Bioresponse of Mammalian Kidney to Implantation of Polymeric Materials

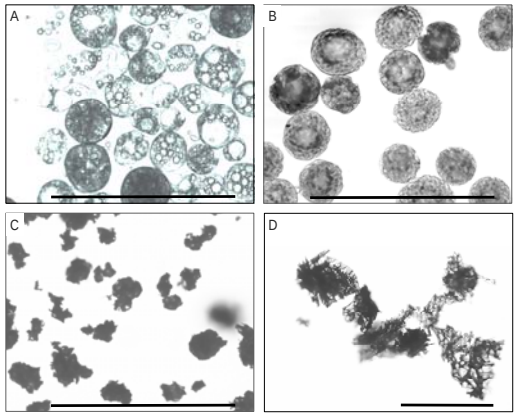
Richard Payne, Toyin Knight, Joydeep Basu, Elias Rivera, Neil Robbins, Darell McCoy, Manuel Jayo, Craig Halberstadt, Deepak Jain

Tengion, Inc., Science & Technology, Winston-Salem, NC

Introduction:

Biomaterials can modulate regenerative outcomes in tissue engineering or regenerative medicine applications by facilitating cell attachment and delivery and by providing a physical substrate for tissue infiltration[1]. This study investigated host tissue responses to intra-renal injection of biomaterials in rodent kidneys to identify candidate biomaterials for forming cell/biomaterial composites with selected regenerative cell populations[2]. Natural and synthetic biomaterials were evaluated in both spherical bead and irregular particle forms. The ultimate goal of this research is to develop Neo-Kidney Augment prototypes that delay the need for dialysis and improve renal function in patients with chronic kidney disease.

Figure 1. Representative images of materials implanted into the parenchyma of rat kidneys to evaluate biocompatibility and safety for cell/material constructs to support tissue regeneration. PLGA beads (A), gelatin beads (B), PLGA particles (C), and gelatin particles (D). Scale bars = 1 mm.



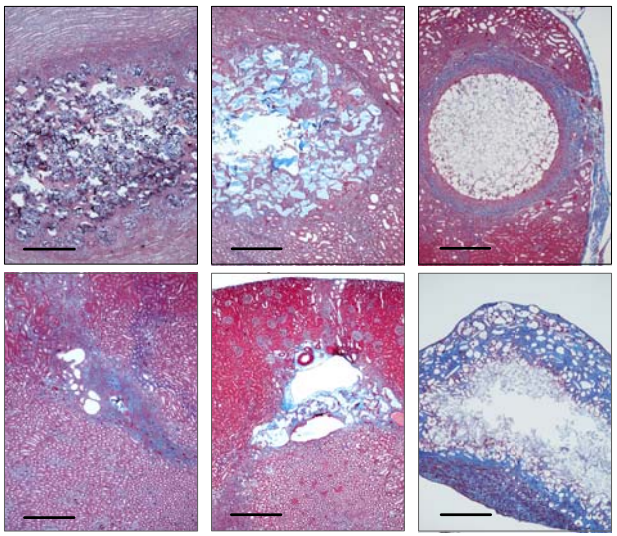
Materials and Methods:

Natural biomaterials included gelatin and hyaluronic acid (HA). Synthetic biomaterials included polycaprolactone (PCL) and poly-lactic-co-glycolic acid (PLGA). Candidate biomaterials were evaluated in two discrete physical conformations: homogenous, spherical beads or heterogeneous and non-uniform particles. PCL and PLGA beads (Figure 1A) were prepared using a modified double emulsion (water/oil/water) solvent extraction method. Gelatin beads (Figure 1B) were purchased (Cultispher-S®, Sigma-Aldrich, St. Louis, MO). PLGA particles were prepared using a solvent casting porogen leaching technique (Figure 1C); gelatin and HA particles were prepared from cross-linked, lyophilized foam (Figure 1D). Following PHS and IACUC guidelines of the Carolinas Medical Center, two injections of 35 µl of loosely packed biomaterials were delivered to the left kidney parenchyma of healthy 3 month old Lewis rats. Histological evaluation of formalin-fixed sections of kidney tissue at 1 and 4 weeks post-injection was conducted to assess biomaterial safety and biocompatibility. The evaluation used a semi-quantitative grading severity scale from 0 (absent) to 4 (marked) of attributes that were identified as those that tend to either promote or inhibit tissue regeneration ("Positive" or "Negative"). Attributes favoring regeneration include tissue/cellular in-growth and neo-vascularization. Inhibitory attributes include inflammation, fibrocellular response, foreign body reaction, evidence of infarction or necrosis, presence of eosinophils, and persistence of biomaterials.

