

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

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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

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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.

Poly(lactic 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 osteoporosis 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

CERTIFICATE OF ORIGINAL AUTHORSHIP	i
Abstract	v
List of Figures and Tables.....	x
List of Publications	xiv
GENERAL INTRODUCTION	xvi
Main Objective.....	xviii
Specific objectives.....	xviii
Significance of study	xviii
Hypothesis	xviii
Thesis layout.....	xviii
References	xix
CHAPTER 1: LITERATURE BACKGROUND	1
1.1 Biomaterials.....	1
1.1.1 Historical background.....	1
1.1.2 Classifications of biomaterials.....	3
1.1.3 Metallic Biomaterials	4
1.1.4 Polymeric Biomaterials	6
1.1.5 Ceramic Biomaterials	7
1.2 Bioglass	9
1.3 Biodegradable polymers.....	13
1.3.1 Naturally occurring polymers	13
1.3.2 Synthetic polymers	14
1.4 Biodegradable polymer composites	15
1.5 Medical Implants infection.....	15
1.6 <i>In vitro</i> Biocompatibility (Cell culture studies).....	17
1.7 Calcium phosphate and drug delivery.....	17
1.8 References	21
CHAPTER 2: PLA THIN FILMS.....	55
2.1 General introduction.....	55
2.2 PLA.....	55
2.3 Study Rationale	58
2.4 Materials and procedures.....	59
2.5 Results and Discussion	60
2.5.1 Thin film by solution casting.....	60
2.5.3 Comparison of Dog bone tensile samples by injection moulding	63
2.6 Summary and Conclusions	65
CHAPTER 3: BIOGLASS.....	70
3.1 Introduction	70

3.2 Production	71
3.2.1 Micro-emulsion techniques	71
3.2.2 Laser spinning techniques.....	72
3.2.3 Gas phase synthesis (Flame spray synthesis).....	73
3.2.4 Sol-gel Bioglass.....	74
3.3 Composites	75
3.4 Applications	77
3.4.1 Maxillofacial and dental repair.....	78
3.4.2 Bone substitutes in orthopaedics and traumatology.....	78
3.4.3 Treatment of bone infections.....	79
3.4.4 Bioactive glass and biodegradable polymer composites	80
3.4.5 Bioactive glasses for wound healing.....	81
3.3 Experimental Work	82
3.3.1 Materials.....	82
3.3.2 Methods.....	82
3.4 Results and Discussions	84
3.4.1 Microstructural evolution and calcinations temperature	84
3.4.2 Thermal analysis	87
3.4.3 Bioglass morphology, particle size and surface area.....	88
3.4.4 Bioglass –polymer composites	89
3.5 Summary and Conclusions	90
3.6 References	91
CHAPTER 4: PLA-BIOGLASS COMPOSITES	104
4.1 Introduction	104
4.2 Experimental Work	105
4.2.1 Materials.....	105
4.2.2 Methods.....	106
4.3 Results and Discussions	107
4.3.1 APTES functionalized bioglass	107
4.3.2 Particle size and morphology of composites.....	109
4.3.3 Mechanical properties of thin film composites	110
4.4 Discussion	116
4.5 Summary and Conclusions	118
4.6 References	119
CHAPTER 5: CORAL AND CONVERSION TO BIOCERAMICS	122
5.1 Introduction	122
5.2 Materials and Methods	124
5.2.1 Materials.....	124
5.2.2 Methods.....	125
5.3.1 Morphology of coral from solid piece to HAp powder.....	127
5.3.2 X-ray Powder Diffraction (XRD)	130

