthopedic implants', *Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology*, vol. 6, no. 5, pp. 475-95.

Kel, D., Gökçe, H., Bilgiç, D., Ağaoğulları, D., Duman, I., Öveçoğlu, M.L., Kayalı, E.S., AKıyıcı, I., Agathopoulos, S. & Oktar, F.N. 2012, 'Production of Natural HA from Land Snails', *Key Eng. Materials* vol. 493, pp. 287-92.

Keledi, G., Hari, J. & Pukanszky, B. 2012, 'Polymer nanocomposites: structure, interaction, and functionality', *Nanoscale*, vol. 4, no. 6, pp. 1919-38.

Kenane, M. & Benzeggagh, M.L. 2009, 'Characterization of Mixed Mode Delamination Growth and Thresholds', in T. Boukharouba, M. Elboujdaini & G. Pluvinage (eds), *Damage and Fracture Mechanics*, Springer Netherlands, pp. 523-30.

Keselowsky, B.G., Collard, D.M. & Garcia, A.J. 2003, 'Surface chemistry modulates fibronectin conformation and directs integrin binding and specificity to control cell adhesion', *J Biomed Mater Res A*, vol. 66, no. 2, pp. 247-59.

Kickelbick, G. 2003, 'Concepts for the incorporation of inorganic building blocks into organic polymers on a nanoscale', *Progress in Polymer Science*, vol. 28, no. 1, pp. 83-114.

Kim, D., Lee, J.S., Barry, C.M.F. & Mead, J.L. 2007, 'Effect of fill factor and validation of characterizing the degree of mixing in polymer nanocomposites', *Polymer Engineering & Science*, vol. 47, no. 12, pp. 2049-56.

Kim, H.W., Lee, H.H. & Chun, G.S. 2008, 'Bioactivity and osteoblast responses of novel biomedical nanocomposites of bioactive glass nanofiber filled poly(lactic acid)', *J Biomed Mater Res A*, vol. 85, no. 3, pp. 651-63.

Kim, H.W., Song, J.H. & Kim, H.E. 2006, 'Bioactive glass nanofiber-collagen nanocomposite as a novel bone regeneration matrix', *J Biomed Mater Res A*, vol. 79, no. 3, pp. 698-705.

Kimura, H., Ogura, Y., Moritera, T., Honda, Y., Wada, R., Hyon, S.H. & Ikada, Y. 1992, 'Injectable microspheres with controlled drug release for glaucoma filtering surgery', *Invest Ophthalmol Vis Sci*, vol. 33, no. 12, pp. 3436-41.

Kivrak, N. & Taş, A.C. 1998, 'Synthesis of Calcium Hydroxyapatite-Tricalcium Phosphate (HA-TCP) Composite Bioceramic Powders and Their Sintering Behavior', *Journal of the American Ceramic Society*, vol. 81, no. 9, pp. 2245-52.

Klevens, R.M., Edwards, J.R., Richards, C.L., Jr., Horan, T.C., Gaynes, R.P., Pollock, D.A. & Cardo, D.M. 2007, 'Estimating health care-associated infections and deaths in U.S. hospitals, 2002', *Public Health Rep*, vol. 122, no. 2, pp. 160-6.

Kokubo, T. & Takadama, H. 2006, 'How useful is SBF in predicting in vivo bone bioactivity?', *Biomaterials*, vol. 27, no. 15, pp. 2907-15.

Kontonasaki, E., Papadopoulou, L., Zorba, T., Pavlidou, E., Paraskevopoulos, K. & Koidis, P. 2003, 'Apatite formation on dental ceramics modified by a bioactive glass', *J Oral Rehabil*, vol. 30, no. 9, pp. 893-902.

Kooten, T.G.V., Klein, C.L., Kohler, H., Kirkpatrick, C.J., Williams, D.F. & Eloy, R. 1997, 'From cytotoxicity to biocompatibility testing in vitro: cell adhesion molecule expression defines a new set of parameters', *Journal of Materials Science: Materials in Medicine*, vol. 8, no. 12, pp. 835-41.

Kreth, J., Hagerman, E., Tam, K., Merritt, J., Wong, D.T., Wu, B.M., Myung, N.V., Shi, W. & Qi, F. 2004, 'Quantitative analyses of Streptococcus mutans biofilms with quartz crystal microbalance, microjet impingement and confocal microscopy', *Biofilms*, vol. 1, no. 4, pp. 277-84.

Kuhne, J.H., Bartl, R., Frisch, B., Hammer, C., Jansson, V. & Zimmer, M. 1994, 'Bone formation in coralline hydroxyapatite. Effects of pore size studied in rabbits', *Acta Orthop Scand*, vol. 65, no. 3, pp. 246-52.

Kundu, B., Lemos, A., Soundrapandian, C., Sen, P.S., Datta, S., Ferreira, J.M.F. & Basu, D. 2010, 'Development of porous HAp and beta-TCP scaffolds by starch consolidation with foaming method and drug-chitosan bilayered scaffold

based drug delivery system', *Journal of Materials Science-Materials in Medicine*, vol. 21, no. 11, pp. 2955-69.

Kuroda, D., Hiromoto, S., Hanawa, T. & Takada, Y. 2002, 'Corrosion Behaviour of Nickel-Free Nitrogen Austenitic Stainless Steel in Simulated Biological Environments', *Materials Transsactions*, vol. 43, no. 12, p. 5.

Lasprilla, A.J.R., Martinez, G.A.R., Lunelli, B.H., Jardini, A.L. & Filho, R.M. 2012, 'Poly-lactic acid synthesis for application in biomedical devices — A review', *Biotechnology Advances*, vol. 30, no. 1, pp. 321-8.

LaVan, D.A., McGuire, T. & Langer, R. 2003, 'Small-scale systems for in vivo drug delivery', *Nat Biotech*, vol. 21, no. 10, pp. 1184-91.

Lee, H.H., Sang Shin, U., Lee, J.H. & Kim, H.W. 2011, 'Biomedical nanocomposites of poly(lactic acid) and calcium phosphate hybridized with modified carbon nanotubes for hard tissue implants', *J Biomed Mater Res B Appl Biomater*, vol. 98B, no. 2, pp. 246-54.

Lee, H.H., Yu, H.S., Jang, J.H. & Kim, H.W. 2008, 'Bioactivity improvement of poly(epsilon-caprolactone) membrane with the addition of nanofibrous bioactive glass', *Acta Biomater*, vol. 4, no. 3, pp. 622-9.

Lee, S. 1997, 'Mode II delamination failure mechanisms of polymer matrix composites', *Journal of Materials Science*, vol. 32, no. 5, pp. 1287-95.

