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Hydroxyapatite/PLA biocomposite thin films for slow drug delivery of antibiotics for the treatment of bone and implant-related infections

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Abstract. Drug delivery systems were developed from coralline hydroxyapatite (HAp) and biodegradable polylactic acid (PLA). Gentamicin (GM) was loaded in either directly to PLA (PLAGM) or in HAp microspheres. Drug loaded HAp was used to make thin film composites (PLAHApGM). Dissolution studies were carried out in phosphate buffered saline (PBS). The release profiles suggested that HAp particles improved drug stabilization and availability as well controlled the release rate. The release also displays a steady state release. *In vitro* studies in human Adipose Derived Stem Cells (hADSCs) showed substantial quantities of cells adhering to hydroxyapatite containing composites. The results suggested that the systems could be tailored to release different clinical active substances for a wide range of biomedical applications.

Introduction

Tissue engineering and advanced medical technologies have been very effective tools to improve the quality of human life. The benefits of bioceramic materials used for the repair and reconstruction of disease or damage afflicted areas of the musculoskeletal system have been broadly implemented [1]. During the last two decades the development of bioceramic composites capable of releasing clinical active materials by reproducible and predictable kinetics has been a clinical and scientific endeavour. Drug release systems have been proved to provide outstanding alternative to conventional clinical therapies. The advancement in both science and biomaterial 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 related diseases. There are also possibilities to develop drug release systems with more efficiency and rational drug delivery platforms featuring up-to-the-minute technological capabilities [2]. Drug delivery technology presents an interesting interdisciplinary challenge for pharmaceutical, chemical engineering, biomaterials and medical communities [3]. 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 with time to the surrounding tissues. Furthermore, it would be advantageous if the material is injectable or alternatively coatable on an implant and most importantly a biodegradable [4]

Usage of biodegradable materials in designing drug release devices addresses these challenges by providing outstanding capability of performing localized and controlled delivery of drugs at different parts of the host body. Biodegradable polymer films loaded with gentamicin have been developed to serve as “coatings” for fracture fixation devices and prevent implant-associated

infections [5]. The use of biodegradable polymer films is advantageous due to its propensity to uptake and release antibiotics, as a consequence of its 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 suits this hypothesis a great deal because of the bioactive nature of ceramic materials, specifically calcium phosphate based materials, which promotes bone tissue growth. Incorporation of bioceramics derived from coral in the polymer will improve not only controlled drug release but also bioactivity and tissue regeneration, especially in orthopaedic and maxillofacial applications.

This research is aimed at developing and testing gentamicin (due to its widely used for the treatment of bacterial infection) loaded hydroxyapatite particles (HAp) within thin film polylactic acid (PLA) biocomposites as slow drug delivery devices for the treatment of bone and implant-related infections.

Experimental

Materials. Coral skeleton samples were obtained from the Great Barrier Reef shoreline, QLD Australia. Gentamicin sulfate, Clodronate (Dichloromethylenediphosphonic acid disodium salt), Chloroform diammonium hydrogen phosphate $(\text{NH}_4)_2\text{HPO}_4$, 98%, and sodium hypochlorite (NaClO), were obtained from Sigma Aldrich, Castle Hill, Australia.

Methods. Coralline materials were hydrothermally converted to hydroxyapatite based on the procedures described in [6, 7]. Drug loading and *in-vitro* drug release from PLAHAp composites followed the procedures described in [6, 8]. Bioceramic composites were produced by solution casting method. Briefly, the film composites were loaded with 10 % (w/w) and the release was studied in buffer solutions at physiological conditions. 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 (because PBS will interfere with ^{31}P quantification) in a temperature controlled water bath shaker running at 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 Cary 100 UV-Vis spectrophotometer (Agilent Technologies, Victoria, Australia, Cary Series UV-Vis Spectrophotometer) at the maximum absorbance of gentamicin-*o*-phthaldialdehyde complex, $\lambda_{\text{max}} = 332$ nm, using procedures described in [5, 9]. 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 min at room temperature. The absorbance, which corresponds to the gentamicin concentration, was then measured at 332 nm.

