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Feedback Controlled Release of Alendronate from Composite Microparticles

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Abstract

Extended bone fractures or fractures coexisting with bone disorders can lead to non-unions where surgical intervention is required. Composite drug delivery systems are being used increasingly more in order to treat such defects locally. Alendronate (ALD), a bisphosphonate extensively used in clinical practice to treat conditions such as osteoporosis has been shown to assist bone fracture healing through its antiresorptive capacity. This study reports the development of a polymeric composite system for the in situ delivery of ALD, which possesses enhanced encapsulation efficiency (EE%) and demonstrates controlled release over a 70 day period. ALD and calcium phosphate (CaP) have been incorporated within poly (lactic-co-glycolic acid) (PLGA) microspheres giving rise to a 70% increase in EE% compared to a control system. Finally, preliminary toxicological evaluation demonstrates a positive effect of the system on pre-osteoblastic cells over 72 hours.

Keywords: Alendronate • Osteoporosis • Encapsulation • Antiresorptive capacity

Introduction

Bone performs a variety of tasks critical to human physiology [1]. Apart from its most commonly acknowledged role as the body's structural component, bone tissue also performs other functions such as protection and support of other internal organs, production of blood cells and storage of mineral salts [2]. Bone tissue possesses some unique properties such as self-remodelling and regeneration via structural and formational tissue restoration [3-6]. Occasionally, damaged or diseased tissue is unable to regenerate and in such cases surgical intervention is required.

In the past, non-healing fractures were addressed surgically using rigid fixation devices which lack biodegradability but also restrict healing [7]. Research then shifted to the development of bone grafts; autografts or allografts [1,2,5]. However, both types of graft are associated with limited availability, prolonged recovery, donor-site morbidity and disease transmittance. There is a clinical need to develop a synthetic alternative [8,9].

Tissue engineering is a multidisciplinary field based on materials science and molecular biology [10]. The field focuses on the development of biological substitutes to replace damaged or diseased tissue or to assist new tissue formation [11]. Within bone tissue engineering, such devices focus on the activation of natural repair mechanisms [5,12]. A vast range of materials have been studied for the formulation of such devices [2,5,7,13] including natural or synthetic polymers and ceramics, either individually or in combination with bioactive compounds (growth factors, proteins etc) and/or cellular components (mesenchymal stem cells). Research has shown that amongst the most successful are composite based devices which incorporate hydrophilic polymers and inorganic mineral like hydroxyapatite (HA). It is suggested that the incorporation of drugs such as bisphosphonates (BPs) could further increase the integration of devices within the host environment and subsequently reduce surgical recovery times [6,14].

Alendronate (ALD) is a type of BP and is clinically used to treat bone disorders such as osteoporosis [15,16]. Recently it has been suggested that the introduction of ALD in osteoporotic bone fractures could improve fracture healing [17]. Alendronate has been found to bind and thus block "… an enzyme in the 3-hydroxy-2-methylglutaryl-CoA (HMG-CoA) reductase pathway (i.e. the mevalonate pathway), thus blocking the prenylation of small GTPases …" as described by Kyllönen [17]. Ultimately, this has a detrimental effect on the function and viability of osteoclasts leading to a net increase in tissue.

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Poly (lactic-co-glycolic acid) (PLGA) is a synthetic copolymer of poly lactic acid (PLA) and poly glycolic acid (PGA) which has been adopted in the production of various therapeutic devices including tissue grafts, surgical sutures, bone tissue engineering scaffolds, and drug carrier systems due its excellent biocompatibility and ability to tune degradation [1,5]; the degradation rate can be tailored based on the ratio of lactic to glycolic acid. PLGA is U.S. Food and Drug Administration (FDA) approved for human treatment and has been prepared into a number of different formulations including scaffolds, hydrogels, nanoparticles, microparticles, and sponges. Consequently, PLGA is an attractive choice for bone regeneration [5].

