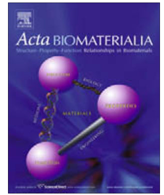
Acta Biomaterialia 6 (2010) 3283–3291

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat



The effect of BMP-2 on micro- and macroscale osteointegration of biphasic calcium phosphate scaffolds with multiscale porosity

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ARTICLE INFO

Article history:
Received 14 December 2009
Accepted 16 February 2010
Available online 20 February 2010

Keywords:
Calcium phosphate
Scaffold
Porosity
Bone ingrowth
Osteointegration

ABSTRACT

It is well established that scaffolds for applications in bone tissue engineering require interconnected pores on the order of 100 μm for bone in growth and nutrient and waste transport. As a result, most studies have focused on scaffold macroporosity (>100 μm). More recently researchers have investigated the role of microporosity in calcium phosphate -based scaffolds. Osteointegration into macropores improves when scaffold rods or struts contain micropores, typically defined as pores less than ~50 μm. We recently demonstrated multiscale osteointegration, or growth into both macropores and intra-red micropores (<10 μm), of biphasic calcium phosphate (BCP) scaffolds. The combined effect of BMP-2, a potent osteoinductive growth factor, and multiscale porosity has yet to be investigated. In this study we implanted BCP scaffolds into porcine mandibular defects for 3, 6, 12 and 24 weeks and evaluated the effect of BMP-2 on multiscale osteointegration. The results showed that given this in vivo model BMP-2 influences osteointegration at the microscale, but not at the macroscale, but not at the macroscale. Cell density was higher in the rod micropores for scaffolds containing BMP-2 compared with controls at all time points, but BMP-2 was not required for bone formation in micropores. In contrast, there was essentially no difference in the fraction of bone in macropores for scaffolds with BMP-2 compared with controls. Additionally, bone in macropores seemed to have reached steady-state by 3 weeks. Multiscale osteointegration results in bone-scaffold composites that are fully osteointegrated, with no 'dead space'. These composites are likely to contain a continuous cell network as well as the potential for enhanced load transfer and improved mechanical properties.
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1. Introduction

A crucial target in the field of bone tissue engineering is the successful regeneration of bone within critical size defects resulting from trauma and disease. This requires the implantation of a bioactive, osteoconductive graft with appropriate mechanical properties. Currently autografts and allografts are the scaffolds of choice in clinical settings [1,2]. Yet neither is optimal, with a major limitation being the amount of graft material available for treatment of large and anatomically complex defects [3,4]. Calcium phosphates (CaPs) with chemical compositions similar to the mineral phase of bone are good candidate materials for the fabrication of synthetic grafts. Calcium phosphate scaffolds have been fabricated using various methods, resulting in a variety of architectures [5–9].

It is well established that synthetic scaffold architecture must include interconnected macroporosity on the order of 100 μm or greater to allow bone ingrowth. Porosity on this length scale is critical for cellular infiltration and nutrient and waste transport, which are necessary for bone formation [10]. Interconnected macroporosity is also important for maximizing bone ingrowth leading to osteointegration and secure graft fixation. Recently the inclusion of microporosity within scaffold rods or struts has gained attention as a positive influence on bone regeneration. Researchers have reported improved osteointegration and increased regenerated bone density and volume and, in some cases, osteoinduction in macropores of scaffolds that possess microporosity [11–13].

In recent work we have focused on microporosity as an important space for bone growth and provided the first definitive example of multiscale osteointegration [14]. Using micro-robotic deposition we fabricated biphasic calcium phosphate (BCP) scaffolds with distinct and independently controlled levels of porosity.

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doi:10.1016/j.actbio.2010.02.026

The combination of scaffold rod spacing and diameter defined the macropore space, while micropores (<10 μm) were present within the scaffold rods. Following implantation of the scaffolds into porcine mandibular defects we observed evidence of bone regeneration within scaffold macropores and micropores. Bone within the interconnected micropores penetrated the nearly 400 μm diameter scaffold rods and osteocyte-like cells were observed within the micropores.

This multiscale osteointegration translated into a scaffold with no 'dead space', or discontinuities of bone, and resulted in a true bone/scaffold composite. Bone formation within micropores has significant implications for improved overall performance of the composite. The bone/scaffold interface area was increased by orders of magnitude over scaffolds without microporosity, resulting in the potential for more effective and more efficient load transfer at the interface. The bone-filled micropores may also provide an effective mechanism to deflect, blunt of slow down crack growth within the scaffold. These characteristics, coupled with the inherent bioactivity and osteoconductivity of CaPs and their similarity in chemical composition to the mineral phase of bone make these scaffolds attractive for repair of large and load bearing defects.

In this work we have evaluated the influence of bone morphogenetic protein-2 (BMP-2) on multiscale osteointegration. BMP-2, one of multiple BMPs in the transforming growth factor-β family, is present during normal bone development and healing and has been implicated in a variety of physiological functions, including osteoinduction [15,16]. BMP-2 is osteoinductive because this growth factor is chemotactic for osteoprogenitors and mesenchymal stem cells and induces their differentiation down the osteogenic lineage leading to bone formation [17,18]. Recombinant human BMP-2 (rhBMP-2) is a clinically useful osteoinductive agent, although, like most proteins, it has a short half-life of a few hours when delivered without a carrier [19]. Therefore, rhBMP-2 is often combined with a biomaterial carrier such as scaffolds and/or degradable microparticles. This results in an increased extent and rate of bone formation in scaffold macropores or ectopic bone formation in vivo [20–24].