Stem Cell attachment. Adult hADSCs tissue culture was conducted under aseptic sterile conditions in a class II laminar flow hood (Clyde-Apac BH2000 series). hADSCs were cultured till sub-confluence at 5×10^4 cells/cm² T25 culture flask (Nunc) in D-MEM Glutmax/F12 (Gibco) with 10% FBS (Invitrogen) and incubated at 37° C at 5% CO₂. Subsequently cells were passaged and diluted to for the seeding density 1×10^4 cells/cm to be placed on PLA and PLAGM for 10 days for continual growth and adherence test. Non-adherent cells are eliminated by replacing the media every 2 days for normal cellular growth. At 10 days media was decanted, cells washed in PBS, fixed in 4% formalin, washed in di-ionized water (dH₂O) and dehydrated for SEM.

Results and Discussion

Drug release from PLA and PLA-HAp composite 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. Fig.1 shows drug release profiles composed of five stages for PLAGM and PLAHApGM thin film composites. It is possible to appreciate different shapes of the release curves depending on the type of drugs and release medium. The release time

was also enough to appreciate significant degradation of the polymer matrix as shown in Fig. 2. The assessment of kinetic release from drug delivery devices provides the confidence to predict the release behaviour before the release systems are realized. The release kinetic study was assessed by model dependent method. Based on number of kinetic models available in literature, the selected model described the overall release of drug from the dosage forms.