Researchers have attempted to deliver ALD to local sites, however achieving controlled release can be challenging due to the high solubility of the drug. In this study, we aimed to create a composite system for the local delivery of ALD to fractures sites via controlled release. This was achieved through the development of (PLGA) and calcium phosphate (CaP) microparticles which were loaded with the drug. CaP was selected in order to prolong the release of ALD which is currently challenging through the formation of a mineral shell which is hypothesised to increase the degradation rate of the system. This system would allow for the delivery of a drug to fracture sites which would lead to a net increase in bone formation. Although out of the scope of the current study, the composite system could be incorporated within a hydrogel system to form an injectable material which could be delivered to the site of injury.

Materials and Methods

Materials

Alendronate Sodium salt (purity ≥ 95%) was purchased from Cayman Chemical. Poly (lactic-co-glycolic acid) 50% was purchased from Lakeshore Biomaterials. Dichloromethane (DCM) was purchased from Fisher Chemicals and nitric acid (HNO_3) from Argos Organics. Poly (vinyl alcohol) (PVA) (molecular weight 31,000-50,000, 98-99% hydrolysed) purchased from Argos Organics. All other chemicals and reagents were purchased from Sigma-Aldrich (UK).

Methods

CaP particles' formation: CaP particles were prepared following a wet precipitation method as described by Mobasherpour [22]. Briefly a 0.29 M aqueous solution of $(NH_4)_2HPO_4$ was added dropwise to 0.24 M aqueous solution of Ca $(NO_3)_2.4H_2O$ at a volume ratio of 1.4 CaP. Synthesis was performed under stirring under atmospheric conditions. Mixing was performed using a homogenizer (IKA® T25 digital, ULTRA TURRAX) and the solution was maintained at pH 10. The precipitate was washed with distilled water and collected by centrifugation at 3000rpm in 15min cycles. Homogenization was performed at different speeds and durations and the size distribution was examined at various time points under atmospheric conditions.

Physicochemical characterization: Size distribution of the CaP particles was performed using Malvern Mastersizer 2000 via light scattering and the surface charge was evaluated using a Malvern Zeta-sizer (Nano-ZS) using the Smoluchowski model. Prior to measurement, samples underwent sonication for one hour to break down any aggregates.

The chemical composition of the CaP particles was determined via Fourier transform infrared spectroscopy (FT-IR) and X-ray fluorescence (XRF). For FT-IR analysis, sample pellets were formed into mechanical mixtures of 1% mineral particles' powder in potassium bromide (KBr). CaP particles were thoroughly dried before analysis. For XRF analysis (50 kV, 300 μA, 25 μm spot size, 20 mbarr vacuum), samples were dried and pressed into pellets.

ALD loaded microspheres: The microspheres (Msps) were prepared using a water-in-oil-in-water (w/o/w) double emulsion-solvent evaporation method [23]. Briefly, 2.25 mL PLGA 50% solution 2% w/v in dichloromethane (DCM) were emulsified with 0.45 mL 10% w/v PVA aqueous solution. For the preparation of ALD-loaded microspheres, ALD was initially dissolved in the inner aqueous phase. For the preparation of CaP-ALD loaded particles, ALD was mixed with CaP particles' aqueous dispersion and allowed to bind for 24 h then mixed with 10% w/v PVA solution. The emulsification was accomplished using an homogenizer (IKA® T25 digital, ULTRA TURRAX) at 8000 rpm for 30 sec. The primary emulsion was further emulsified using a magnetic stirrer with 42.2 mL 10% w/v PVA aqueous solution for 4 h until the organic phase fully evaporated. Microspheres were washed with water to remove residual PVA and non-encapsulated drug and collected using centrifugation (JOUAN C422 centrifuge) at 4000 rpm using 30 min cycles. The process was repeated until the supernatant was clear and transparent. Finally, the microspheres were dried under vacuum at room temperature for 24-48 h (Edwards High Vacuum, V&IP, Sussex).

Microspheres physicochemical characterization

Drug encapsulation: Spectrophotometric evaluation (CE 7500 Double Beam UV/Visible Spectrophotometer) of the polymeric microspheres was carried out to calculate ALD content. The method used was based on the formation of an ALD - Cu (II) chromophoric complex absorbing at 240 nm according to the work described by Ostovic [18,24,25].