LeGeros, R.Z. 1988, 'Calcium phosphate materials in restorative dentistry: a review', *Adv Dent Res*, vol. 2, no. 1, pp. 164-80.

LeGeros, R.Z. 2002, 'Properties of osteoconductive biomaterials: calcium phosphates', *Clin Orthop Relat Res*, no. 395, pp. 81-98.

LeGeros, R.Z. 2008a, 'Calcium phosphate-based osteoinductive materials', *Chem Rev*, vol. 108, no. 11, pp. 4742-53.

LeGeros, R.Z. 2008b, 'Calcium Phosphate-Based Osteoinductive Materials', *Chemical Reviews*, vol. 108, no. 11, pp. 4742-53.

LeGeros, R.Z., LeGeros, J.P., Daculsi, G. & Kijkowaka, R. 1995, *Encyclopedic Handbook of Biomaterials and Bioengineering*, vol. 2, Marcel Dekker, New York.

Lei, B., Shin, K.H., Noh, D.Y., Koh, Y.H., Choi, W.Y. & Kim, H.E. 2012, 'Bioactive glass microspheres as reinforcement for improving the mechanical properties and biological performance of poly(e-caprolactone) polymer for bone tissue

regeneration', *Journal of Biomedical Materials Research Part B-Applied Biomaterials*, vol. 100B, no. 4, pp. 967-75.

Lemons, J.E. 1996, 'Ceramics: past, present, and future', *Bone*, vol. 19, no. 1 Suppl, pp. 121s-8s.

Li, G., Feng, S. & Zhou, D. 2011, 'Magnetic bioactive glass ceramic in the system CaO-P2O5-SiO2-MgO-CaF2-MnO2-Fe2O3 for hyperthermia treatment of bone tumor', *J Mater Sci Mater Med*, vol. 22, no. 10, pp. 2197-206.

Li, H., Chen, S., Wu, Y., Jiang, J., Ge, Y., Gao, K., Zhang, P. & Wu, L. 2012, 'Enhancement of the osseointegration of a polyethylene terephthalate artificial ligament graft in a bone tunnel using 58S bioglass', *International Orthopaedics*, vol. 36, no. 1, pp. 191-7.

Li, R., Clark, A.E. & Hench, L.L. 1991, 'An investigation of bioactive glass powders by sol-gel processing', *Journal of Applied Biomaterials*, vol. 2, no. 4, pp. 231-9.

Li, S., Ma, W., Zhou, Y., Chen, X., Ma, M., Xu, Y., Ding, Z. & Wu, X. 2013, '3-aminopropyltriethoxysilanes Modified Porous Silicon as a Voltammetric Sensor for Determination of Silver Ion', *International Journal of Electrochemical Science*, vol. 8, no. 2, p. 11.

Li, Y., Liu, Y.Z., Long, T., Yu, X.B., Tang, T.T., Dai, K.R., Tian, B., Guo, Y.P. & Zhu, Z.A. 2013, 'Mesoporous bioactive glass as a drug delivery system: fabrication, bactericidal properties and biocompatibility', *J Mater Sci Mater Med*, vol. 24, no. 8, pp. 1951-61.

Li, Y., Yang, C., Zhao, H., Qu, S., Li, X. & Li, Y. 2014, 'New Developments of Ti-Based Alloys for Biomedical Applications', *Materials*, vol. 7, no. 3, pp. 1709-800.

Liechty, W.B., Kryscio, D.R., Slaughter, B.V. & Peppas, N.A. 2010a, 'Polymers for drug delivery systems', *Annu Rev Chem Biomol Eng*, vol. 1, pp. 149-73.

Liechty, W.B., Kryscio, D.R., Slaughter, B.V. & Peppas, N.A. 2010b, 'Polymers for Drug Delivery Systems', *Annual Review of Chemical and Biomolecular Engineering*, vol. 1, no. 1, pp. 149-73.

Liesegang, R.E. 1896, 'Ueber einige Eigenschaften von Gallerten', *Naturwissenschaftliche Wochenschrift,*, vol. 11, no. 30, pp. 353 - 62.

Lim, G.K., Wang, J., Ng, S.C. & Gan, L.M. 1996, 'Processing of fine hydroxyapatite powders via an inverse microemulsion route', *Materials Letters*, vol. 28, no. 4–6, pp. 431-6.

Lim, G.K., Wang, J., Ng, S.C. & Gan, L.M. 1999, 'Formation of Nanocrystalline Hydroxyapatite in Nonionic Surfactant Emulsions', *Langmuir*, vol. 15, no. 22, pp. 7472-7.

Lin, C., Mao, C., Zhang, J., Li, Y. & Chen, X. 2012, 'Healing effect of bioactive glass ointment on full-thickness skin wounds', *Biomedical Materials*, vol. 7, no. 4, p. 045017.

Lin, F.-H., Liao, C.-J., Chen, K.-S. & Sun, J.-S. 1998, 'Preparation of high-temperature stabilized β-tricalcium phosphate by heating deficient hydroxyapatite with Na4P2O7 · 10H2O addition', *Biomaterials*, vol. 19, no. 11–12, pp. 1101-7.

Lin, F.-H., Liao, C.-J., Chen, K.-S., Sun, J.-S. & Lin, C.-P. 2001, 'Petal-like apatite formed on the surface of tricalcium phosphate ceramic after soaking in distilled water', *Biomaterials*, vol. 22, no. 22, pp. 2981-92.

Lindfors, N.C. 2009, 'Treatment of a recurrent aneurysmal bone cyst with bioactive glass in a child allows for good bone remodelling and growth', *Bone*, vol. 45, no. 2, pp. 398-400.

Lindfors, N.C., Hyvonen, P., Nyyssonen, M., Kirjavainen, M., Kankare, J., Gullichsen, E. & Salo, J. 2010, 'Bioactive glass S53P4 as bone graft substitute in treatment of osteomyelitis', *Bone*, vol. 47, no. 2, pp. 212-8.

Liu, A., Hong, Z., Zhuang, X., Chen, X., Cui, Y., Liu, Y. & Jing, X. 2008, 'Surface modification of bioactive glass nanoparticles and the mechanical and biological properties of poly(L-lactide) composites', *Acta Biomater*, vol. 4, no. 4, pp. 1005-15.

Liu, H. & Webster, T.J. 2010a, 'Ceramic/polymer nanocomposites with tunable drug delivery capability at specific disease sites', *Journal of Biomedical Materials Research Part A*, vol. 93A, no. 3, pp. 1180-92.

