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Microstructural control of modular peptide release from microporous biphasic calcium phosphate

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ABSTRACT

Drug release from tissue scaffolds is commonly controlled by using coatings and carriers, as well as by varying the binding affinity of molecules being released. This paper considers modulating synthetic peptide incorporation and release through the use of interconnected microporosity in biphasic calcium phosphate (BCP) and identifies the microstructural characteristics important to the release using experiments and a model of relative diffusivity. First, the release of three modular peptides designed to include an osteocalcin-inspired binding sequence based on bone morphogenic protein-2 (BMP-2) was compared and one was selected for further study. Next, the incorporation and release of the peptide from four types of substrates were compared: non-microporous (NMP) substrates had no microporosity; microporous (MP) substrates were either 50% microporous with 5 μm pores (50/5), 60% microporous with 5 μm pores (60/5), or 50% microporous with 50 μm pores (50/50). Results showed that MP substrates incorporated significantly more peptide than NMP ones, but that the three different microporous substrates all incorporated the same total amount of peptide. NMP had a markedly lower release rate compared to each of three of the MP samples, though the initial burst release was the highest. The initial release and the release rate for the 60/5 samples were different from the 50/50, though they were not statistically different from the 50/5. The model indicated that the pore interconnection to pore size ratio, affecting the constriction between pores, had the greatest influence on the calculated relative diffusivity. While the model was consistent with the trends observed experimentally, the quantitative experimental results suggested that to attain an appreciable difference in release characteristics, both pore size and pore fraction should be changed for this system. These results contribute to rational scaffold design by showing that microstructure, specifically microporosity, can be used to modulate drug release.

1. Introduction

Controlled drug delivery in engineered scaffolds is of significant interest to the fields of tissue engineering and regenerative medicine, among others. The local delivery of antibiotics and growth factors is advantageous as systemic delivery often requires larger doses and targets multiple regions [1]. Several approaches have been taken in bone tissue engineering to control the release rate of locally delivered drugs including the use of drug-laced scaffold coatings [1,2], exploiting the native mineral affinity of growth factors [3], tailoring the affinity of synthetic peptides [4,5], and impregnating scaffolds with polymer-drug matrices [6]. These approaches either rely on additives - coatings, carriers, peptides, or matrices – or they do not provide a mechanism to control the release rate. In this paper, we demonstrate that microstructure can also modulate the release of therapeutic molecules, like drugs or proteins, in calcium phosphate (CaP)-based scaffolds. In particular, we are interested in a biphasic calcium phosphate (BCP) system consisting of hydroxyapatite (HA) and β-tricalcium phosphate (β-TCP) scaffolds and substrates.

Previous studies from our group have shown that protein-mineral affinity can be systematically adjusted to control protein binding and release [4,5,7–12]. However, this approach relies solely on the differential mineral-binding affinity. Here we hypothesized that the incorporation and release of a particular synthetic peptide could also be controlled by modifying the microstructure of a biomaterial, specifically by modifying the interconnected microporosity. In previous work, we

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demonstrated that cells could be drawn into a microporous substrate in vitro and into BCP scaffolds with microporous rods in vivo [13]. We further showed that use of capillary forces in this manner significantly enhances the bone distribution and depth of bone growth in vivo [14]. These designed capillary forces can also be used to draw in endogenous or exogenous molecules that can enhance bone regeneration. Here we aim to identify the microstructural parameters affecting protein release from the microporous substrates so that a scaffold could be designed to have a controlled release rate, while still drawing in the appropriate cells and molecules via capillary forces.

This paper has three objectives. The first is compare the release of three modular peptides designed to include an osteocalcin-inspired HA-binding sequence, based on the osteoinductive protein bone morphogenic protein-2 (BMP-2). Here, we refer to the peptides used in this study as modular bone morphogenetic peptide, or mBMP. These three peptides, mBMP-0Gla, mBMP-1Gla, and mBMP-3Gla, have varying affinity to HA and use of such peptides allows for variation in release without changing the biomaterial substrate. We select one of these peptides for the second part of the study, with the objective of showing that the incorporation and release of such peptides can also be controlled through the modification of substrate microstructure. The third objective is to identify the microstructural characteristics that influence incorporation and release. To that end, we quantify peptide incorporation and subsequent release in solid, or non-microporous (NMP), BCP substrates and in microporous (MP) BCP substrates for three different microstructures. Through comparison of microstructural characteristics that influence the relative diffusivity, we identify the dominant microstructural characteristics influencing release. These results contribute to scaffold design by showing that microstructure can be used to control drug or molecule release.