5.3.3	Fourier transform infrared spectroscopy (FTIR).....	130
5.3.4	Specific surface area, pore size and pore size distribution.....	131
5.3.5	Inductively coupled plasma-mass spectroscopy (ICP-MS).....	132
5.3.6	Thermal analysis (DTA/TGA).....	133
5.3.7	Microstructural evolution during mechano-chemical conversion.....	134
5.4	Discussions.....	137
5.5	Summary and Conclusions.....	140
5.6	References.....	141
CHAPTER 6:	DRUG LOADING AND RELEASE STUDY.....	145
6.1	Introduction.....	145
6.1.1	Release kinetics.....	148
6.2	Materials and Methods.....	148
6.2.2	Methods.....	148
6.2.3	<i>In vitro</i> drug release: Theoretical Mechanism.....	152
6.3	Results and Discussions.....	154
6.3.1	Drug loading to HAp.....	154
6.3.2	Antibacterial Efficacy Test.....	156
6.3.3	Morphological study of gentamicin and bisphosphonate release.....	156
6.3.4	<i>in-vitro</i> gentamicin and bisphosphonate release in PBS and Tris-Cl buffer respectively.....	160
6.3.5	Release kinetics-(Gentamicin).....	165
6.3.6	Release kinetics - Bisphosphonate.....	167
6.4	Summary and Conclusions.....	169
6.5	References.....	169
CHAPTER 7:	STEM CELLS AND BIOLFIM STUDY.....	174
7.1	Introduction.....	174
7.1.1	Stem cells.....	174
7.1.2	Bacteria and biofilm.....	176
7.2	Materials and Methods.....	179
7.2.1	Materials.....	179
7.2.2	Methods.....	179
7.3	Results and Discussions.....	181
7.3.1	Biofilm.....	181
7.3.2	Cell attachment and morphology.....	185
7.4	Summary and Conclusions.....	187
7.5	References.....	188
CHAPTER 8:	GENERAL SUMMARY AND CONCLUSIONS.....	222
8.1	Effects of drying techniques of PLA films and improvement of interfacial properties of PLA-Bioglass composites.....	222
8.2	Drug loading and release from PLA-HAp thin film composites.....	223

8.3 Biocompatibility in vitro and biofilm behavior of PLA-HAp thin film composites.....223

List of Figures and Tables

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). 11

Figure 2: Sequence of interfacial reactions involved in forming a bond between tissue ad bioactive ceramic (Hench 1998)..... 13

Figure 3: Optical microscopy pictures of PLA films dried in the air and vacuum on glass substrate..... 61

Figure 4: Comparison of mechanical tensile strength for different drying techniques (n=5)..... 62

Figure 5: The effect of thickness on films tensile strength and elongation 63

Figure 6: Comparison of thin films and dog-bone samples a) Tensile Strength b) Percentage Elongation 64

Figure 7: Tensile fracture morphology of a&b) dog-bone b) thin film showing riverlines..... 64

Figure 8: Flexural fracture morphology of a&b) PLA V-notched samples 65

Figure 9: XRD patterns of 55S5 thermally treated at 700, 750, 800, 850, 900, 1000 and 1050 °C..... 85

Figure 10: FT-IR spectra of bioglass calcined at 700, 750, 800, 850, 900, 1000, and 1050 °C for 3 hrs..... 86

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. 87

Figure 12: a) Particle size analysis b) SEM picture of sol-gel bioglass 55S5 C) High magnification SEM of 55S5 revealing nanoparticles 89

Figure 13: FTIR spectrum of bioglass and bioglass treated with APTES.....108

Figure 14: Schematic diagram showing the process of attaching APTES on bioglass surfaces.....108

Figure 15: SEM image of PLA-Bioglass composites revealing agglomerated bioglass in PLA matrix (a) 26KX (b) 100KX.	109
Figure 16: SEM micrograph of PLA-1% APTES treated bioglass composite at different magnifications a) 26KX and b) 100KX.....	110
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).....	111
Figure 18: SEM micrograph of tensile fractured pure PLA film showing the riverlines at the edge and the surface of the thin film.	113
Figure 19: SEM micrograph of fractured PLA-untreated bioglass composite showing agglomerated particles and related pore within PLA matrix (2KX).114	
Figure 20: SEM micrograph fractured treated bioglass PLA composite at different magnifications: a) 2 KX b) 5KX.....	115
Figure 21: Coral conversion to calcium phosphate materials through hydrothermal and mechano-chemical techniques	125
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.....	128
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.....	129
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.....	129
Figure 25: XRD patterns of HAp derived coral by hydrothermal (HAp-HT) and mechano-chemical (HAp-MC) techniques compared to coral before conversion.	130
Figure 26: FTIR spectra of coral and HAp derived coral from hydrothermal (HAp-HT) and mechano-chemical (HAp-MC) conversion techniques.	131
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	132