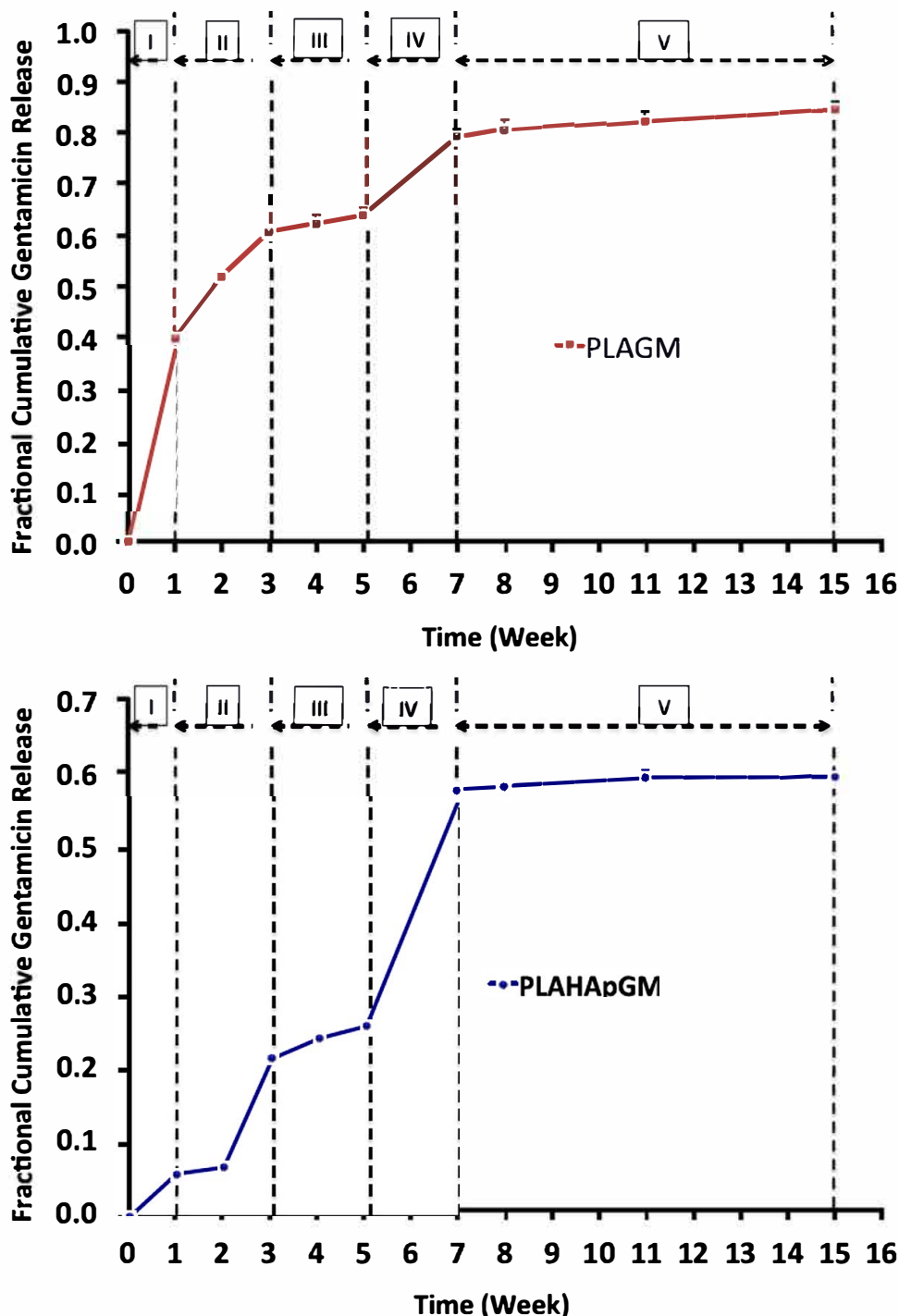


Figure 1: Release profile of gentamicin from PLAGM and PLAHApGM thin films

In the release profiles of drugs from degradable polymer matrix the most sustained release part is by degradation of the polymer matrix. Table 1 shows time range of each release stage for 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

assumptions that the drug is homogeneously distributed in the matrix and therefore homogeneously release of the drugs from the matrix.

Table 1: Specific time frames for different release stages and their numerical values for gentamicin (Five stages)

Name of Drug	Stages	t_0 (w)	t_f (w)
Gentamicin	Stage I (burst ext. release)	0	1
	Stage II (Internal release)	1	3
	Stage III (lag phase)	3	5
	Stage IV (2 nd burst int. release)	5	7
	Stage V (Internal release)	7	15

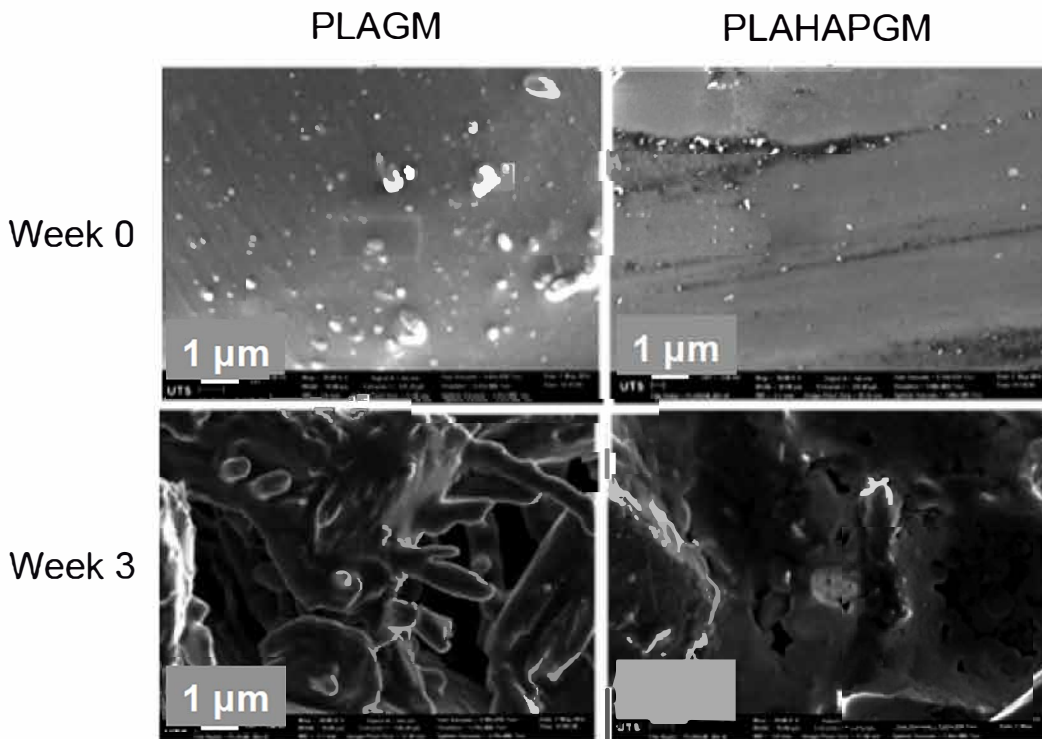


Figure 2: SEM picture of gentamicin loaded PLAGM and PLAHApGM before and after three weeks of drug release in PBS revealing degraded morphologies.

