Effect of Tricalcium Phosphate Incorporation in Poly(ε-caprolactone) Scaffolds for Bone Tissue Engineering

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Introduction:

Bone grafting is widely used to treat acute fractures, fracture non-unions, and bone defects. Autogenous cancellous bone, the gold standard for treatment, provides an osteoconductive and osteoinductive matrix for bone re-growth but has numerous shortcomings, such as availability, morbidity of graft harvest, and infection risk. Using a technique known as Three Dimensional Printing (3DP™, MIT, Cambridge, MA), we can create biodegradable polymeric scaffolds with the defined architecture and internal geometry allowing for cellular migration, proliferation, and deposition of bone matrix throughout the scaffold interior. Incorporation of tricalcium phosphate (TCP) particles in the scaffold walls provides osteoinductive signals to enhance the rate of new bone formation. *In situ* perfusion of bone marrow aspirate into the scaffold provides an exogenous source of osteogenic stem cells and progenitor cells to further enhance the bone formation rate. We investigated two scaffolds – with and without TCP – to assess the effect of the osteoinductive material on scaffold efficacy *in vivo* utilizing a canine femur defect model.

Methods:

Scaffold construction: Synthetic matrix implants were prepared as 9.5 (mm) diameter x 10 (mm) cylinders and internal waffle architecture, with channel size of 1000 (microns) and wall thickness of 500 (microns), using 3DP™. 3DP™ creates three-dimensional objects as a series of layers built up in a step-by-step fashion. Poly(ε-caprolactone) [PCL], sucrose, lactose, and TCP particles of diameter < 106 (microns were blended prior to printing. Control scaffolds were prepared with a blend of 20% PCL and 40% each sucrose and lactose, by weight. The +TCP blend was 20% PCL, 20% TCP, and 30% each sucrose and lactose. Water was used as the binder, causing partial dissolution of the sucrose and lactose. After drying and free powder removal, scaffolds were placed in a chloroform vapor chamber, binding the PCL particles. Scaffolds were soaked in a water bath for 6 hours to remove the excipients. Finally, scaffolds were sterilized with ethylene oxide.

Scaffold Preparation, Implantation, and Analysis: Bone marrow was aspirated from the proximal humerus of canine subjects. Marrow was passed through the matrices at a constant linear flow rate of 25mm/min. Aliquots of initial and effluent marrow were collected. Cell counts and CTP assays were performed on all samples. A volume-based loading concentration of 4X, relative to total matrix volume, was used. The left proximal femur was exposed from a lateral sub-bicep incision and four 1.0 (cm) diameter and 1.5 (cm) deep drill hole defects were created. Loaded matrices were implanted into the defect and capped to prevent displacement. Animals were euthanized at 4 weeks. Defect tissue was processed for undecalcified histology. Bone formation in the graft site was assessed using microCT before sectioning. Total area fraction of bone formation as well as mineral density were primary outcome parameters.

Results:

Scaffold Construction: Control and +TCP scaffolds had wall void volumes of 18±5 and 38±4%, respectively, and a total void volume of greater than 90% for both groups. SEM analysis of the scaffolds indicated complete clearing of unbound PCL and TCP particles, and tight control of internal architecture.

Implantation: Prior studies indicated little enhancement of scaffold performance for perfusion concentrations of > 4x, but slight improvement in bone formation when marrow clot formation was allowed before implantation. Control scaffolds exhibited 2.0±0.5 fold concentration of marrow cells retained, with 3.4±0.4 fold concentration of connective tissue progenitor cells (CTPs) in the matrix. Analysis with microCT indicated less than 15% bone volume at scaffold perimeter, with less than 5% at the interior for all scaffolds.

The scaffolds containing TCP (+TCP) exhibited 3.3±1 fold concentration of total marrow cells retained, with 4.4±0.9 fold concentration of CTPs. Analysis with microCT indicated significantly greater bone formation, with the outer 2 (mm) of the scaffold occupied by bone at density greater than 20%, and the interior at 10% bone density.

Conclusions:

The use of synthetic polymeric materials for bone tissue engineering applications is problematic due to their lack of any significant osteoinductive properties. Previous work had indicated that altering the scaffold architecture to produce a more osteoconductive environment, and concentration of CTPs in the scaffolds by up to 12 fold, were not sufficient to significantly enhance the rate of bone formation in these synthetic matrices. 3DP™ allows the flexibility of incorporating other materials into the scaffolds to enhance osteoinductivity. In this study the inclusion of TCP, the mineral component of bone matrix, proved efficacious in significantly increasing the rate of new bone formation *in vivo*. An alternative, currently under investigation in our labs, is to alter the surface properties of the polymeric component to create selectivity for adhesion and proliferation of progenitor cells. This approach would eliminate the need for inclusion of additional synthetic materials that may remain in the matrix long after their osteoinductive properties are necessary.