Liu, H.N. & Webster, T.J. 2010b, 'Ceramic/polymer nanocomposites with tunable drug delivery capability at specific disease sites', *Journal of Biomedical Materials Research Part A*, vol. 93A, no. 3, pp. 1180-92.

Liu, P., Luo, Q., Guan, Y. & Zhang, Y. 2010, 'Drug release kinetics from monolayer films of glucose-sensitive microgel', *Polymer*, vol. 51, no. 12, pp. 2668-75.

Liu, X., Xie, Z.P., Zhang, C.Q., Pan, H.B., Rahaman, M.N., Zhang, X., Fu, Q. & Huang, W.H. 2010, 'Bioactive borate glass scaffolds: in vitro and in vivo evaluation

for use as a drug delivery system in the treatment of bone infection', *Journal of Materials Science-Materials in Medicine*, vol. 21, no. 2, pp. 575-82.

Lou, X., Detrembleur, C. & Jérôme, R. 2003, 'Novel Aliphatic Polyesters Based on Functional Cyclic (Di)Esters', *Macromolecular Rapid Communications*, vol. 24, no. 2, pp. 161-72.

Lovell-Badge, R. 2007, 'Many ways to pluripotency', *Nat Biotech*, vol. 25, no. 10, pp. 1114-6.

Ma, J., Chen, C., Wang, D., Meng, X. & Shi, J. 2010, 'In vitro degradability and bioactivity of mesoporous CaO-MgO-P₂O₅-SiO₂ glasses synthesized by sol–gel method', *Journal of Sol-Gel Science and Technology*, vol. 54, no. 1, pp. 69-76.

Macha, I.J., Ben-Nissan, B. & Milthorpe, B. 2014, 'Improvement of Elongation in Nanosurface Modified Bioglass/PLA Thin Film Composites', *Current Nanoscience*, vol. 10, no. 2, pp. 200-4.

Macha, I.J., Boonyang, U., Cazalbou, S., Ben-Nissan, B., Charvillat, C., Oktar, F.N. & Grossin, D. 2015a, 'Comparative study of Coral Conversion, Part 2: Microstructural evolution of calcium phosphate', *Journal of the Australian Ceramic Society* vol. 52, no. 2.

Macha, I.J., Boonyang, U., Cazalbou, S., Ben-Nissan, B., Charvillat, C., Oktar, F.N. & Grossin, D. 2015b, 'Comparative study of Coral Conversion, Part 2: Microstructural evolution of calcium phosphate', *Journal of the Australian Ceramic Society* vol. 51, no. 2, pp. 149-59.

Macha, I.J., Cazalbou, S., Ben-Nissan, B., Harvey, K.L. & Milthorpe, B. 2015, 'Marine structure derived calcium phosphate-polymer biocomposites for local antibiotic delivery', *Mar Drugs*, vol. 13, no. 1, pp. 666-80.

Macha, I.J., Cazalbou, S., Shimmon, R., Ben-Nissan, B. & Milthorpe, B. 2015a, 'Development and dissolution studies of Bisphosphonate (Clodronate) containing hydroxyapatite/PLA biocomposites for slow drug delivery ', *Journal of Tissue Engineering and Regenerative Medicine*.

Macha, I.J., Cazalbou, S., Shimmon, R., Ben-Nissan, B. & Milthorpe, B. 2015b, 'Development and dissolution studies of bisphosphonate (clodronate)-containing hydroxyapatite–polylactic acid biocomposites for slow drug

delivery', *Journal of Tissue Engineering and Regenerative Medicine*, pp. n/a-n/a.

Macha, I.J., Ozyegin, L.S., Chou, J., Samur, R., Oktar, F.N. & Ben-Nissan, B. 2013, 'An Alternative Synthesis Method for Di Calcium Phosphate (Monetite) Powders from Mediterranean Mussel (Mytilus galloprovincialis) Shells', *Journal of The Australian Ceramic Society*, vol. 49, no. 2, pp. 122-8.

Macha, I.J., Ozyegin, L.S., Oktar, F.N. & Ben-Nissan, B. 2015, 'Conversion of Ostrich Eggshells (Struthio camelus) to Calcium Phosphates', *Journal of The Australian Ceramic Society* vol. 51, no. 2, pp. 125-33.

Mah, T.-F., Pitts, B., Pellock, B., Walker, G.C., Stewart, P.S. & O'Toole, G.A. 2003, 'A genetic basis for Pseudomonas aeruginosa biofilm antibiotic resistance', *Nature*, vol. 426, no. 6964, pp. 306-10.

Marshall, B.C. & Hazl, L. 2011, *Cystic Fibrosis Patient Registry Annual Data Report*, Cystic Fibrosis Foundation, Bethesda, Maryland USA.

Mauger, J.W., Chilko, D. & Howard, S. 1986, 'On the Analysis of Dissolution Data', *Drug Development and Industrial Pharmacy*, vol. 12, no. 7, pp. 969-92.

McCutcheon, A.L., Kannangara, G.S.K., Wilson, M.A. & Ben-Nissan, B. 2004, 'Preliminary analysis of pore distributions using NMR in natural coral and hydrothermally prepared hydroxyapatite', *Journal of Materials Science*, vol. 39, no. 18, pp. 5711-7.

McGee, D.C. & Gould, M.K. 2003, 'Preventing Complications of Central Venous Catheterization', *New England Journal of Medicine*, vol. 348, no. 12, pp. 1123-33.

Meejoo, S., Maneeprakorn, W. & Winotai, P. 2006, 'Phase and thermal stability of nanocrystalline hydroxyapatite prepared via microwave heating', *Thermochimica Acta*, vol. 447, no. 1, pp. 115-20.

Mehdikhani-Nahrkhalaji, M., Fathi, M.H., Mortazavi, V., Mousavi, S.B., Hashemi-Beni, B. & Razavi, S.M. 2012, 'Novel nanocomposite coating for dental implant applications in vitro and in vivo evaluation', *J Mater Sci Mater Med*, vol. 23, no. 2, pp. 485-95.

Merrer, J., De Jonghe, B., Golliot, F. & et al. 2001, 'Complications of femoral and subclavian venous catheterization in critically ill patients: A randomized controlled trial', *JAMA*, vol. 286, no. 6, pp. 700-7.

Merwin, G.E. 1990, 'Review of Bioactive Materials for Otologic and Maxillofacial Applications', in T. Yamamuro, L.L. Hench & J.Wilson (eds), *Handbook of Bioactive Ceramics*, vol. 1, CRC Press, Boca Raton, Florida, p. 323.