Table 1: Modelled dissolution characteristics of the mean dissolution profile

Model	Model expression		PLAGM	PLAHApGM
Korsmeyer-Peppas	$F = kt^n$	r^2	0.992	0.962
		n	0.282	1.315

The release of gentamicin from these devices seem to follow semi-empirical equation describe by Korsmeyer-Peppas model. Nevertheless, the ‘n’ coefficient obtained for PLAGM and PLAHApGM (Table 2), indicates that somehow a number of different mechanisms might control the release. Thus, the release of gentamicin contained from PLA matrix seems to be mainly controlled by diffusion whereas for PLAHApGM is possibly mixture of diffusion, super case II mechanism and possible combination of other mechanisms of transport which control drug release.

Fig. 3 presents SEM pictures showing *in vitro* studies using stem cell investigations. The morphology and attachment of (hADSC) seeded on PLA thin film composites. The results show high density of cellular attachment on hydroxyapatite containing composites such as PLAHAp and PLAHApGM samples but none on PLA and PLAGM (not shown) composites. PLA has an alkyl

pendant group (CH_3 -) in its backbone, which makes the polymer more hydrophobic, and tends to denaturalize proteins responsible for cell binding and adhesion, while hydroxyapatite is osteoconductive material. Gentamicin has NH_2 - group, which with CH_3 - on the polymer backbone reduce any chance for protein binding on the surface. This was evident because these samples (PLA and PLGM) do not show any cell on their surfaces.

