**BMP-2 increases cell density in micropores but not bone volume in macropores in biphasic calcium phosphate scaffolds**

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**Statement of Purpose:** For years researchers have reported that the minimum pore size for bone ingrowth in a scaffold is ~100 μm. In this work, we show that bone grows not only into the macropores, defined as pores >100 μm, but also into the micropores of biphasic calcium phosphate (BCP) scaffolds. Micropores, for this work, are defined as pores <10μm. Other research has shown that BMP-2 increases osteoconductivity for a range of bone scaffolds. However, the interaction between BMP-2 and microporosity has not been investigated. The purpose of this work was to determine the influence of BMP-2 on multiscale osteointegration in BCP scaffolds containing macroporosity between scaffold rods and microporosity within rods (Fig. 1).

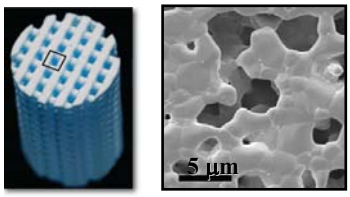


**Figure 1**. Scaffold showing macropore outlined in black (left) and microporosity in the rods (right)

**Methods:** BCP scaffolds were fabricated by robotic deposition, sintered at 1300C for 2 hours, machined into cylinders 5 mm diameter and 8 mm tall, and sterilized by autoclave. Micropore size was determined using mercury intrusion porosimetry (MIP). Gelatin microparticles were fabricated using a water-in-oil emulsion technique and sterile rhBMP-2 (R&D Systems, Minneapolis, MN) was added to lyophilized particles. Six 5mm bicortical defects were created in each animal in the ramus of the mandible, with three per side. Scaffolds were retrieved after 3, 6, 12, and 24 weeks. All animal experiments conformed to the University of Illinois Institutional Animal Care and Use Committee guidelines. Excised samples were imaged using microcomputed tomography (micro-CT) then hard plastic embedded, cut, polished, and stained using Sanderson's Rapid Bone stain and acid fuschin counter stain according to standard methods. Cells were counted from six fields of view, each from different rods, per sample. Between four and seven scaffolds were evaluated with the exception that only two samples at 6 weeks in the BMP group were evaluated for cell density. Bone volume fraction was calculated using a custom, automated segmentation program. Statistical differences were evaluated using ANOVA and post-hoc t-test or Tukey's tests with significance level of $p<0.05$.

**Results:** The scaffolds consist of layered microporous BCP rods. Space between and within the rods constitutes macro and microporosity, respectively (Fig. 1). The rod diameter is 394 μm with center-to-center in-plane spacing of 359 μm and out-of-plane of 252 μm. The micropore size is 5.31 ± 4.07 μm with an average pore connection size of 2.15 μm.

Scaffolds containing rhBMP-2 (BMP) showed significantly greater cell density for all time points compared to control (No BMP), Fig. 2. Cell density within a treatment group was greater at three weeks compared to any other time point and greater for the BMP group at 12 weeks compared to 24. The volume fraction of bone filling macropores at 12 weeks for the No BMP group was greater than the BMP one. There were no other significant differences for macropore bone fill.

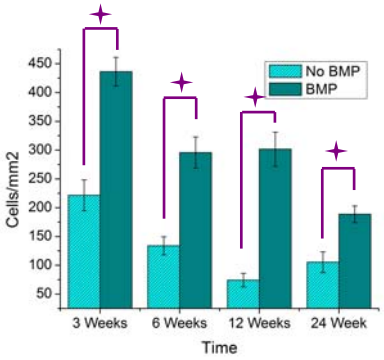


**Figure 2**. Cell density in microporous scaffold rods for BMP-2 was higher in scaffolds containing BMP-2 as compared to control ($p<0.05$). Significances within a treatment are described in the text.

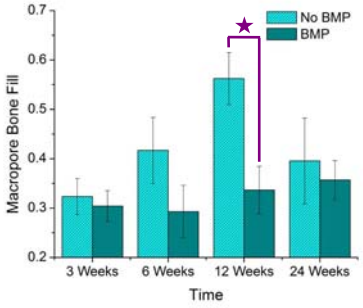


**Figure 3**. Fraction of macropore space filled by bone. The control had more bone at 12 weeks compared to the BMP group ($p<0.05$). There were no other statistically significant differences.

**Conclusions:** The results demonstrate that BMP-2 provides significant benefit for integration at the *micro*scale, but not at the macroscale for this scaffold system and animal model. Importantly, BMP-2 is not *required* for cell migration and bone formation in micropores. The latter may be a novel indicator of the osteoinductive microenvironment provided by the combination of the macro and microporosity for these scaffolds. Finally, these results debunk the concept of a 100 μm minimum pore size for bone ingrowth that has been accepted for many years.