2. Materials and methods

2.1. Substrate fabrication

Substrates were fabricated using directed deposition, a layer by layer printing process, described previously [15–17]. Here, we aimed to have solid substrates that had been fabricated by directed deposition to be consistent with other work that used deposition. This is also a simple and efficient way to make thin samples of a particular geometry. Briefly, HA powder is calcined at 1100 °C for 10 h then ball milled in order to break up particle aggregates. To make the "ink" for deposition, HA powder is combined with additives in order to tune the rheological properties of the ink for printing. The mass of HA powder in a batch determines the specific amount of additives, which include deionized (DI) water, an anti-foaming reagent, gelling reagents, and surfactants [16,17]. The viscosity of the ink is adjusted by adding the base NH_4OH until the ink is viscous enough to hold its shape when extruded. The nozzle diameter used for deposition of these "solid" substrates was 610 μm and the substrates were deposited in 23 rows, 23 columns, and 2 layers, with a center-to-center rod and layer spacing of 450 μm. The rod and layer spacing were manipulated such that substrates were macroscopically solid rather than being macroporous scaffolds like what have been used in our previous in vivo studies [15,18–21]. After deposition, the substrates were sintered at 1300 °C for 2 h, which yielded the final substrate composition of 13% β-TCP and 87% HA [18]. Substrates had post-sintered dimensions of 1.0 cm × 1.0 cm × 0.079 cm.

Sacrificial poly-methyl methacrylate (PMMA) microspheres can be added to the ink in order to control micropore size and fraction, with pore size determined by the size of the PMMA porogen [22]. In this work, and our previous work [e.g. 13,18,21,23], micropores are pores that are less than approximately 50 μm that are generated in a controlled way, using the PMMA microspheres. We recognize that in some fields pores of this size are called macropores. However, to be consistent with our previous work, we maintain the terminology as stated here. Non-microporous and three microporous substrates were used in this study. Non-microporous substrates were named NMP. Microporous substrates were either named MP as a family, or according to their nominal percent of microporosity, determined by the volume fraction of PMMA porogen relative to the volume of the ceramic powder, and the average PMMA bead size, i.e. using the notation percent porogen/bead size. The PMMA bead size was measured by light scattering (Horiba Laser Scattering Particle Size Distribution Analyzer LA-950) prior to incorporation into the ink. The MP substrates were 50% porogen with nominally 5 μm PMMA beads (50/5), 60% porogen with the same 5 μm beads (60/5), and 50% porogen with nominally 50 μm beads (50/50).

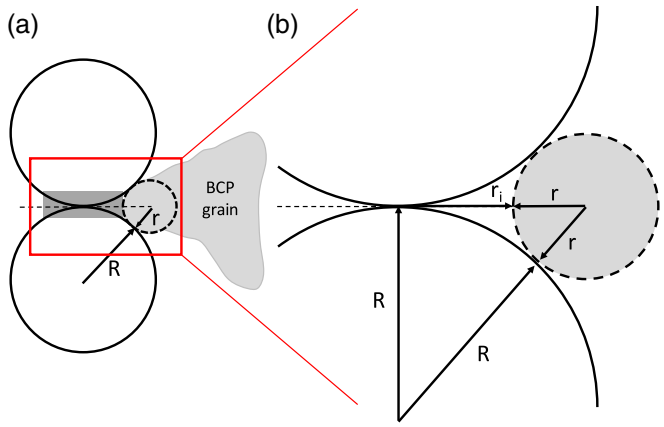

Fig. 1. 2D schematic showing the relationship between the pores generated by the PMMA beads, the adjacent BCP grain, and the pore interconnections. (a) Depiction of two pores created by spherical PMMA beads and a BCP grain. (b) An inset of (a) showing more detail of the interconnection region. 2R is the diameter of the pore that remains after the sacrificial PMMA spheres volatilize during sintering; 2r is the diameter of the grain adjacent to these pores; $2r_i$ is the diameter of the interconnection between pores, also referred to as the interconnection size.

2.2. Substrate characterization

The microstructure of each of the four types of substrates, NMP, 50/5, 60/5, 50/50, was characterized by Scanning Electron Microscopy (SEM) to measure pore size generated by the PMMA microspheres or by incomplete sintering; Mercury Intrusion Porosimetry (MIP) for pore access diameter and pore fraction; and nitrogen BET for specific surface area (SSA). For SEM, samples were coated with Au-Pd and imaged in cross-section at a voltage of 5 kV–25 kV (JEOL JSM 6060LV). Pore diameter was measured from approximately 100 pores from at least three SEM images for the three MP and the NMP sample types using ImageJ

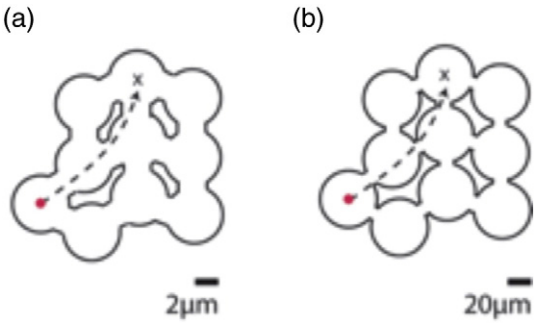

Fig. 2. Illustration of tortuosity in a porous microstructure. (a) Schematic of the microstructure with the 5 μm diameter pores with interconnection radius of approximately 1 μm. The shortest path from the dot to the "X" is indicated by the dashed line. (b) Schematic of the 50 μm diameter pores with interconnection radius of approximately 3 μm. The shortest path to the "X" is identical to the shortest path in the 5 μm pore schematic. The tortuosity is the same for these two microstructures with the same pore arrangement and fraction, but different pore size. Note the different scale bars in (a) and (b) and the different ratio of pore size to interconnection size.