Middleton, J.C. & Tipton, A.J. 2000, 'Synthetic biodegradable polymers as orthopedic devices', *Biomaterials*, vol. 21, no. 23, pp. 2335-46.

Milev, A., Kannangara, G.S.K. & Ben-Nissan, B. 2003, 'Morphological Stability of Plate-Like Hydroxyapatite', *Key Engineering Materials*, vol. 240-242, pp. 481-4.

Mohammadi, G., Barzegar-Jalali, M., Valizadeh, H., Nazemiyeh, H., Barzegar-Jalali, A., Siahi Shadbad, M.R., Adibkia, K. & Zare, M. 2010, 'Reciprocal powered time model for release kinetic analysis of ibuprofen solid dispersions in oleaster powder, microcrystalline cellulose and crospovidone', *J Pharm Pharm Sci*, vol. 13, no. 2, pp. 152-61.

Mohn, D., Zehnder, M., Imfeld, T. & Stark, W.J. 2010, 'Radio-opaque nanosized bioactive glass for potential root canal application: evaluation of radiopacity, bioactivity and alkaline capacity', *International Endodontic Journal*, vol. 43, no. 3, pp. 210-7.

Moncada, E., Quijada, R. & Retuert, J. 2007, 'Nanoparticles prepared by the sol–gel method and their use in the formation of nanocomposites with polypropylene', *Nanotechnology*, vol. 18, no. 33, p. 335606.

Mota, J., Yu, N., Caridade, S.G., Luz, G., Gomes, M.E., Reis, R.L., Jansen, J.A., Walboomers, X.F. & Mano, J.F. 2012, 'Chitosan/bioactive glass nanoparticle composite membranes for periodontal regeneration', *Acta Biomater*.

Nair, L.S. & Laurencin, C.T. 2007, 'Biodegradable polymers as biomaterials', *Progress in Polymer Science*, vol. 32, no. 8–9, pp. 762-98.

Nam, K., Watanabe, J. & Ishihara, K. 2004, 'Modeling of swelling and drug release behavior of spontaneously forming hydrogels composed of phospholipid polymers', *International Journal of Pharmaceutics*, vol. 275, no. 1–2, pp. 259-69.

Navarro, M., Michiardi, A., Castaño, O. & Planell, J.A. 2008, 'Biomaterials in orthopaedics', *Journal of The Royal Society Interface*, vol. 5, no. 27, pp. 1137-58.

Nelson, C.L., Hickmon, S.G. & Skinner, R.A. 1997, 'Treatment of experimental osteomyelitis by surgical debridement and the implantation of bioerodable, polyanhydride-gentamicin beads', *Journal of Orthopaedic Research*, vol. 15, no. 2, pp. 249-55.

Nelson, C.L., McLaren, S.G., Skinner, R.A., Smeltzer, M.S., Thomas, J.R. & Olsen, K.M. 2002, 'The treatment of experimental osteomyelitis by surgical debridement and the implantation of calcium sulfate tobramycin pellets', *J Orthop Res*, vol. 20, no. 4, pp. 643-7.

Niemelä, T., Niiranen, H., Kellomäki, M. & Törmälä, P. 2005, 'Self-reinforced composites of bioabsorbable polymer and bioactive glass with different bioactive glass contents. Part I: Initial mechanical properties and bioactivity', *Acta Biomaterialia*, vol. 1, no. 2, pp. 235-42.

Niiranen, H. & To¨rma¨la¨, P. 1999, 'Bioabsorbable polymer plates coated with bioactive glass spheres', *Journal of Materials Science: Materials in Medicine*, vol. 10, no. 12, pp. 707-10.

Noh, H. & Vogler, E.A. 2007, 'Volumetric interpretation of protein adsorption: Competition from mixtures and the Vroman effect', *Biomaterials*, vol. 28, no. 3, pp. 405-22.

Norton, M.R. & Wilson, J. 2002, 'Dental implants placed in extraction sites implanted with bioactive glass: human histology and clinical outcome', *Int J Oral Maxillofac Implants*, vol. 17, no. 2, pp. 249-57.

Oktar, F., Tuyel, U., Demirkol, N., Gunduz, O., Samur, R., Kannan, S. & Agathopoulos, S. 2010, 'A new safe method to produce bioceramic nano-powders from nacre venus verrucosa', paper presented to the *Artificial Organs Conference, FYROM, 2010*.

Palacio, M.L. & Bhushan, B. 2012, 'Bioadhesion: a review of concepts and applications', *Philos Trans A Math Phys Eng Sci*, vol. 370, no. 1967, pp. 2321-47.

Pascaud, P., Bareille, R., Bourget, C., Amedee, J., Rey, C. & Sarda, S. 2012, 'Interaction between a bisphosphonate, tiludronate and nanocrystalline apatite: in vitro viability and proliferation of HOP and HBMSC cells', *Biomed Mater*, vol. 7, no. 5, p. 054108.

Pereira, M.M. & Hench, L.L. 1996, 'Mechanisms of hydroxyapatite formation on porous gel-silica substrates', *Journal of Sol-Gel Science and Technology*, vol. 7, no. 1-2, pp. 59-68.

Pernaa, K., Koski, I., Mattila, K., Gullichsen, E., Heikkila, J., Aho, A. & Lindfors, N. 2011, 'Bioactive Glass S53P4 and Autograft Bone in Treatment of Depressed Tibial Plateau Fractures - A Prospective Randomized 11-year Follow-Up', *J Long Term Eff Med Implants*, vol. 21, no. 2, pp. 139-48.

Pirkey, E.L. & Hurt, J. 1959, 'Roentgen Evaluation of the Soft Tissues in Orthopedics', *American Journal of Roentgenology Radium Therapy and Nuclear Medicine*, vol. 82, no. 2, pp. 271-6.

Pratsinis, S.E. 1998, 'Flame aerosol synthesis of ceramic powders', *Progress in Energy and Combustion Science*, vol. 24, no. 3, pp. 197-219.

Prior, S., Gamazo, C., Irache, J.M., Merkle, H.P. & Gander, B. 2000, 'Gentamicin encapsulation in PLA/PLGA microspheres in view of treating Brucella infections', *Int J Pharm*, vol. 196, no. 1, pp. 115-25.

Qian, J.M., Kang, Y.H., Wei, Z.L. & Zhang, W. 2009, 'Fabrication and characterization of biomorphic 45S5 bioglass scaffold from sugarcane', *Materials Science & Engineering C-Biomimetic and Supramolecular Systems*, vol. 29, no. 4, pp. 1361-4.

