Healing of Rat Calvarial Defects With Nanohydroxyapatite/Poly(Ester-urethane) Scaffolds Loaded With rhBMP-2.

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Disclosures:

Introduction: A variety of materials have been used to construct scaffolds for tissue-engineering applications, but their press-fitting properties in complex defects are seldom reported. A novel elastomeric nanosized hydroxyapatite-incorporated poly(ester-urethane) (nHA/PU) scaffold was developed and shown to adsorb proteins [1] and promote angiogenesis [2-4] within the scaffold. However, limited in vivo studies have been performed to investigate whether nHA/PU scaffolds can effectively enhance bone regeneration. The objective of this study is to determine the effectiveness of nHA/PU scaffold as a carrier of rhBMP-2 (R&D Systems) for guided bone regeneration. We hypothesize that the presence of rhBMP-2 on the nHA/PU scaffold surface will enhance bone repair.

Methods: Scaffold preparation. The scaffold was prepared using a salt-leaching/phase-inverse process as previously described [5]. The scaffold contains 10% by weight nHA, and 90% w:w PU synthesized by polycondensation of 1,6-hexamethylene diisocyanate, poly(ε-caprolactone) diol and 1,4,3,6-dianhydro-D-sorbitol. For this study, the nHA/PU scaffolds were prepared with a circular dimension of 5.5 mm and 1 mm thickness, with pore sizes of 90 to 300 μm. Surgical preparation. All animal procedures were carried out in accordance with the guidelines of the Canadian Council on Animal Care (CCAC). A 5-mm circular defect was made on each parietal bone of anesthetized Sprague-Dawley rats (aged 11-12 weeks) as described in other studies [6, 7]. No-protein control scaffolds (nHA/PU) or nHA/PU scaffolds coated with 1 μg of rhBMP-2 (nHA/PU/rhBMP-2) were inserted into the calvarial defects. Analysis. At intervals of 2 weeks, the rats were imaged with a live-animal microCT scanner (GE eXplore Locus) to quantify mineral deposition within the defect sites. In addition, at 6 and 12 weeks, implants and surrounding bone were also recovered and fixed for histological analysis. Mechanical testing. At 6 and 12 weeks, specimens were prepared for micro-indentation and push-out tests to determine the mechanical properties and osteointegration of the newly formed bone. Statistics. Quantitative data were expressed as means ± standard error. Statistical analysis among groups was performed using two-way analysis of variance (ANOVA) and p<0.05 was considered to be statistically significant.

Results: At 12 weeks, the nHA/PU/rhBMP-2 implants were filled with apparent new bone, whereas for the nHA/PU group apparent bone formation was minimal (Fig 1). Using quantitative microCT analysis, addition of rhBMP-2 to the scaffolds enhanced bone formation (approximately 4-fold) compared to control (Fig 2). Histological sections stained with H&E and Masson trichrome also agree with microCT data showing higher bone formation in the nHA/PU/rhBMP-2 group compared to control (data not shown). Furthermore, the newly formed bone in the nHA/PU/rhBMP-2 group has a higher stiffness and required more load to fracture the implant-bone interface compared to the control group. However, these values are still lower compared to native bone (Fig 3).

Discussion: The addition of rhBMP-2 to nHA/PU scaffolds significantly enhanced in vivo bone repair. Based on microCT and histological analysis, the new in-growth bone appears to form from the host bone adjacent to the dura membrane. No major evidence of inflammation was seen in any of the groups, which indicate that the nHA/PU scaffold is biocompatible and can be used as a bioactive protein carrier. Mechanically, however, the properties of the newly formed bone do not reach that of undamaged bone in the 12 weeks post-surgery period. Of note, nHA/PU scaffolds bind rhBMP-2 well and slowly release the growth factor over time (85% of the rhBMP-2 was still bound to the scaffold at 72 h; data not shown).

Significance: This study demonstrates that nHA/PU scaffolds are an effective carrier for rhBMP-2 for use in bone-regeneration therapy. Our ongoing study will look at the effectiveness of the scaffold as a carrier for various bioactive proteins and whether addition of mesenchymal stromal cells further enhances bone repair.

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Figure 1. Maximum Intensity Projection (MIP) and isosurface images of rats at 0, 2, 6 and 12 weeks. MicroCT images indicate that the presence of rhBMP-2 enhanced bone formation in the calvarial defects. At 6 weeks bone formation is apparent in the nHA/PU/rhBMP-2 group, with density of the newly formed bone increasing over time. The image on the left depicts the region of interest (ROI) in yellow chosen for analysis.
Figure 2. Quantitative microCT analysis results showing (A) bone mineral content (BMC), (B) new bone volume, (C) percentage of bone-to-tissue volume and (D) percentage of cortical bone volume are enhanced in rhBMP-2/hHA/PU groups compared to nHA/PU group at 6 and 12 weeks. Data represents mean ± standard error (n=20 for 2 and 6 weeks, n=12 for 12 weeks, *p<0.05, **p<0.01, ***p<0.001).
Figure 3. Mechanical test of rat calvaria at 6 and 12 weeks. (A) Micro-indentation test indicated that the young’s modulus of newly formed bone in rhBMP-2/nHA/PU group were significantly higher compared to nHA/PU group at both time points. (B) Push-out test showed a significant difference in load of fracture for rhBMP-2/nHA/PU group compared to nHA/PU group. The bone-control was done on undamaged bone. Data represents mean ± standard error (micro-indentation test: $n=6$ for bone groups and $n=8$ and 5 for all other groups at 6 and 12 weeks respectively, push-out test: $n=5$ for no scaffold group and $n=7$ for all other groups; *$p<0.05$, **$p<0.01$, ***$p<0.001$).