Healing Bone Using a Biomimetic Tubular Sintered Microsphere Matrix: *In Vitro* Polymer Evaluation and *In Vivo* Bone Regeneration

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Statement of Purpose: Biodegradable polymeric microspheres have been used to fill bone defects or joined to form porous, three-dimensional scaffolds able to support bone regeneration *in vivo* (1-2). To advance bone repair using sintered microsphere technology, a tubular sintered microsphere matrix was designed (Figure 1). By leaving the center of the matrix void, this scaffold more closely mimics the bone marrow cavity observed in native bone.

Previous studies by our laboratory, using an 85:15 poly(lactide-*co*-glycolide) (PLAGA) sintered microsphere matrix, demonstrated bridging bone on the external surface of scaffold at 8 weeks post-implantation; however, the majority of the matrix remained (2). To hasten scaffold degradation, without compromising mechanical properties, a higher molecular weight PLAGA material comprised of 20% glycolic acid was selected. We hypothesized that tubular scaffolds fabricated from 80:20 PLAGA ($\overline{Mw}$ 150,000) would demonstrate increased initial mechanical properties and a faster degradation profile as compared to scaffolds fabricated using 85:15 PLAGA ($\overline{Mw}$ 90,000).

In vitro scaffold degradation was monitored for 6 months. The scaffolds are being evaluated *in vivo* for their ability to support bone regeneration in a critical-sized ulnar defect model in rabbit. Long-term characterization of the scaffold's degradation profile, mechanical properties, and *in vivo* performance are necessary to evaluate its potential for transfer from the research laboratory to the clinic.

Materials and Methods:

Scaffold Fabrication: Polymeric microspheres 600-1000μm in diameter were fabricated using a solvent evaporation technique (1), loaded into a stainless steel mold, and sintered to form porous tubular scaffolds.

Scaffold Degradation Study: Scaffolds were individually placed in centrifuge tubes with 5ml PBS supplemented with 1% antibiotics, pH = 7.4. The tubes were in a shaker water bath at 37ºC, 250RPM. Each week, the pH of the degradation solution was measured (n=6) and the solution replaced with fresh degradation media.

Quantitative total pore area and median pore diameter measurements of the scaffold specimens (n=6), were determined bi-weekly using mercury porosimetry (Autopore III, Micromeritics) (3).

Scaffold specimens (n=6) at a length to diameter 2:1 were used for compression testing on a weekly basis. An Instron Testing Apparatus (model 5544, Instron) was operated at a ramp speed of 1 mm/min to implant failure (4). For each specimen, compressive modulus, compressive strength, maximum compressive load, and energy absorbed at failure were measured.

To quantify polymer degradation, matrices (n=6) were dissolved in tetrahydrofuran at a concentration of 1%(w/v) on a weekly basis. The solution was analyzed using Gel Permeation Chromatography (model 1100, Hewlett Packard) equipped with a Zorbax PSM 1000S, 300S, or 60S column.

Critical-Sized Ulnar Defect Model: Institutional ACUC approved all experiments. 80:20 or 85:15 PLAGA tubular sintered microsphere scaffold was implanted in a critical-sized (15mm) New Zealand White rabbit ulnar defect model. Healing is scheduled to progress over 24 weeks. Results will include quantitative densitometry, microcomputed tomography, and histological stain.

Results and Discussion:

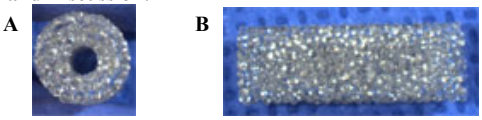
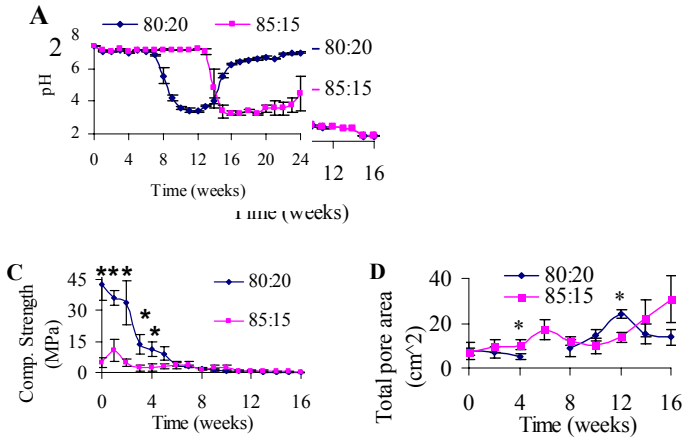