Quintero, F., Dieste, O., Pou, J., Lusqui˜nos, F. & Riveiro, A. 2009, 'On the conditions to produce micro- and nanofibres by laser spinning', *J. Phys. D: Appl. Phys.*, vol. 42, pp. 1-10.

Quintero, F., Mann, A.B., Pou, J., Lusquinos, F. & Riveiro, A. 2007, 'Rapid production of ultralong amorphous ceramic nanofibers by laser spinning', *Applied Physics Letters*, vol. 90, no. 15, pp. 153109-3.

Quintero, F., Pou, J., Comesaña, R., Lusquiños, F., Riveiro, A., Mann, A.B., Hill, R.G., Wu, Z.Y. & Jones, J.R. 2009, 'Laser Spinning of Bioactive Glass Nanofibers', *Advanced Functional Materials*, vol. 19, no. 19, pp. 3084-90.

Quintero, F., Pou, J., Lusquiños, F. & Riveiro, A. 2007, 'Experimental analysis of the production of micro- and nanofibres by Laser Spinning', *Applied Surface Science*, vol. 254, no. 4, pp. 1042-7.

Rahman, I.A. & Padavettan, V. 2012, 'Synthesis of Silica Nanoparticles by Sol-Gel: Size-Dependent Properties, Surface Modification, and Applications in Silica-

Polymer Nanocomposites—A Review', *Journal of Nanomaterials*, vol. 2012, p. 15.

Ramires, P.A., Wennerberg, A., Johansson, C.B., Cosentino, F., Tundo, S. & Milella, E. 2003, 'Biological behavior of sol-gel coated dental implants', *J Mater Sci Mater Med*, vol. 14, no. 6, pp. 539-45.

Rantakokko, J., Frantzen, J.P., Heinanen, J., Kajander, S., Kotilainen, E., Gullichsen, E. & Lindfors, N.C. 2012, 'Posterolateral Spondylodesis Using Bioactive Glass S53p4 and Autogenous Bone in Instrumented Unstable Lumbar Spine Burst Fractures', *Scandinavian Journal of Surgery*, vol. 101, no. 1, pp. 66-71.

Rao, M.S.a.K.P. 2002, 'Preparation, characterization and in vitro release of gentamicin from coralline hydroxyapatite-gelatin composite microspheres', *Biomaterials*, vol. 23, no. 15, pp. 3175-81.

Rey, C., Marsan, O., Combes, C., Drouet, C., Grossin, D. & Sarda, S. 2014, 'Characterization of Calcium Phosphates Using Vibrational Spectroscopies', in B. Ben-Nissan (ed.), *Advances in Calcium Phosphate Biomaterials*, vol. 2, Springer Berlin Heidelberg, pp. 229-66.

RM, G., AR, J. & A., J. 2011, 'Internal fixation: a historical review', *Clin Podiatr Med Surg*, vol. 28, no. 4, pp. 607-18.

Rothstein, S.N., Federspiel, W.J. & Little, S.R. 2008, 'A simple model framework for the prediction of controlled release from bulk eroding polymer matrices', *Journal of Materials Chemistry*, vol. 18, no. 16, pp. 1873-80.

Rothstein, S.N., Federspiel, W.J. & Little, S.R. 2009, 'A unified mathematical model for the prediction of controlled release from surface and bulk eroding polymer matrices', *Biomaterials*, vol. 30, no. 8, pp. 1657-64.

Ryu, H.-S., Youn, H.-J., Sun Hong, K., Chang, B.-S., Lee, C.-K. & Chung, S.-S. 2002, 'An improvement in sintering property of β-tricalcium phosphate by addition of calcium pyrophosphate', *Biomaterials*, vol. 23, no. 3, pp. 909-14.

Saboori, A., Rabiee, M., Mutarzadeh, F., Sheikhi, M., Tahriri, M. & Karimi, M. 2009, 'Synthesis, characterization and in vitro bioactivity of sol-gel-derived SiO2-CaO-P2O5-MgO bioglass', *Materials Science & Engineering C-Biomimetic and Supramolecular Systems*, vol. 29, no. 1, pp. 335-40.

Sakai, K., Kawano, H., Iwami, A., Nakamura, M. & Moriguchi, M. 2001, 'Isolation of a thermophilic poly-L-lactide degrading bacterium from compost and its enzymatic characterization', *J Biosci Bioeng*, vol. 92, no. 3, pp. 298-300.

Sakka, S. 1985, 'Glasses and glass-ceramics from gels', *J. Non-Cryst. Solids*, vol. 73, p. 651.

Sampath, S.S., Garvin, K. & Robinson, D.H. 1992, 'Preparation and characterization of biodegradable poly(l-lactic acid) gentamicin delivery systems', *International Journal of Pharmaceutics*, vol. 78, no. 1–3, pp. 165-74.

Saska, S., Barud, H.S., Gaspar, A.M., Marchetto, R., Ribeiro, S.J. & Messaddeq, Y. 2011, 'Bacterial cellulose-hydroxyapatite nanocomposites for bone regeneration', *Int J Biomater*, vol. 2011, p. 175362.

Scheele, C.W. 1980, 'Kgl. Vetenskaps-Academiens nya Handlingar', *Stockholm*, vol. 1, pp. 116-24.

Seal, B.L., Otero, T.C. & Panitch, A. 2001, 'Polymeric biomaterials for tissue and organ regeneration', *Materials Science and Engineering: R: Reports*, vol. 34, no. 4–5, pp. 147-230.

Sepulveda, P., Jones, J.R. & Hench, L.L. 2001, 'Characterization of melt-derived 45S5 and sol-gel-derived 58S bioactive glasses', *Journal of Biomedical Materials Research*, vol. 58, no. 6, pp. 734-40.

Severino, P., Santana, M.H.A., Malmonge, S.M. & Souto, E.B. 2011, 'Polymers for Drug Delivery Systems Formulations', *Polimeros-Ciencia E Tecnologia*, vol. 21, no. 5, pp. 361-8.

Shields, R.K., Martello, J.L. & Potoski, B.A. 2009, 'Is Vancomycin Ototoxicity a Significant Risk?', *Antimicrobial Agents and Chemotherapy*, vol. 53, no. 10, pp. 4572-3.

Shih, C. 1995, 'Chain-end scission in acid catalyzed hydrolysis of poly (d,l-lactide) in solution', *Journal of Controlled Release*, vol. 34, no. 1, pp. 9-15.

Siepmann, J. & Siepmann, F. 2008, 'Mathematical modeling of drug delivery', *International Journal of Pharmaceutics*, vol. 364, no. 2, pp. 328-43.