Results:

Histological evaluation was performed on biomaterial candidates to evaluate their suitability for use in a NeoKidney Augment product. Representative images of kidneys harvested 1 week and 4 weeks post-implantation were made from sections stained with Masson's Trichrome (Figure 2). Materials composed of polymers of natural origin, such as gelatin (left) and HA (center) were associated with milder fibro-cellular response and chronic inflammation, and greater cellular in-growth, neo-vascularization, biomaterial degradation, and necessary inflammation required for tissue healing and integration when compared to the synthetic biomaterials, such as PLGA (not shown) and PCL (right – note organized fibrous encapsulation at 1 week and evidence of necrosis and marked fibrosis at 4 weeks).

Figure 2: Representative histology images of materials implanted into rat kidney and harvested 1 week (Top) and 4 weeks (Bottom) post-implantation. Left - Gelatin beads, Center - HA particles, Right - PCL beads. Masson's Trichrome, 40X. Scale bars = 1mm. For the PCL, note lack of tissue integration and fibrous encapsulation at 1 week and substantial fibrotic and necrotic outcome at 4 weeks. Conversely, Gelatin and HA exhibited tissue integration and minimal fibrosis at 1 week, and significant biomaterial resorption and integration at 4 weeks, consistent with acceptable biosafety profile and potential for supporting regenerative outcomes.



Histological evaluation scoring. Overall scores were calculated as the ratio of % Positive to % Negative response (the higher the overall score the more likely the biomaterial will promote regeneration when combined with the selected regenerative cells as part of a NeoKidney Augment prototype). Scores were averaged by material composition (Figure 3) or by material form (Figure 4). Values are represented as mean±SD.

Figure 3. The synthetic materials (PLGA and PCL) scored the lowest, and gelatin materials generally scored higher than HA materials. This trend is more pronounced at the 4 week time point. N=3 for all groups.

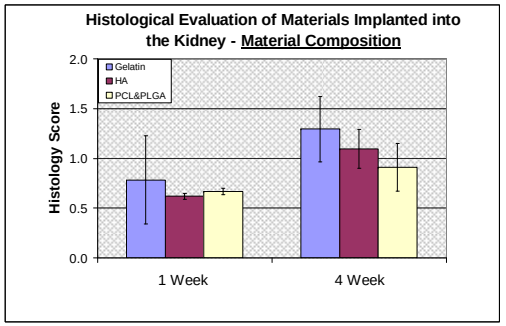
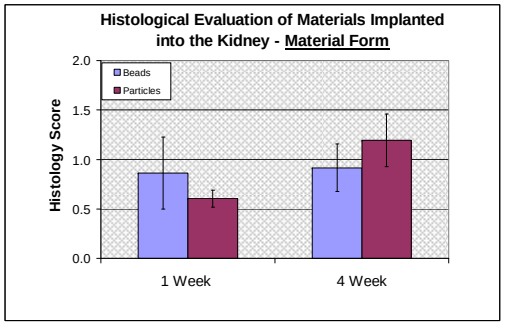


Figure 4: There was no consistent trend on the effect of the form of the implant material (beads or particles) on the evaluation score. For the beads N=3, for particles N=6.



Conclusions:

- Biomaterials of natural origin promoted neo-vascularization, had minimal inflammatory response to degradation and integrated into the native tissues (i.e. gelatin and HA).
- Biomaterials of synthetic origin elicited a chronic inflammatory reaction with fibrosis and limited integration into native tissues (i.e. PCL and PLGA)
- The physical form (bead or particle) of either synthetic or natural materials did not appear to have an effect on the tissue responses
- Natural biomaterials may serve as acceptable support for kidney tissue regeneration.

References:

1. Basu J. Trends Biotechnol. 2010; 28 (10): 526-33
2. Presnell SC. Tissue Eng Part C Methods. 2011 Mar;17(3):261-73.

Acknowledgements:

We thank Kim Mihalko (Carolinas Medical Center) for animal surgeries.