Amorphous Calcium Phosphate Blended Polymer Coatings for Biomedical Implants

Eben Adarkwa & Salil Desai
Department of Industrial & Systems Engineering
Engineering Research Center for Revolutionizing Metallic Biomaterials
North Carolina A&T State University
Greensboro, NC, 27411, USA

John Michael Ohodnicki, Abhijit Roy, Boeun Lee, and Prashant N. Kumta
Department of Bioengineering
McGowan Institute for Regenerative Medicine
University of Pittsburgh,
Pittsburgh, PA, 15219, USA

Abstract

The osseointegration of biomedical implants within the host site is a key aspect to early recovery of patients. In this research report we studied the feasibility of depositing polymeric coatings embedded with a novel amorphous calcium phosphate (ACP) formulation using the direct-write inkjet method. Biodegradable polymers that include polycaprolactone (PCL) and poly (lactic-co-glycolic) acid (PLGA) were blended with varying loading concentrations of a proprietary ACP. The direct-write inkjet method was used to deposit 10 and 20 layers of respective materials on polished titanium (Ti) substrates. Surface morphology and chemical composition of the coatings were characterized by optical microscopy and Fourier transform infrared spectroscopy (FTIR), respectively. PCL-ACP composite films showed homogeneous deposition, whereas the PLGA-ACP coatings showed regions coated with bare PLGA polymer without the presence of ACP. The Alamar blue assay was employed to assess the metabolic activity (viability) of the MC3T3 osteoblast precursor cells. Further, an in-vitro live-dead assessment using MC3T3 cells was conducted for cyto-compatibility. This research lays a foundation for developing calcium phosphate containing polymeric composite coatings for orthopedic applications.

Keywords
Amorphous calcium phosphate, biomedical implants, direct write inkjet, osseointegration, polymeric coatings.

1. Introduction

Surface modification is often implemented on a medical implant device to enhance its surface texture, biocompatibility, wear resistance, corrosion resistance, performance and therapeutic effectiveness [1-3]. The careful choice and application of materials at the implant interface is a key to its success. The incorporation of drugs or growth factors within polymeric encapsulation on metallic implants not only serves as a medium of bio-agent delivery but also provides surface modification properties to improve the biocompatibility, and performance of the implant device [4].

To change the surface characteristics of a medical implant device, various surface modification/coating techniques have been developed thus far. The application of multilayered coatings have been proven to be effective in providing temporal release of different growth and healing agents when encapsulated within biodegradable polymeric thin films. In this research, we proposed the use of direct-write (inkjet) printing system as a coating technique. In the field of polymer deposition, inkjet technology has several advantages [5] making it an ideal technique for coating medical devices. The problems associated with conventional polymer/drug loading coating techniques are numerous. They range from the lack to precisely control and maintain drug concentration from device to device. Other issues include recurrent webbing between the struts, inability to vary drug distribution in a
controlled manner for a specific drug loading profile, and the inability to control the local density of the drug [6]. Furthermore, issues with cost also exist as wastage of very expensive active compounds during coating is a major problem with most of these conventional techniques. The use of the drop-on-demand inkjet printing eliminates all these aforementioned problems associated with the conventional coating techniques. Inkjet printing offers the ability to optimize jetting parameters to suit different coating applications towards precise and control deposition of polymer. As stated by Cooley et al. [7], “Inkjet based deposition requires no tooling, is non-contact, and is data-driven; no masks or screens are required; the printing information is created directly from CAD information stored digitally. Being data driven, it is flexible. As an additive process with no chemical waste, it is environmentally friendly and cost effective”. Furthermore, it offers the ability to vary local thickness or density of the polymer/drug to achieve different release kinetics behaviors at target specific locations.