Singh, R. & Dahotre, N.B. 2007, 'Corrosion degradation and prevention by surface modification of biometallic materials', *J Mater Sci Mater Med*, vol. 18, no. 5, pp. 725-51.

Singh, S., Bhardwaj, P., Singh, V., Aggarwal, S. & Mandal, U.K. 2008, 'Synthesis of nanocrystalline calcium phosphate in microemulsion—effect of nature of surfactants', *Journal of Colloid and Interface Science*, vol. 319, no. 1, pp. 322-9.

Smeaton, W.A. 1986, 'Carl Wilhelm Scheele (1742–1786)', *Endeavour*, vol. 10, no. 1, pp. 28-30.

Södergård, A. & Stolt, M. 2002, 'Properties of lactic acid based polymers and their correlation with composition', *Progress in Polymer Science*, vol. 27, no. 6, pp. 1123-63.

Sokolsky-Papkov, M., Agashi, K., Olaye, A., Shakesheff, K. & Domb, A.J. 2007, 'Polymer carriers for drug delivery in tissue engineering', *Advanced Drug Delivery Reviews*, vol. 59, no. 4–5, pp. 187-206.

Soundrapandian, C., Basu, D., Sa, B. & Datta, S. 2011, 'Local drug delivery system for the treatment of osteomyelitis: In vitro evaluation', *Drug Development and Industrial Pharmacy*, vol. 37, no. 5, pp. 538-46.

Soundrapandian, C., Datta, S., Kundu, B., Basu, D. & Sa, B. 2010, 'Porous Bioactive Glass Scaffolds for Local Drug Delivery in Osteomyelitis: Development and In Vitro Characterization', *Aaps Pharmscitech*, vol. 11, no. 4, pp. 1675-83.

Stark, W.J., Madler, L., Maciejewski, M., Pratsinis, S.E. & Baiker, A. 2003, 'Flame synthesis of nanocrystalline ceria-zirconia: effect of carrier liquid', *Chemical Communications*, no. 5, pp. 588-9.

Stephens, D., Li, L., Robinson, D., Chen, S., Chang, H., Liu, R.M., Tian, Y., Ginsburg, E.J., Gao, X. & Stultz, T. 2000, 'Investigation of the in vitro release of gentamicin from a polyanhydride matrix', *J Control Release*, vol. 63, no. 3, pp. 305-17.

Stewart, P.S. 2002, 'Mechanisms of antibiotic resistance in bacterial biofilms', *International Journal of Medical Microbiology*, vol. 292, no. 2, pp. 107-13.

Suchanek, W. & Yoshimura, M. 1998, 'Processing and properties of hydroxyapatite-based biomaterials for use as hard tissue replacement implants', *Journal of Materials Research*, vol. 13, no. 01, pp. 94-117.

Sun, Y., Guo, G., Tao, D. & Wang, Z. 2007, 'Reverse microemulsion-directed synthesis of hydroxyapatite nanoparticles under hydrothermal conditions', *Journal of Physics and Chemistry of Solids*, vol. 68, no. 3, pp. 373-7.

Sungsanit, K. 2011, 'Rheological and Mechanical Behaviour of Poly(Lactic Acid)/Polyethylene Glycol Blends', RMIT University.

Tacconelli, E., Smith, G., Hieke, K., Lafuma, A. & Bastide, P. 2009, 'Epidemiology, medical outcomes and costs of catheter-related bloodstream infections in intensive care units of four European countries: literature- and registry-based estimates', *Journal of Hospital Infection*, vol. 72, no. 2, pp. 97-103.

Talal, A., Waheed, N., Al-Masri, M., McKay, I.J., Tanner, K.E. & Hughes, F.J. 2009, 'Absorption and release of protein from hydroxyapatite-polylactic acid (HA-PLA) membranes', *J Dent*, vol. 37, no. 11, pp. 820-6.

Talha, M., Behera, C.K. & Sinha, O.P. 2013, 'A review on nickel-free nitrogen containing austenitic stainless steels for biomedical applications', *Mater Sci Eng C Mater Biol Appl*, vol. 33, no. 7, pp. 3563-75.

Tang, Q.L., Zhu, Y.J., Duan, Y.R., Wang, Q., Wang, K.W., Cao, S.W., Chen, F. & Wu, J. 2010, 'Porous nanocomposites of PEG-PLA/calcium phosphate: room-temperature synthesis and its application in drug delivery', *Dalton Transactions*, vol. 39, no. 18, pp. 4435-9.

Thanh, N.T.K. & Green, L.A.W. 2010, 'Functionalisation of nanoparticles for biomedical applications', *Nano Today*, vol. 5, no. 3, pp. 213-30.

Thevenot, P., Hu, W. & Tang, L. 2008, 'SURFACE CHEMISTRY INFLUENCE IMPLANT BIOCOMPATIBILITY', *Current topics in medicinal chemistry*, vol. 8, no. 4, pp. 270-80.

Thompson, I.D. & Hench, L.L. 1998, 'Mechanical properties of bioactive glasses, glass-ceramics and composites', *Proceedings of the Institution of Mechanical Engineers Part H-Journal of Engineering in Medicine*, vol. 212, no. H2, pp. 127-36.

Tian, H., Tang, Z., Zhuang, X., Chen, X. & Jing, X. 2012, 'Biodegradable synthetic polymers: Preparation, functionalization and biomedical application', *Progress in Polymer Science*, vol. 37, no. 2, pp. 237-80.

Tirrell, M., Kokkoli, E. & Biesalski, M. 2002, 'The role of surface science in bioengineered materials', *Surface Science*, vol. 500, no. 1–3, pp. 61-83.

Tjong, S.C. 2006, 'Structural and mechanical properties of polymer nanocomposites', *Materials Science and Engineering: R: Reports*, vol. 53, no. 3–4, pp. 73-197.

Tokgozoglu, A.M. 1998, 'The use of bioresorbable implants in orthopaedics', *Biomedical Science and Technology*, pp. 197-203.

Törmälä, P. 1992, 'Biodegradable self-reinforced composite materials; Manufacturing structure and mechanical properties', *Clinical Materials*, vol. 10, no. 1–2, pp. 29-34.

Tsigkou, O., Hench, L.L., Boccaccini, A.R., Polak, J.M. & Stevens, M.M. 2007, 'Enhanced differentiation and mineralization of human fetal osteoblasts on PDLLA containing Bioglass® composite films in the absence of osteogenic supplements', *Journal of Biomedical Materials Research Part A*, vol. 80A, no. 4, pp. 837-51.

Tsukrov, I. & Kachanov, M. 1997, 'Stress concentrations and microfracturing patterns in a brittle-elastic solid with interacting pores of diverse shapes', *International Journal of Solids and Structures*, vol. 34, no. 22, pp. 2887-904.