Drug encapsulation in solid microspheres: 2 mg of particles were dispersed in 0.5 mL DCM. 2 mL nitric acid (HNO_3) 1.5 mM was added and the mixture was stirred at 160 rpm for 20 min. The aqueous phase was filtered, and 1 mL was diluted with 1 mL 1.5 mM HNO_3 and 2 mL Cu^{+2} 5 mM solution in 1.5 mM HNO_3. The mixture was vortexed for 30 sec and left in the dark for 20 min. The sample's absorbance was measured at 240 nm and used for calculating the ALD concentration. A calibration equation as used to calculate the ALD loading% of the and consequently the EE% of the final formulation using the equations provided below.

$$Drug\ Load\% = \left({}^{ALD\ mass}/_{msps\ mass}\right) \times 100$$

$$EE\% = \left({}^{Experimental\ ALD\ content}/_{Theoretical\ ALD\ content}\right) \times 100$$

The theoretical ALD content was based on the initial ALD concentration used for the preparation of the microspheres (msps).

Size distribution and morphology: The size distribution of the fabricated microspheres was determined using optical microscopy (ZEISS optical microscope) and a particle sizer (Malvern Mastersizer 2000). Particle morphology was further evaluated using scanning electron microscopy (SEM) (JEOL JSM-6060LV). For SEM imaging samples were dried, mounted on aluminium stubs using double sided adhesive tape and then subjected toplatinum sputtering. Micrographs were further examined using an image processing software (ImageJ). Density was examined using a helium pycnometer (AccuPyc II 1340).

In vitro release: Particle dispersions in high performance liquid chromatography (HPLC)-grade distilled water were prepared at a concentration of 1 mg/mL and kept at 37°C under 100 rpm shaking using a temperature controlled shaker (Gallenkamp coated orbital incubator). Sampling to measure ALD release was performed at regular time points. Dispersions were centrifuged at 4000 rpm for 30 min and 1 mL supernatant was drawn and used to measure ALD concentration as previously described. HPLC-grade distilled water was added to the dispersion to reach the original volume. Spectrophotometric evaluation (CE 7500 Double Beam UV/Visible Spectrophotometer) of the polymeric microspheres was used to calculate ALD content. The method used was based on the formation of an ALD - Cu (II) chromophoric complex absorbing at 240 nm. In solution, alendronate bonds copper ions via chelate bonding. Each alendronate molecule binds to one copper ion leading to the formation of a chromophore that absorbs photons at the ultraviolet spectrum. This property is exploited for the quantification analysis of alendronate.

Biocompatibility evaluation: Preliminary toxicity experiments were conducted in order to assess biocompatibility. MC-3T3 cells (ATCC) were cultured in culture media; 84.6% v/v Alpha Modified Minimum Essential Medium Eagle (A-MEM), 10% v/v Fetal bovine serum (FBS), 2% v/v L-glutamine (200mM), 2.4% v/v 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (1 M) and 1% v/v penicillin/streptomycin solution. Cells were initially grown in polystyrene tissue culture flasks and then transferred to 96-well plates at a concentration of 4×10^4 cells per well to be treated with the polymeric particles. Cells were cultured at 37°C under 5% CO_2 atmosphere and all procedures were conducted under aseptic conditions. Particles were sterilized by overnight exposure to ultraviolet radiation.

Metabolic activity evaluation: The impact of the particles on metabolic activity was assessed using the AlamerBlue® assay. Briefly, reagent solution was added to the culture at 10% v/v and plates were incubated at 37°C for 4 h. The fluorescence was measured at an excitation of 540 nm and emission of 620 nm. The results were expressed as a percentage of the value corresponding to untreated cells.

$$Cell\ Viability\ \% = \frac{Fluorescent\ Intensity\ of\ cells\ growing\ in\ the\ presence\ of\ particles}{Fluorescent\ Intensity\ of\ cells\ growing\ in\ plain\ media} * 100$$

Statistical analysis: The Thompson Tau analysis was used to determine outliers and the average value and standard deviation of the multiple replicates were calculated. Two-tailed unpaired Student's T-test was performed to establish statistically significant differences, $p \leq 0.05$ are denoted by * and $p \leq 0.01$ are denoted by **.

Results

CaP particles: CaP particles were prepared under different conditions of mixing. The impact of aging on the physical characteristics of the particles was examined. The effects of different homogenization parameters (speed and duration) on particle size distribution are presented in Table 1.