Figure 28: Thermal analysis of coral and HAp derived coral.....	134
Figure 29: SEM images showing morphology of microstructural evolution in the first 3 hours.	136
Figure 30: Solubility isotherms for differing calcium phosphate forms versus pH (Modified using our data from (Wang & Nancollas 2008))......	139
Figure 31: Drug loading and release study of gentamicin and bisphosphonate	150
Figure 32: SEM picture showing coral (a) before loading (b) after loading with gentamicin and (c) after loading with Bisphosphonate	154
Figure 33: FTIR spectra of drug loaded PLA confirming non-denaturation of drugs in the PLA matrix after loading (a) Gentamicin and (b) Bisphosphonate	155
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.....	157
Figure 35: SEM pictures of PLAGM, PLAHApGM and PLAHAp composites revealing the degraded morphology after 1st and 3rd weeks in PBS solution.	158
Figure 36: Structure of PLA drug release devices in sink conditions comparing before and after 7 weeks of drug release.	159
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°C and 100 rpm) for eleven weeks and.....	161
Figure 38: CLSM of 1-day old static grown biofilm of <i>S. aureus</i> and <i>P. aeruginosa</i> on a) PLA b) PLAGM c) PLAHAp and d) PLAHApGM showing their distributions. a, b and d for <i>S. aureus</i> reveals large and high micro-colonies while b shows single cells and small cell clusters cover the surface on of the film. <i>P. aeruginosa</i> shows more growth and wider coverage of the surface than <i>S. aureus</i>	183

Figure 39: CLSM of 5-day old static grown biofilm of <i>S. aureus</i> and <i>P. aeruginosa</i> on a) PLA b) PLAGM c) PLAHAp and d) PLAHApGM showing their distributions. a, b and d for <i>S. aureus</i> reveals large and high micro-colonies while b shows single cells and small cell clusters cover the surface on of the film. <i>P. aeruginosa</i> b and d shows more coverage of the surface by biofilm compared to a and b.....	184
Figure 40: SEM picture of stem cell cultured PLA thin film composites for 10 days, showing attachment and morphology of cells.....	186
Figure 41: SEM picture of cell cultured samples coated with polylysine a) PLA b) PLAGM	187
Table 1: Existing calcium orthophosphates (Macha et al. 2013)	9
Table 2. Summary of the experimental and calculated elastic modulus of samples	116
Table 3: Calcium phosphate materials.....	123
Table 4: Trace elements by ICP-MS of coral and HAp derived coral	133
Table 5: Quantification for HAp derived coral by orthophosphoric phosphate solution experiment showing the amount of transformed phases and crystal growth of HAp	135
Table 6: Quantification for HAp derived coral by ammonium phosphate solution experiment showing the amount of transformed phases and crystal growth of HAp.....	135
Table 7: Specific time frames for different release stages and their numerical values for bisphosphonate (Three stages) and gentamicin (Four stages).....	163
Table 8. Modelled dissolution characteristics of the mean dissolution profile.	165
Table 9. Modelled dissolution characteristics and difference and similarity factors of PLA film and PLAHAp composites loaded with gentamicin.....	166
Table 10: Biomass, average thickness, roughness coefficient and surface to biovolume ratio of biofilm of <i>S. aureus</i> and <i>P. aeruginosa</i> on PLA thin film composites after 24 hours.....	182
Table 11: Biomass, average thickness, roughness coefficient and surface to biovolume ratio of biofilm of <i>S. aureus</i> and <i>P. aeruginosa</i> on PLA thin film composites after 5 days.....	183

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)
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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.

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