Ustariz-Peyret, C., Coudane, J., Vert, M., Kaltsatos, V. & Boisramene, B. 2000, 'Labile conjugation of a hydrophilic drug to PLA oligomers to modify a drug delivery system: cephradin in a PLAGA matrix', *Journal of Microencapsulation*, vol. 17, no. 5, pp. 615-24.

Valenzuela, F., Covarrubias, C., Martinez, C., Smith, P., Diaz-Dosque, M. & Yazdani-Pedram, M. 2012, 'Preparation and bioactive properties of novel bone-repair bionanocomposites based on hydroxyapatite and bioactive glass nanoparticles', *J Biomed Mater Res B Appl Biomater*, vol. 100, no. 6, pp. 1672-82.

Vedantham, K., Swet, J.H., McKillop, I.H. & El-Ghannam, A. 2012, 'Evaluation of a bioresorbable drug delivery system for the treatment of hepatocellular carcinoma', *Journal of Biomedical Materials Research Part A*, vol. 100A, no. 2, pp. 432-40.

Vendra, V.K., Wu, L. & Krishnan, S. 2015, 'Polymer Thin Films for Biomedical Applications', in C.S.S.R. Kumar (ed.), *Nanotechnologies for the Life Sciences*, vol. 5, WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany, p. 54.

Vermonden, T., Censi, R. & Hennink, W.E. 2012, 'Hydrogels for Protein Delivery', *Chemical Reviews*, vol. 112, no. 5, pp. 2853-88.

Verne, E., Vitale-Brovarone, C., Bui, E., Bianchi, C.L. & Boccaccini, A.R. 2009, 'Surface functionalization of bioactive glasses', *J Biomed Mater Res A*, vol. 90, no. 4, pp. 981-92.

Vert, M., Doi, Y., Hellwich, K.-H., Hess, M., Hodge, P., Kubisa, P., Rinaudo, M. & Schué, F. 2012, 'Terminology for biorelated polymers and applications (IUPAC Recommendations 2012)', *Pure and Applied Chemistry*, vol. 84, no. 2, p. 34.

Vert, M., Li, S. & Garreau, H. 1991, 'More about the degradation of LA/GA-derived matrices in aqueous media', *Journal of Controlled Release*, vol. 16, no. 1–2, pp. 15-26.

Vidal, C.V. & Muñoz, A.I. 2009, 'Effect of thermal treatment and applied potential on the electrochemical behaviour of CoCrMo biomedical alloy', *Electrochimica Acta*, vol. 54, no. 6, pp. 1798-809.

Vollenweider, M., Brunner, T.J., Knecht, S., Grass, R.N., Zehnder, M., Imfeld, T. & Stark, W.J. 2007, 'Remineralization of human dentin using ultrafine bioactive glass particles', *Acta Biomaterialia*, vol. 3, no. 6, pp. 936-43.

von Eiff, C., Jansen, B., Kohnen, W. & Becker, K. 2005, 'Infections associated with medical devices: pathogenesis, management and prophylaxis', *Drugs*, vol. 65, no. 2, pp. 179-214.

Wang, G., Liu, S.-J., Ueng, S.W.-N. & Chan, E.-C. 2004, 'The release of cefazolin and gentamicin from biodegradable PLA/PGA beads', *International Journal of Pharmaceutics*, vol. 273, no. 1–2, pp. 203-12.

Wang, L. & Nancollas, G.H. 2008, 'Calcium Orthophosphates: Crystallization and Dissolution', *Chemical reviews*, vol. 108, no. 11, pp. 4628-69.

Wang, M., Hench, L.L. & Bonfield, W. 1998, 'Bioglass/high density polyethylene composite for soft tissue applications: preparation and evaluation', *J Biomed Mater Res*, vol. 42, no. 4, pp. 577-86.

Wiegand, C. & Hipler, U.-C. 2010, 'Polymer-based Biomaterials as Dressings for Chronic Stagnating Wounds', *Macromolecular Symposia*, vol. 294, no. 2, pp. 1-13.

Willerth, S.M. & Sakiyama-Elbert, S.E. 2007, 'Approaches to neural tissue engineering using scaffolds for drug delivery', *Advanced Drug Delivery Reviews*, vol. 59, no. 4–5, pp. 325-38.

Wren, A.W., Coughlan, A., Hassanzadeh, P. & Towler, M.R. 2012, 'Silver coated bioactive glass particles for wound healing applications', *J Mater Sci Mater Med*, vol. 23, no. 5, pp. 1331-41.

Wu, C., Fan, W., Chang, J. & Xiao, Y. 2013, 'Mesoporous bioactive glass scaffolds for efficient delivery of vascular endothelial growth factor', *J Biomater Appl*, vol. 28, no. 3, pp. 367-74.

Xie, F., Ji, S. & Cheng, Z. 2015, 'In vitro dissolution similarity factor (f2) and in vivo bioequivalence criteria, how and when do they match? Using a BCS class II drug as a simulation example', *European Journal of Pharmaceutical Sciences*, vol. 66, no. 0, pp. 163-72.

Xu, C., Zhang, Y., Cheng, G. & Zhu, W. 2008, 'Pitting corrosion behavior of 316L stainless steel in the media of sulphate-reducing and iron-oxidizing bacteria', *Materials Characterization*, vol. 59, no. 3, pp. 245-55.

Yamamoto, A., Honma, R. & Sumita, M. 1998, 'Cytotoxicity evaluation of 43 metal salts using murine fibroblasts and osteoblastic cells', *Journal of Biomedical Materials Research*, vol. 39, no. 2, pp. 331-40.

Yamamoto, A., Kohyama, Y. & Hanawa, T. 2002, 'Mutagenicity evaluation of forty-one metal salts by the umu test', *Journal of Biomedical Materials Research*, vol. 59, no. 1, pp. 176-83.

Yamamoto, A., Mishima, S., Maruyama, N. & Sumita, M. 1998, 'A new technique for direct measurement of the shear force necessary to detach a cell from a material', *Biomaterials*, vol. 19, no. 7–9, pp. 871-9.

Yang, C., Li, Y.Y., Meng, L.E., Chen, J.X. & Huang, Y. 2007, 'Long-term observation for osseointegriation of calcium 'phosphate sol-gel coating on Ti-6Al-4V implants', *Chin Med Sci J*, vol. 22, no. 3, pp. 183-6.

