Evaluation of Biodegradable Hydrogels in a Femoral Bone Plug Model

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Statement of Purpose

Poly(β-amino ester) (PBAE) hydrogels are of interest because of their controllable degradation rate and mechanical properties, and the simplicity of their synthesis [e.g., Adv Mater 18:2614, 2006]. PBAE macromers and hydrogels have been investigated as drug delivery devices for applications including gene therapy and scaffolds for soft and hard tissues. PBAEs have *in vitro* toxicity similar to common implantable materials such as PLGA, and cells were capable of growing on their surfaces [e.g., Acta Biomater 7:1956, 2011]. PBAE hydrogels have potential utility for orthopedic applications, but there is little information on the *in vivo* effects of these materials in bone. Therefore, the aim of this study was to investigate the degradation behavior of, and the tissue response to, PBAE hydrogel implants in non-critically sized femoral defects in rats.

Methods

Hydrogel Plug Formation: Diethylene glycol diacrylate (A) and poly(ethylene glycol) 400 diacrylate (H) were used with isobutylamine (6) to create A6, H6, and AH6 (2:1 A to H) degradable hydrogels [Polymer 54:4422, 2013]. Cylindrical gels were made using a chemical polymerization method with ammonium persulfate and tetramethylethylenediamine as initiator. Tygon tubing with an inner diameter of 3.5 mm was used as a mold. Samples were disinfected in 70% ethanol.

Surgical procedure: Animal studies were conducted in accordance with a protocol approved by the Institutional Animal Care and Use Committee. Samples were press fit into 2.5 mm diameter unicortical defects in the distal femur of male Sprague-Dawley rats. Each leg was randomly assigned to receive one of the following implants: H6, AH6, A6, or control (empty). Femurs were harvested at increasing times post-implantation and immediately fixed in 10% neutral buffered formalin.

Evaluation: Microcomputed tomography (microCT) was used to monitor the extent of bone tissue regeneration and defect closure with and without PBAE plugs. Using a Scanco Medical μCT-40 scanner, specimens were evaluated using a 36 μm voxel size. Following microCT analysis, representative samples from each experimental group were characterized for bone ingrowth and signs of inflammation using undecalcified histology.

Results

The bone ingrowth trends between histological sections and microCT cut planes were in close agreement. Figure 1 shows images of the defects during material degradation and bone healing. Similar growth patterns were observed for empty defects and those filled with H6 or AH6, with little or no visible bone tissue within the defect site until 2 weeks post-surgery. Bone growth began from the defect margins, or the edges of the embedded hydrogel plugs. Bone growth followed this pattern even in empty defects, with new bone first seen at the contours of the defect edges. Through 8 weeks, this bone tissue organized into a more trabecular structure and grew toward the absent portion of the cortical shell, eventually re-forming the missing cortex. This pattern was consistent for empty defects as well as those filled with H6 or AH6. H6 was fully degraded within 1 week, AH6 was fully degraded by 2 weeks, and A6 remained present through 12 weeks. In A6-filled defects, the unstained outline of the implant was clearly visible at 8 and 12 weeks, however there were clear signs of bone growth around the edges of the implant. In no case did the bone tissue in PBAE-filled defects differ visually from that found in empty defects.

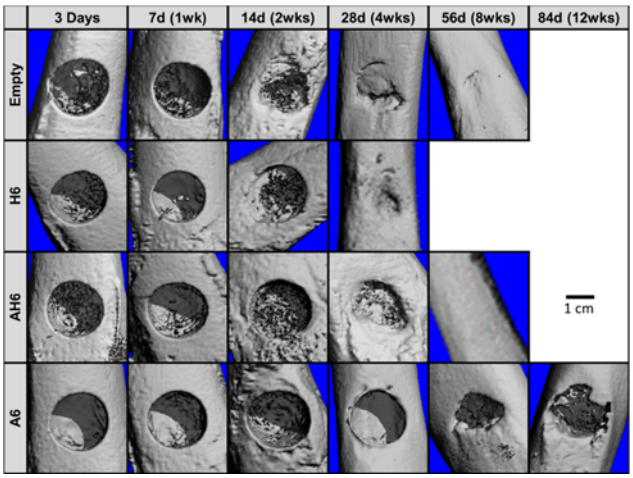

Figure 1. Representative microCT reconstructions of defects from each treatment group.

Conclusions

The aim of this study was to investigate biological responses when biodegradable PBAE hydrogels were implanted in non-critically sized femoral defects in rats. Three different PBAE hydrogels were fabricated to compare a wide range of degradation times. Both microCT and histological analysis of bone formation in the defects revealed similar growth patterns for empty defects and those filled with H6 or AH6, which may indicate that these two faster degrading PBAE hydrogels do not interfere with normal bone repair. Importantly, the lack of any obvious signs of inflammation or fibrous encapsulation suggest that these materials remain inert as they degraded. Interestingly, there were no signs of fibrous encapsulation in the A6-filled defects even through 12 weeks. In fact, the A6-filled defects showed bone growth along all margins of the defect where hydrogel resided, suggesting that PBAE hydrogels may exhibit osteoconductive properties allowing for new bone to grow on the surface of the gels.

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