Complications as a result of osteoporosis are an important healthcare problem [8]. Although osteoporosis has been studied for several decades, the effective integration of an implant device with the bone structure is yet to be addressed [9]. This research employs a direct-write inkjet printing technique for surface modification of titanium (Ti) alloy substrates. We employ polymeric materials embedded with nanoparticles for the targeted release of bioactive agent to promote bone formations. The use of crystalline hydroxyapatite (HA) particles has been researched earlier [10]. However, due to its stable and hydrophobic nature in physiological fluids, its release and efficacy for bone healing is limited [11]. The purpose of this study is to investigate the effect of incorporating amorphous calcium phosphate (ACP) nanoparticles within polymeric coatings for its targeted release to promote osseointegration. Polymers such as poly (lactic-co-glycolic) acid (PLGA) and polycaprolactone (PCL) are chosen based on differences in their dissolution rates. A novel composition of the ACP is formulated towards effective proliferation and differentiation of osteoblasts. The highly biodegradable nature of ACP results in the faster release of embedded bioactive agents such as bone morphogenic protein-2 (BMP-2) [8]. The direct-write coating method is incorporated to deposit multilayers thin films of these polymeric films with bone pro-healing agents for orthopedic applications such as fixation screws and plates. Based on a detailed literature review, the use of direct-write inkjet coating technique and a proprietary ACP formulation stands novel based on the application and approach of inducing bioactive/growth agents release profiles of embedded biological agents.

2. Methodology

2.1 Materials

Nanoparticles of amorphous calcium phosphate (ACP) were synthesized by controlled precipitation using water soluble calcium and phosphate salts. These nanoparticulates of ACP are expected to possess similar size and chemistry to the major inorganic components of the human bone. Biodegradable polymers (PLGA and PCL) and solvent (2,2,2-trifluoroethanol (TFE)) were obtained from Sigma-Aldrich.. A thin Ti metallic sheet was cut into 10mm x 10mm coupons and used as substrates for the purpose of depositing the embedded polymeric materials. The JetLab® 4 DOD Inkjet Printing System (MicroFab Technologies Inc., Plano, TX) was employed for coating the Ti substrates with the various polymeric solutions. MC3T3 cells were sourced to assess cellular viability based on the different ACP embedded polymeric coatings for osseointegration studies.

2.2 Coating Preparation

Different formulations of PCL and PLGA polymers were prepared by dissolving the polymers in TFE solvent and stirring for 2 hours. The concentrations of both PLGA and PCL solutions used for the coating process were fixed at 1%w/v. These biopolymer solutions were further blended with ACP at 0.5%w/v and 1%w/v concentrations. The resultant polymer/ACP solutions were then stirred for 2 hours and further sonicated for 4 hours to obtain a completely homogeneous mixture before coating the Ti substrates.

Titanium (Ti) substrates underwent a cleaning procedure. The pre-cleaning treatment of Ti coupon substrates involved an initial rinsing of the coupon substrates with ethanol to remove organic surface impurities followed by further rinsing with excess distilled water. The rinsed Ti substrates were then dipped and washed in 3mol L⁻¹ of nitric acid in water for degreasing. After that the substrates were washed with excess of deionized water to remove the acids at the surface and then dried. The mechanical polishing process consisted of using a 1200 grit size paper to eliminate surface adhered impurities. The polished surfaces were finally rinsed using deionized water and the samples were air dried and stored in a cleanroom.
A custom built direct-write inkjet system (JetLab® DOD) was employed to deposit 10 and 20 coatings layers of different polymeric formulations (shown in Figure 1). A 50μm orifice nozzle was used within the piezoelectric micro-valve for the coating process. The jetting process parameters were optimized to ensure a consistent deposition of the coatings layers. The optimal coating process conditions for a combination of the selected polymer and ACP concentration were determined.

Figure 1: Custom 3D direct-write inkjet equipment for deposition of polymeric formulations on Ti substrate

The experimental design for the different polymers and ACP concentrations are shown in Table 1. A $2^3$ factorial design of experiments was employed. The run sequence for the coating process was determined randomly and each experimental run was replicated five times (n=5) to enable the variability associated with the experimental units to be estimated. A total of forty (N=40) samples were prepared for both characterization and in-vitro studies. Two samples (n=2) from each experimental run were used for coating characterization studies (optical microscopy and FTIR) whereas the other three samples (n=3) were used for in-vitro viability and cyto-compatibility assessment using MC3T3 cells. Bare Ti substrate and tissue culture polystyrene (TCPS) samples were used as controls.

