Calcium alginate porogens for the development of an injectable paste to treat traumatic fractures

Buchanan, R.[a,b] ; Murphy, M.[b]; Yoon, D.[c]; Henslee, A.[c]; Nair, M.[c]; Kasper, K.[c]; Simmons, P.[d]; Mikos, A.[c]; Ferrari, M.[a,b,c]; Tasciotti, E.[b]

[a]The University of Texas at Austin, Austin, TX [b]The Methodist Hospital Research Institute, Houston, TX [c]Rice University, Houston, TX [d]The University of Texas Health Science Center at Houston , Houston, Texas

Statement of Purpose: Porosity of tissue engineering scaffolds is essential for tissue in-growth, vascularization and nutrient supply (Jones JR. *Journal of European Ceramic Society*. 2009;9:1275-1281), yet high porosity inevitably compromises the mechanical properties of the material. Therefore, we developed an *in situ* crosslinkable, injectable putty using an alginate porogen delivery system for enhanced bone regeneration. Alginate porogens serve three functions: 1) to provide immediate mechanical stability to the scaffold 2) to protect and deliver cells and biological molecules essential for accelerating the regenerative process: chemokines and mitogens contained in platelet-rich plasma (PRP) and poly(lactic-*co*-glycolic acid) (PLGA) microparticles for sustained delivery of differentiating factors and 3) to create interconnected pores within a polymeric scaffold *in vivo* to invoke the infiltration of regenerating tissue.

Methods: To create sodium alginate porogens sized 200-500µm, an insoluble calcium complex was dispersed in an aqueous phase containing sodium alginate and bioactive components (Fig 1 left Panel) The aqueous phase was added to the oil phase with a surfactant present and continuous stirring to form a stable emulsion (Center Panel) An oil soluble acid was then added to the mixture reducing the pH and triggering the release of calcium ions from the calcium complex to initiate gelation of the formed microspheres (Right Panel).

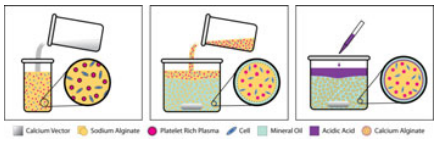

Figure1. Scheme production of calcium alginate beads by internal gelation/emulsion

To evaluate the mechanical integrity the porogens contribute to a polymeric construct, Poly (propylene fumarate) (PPF)/alginate scaffolds were created using Teflon molds. Briefly, the PPF monomer was diluted with N-vinyl-2-pyrrolidone prior to dispersion. A mixture of 80% alginate porogens in PPF (w/w) was mechanically stirred. Benzoyl peroxide was then added to initiate the crosslinking of PPF along with N, N-Dimethyl-p-toluidine to accelerate the reaction. The mixture was then poured into the Teflon mold and placed at 60 degrees Celsius overnight to accelerate crosslinking.

Calcium alginate beads were synthesized by the emulsion process described above with the incorporation of CSFE stained rat mesenchymal stem cells (25µm staining solution) into the aqueous alginate phase. Cells were resuspended in the alginate/PRP solution at 2 million cells per ml. The emulsion was then performed as described above thereby creating alginate and PRP beads encapsulating live cells.

Alginate beads with cells, PRP and PLGA microparticles loaded with BMP-2 were gelled together with fibrinogen and thrombin to form 8mm diameter x 1.5mm thick scaffolds and implanted subcutaneously on the backs of Lewis Rats to confirm biocompatibility, biodegradation, angiogenic and osteogenic potential.

Results: Mechanical testing using ASTM standards was performed on crosslinked scaffolds (Fig 2) to determine whether the incorporation of alginate porogens provided temporary mechanical stability by filling the voids that would otherwise be present with pre-fabricated porous scaffolds. Results showed the porogens provide an 8-fold increase in mechanical strength (1.065MPa to 8.835MPa) compared to pre-fabricated PPF porous scaffolds by temporarily filling the voids until they are degraded by the physiological environment. Furthermore, the elastic modulus of the porogen composite (281.9 MPa) provides a significantly closer match to that of trabecular bone within the vertebral body (165-291MPa) (M.A.K. Liebschner. *Biomaterials*. 2004; 25: 1697-1714)
than the current PMMA standard (48-76 MPa). The ability of the alginate porogen to encapsulate cells and maintain viability upon incorporation into the PPF matrix as well as to preserve growth factor bioactivity was confirmed (Fig 3). Lastly, an *in vivo* study has shown significant vascularization of the scaffold at 2 weeks.

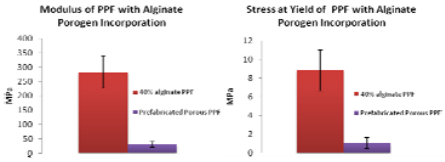

Figure 2. Elastic Modulus (left) and stress at offset yield (right) of PPF/Alginate composite scaffolds compared to prefabricated porous PPF scaffolds

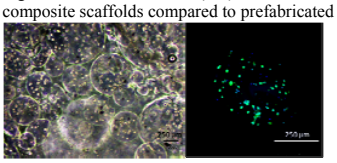

Figure 3. Optical image (left) of cells encapsulated within alginate beads. Confocal microscopy image (right) displaying CSFE stained mesenchymal stem cells (green) and alginate stained with Draq-5 (blue)

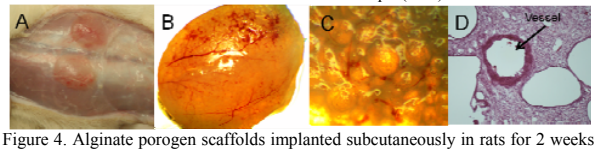

Figure 4. Alginate porogen scaffolds implanted subcutaneously in rats for 2 weeks (A-C) H&E stain of scaffold slice (D)

Conclusions: The mechanical properties comparable to those of trabecular bone, together with the ability of alginate to preserve the biological activity of its components within the putty make this system an ideal candidate for fracture repair. Through the fabrication and optimization of the various components, a matrix has been created with the necessary mechanical reinforcement, cell viability, and staggered release essential for optimal fracture healing. A continuation of the material optimization and bone growth characterization will occur, including an evaluation of bone ectopic development at 4 and 8 weeks.