This is the first report of the effect of BMP-2 on cellular activity and bone formation within bone scaffold micropores (<10 μm). Because BMP-2 has been shown to enhance bone formation in scaffold macropores, our goal was to characterize its effect on multiscale osteointegration. The addition of BMP-2 to BCP scaffolds could result in a superior bone tissue engineering construct useful for the treatment of critical size bone defects. BCP scaffolds with multiscale porosity were implanted into porcine mandibular defects with or without rhBMP-2 for 3, 6, 12 and 24 weeks. Measures of micro- and macroscale osteointegration included cell density within BCP scaffolds rods and the volume fraction of bone in the macropore space, respectively. Consistent with our previous results, we observed bone and cells within micropores, even in the control group that did not contain rhBMP-2. As there must be a strong driving force for cells to migrate into the micropores, this may be a novel indicator of scaffold osteoinductivity. Possible in vivo modifications that lead to cell migration and bone formation in micropores are also discussed.

2. Materials and methods

2.1. Biphasic calcium phosphate scaffold fabrication and characterization

BCP scaffolds with multiscale porosity were fabricated according to methods described previously [25–27]. Briefly, deionized water and Darvan 821A (R.T. Vanderbilt, Norwalk, CT) were mixed and the solution pH adjusted to 10 with 5 M NH_4OH. Commercially available hydroxyapatite (HA) powder (Riedel-de Haen, Seezle, Germany) was added and mixed by sonication and by using a paint shaker. The suspension was centrifuged and then the solids loading was determined by drying a known mass of slurry. To achieve the targeted solids loading of 55%, appropriate amounts of polymethylmethacrylate (PMMA) microspheres (Matsumoto Microsphere M-100, Tomen America, New York, NY) (fugitive porogen), water, Methocel (Dow Chemical Co., Midland, MI) and 1-octanol (Fisher Scientific, Pittsburgh, PA) were added. The suspension viscosity was increased by decreasing the colloid pH through the addition of 1 M HNO_3. Lastly, polyethyleneimine (PEI) (Polysciences, Inc., Warrington, PA) was added as a gelling agent. Lattices with orthogonal rods were fabricated using micro-robotic deposition [25]. The lattices were heated to burn out organic binders and then were densified via sintering at 1300 °C for 2 h. Finally, lattices were embedded in machinist's wax and machined into cylindrical scaffolds 5 mm in diameter and 8 mm in height. The wax was burned out of scaffold pores by heating for 1 h at 525 °C. Scaffolds were autoclaved for sterilization prior to implantation.

Rod spacing and diameter, which are used to characterize macropore size and geometry, were measured using micro-computed tomography (micro-CT) images of the as-fabricated BCP scaffolds. Measurements were made using standard tools in Amira® (Mercury Computer Systems, Berlin, Germany). Micropore size was determined using backscatter electron (BSE) mode scanning electron microscopy (SEM) micrographs (Phillips XL30 ESEM-FEG, FEI Company, Hillsboro, OR) and the linear intercept method [28], typically used for measuring grain size in polycrystalline materials. Details have been described by Cordell [29]. Micropore size and pore interconnection size were evaluated by mercury intrusion porosimetry [13] using a Micrometrics Autopore II 9220 (Micrometrics Instrument Corp., Norcross, GA).

2.2. Gelatin microparticle fabrication and incorporation into scaffolds

Gelatin microparticles for growth factor delivery were fabricated through a water in oil emulsion as described previously [30]. Pharmaceutical grade basic gelatin (isoelectric point 9) (Nitta Gelatin Corp., Osaka, Japan) was dissolved in water to yield a 10 wt% gelatin solution, which was added to 40 °C olive oil in a solution to oil volume ratio of 1:35. The emulsion was created at 4 °C using a motorized stirrer. The resulting microparticles were washed in cold acetone and 20–50 μm diameter particles were isolated using stainless steel sieves. Microparticles were cross-linked with 500 μl of 25% glutaraldehyde in 0.1% Tween 80. Cross-linking was quenched with 100 mM glycine and microparticles were washed in ultrapure water prior to lyophilization.

rhBMP-2 (R&D Systems, Minneapolis, MN) was reconstituted in sterile 4 mM HCl with 0.1% bovine serum albumin (BSA) at a concentration of 0.5 mg ml^{-1}. The rhBMP-2 was allowed to infiltrate the basic gelatin microparticles at a ratio of 10 μl rhBMP-2 solution to 1 mg gelatin microparticles for 2 h at room temperature. At this ratio the gelatin particles were undersaturated [31]. The final dose per scaffold was 5 μg rhBMP-2 per mg gelatin. The microparticles were resuspended in 2 wt% uncross-linked gelatin at a concentration of 1 mg 45 μl^{-1}. The 45 μl of solution was injected into the top face of the scaffolds, resulting in macropore infiltration. Scaffolds were maintained at 4 °C until implantation.

2.3. In vivo study

2.3.1. Experimental design

Six-month-old 80 kg male Yorkshire pigs were used in the study. Two experimental groups were defined with one treatment group per animal. The control group consisted of BCP scaffolds only and the 'BMP' group consisted of BCP scaffolds containing basic

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