Table 1: Experimental design for in-vitro cellular viability and cyto-compatibility assessments

<table>
<thead>
<tr>
<th>Sample code used for tissue culture plot</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Ti-1% PCL-0.5% ACP-20 layers</td>
<td></td>
</tr>
<tr>
<td>2. Ti-1% PCL-0.5% ACP-10 layers</td>
<td></td>
</tr>
<tr>
<td>3. Ti-1% PLGA-0.5% ACP-20 layers</td>
<td></td>
</tr>
<tr>
<td>4. Ti-1% PLGA-0.5% ACP-10 layers</td>
<td></td>
</tr>
<tr>
<td>5. Ti-1% PCL-1% ACP-20 layers</td>
<td></td>
</tr>
<tr>
<td>6. Ti-1% PCL-1% ACP-10 layers</td>
<td></td>
</tr>
<tr>
<td>7. Ti-1% PLGA-1% ACP-20 layers</td>
<td></td>
</tr>
<tr>
<td>8. Ti-1% PLGA-1% ACP-10 layers</td>
<td></td>
</tr>
<tr>
<td>Ti</td>
<td>Polished Ti-substrates, positive control</td>
</tr>
<tr>
<td>Culture dish</td>
<td>Tissue culture plastics (well plates), positive control</td>
</tr>
</tbody>
</table>
2.3 Coating Characterization
The morphology of the different coatings surfaces was studied using the optical microscopy (Keyence VHX 600K Digital Microscope). The Fourier transform infrared (FTIR) spectroscopy was performed on the sample powders as well as on the obtained films using a Nicolet 6700 spectrophotometer (Thermo Electron Corporation) using a diamond ATR Smart orbit. Spectra were obtained at 1.0 cm$^{-1}$ resolution averaging 32 scans to investigate and confirm the presence of ACP within the polymeric coatings.

2.4 Cytocompatibility tests
The influence of factors such as polymer type, ACP concentration, and coating thickness on Mouse osteoblast was investigated. Murine osteoblast cell line, MC3T3-E1, was obtained from ATCC (Manassas, VA). Cells were cultured under 37 °C, 5% CO2, and 95% relative humidity in minimum essential medium alpha (MEM-α, Gibco, Grand Island, NY) containing 10% fetal bovine serum (FBS, Atlanta Biologicals, Lawrenceville, GA) and penicillin streptomycin (P/S, Gibco, Grand Island, NY). Cells at third to seventh passage were used in this experiment. All the substrates were sterilized under UV for at least 60 min. These sterilized substrates were placed in 12 well plates following which cells were seeded on them at a concentration of 120,000 cells/well. 1 milliliter of media per cm$^2$ of surface area was used and the culture media was changed daily. The effect of ACP concentration and sustained release of calcium and phosphate via the polymeric coatings on the osteoblast viability was evaluated using the Alamar blue assay. This bioassay is designed to measure quantitatively the viability of various human and animal cell lines [12-14]. Cell viability on these coated substrates was also assessed using live/dead staining (Invitrogen, Live/Dead Staining Kit). The live and dead cells were visualized at day 3 post seeding using Fluorescence microscope (Olympus-CKX41).

3. Results and Discussion
3.1 Coating integrity
The PCL coatings displayed uniform deposition pattern and adherence with the Ti substrate as seen in Figure 2(a). However, the PLGA coatings had random deposition patterns as seen in Figure 2(b). The PLGA coatings show spots on the Ti substrate which represent regions coated with bare PLGA polymer without the presence of ACP. This can be attributed to the precipitation and saturation of the ACP within the coated regions.

Figure 2: Optical micrographs of (a) PCL-ACP coatings and (b) PLGA-ACP coatings
3.2 Chemical composition

Figure 3(a&b) shows the FTIR spectrum of PCL-ACP and PLGA-ACP coatings, respectively. The respective figures also include the FTIR spectrum for both of the virgin polymers and ACP for comparative analysis.