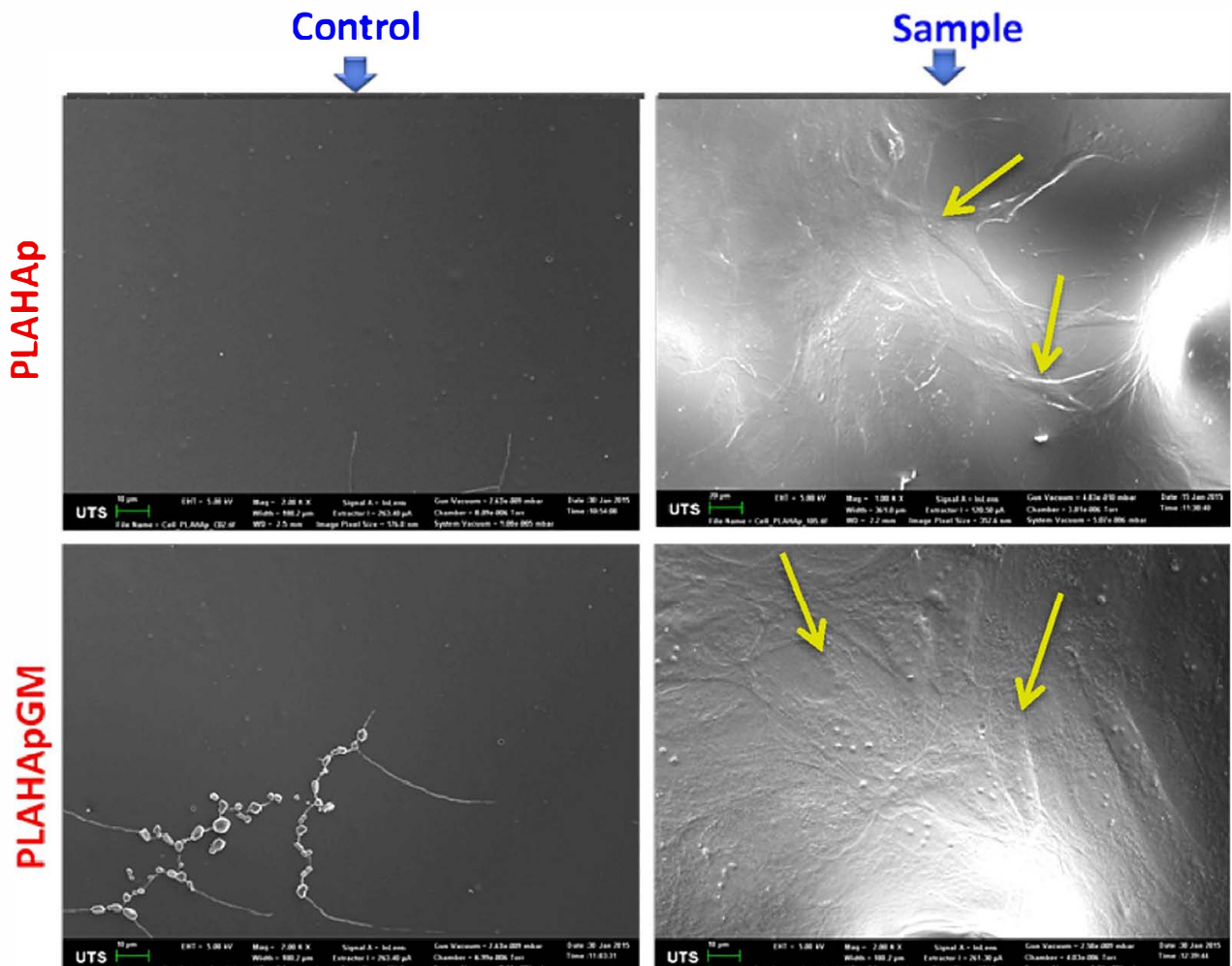


Figure 3: SEM pictures of stem cell cultured PLA thin film composites for 10 days, showing attachment and morphology of cells.

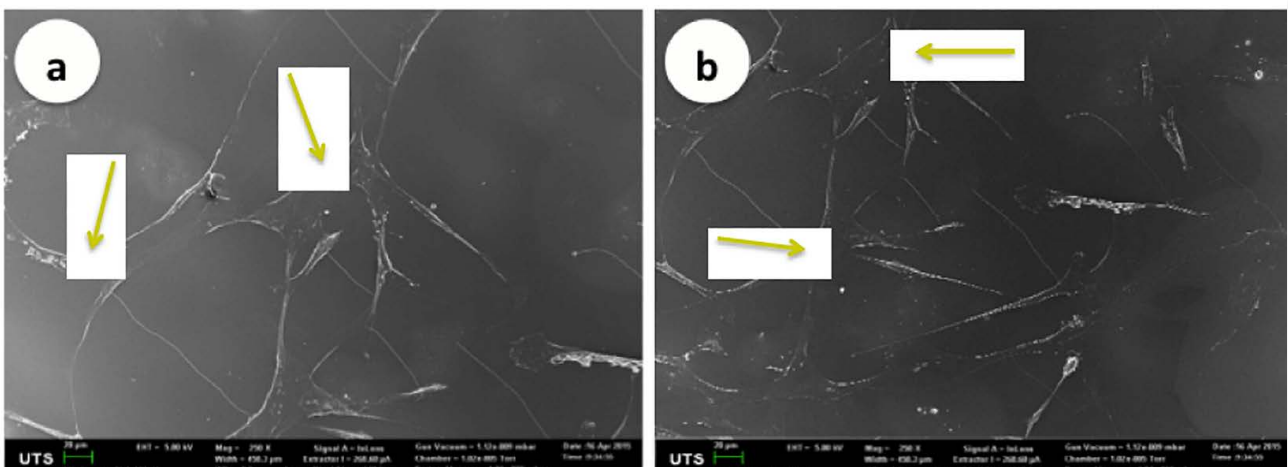


Figure 4: SEM picture of cell cultured samples coated with polylysine a) PLA b) PLGM

In order to confirm the lack of protein adsorption on the surface of PLA and PLAGM samples, we coated them with poly-L-lysine (Sigma Aldrich, Australia) 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 (Fig. 4).

Conclusions

Controlled drugs release successfully achieved for gentamicin containing composites with defined dissolution kinetics. This *in-vitro* study shows the potential of PLA-bioceramic film composites as effective local drug delivery devices for biomedical applications. In addition, they can generally be used in surgery to prevent infections caused by bacterial.

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