Under 7000 rpm homogenization speed, an increase in homogenization time leads to a decrease in surface weighted average (SWA) and d^{50} of the particle distribution. Longer homogenization times led to a 56% reduction of the SWA diameter of the population. Aging the samples under low stirring under bench conditions leads to a decrease in size at an average of 5.5 μm, regardless of the initial mixing duration. When particles were prepared by mixing at 10000 rpm, size distribution remained stable (ave. SWA = 2.7 μm), regardless of mixing or aging duration.

The size distribution of samples 3 and 4 were stable for 48 h, their stability was further examined as presented in Figure 1. Sample 3 parameters were chosen for the continuation of the study. Particle density was evaluated using a helium

Table 1. Influence of different homogenization parameters on Ca/P particle size distribution during time (revolutions per minute (rpm), surface weighted average (SWA)) (n=5).

Samples	Homogenization parameters		Time (hours)	Size distribution (μm)	
	Speed (rpm)	Duration (min)		SWA	Median Diameter (d^{50})
1	7000	10	-	12.4 +/- 2.274	13.8 +/- 2.354
			48	5.1 +/- 0.349	8.9 +/- 5.322
2	7000	30	-	7 +/- 1.948	9.1 +/- 3.398
			48	6.0 +/- 0.093	7.9 +/- 0.066
3	10000	30	-	2.8 +/- 0.163	3.1 +/- 0.210
			48	2.6 +/- 0.009	2.8 +/- 0.006
4	10000	60	-	2.9 +/- 0.239	3.2 +/- 0.407
			48	2.4 +/- 0.332	2.6 +/- 0.057

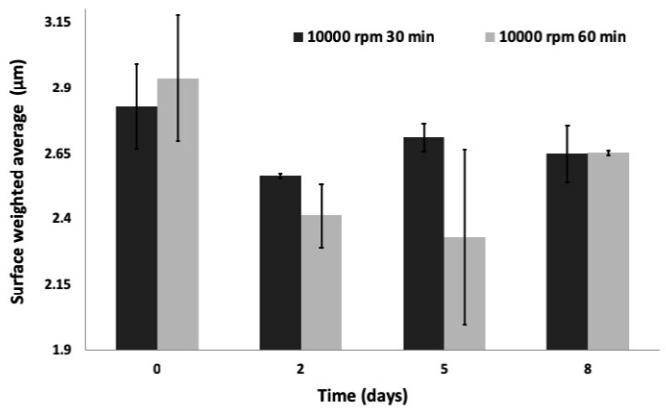

Figure 1. Ca/P particles' size stability over time under bench conditions. Although statistically significant, the size changes observed due to aging were not significant in magnitude with the maximum difference observed being 0.6 µm.

Table 2. Effect of microparticle chemical composition on particle density (n=5)(SA HA: commercially available HA).

Sample	Density (g/cm^3)	
	Mean	SD
SA HA	2.38	0.01
CaP	3.14	0.02
PLGA microspheres	1.51	0.13
CaP loaded PLGA microspheres	1.53	0.01
10% ALD loaded PLGA microspheres	1.48	0.02
10% ALD-CaP loaded PLGA microspheres	1.99	0.06

pycnometer (Table 2) but no significant difference was observed between commercial HA and the CaP particles prepared.

To verify the formation of CaP particles as HA, chemical analysis was performed via FTIR comparing the particles with commercially purchased HA (Figure 2).

The two spectra display the same major peaks. By comparing these spectra with the literature, we can identify the peaks corresponding to OH^- (stretching vibration from 3600 – 2600 cm^{-1}, particularly close to 3570 cm^{-1}) [10,26–29] and the peaks corresponding to PO_4^{3-} around 1100-960 cm^{-1} and 600-560 cm^{-1}. This analysis supports the hypothesis that the CaP particles prepared are HA. The same samples were also analysed using XRF to further determine the phase of calcium phosphate present (Figure 3).

Physical characteristics: The encapsulation of ALD, CaP or their conjugate does not affect the size distribution of the polymeric particles as observed in Figure 4. Encapsulation of ALD appears to have a significant effect on the morphology of the particles; samples of PLGA microspheres demonstrate a small number of porous or damaged structures, whilst ALD loaded microspheres appear to be comprised mostly of highly porous spheres and the incidence of damaged particles increased significantly. The incorporation of ALD in the form of a mineral composite is not accompanied by the same porous structures. In Figure 4d the structures observed are predominantly spherical with a smooth surface similar to those in Figure 4b.