![FTIR spectra](image)

Figure 3: Fourier transform infrared spectroscopy (FTIR) of (a) PCL-ACP coatings and (b) PLGA-ACP coatings

Figure 3 (a) shows absorbance peaks that are superimposed for PCL and ACP within the sample 1%PCL 1%ACP 20 layers. This confirms the presence of the PCL polymer (C-H ~ 2850 cm⁻¹, C=O ~ 1750 cm⁻¹) and ACP phase within the coatings. Similarly, figure 3(b) shows absorbance peaks that are superimposed for PLGA and ACP within the sample 1%PLGA 1%ACP 20 layers, thereby confirming the presence of the PLGA polymer (C-H ~2997 cm⁻¹, C=O ~ 1695 cm⁻¹) and ACP phase within the coatings. In addition, the ACP peaks (PO₄³⁻ group ~ 1000 cm⁻¹ and 560 cm⁻¹, CO₃²⁻ group ~ 1640 cm⁻¹) [14] are detected within the blended PCL-ACP and PLGA-ACP coatings.

3.3 Cytocompatibility tests

![Cell viability graph](image)

Figure 4: In-vitro viability assessment using MC3T3 Cells after 24hrs (Day 1)

Figure 4 shows the in-vitro viability assessment using MC3T3 cells at 24 hours post seeding. Based on sample codes as shown in Table 1 it is evident that all experimental samples (coatings) displayed higher cellular viability (around
100%) after 24hrs (day 1) as compared to the positive controls. These results demonstrate that these polymeric coatings are cyto-compatible and presence of ACP-polymer composite films does not affect the cell attachment and viability.

3.4 In-vitro cyto-compatibility assessment

In order to confirm the cellular viability data, we visualized the viability of the cells by fluorescence image using live/dead staining. Figure 5 shows the live-dead cells at 72 hrs (day 3) for different polymeric coatings and positive controls. The PCL-ACP coatings showed cellular attachment comparable to the positive controls. The PCL-ACP coatings with 10 layers showed better cellular attachment (green) as compared to 20 layers. This can be attributed to the rapid release of the ACP during the burst phase from within the 10 layers as compared to the steady-state release of ACP within the 20 layer coatings. However, the PLGA-ACP coatings displayed regions with dead cells (red spots) where the ACP phase was absent. We can correlate the PLGA-ACP cyto-compatibility results with optical micrographs as shown in Figure 2(b) which shows PLGA-ACP coatings with regions of PLGA polymer without the ACP phase on the Ti substrate. In addition, the PLGA-ACP coatings with 10 layers show higher number of live cells as compared to 20 layer coatings. The PLGA-ACP coatings follow analogous release behavior of elemental ACP as compared to the PCL-ACP coatings. Thus, PCL coatings had consistent uniformity and comparable cyto-compatibility as compared to positive controls over PLGA-ACP coatings.

![Figure 5: MC3T3-E1 cells were seeded onto uncoated and coated Ti-substrates, and tissue culture polystyrene plate, incubated for 72 h, and double-stained to be green for live cells and red for dead cells. The scale bar for all the images is 200µm.](image)

4. Conclusion

In this research the direct-write inkjet method was employed to deposit bioactive organic-inorganic composite thin films on Ti substrates. Optimal jetting conditions suitable for both the polymer types were used for coating multilayer polymeric thin films. PCL and PLGA biopolymers were blended with nanostructured amorphous calcium phosphate phase for promoting rapid osseointegration of biomedical implants. The direct-write process enabled precise control on the drop placement and thereby the thickness of these films to obtain tunable release of the ACP. Optical microscopy revealed that PCL-ACP coatings had uniform deposition patterns whereas the PLGA-ACP coatings displayed precipitation of ACP rich patches on the Ti substrate. The FTIR analysis confirmed the presence of both the polymers and ACP phase within the multilayered thin films. Cell viability data showed higher or comparable MC3T3 cell proliferation on the polymeric coatings as compared to Ti substrate and TCPS controls.
Furthermore, the in-vitro live-dead assessment indicated higher cell attachments for coatings with 10 layers (burst release) as compared to the 20 layers (steady-state). PCL-ACP coatings displayed better cell attachment and viability results compared to PLGA-ACP coatings. This research builds the foundation for incorporating bioactive agents within polymeric coating to efficiently regenerate bone structure that interface with biomedical implants.

Acknowledgements
The authors would like to thank the NSF Engineering Research Center for Revolutionizing Metallic Biomaterials (NSF-EEC award 0812348) for support towards this research.

References