Zammarano, M., Maupin, P.H., Sung, L.P., Gilman, J.W., McCarthy, E.D., Kim, Y.S. & Fox, D.M. 2011, 'Revealing the interface in polymer nanocomposites', *Acs Nano*, vol. 5, no. 4, pp. 3391-9.

Zhang, J., Wang, M., Cha, J.M. & Mantalaris, A. 2009, 'The incorporation of 70s bioactive glass to the osteogenic differentiation of murine embryonic stem cells in 3D bioreactors', *Journal of Tissue Engineering and Regenerative Medicine*, vol. 3, no. 1, pp. 63-71.

Zhou, Q., Pramoda, K.P., Lee, J.M., Wang, K. & Loo, L.S. 2011, 'Role of interface in dispersion and surface energetics of polymer nanocomposites containing hydrophilic POSS and layered silicates', *J Colloid Interface Sci*, vol. 355, no. 1, pp. 222-30.

Zhou, Y., Li, H., Lin, K., Zhai, W., Gu, W. & Chang, J. 2012, 'Effect of heat treatment on the properties of SiO(2)-CaO-MgO-P (2)O (5) bioactive glasses', *J Mater Sci Mater Med*.

Zhou, Z.H., Ruan, J.M., Zou, J.P., Zhou, Z.C. & Shen, X.J. 2007, 'Bioactivity of bioresorbable composite based on bioactive glass and poly-L-lactide', *Transactions of Nonferrous Metals Society of China*, vol. 17, no. 2, pp. 394-9.

Zhu, G., Mallery, S.R. & Schwendeman, S.P. 2000, 'Stabilization of proteins encapsulated in injectable poly (lactide- co-glycolide)', *Nat Biotech*, vol. 18, no. 1, pp. 52-7.

Zhu, M., Wang, H., Liu, J., He, H., Hua, X., He, Q., Zhang, L., Ye, X. & Shi, J. 2011, 'A mesoporous silica nanoparticulate/beta-TCP/BG composite drug delivery system for osteoarticular tuberculosis therapy', *Biomaterials*, vol. 32, no. 7, pp. 1986-95.

CHAPTER 8

GENERAL CONCLUSIONS

CHAPTER 8: GENERAL SUMMARY AND CONCLUSIONS

In this research, bioactive PLA based thin film composites for biomedical applications were successful developed and characterized. It also entailed the use of marine structures because they are biogenic, have unique architecture and are readily available. Most of the marine structures are composed of calcium carbonate (aragonite or calcite) and can be converted into calcium phosphate materials by simple chemical exchange. The marine structure, coralline materials, was converted to HAp and used with PLA for thin film composites. The use of PLA thin films for biomedical applications has clinical advantages due to low material cost, its architecture due to their interconnected porosity, faster processing times, the ability to conform into different shapes, availability of large surface area, the improvement of properties especially mechanical and the possibility of being able to control drug release.

8.1 Effects of drying techniques of PLA films and improvement of interfacial properties of PLA-Bioglass composites

The modified drying techniques have been suggested to improve mechanical properties of thin film PLA. It was suggested that by controlling the evaporation rate of the solvent from the polymer controls both the topography and the mechanical properties of the film. These techniques have successful improved tensile strength of PLA film by 300% when at high drying rate (under vacuum) and 200% at low drying rate (in desiccator). Bioglass particles were successful modifies using APTES and used for PLA composites. The results suggested the improvements of the percentage tensile elongation of PLA-Bioglass thin film composites. The morphology of the fracture surfaces showed that treated bioglass composites had stronger interfacial bonding at the PLA interface than untreated bioglass composites. The results suggest that by nano-surface treating the bioglass with 1% APTES significantly influences the percentage elongation of the PLA/bioglass composite at fracture. SEM shows more agglomeration of untreated bioglass within the composite.

8.2 Drug loading and release from PLA-HAp thin film composites

Coralline materials were characterized and then successfully converted to HAp in two different techniques. Hydrothermally, coral converts into HAp in ammonium phosphate solutions with preservation of sample form and morphology indication topotactic iron exchange reaction mechanisms. Mechano-chemical conversion of coral to HAp follows a topotactic reaction mechanism under ammonium phosphate solution and dissolution-recrystallization using orthophosphoric acid phosphate solution. HAp derived coral has the wide potential in the medical field especially in orthopaedic and tissue engineering.

The study of PLA–calcium phosphate thin film composites reveals possible potential applications of these devices in the biomedical field as drug delivery systems. The flexibility they provide allows them to conform into any desired clinical shape and size. Incorporation of hydroxyapatite in the matrix, has the added advantages of controlled drug release, improves drug encapsulation efficiency, increases drug stability and maintenance of bioactivity and continuous supply of calcium Ca^{2+} and phosphate PO_4^{2-} ions which can assist in bone regeneration and repair. The devices showed the ability to uptake and release gentamicin and bisphosphonate, which could be used for the treatment of bacterial infections and osteoporosis respectively. The systems also showed the potential for prolonged release of these drugs. The loading of a drug onto HAp particles induces a significant decrease of the release period, for both gentamicin and bisphosphonate, permitting the therapeutic efficacy of composite biomaterial locally to be extended. Depending on the degree of clinical dosage required, the devices developed could be tuned to release the drug in both short and prolonged periods of time.

8.3 Biocompatibility in vitro and biofilm behavior of PLA-HAp thin film composites

Cell attachment and proliferation on synthetic biomaterials is an important step for the materials biocompatibility. PLAHAp and PLAHApGM display strong affinity to hADSC due to the presence of HAp in a polymer matrix. Depending on the application, lack of cells attachment on PLA and PLAGM is not a problem since PLA is biodegradable and in most applications it degrades over time and is replaced

with tissue. Polylysine coating on PLA and PLAGM reveals the possibility of surface treatments in case cell attachment is of medical importance. Other surface methods like plasma treatments could also be employed to render the surface suitable for protein binding that enhance cells' attachment.

S. aureus and P. aerigonas bacterial strains used to study biofilm on the surfaces of the developed devices play a significant role in the medical context. They are responsible for most of the medical implant-related infections. The activities of these organisms have resulted in a growing interest in their physiology and molecular biology performance especially in biofilm formations. In this study, it appears that biofilm development occurs differently on the same surface under different strains. Since the biofilm study was preliminary, it is suggested that an alternative model for biofilm development (flow chamber biofilms) would give the other side of biofilm development, a phenomenon that is close to *in-vivo* settings. Reduction of the medical device-related infections lies on the ability to develop medical devices with antimicrobial surface modified materials. However, further investigations are still needed to widen our current understanding on the biofilms and their prevention mechanisms in order to achieve the minimum number of medical device related infection incidents.