As porous structures were observed, the density of different compositions was examined using a helium pycnometer. The true density (g/cm^3) of the samples is presented in Table 2.

The addition of CaP particles within the PLGA polymeric spheres does not seem to have a significant effect on the density of the structures. The encapsulation of ALD decreases density and thus increases porosity of the spheres which is in accordance with the morphological evaluation of their surface (Figure 4c). The encapsulation of ALD in the form of an ALD-CaP conjugate leads to an increase in density. It was also observed that the density of the ALD-CaP – PLGA composites was higher than that of PLGA or CaP loaded PLGA microspheres.

Encapsulation Efficiency (EE%): ALD is highly hydrophilic and consequently not readily retained by polymeric structures such as PLGA microspheres. As observed in Figure 5, the entrapment efficiency of ALD by polymeric spheres is low regardless of the initial loading. When initial loading was 10% ALD and 10% ALD CaP, EE% was 8% and 14% respectively. 20% ALD and 20% ALD CaP gave rise to 11.5% and 20.5% EE%. An increase in loading from 10% to 20% does not have a significant effect on the EE%. Although there is large deviation between samples,

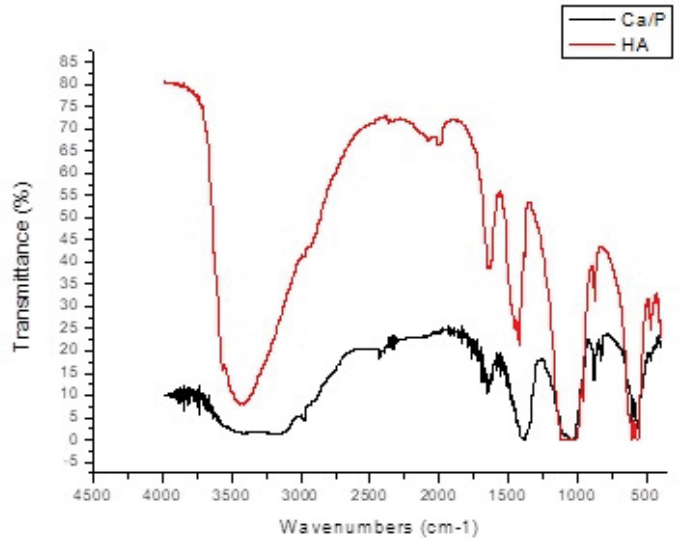

Figure 2. FT-IR spectra of commercial HA and Ca/P particles prepared following the Mobasherpour protocol.

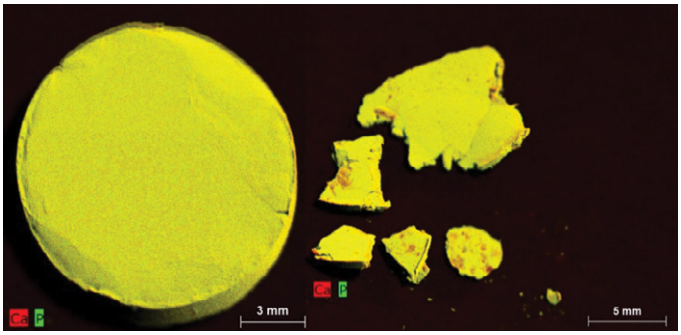

Figure 3. XRF maps of (a) commercial HA and (b) Ca/P particles. Quantitative analysis of the two compositions showed similar calcium to potassium ratio (data not shown).

