INTRODUCTION:

There is a pressing need for the development of more effective strategies to repair critical-sized bone defects in patients who have had volumetric loss due to disease, trauma or resections secondary to tumor surgery. Currently, the most common methods to repair critical-sized bony defects are the use of autografts and allografts, which are limited by the lack of supply (autografts) or are associated with high failure rates (allografts). An alternative would be synthetic bone grafts that can be conveniently and securely fitted into a defect, serve as an osteoconductive scaffold for new bone growth and deliver therapeutic agents to expedite graft healing. Many existing synthetic bone substitutes are either brittle ceramics or weak gel foams. They generally exhibit poor surgical handling characteristics and inadequate structural and biochemical properties. Towards this end, we have developed a promising osteoconductive composite bone substitute named FlexBone. FlexBone is a synthetic hydrogel-biomineral composite prepared by crosslinking poly(2-hydroxyethyl methacrylate) (pHEMA) in the presence of 50% HA or 25% HA-25% TCP. Despite its high mineral content, FlexBone exhibits elastomeric properties under physiological conditions and can withstand repetitive, moderate (MPa) compressive loadings without exhibiting brittle fractures. We further demonstrated that antibiotics and recombinant proteins can be encapsulated with FlexBone and locally released in a sustained, rather than burst-release, manner in vitro (data not shown), making FlexBone an ideal carrier to deliver therapeutic agents to expedite the repair of hard-to-heal defects. The purpose of this study was to first examine the use of FlexBone containing 50% HA or 25% HA-25% TCP in the repair of 5-mm critical femoral defects in rats without exogenous osteogenic growth factor. The clinical rationale was to determine the potential of FlexBone as an osteoconductive scaffold to facilitate the healing of segmental defects in patients where delivery of factors such as BMPs would be contraindicated (e.g. tumor resections patients). An additional purpose of the study was to evaluate the effect of FlexBone delivery of an osteogenic growth factor (rhBMP-2/7) on the graft healing.

METHODS:

FlexBone Preparation: FlexBone (50%HA or 25%HA-25%TCP) was prepared as previously described. 2 Graft (8×3.2×4 mm) was drilled with orthogonal drill holes for marrow penetration, and sterilized in 70% ethanol and equilibrated with water prior to use.

Study Design and Surgical Procedure: FlexBone containing 50%HA or 25%HA-25%TCP were implanted in 5-mm rat femoral defects with or without exogenous BMP-2/7, and osteointegration of the grafts over time were compared among the experimental groups and with the no-graft control. All animal procedures were approved by the IACUC. Briefly, the shaft of the femur of male Charles River SASCO-SD rats (289-300 g) were exposed by a combination of sharp and blunt dissection and the periosteum was removed. A specially designed radiolucent PEEK internal plate fixator was secured to the exposed femur with 4 bicortical screws. A mid-diaphyseal 5-mm defect was then created using an oscillating Hall saw with parallel blades, followed by thorough irrigation of the defect and the press-fitting of a FlexBone graft with or without rhBMP-2/7 (400-ng in 4-µm). Rats were radiographed post-op to ensure graft positioning, and every 2 weeks thereafter to monitor the mineralized callus formation over time. The rats were euthanized at 4 days, 2, 4, 6, 8 and 12 weeks for histologic analysis. In a subset of 12 week rats, fresh frozen explanted femurs were evaluated using MicroCT and then tested to failure in torsion. A total of 115 defects were generated in 58 rats: 5 groups (4 exp. groups + 1 no-graft control group) of 6 time points×3(N=3, histology)+11 time points×5(N=5, MicroCT & torsion tests) = 115 defects.

Histology, Microscopy, Torsion Tests and MicroCT: To evaluate cellularity, new bone formation, remodeling and vascularization of the FlexBone over time, histochemical and immunohistochemical staining of the explants for the osteogenic differentiation marker alkaline phosphatase (ALP), osteoclast lineage marker TRAP, chondrocytes (by toluidine blue), and endothelial cell surface marker PECAM-1 were performed on paraffin sections. H&E staining was carried out to distinguish cell nuclei and collagen matrices. Polarized light microscopy was used to assess the orientation of collagen fibrils and thus the maturity of new bone formation. Fresh frozen explants were scanned on a cone-beam xKplode Lowet SP microCT system. The effective voxel size of the reconstructed images was 18×18×18 µm3. Images were globally thresholded and analyzed to measure callus volume, bone volume, bone to callus volume, bone mineral content, tissue mineral density, and polar moment of inertia. Following MicroCT, both ends of the explants were potted in aluminum pots with molten bismuth and mounted in a custom mini-torsion tester. PEEK fixators were removed to expose the graft-bone interfaces before the explants were loaded to failure (0.5 μ/sec) to determine the structural stiffness and failure torque.

RESULTS:

The elasticity of FlexBone enabled its convenient and stable implantation into the 5-mm femoral defects. The grafts containing 50%HA were more resistant to crack formation during the surgical handling than those containing 25%HA-25%TCP. Bone marrow infiltrated the drill hole cavities of the graft by 4 days with or without rhBMP-2/7. Callus mineralization was detected in all experimental groups 2-4 weeks post-op, with those supplemented with rhBMP-2/7 exhibiting more pronounced mineralization. Endochondral bone formation was observed within the callus bridging over FlexBone, at the FlexBone-cortical bone interface, and within the drill holes with or without the supplement of exogenous rhBMP-2/7. A high level of ALP and TRAP activities was detected at the FlexBone-new bone interface (Fig. 1A-C). The extensive new bone formation and graft remodeling activities were also accompanied by the neovascularization of the healing callus. While the no-graft control group remained un-bridged by 12 weeks, all experimental groups exhibited extensive osteointegration as supported by MicroCT analysis and torsion tests, with fully mineralized callus observed in the BMP-2/7-treated group (Figs. 1D-F).

ACKNOWLEDGEMENTS:

CTS Pilot Project Program (UMMS); IR01AR055615 (NIAMS).

REFERENCES: