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.

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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.

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

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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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