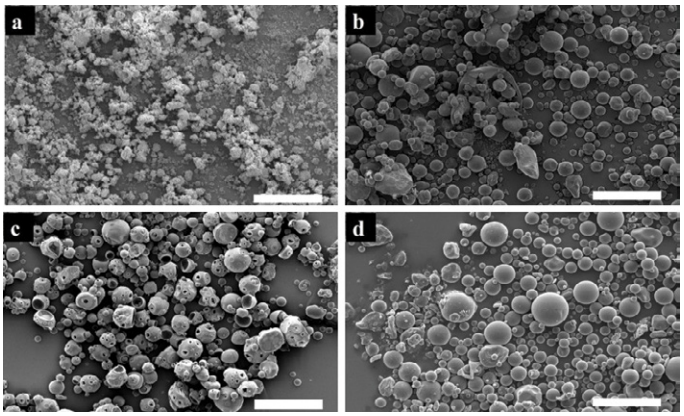

Figure 4. Scanning electron microscopy is used to examine the morphology of particles (a) Ca/P particles, (b) poly(lactic-co-glycolic acid) particles (10.525 µm), (c) ALD loaded polymeric particles (10.6 µm) and (d) ALD-Ca/P loaded PLGA particles (9.13 µm). Scale bar 50 µm.

the incorporation of ALD in the form of a CaP conjugate leads to a statistically significant increase in the EE% which is further enhanced when combined with the increase of the initial loading from 10% to 20%.

In vitro release: It is important to note that release studies were performed in distilled water instead of a PBS (Phosphate-buffered saline), a common buffer solution used in release studies. This decision was based on the fact that the method used for the quantitative analysis of ALD is based on the formation of a chromophoric complex between ALD and copper ions. Cu (II) ions bind to PO_4^{-3} ions, present in PBS, creating a low solubility chromophoric compound that would interfere with the analysis [30,31]. In the case of CaP-containing structures this issue is overcome by the fact that the same mass of CaP particles is used for the formation of ALD loaded

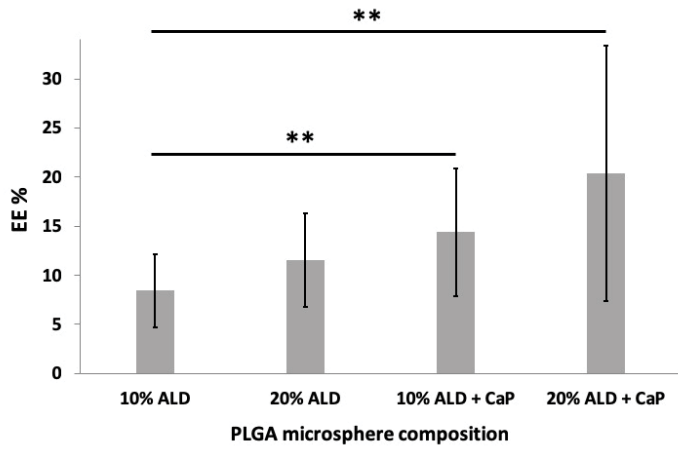

Figure 5. The effect of Ca/P addition on encapsulation efficiency (EE%) of ALD within PLGA microspheres under different initial ALD loadings (10% and 20% w/w of PLGA). Six replicates were performed for each composition (**p ≤ 0.01).

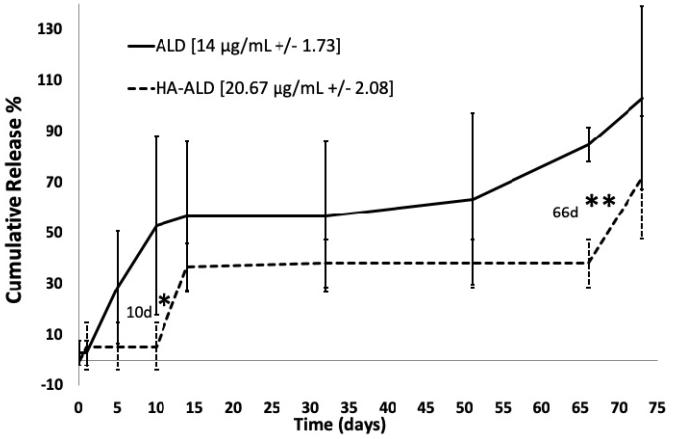

Figure 6. Cumulative release of ALD from PLGA microspheres in vitro. Particles' suspension is set at a concentration of 1 mg/mL in HPLC-grade distilled water, 37°C, suspensions are shaken at a speed of 100rpm. Final loading of ALD is 14 ug/mL (+/- 1.73) for ALD-loaded PLGA particles and 20.67 ug/mL (+/- 2.08) for ALD-HA- loaded PLGA particles (* p ≤ 0.05 and **p ≤ 0.01).