Figure 1: *Scaffold Fabrication:* Photograph showing the (**A**) top view and (**B**) side view of the 85:15 PLAGA tubular sintered microsphere matrix. The scaffold dimensions are 5mm diameter x 15mm length. The tubular design may overcome the limitations of nutrient and waste exchange at the center of the matrix during *in vivo* implantation.

Figure 2: *Scaffold Degradation:* Over the first 7 weeks, the 80:20 PLAGA scaffold experienced a large drop in molecular weight (Fig 2B). This loss directly translated to a loss of mechanical properties (Fig 2C, $p<0.05$). During this time, water entered the polymer material and fragmented the weaker ester bonds. After week 7, the monomer products of degradation that are acidic in nature registered a drop in pH (Fig 2A). By week 15, the amorphous regions of the 80:20 PLAGA were degraded. At weeks 16-24, the pH remained near neutral as the crystalline regions of the polymer resisted degradation. However, as the scaffold had already undergone significant bulk degradation, the mechanical properties remain compromised.



In contrast, the 85:15 PLAGA scaffold underwent a steady linear drop in molecular weight (Fig 2B), which corresponded to a steady decrease in mechanical properties (Fig 2C). The pH drop occurred at week 14, which was approximately double in time to that observed by the 80:20 PLAGA (Fig 2A). The degradation solution remained acidic throughout the remainder of the study as monomers were continually released. As the scaffolds degraded, both indicated an increase in pore area (Fig 2D, $p<0.05$). This corresponds to an increase in area available for newly regenerating tissue.

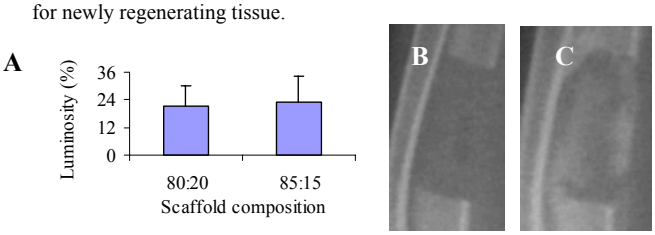


Figure 3: *Critical-Sized Ulnar Defect Model:* Percent increase in luminosity from 0 to 4 weeks for scaffolds fabricated from 80:20 or 85:15 PLAGA (Fig 3A). Fig 3B and 3C are radiographs of 80:20 PLAGA scaffold implanted in the rabbit ulna at 0 and 4 weeks post-implantation, respectively. Note the formation of mineralized tissue is restricted to the scaffold and the center remains devoid of mineralized tissue.

Conclusion: A higher glycolide content in PLAGA will hasten material degradation and provide an increased area for new bone formation. A higher molecular weight will contribute to an increase in the mechanical properties, so that the compressive properties of the scaffold will not be compromised prior to the formation of mineralized tissue at the defect site. *In vitro* degradation studies indicated scaffolds fabricated from 80:20 PLAGA showed significantly greater mechanical properties as compared to 85:15 PLAGA during the first 4 weeks. The

molecular weight and pH measurements indicated the higher glycolide content of the 80:20 PLAGA contributed to the polymer showing signs of degradation at 4 weeks, while the 85:15 PLAGA showed similar signs at 14 weeks.

One rabbit was euthanized and removed from the study due to a radial fracture. All other rabbits are at 14 weeks post-implantation and in good health. While the optimal bone regeneration matrix would likely include the use of regenerative agents, such as growth factors, autologous cells, or genetically-modified cells, the goal of this project was to evaluate regeneration comparing the two PLAGA ratios.

References:

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