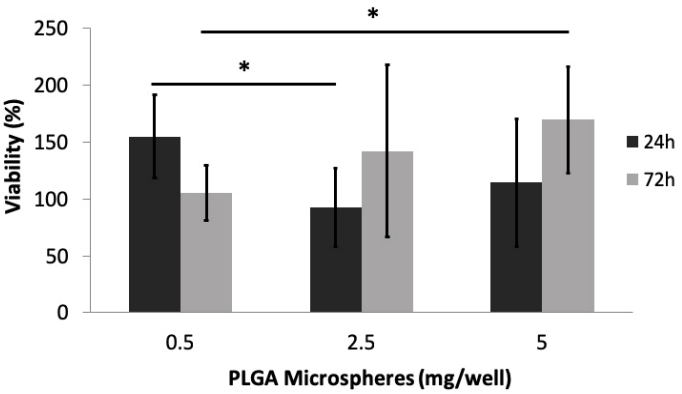

Figure 7. MC-3T3 cellular viability % in the presence of polymeric microparticles was evaluated over a period of 5 days (*p ≤ 0.05).

samples and empty samples used as blank thus the effect of the presence of PO_4^{-3} is subtracted from the final concentration calculations.

Figure 6 demonstrates that 50% of ALD is released within the first ten days from ALD loaded PLGA microspheres. However, there is little to no burst release from the ALD-CaP-loaded PLGA microspheres. A short burst release was observed in the following five days and the cumulative release remains stable for fifty days. The majority of ALD is released within three months. Statistical analysis showed that there is statistically significant difference in the release from the two compositions after ten days and after two months.

Cytotoxicity evaluation: For a period of two days, cellular viability % remains just above 100% regardless of the microparticles composition and concentration. After four days of culture, the cells grown in the presence of microparticles at a concentration of 2 mg/mL exhibited a decrease in viability but significant variations between replicates leads to an absence of statistical difference. Other studies have shown that, although PLGA is generally inert, pre-osteoblasts have not only shown good viability when cultured on PLGA scaffolds but also have exhibited bone-related protein expression and a fibril extracellular matrix formation [27]. Protein expression is enhanced by the presence of HA due to its osteoinductive properties. It has also been suggested that the impact of HA could be caused by the roughness of the mineral and increase in the hydrophilicity of systems based on hydrophobic polymers like PLGA. Additionally, previous co-culture of chondrocytes and osteoblasts in the presence of PLGA and PLGA-bioglass microspheres have shown collagen deposition within the first 10 days with no significant statistical differences between the two systems proving the positive effect of PLGA in the growth and functionality of bone cells [32]. The viability % increase over 100% in comparison to untreated cells could be an effect of the physical presence of particles increasing the surface area available for cell growth. According to Fu et al., the provision of biocompatible environment in the vicinity of small cellular clusters can lead to aggregation of the cells in that area and increase in proliferation rate [27]. As alamar blue is a metabolic activity assay, it is limited by the fact that does not differentiate between increase in number of cells or increase in metabolic activity which could suggest that the increased signal over the first few days of culture could be because of increase in cell number and not due to change of metabolic activity. Preliminary toxicological evaluation showed that the introduction of particles has no negative impact on cell viability of pre-osteoblasts.

Discussion

CaP particles

In previous studies, it has been demonstrated that CaP particle preparation parameters not only affect the size and morphology of the particles but also the phase of the mineral ranging from amorphous hydroxyl apatite to brushite and finally to highly crystalline HA [22]. Specifically, high temperature post-formulation treatment leads to an increase in crystallinity and particle size. In order to obtain a significant loading of CaP particles in PLGA microspheres, the size difference between the two structures needs to be maximized. For our formulations, we examined several preparation parameters (mixing speed, mixing duration and aging duration) in order to obtain the minimum average particle diameter and obtain a narrow size distribution. For these reasons, the preparation was conducted at room temperature and post-formulation heat treatment was avoided to minimize crystal growth.

It was concluded that size distribution of the particles is affected by preparation parameters, predominately, lower mixing speeds and aging leads to a decrease in size. As agglomerates have been shown to form through cold welding from very fine particles, it was not something that could be easily avoided. In order to decrease the effect that agglomerates have on further usage of the particles, formulations were sonicated prior to utilization and characterization. Chemical analysis of those particles and comparison with commercially available HA via FTIR and XRF analysis, shows similar chemical composition and the fact that the CaP ratio is similar between samples, supports the identification of these particles as CaP in the phase of HA. Our study also focused on decreasing size and crystallinity due to the fact that highly crystalline HA particles appear to have lower solubility [33] which would hinder the release of the ALD from the CaP particles after they are released from the PLGA microspheres.

Size, morphology and phase of CaP particles have also been linked to an inflammatory response [34,35]. It has recently been shown that different shapes and sizes of HA particles can influence the recruitment and response of immune cells and signalling molecules. Specifically, smaller particles, less than 10 µm, have been associated with immunological responses; larger particles are less toxic to the surrounding tissue post injection. The particles formulated in this study were in that range predominantly to increase incorporation in the PLGA microspheres so this could be an issue in in vivo administration as it can hinder bone remodelling so it needs to be examined further. Additionally, Lebre's [35] study also showed that although needle like fairly sharp particles can have negative effect on cells as they can damage cellular membrane, whereas smooth spherical particles did not have the same effects. The particles reported in this research are spherical and therefore should avoid such an immune response.

The incorporation of ALD into PLGA microspheres in the form of CaP conjugates could provide a secondary release mechanism; as HA is soluble in low pH, it makes it an ideal vector for delivering osteoclast targeting ALD. Local pH in the lacuna

created between osteoclast cells and the bone surface is in the range of 4–5 due to the acidic excretions of osteoclasts [33]. Therefore, after the release of CaP particles from PLGA microspheres packed in the fracture, CaP particles are free to bind on the existing bone surface due to high affinity of these minerals to bone mineral. The proposed mechanism is as CaP particles dissolve in the lacuna, ALD is gradually released. Once ALD is released into the lacuna, endocytosis is facilitated by the high permeability of the osteoclast ruffled membrane. Post-endocytosis, ALD hinders osteoclast activity and consequently the release of ALD from the particles is based on a "feedback" mechanism.

ALD loaded PLGA microspheres

The chemical composition of the particles significantly affects their physical structure. All compositions led to spherical structures of a similar size distribution but the surface morphology and also the bulk density of the spheres varied. Surface morphology, roughness and density can all have an effect on the response of a drug delivery system, with the host tissue and can also play an important role in the delivery mechanism of the entrapped drug. The incorporation of ALD in the form of a CaP conjugate led to a statistically significant improvement in the encapsulation efficiency (70% increase) of ALD. This is explained by the fact that ALD as a bisphosphonate exhibits a high affinity to bone mineral like HA [36,37]. Also, the formulation of less porous structures leads to decrease in the loss of ALD during fabrication due to diffusion from the inner to the outer aqueous phase. The highly porous structure of ALD-loaded particles explains the initial burst release observed in the first 10 days of the releases studies of this system as well as in similar systems [15]. On the contrary, the addition of ALD as a conjugate with CaP particles leads to significant decrease in porosity which is used as a way to decrease ALD release via diffusion through the polymeric structure. Most importantly the study showed that this composite system provides a controlled and prolonged release of ALD that could assist bone healing over a period of 70 days. Finally, preliminary toxicological experiments displayed no significant negative impact on the growth of MC-3T3 cells, a pre-osteoblastic cell line, in the presence of such structure. Preliminary data suggests that the presence of these structures could promote the growth of these cells, which is explained by the osteoconductive capacity of these materials. The above work supports the safety of these structures in cellular environments.

Conclusion

This study aimed to develop a composite drug delivery system which has the ability to deliver ALD to fracture sites in a controlled manner. The system composed of PLGA ALD-CaP has demonstrated controlled release over 70 days, with increased encapsulation efficiency upon the addition of CaP. Future work will look at developing an injectable carrier system which will allow the delivery of the particles to the sites of injury. Extensive studies to determine osteoblast and osteoclast activity as well as investigating the in vivo effects of the particles on the healing of critical sized defects will also be conducted.

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