Animal Models for Biomaterials Screening and Neo-Kidney Augment Prototype Evaluation in the Kidney

Richard Payne, Toyin Knight, Joydeep Basu, Elias Rivera, Kim Mihalko*, Neil Robbins, Darell McCoy, Craig Halberstadt and Deepak Jain.

Tengion Inc., Winston-Salem, NC, USA; *Carolinas Medical Center, Charlotte, NC, USA.

Statement of Purpose: Chronic kidney disease (CKD) is continued loss of renal function over time. Current renal therapies include dialysis and kidney transplant. An unmet need exists for new treatments to restore renal function thus delaying dialysis/transplant. We have identified populations of tubular epithelial cell-enriched primary renal cells (Selected Renal Cells, SRC) that positively impact aspects of disease phenotype in rodent CKD models. The addition of biomaterials may facilitate cellular engraftment by enhancing product stability and allowing for targeted delivery. Here, we report on development of Neo-Kidney Augment (NKA) product prototypes, comprised of SRC/biomaterial combination constructs which facilitate regeneration of kidney tissue. A rational, iterative series of animal models was used to identify appropriate SRC/biomaterial candidates. Biomaterials identified from literature were initially screened for bio-response in a healthy rodent kidney model, then further evaluated within progressively more diseased rodent models of CKD. Final evaluation of the NKA product prototypes was performed in a clinically relevant large animal model of CKD.

Methods: Identification of biomaterials appropriate for renal tissue engineering was accomplished by iterative screening and evaluation of bio-response to implantation within parenchyma of healthy kidney as follows: (1) Healthy adult rodents (*n*=200) were used to identify biomaterials associated with minimal inflammatory and fibrotic outcomes, cellular infiltration and neo-kidney tissue induction. Advantages of this model include availability, cost, and ease of generation, limited histological endpoints. (2) Removal of single kidney from rodents (hemi-nephrectomy/Hemi-Nx) eliminates any confounding effects or compensation from the contra-lateral kidney. Hemi-Nx rodents (*n*=20) were injected with NKA prototypes within renal parenchyma of remnant kidney. Physiological indices derived from whole blood, serum and urine chemistries were evaluated at 2 and 4 week time points post-implantation. Animals were sacrificed at 4 weeks post-injection and remnant kidney examined histologically for evidence of inflammatory or fibrotic bio-response. Advantages of Hemi-Nx model are similar to healthy kidney model. (3) 5/6th nephrectomy (5/6-Nx) model consists of removal of one kidney and 2/3 of remnant kidney mimicking aspects of CKD in humans, permitting evaluation of bio-response to implantation of NKA prototypes with clinical relevance. 5/6-Nx rodents (*n*=20) were implanted and evaluated as described above for Hemi-Nx rodents. (4) This reduced mass model was adapted to a large animal, allowing for material to be implanted using clinically relevant dosing strategies. 5/6-Nx canines (n=4) were implanted and evaluated as described above for 5/6-Nx rodents.

Results: Of the biomaterial candidates examined, implantation of gelatin-based hydrogels within healthy rodent renal parenchyma was associated with minimal fibrotic and inflammatory outcomes and concomitant neovascularization, cell and tissue infiltration and biomaterial degradation within 4 weeks post-implantation; by 8 weeks, neo-kidney tissue was observed, suggesting regenerative response induction in vivo (Illustrative results shown in Figure 1). Implantation of NKA prototypes within remnant renal parenchyma of hemi- or 5/6-Nx rodent kidneys presented minimal evidence of inflammatory, necrotic or fibrotic bio-response and did not significantly affect key renal physiological indices. NKA treatment appears to be well tolerated in the canine 5/6-Nx model; no morphological alterations were observed in the tubular or glomerular compartments other than expected renal compensatory hypertrophy (Illustrative results shown in Figure 2).

Conclusions: A rational, iterative approach to biomaterial screening and prototype evaluation was employed during the development of NKA product. Initial screening of biomaterials in healthy rodent kidneys demonstrated that gelatin-based hydrogels are biocompatible and elicited minimal inflammatory response. Next, testing of NKA product prototypes within remnant renal parenchyma of diseased rodent kidneys, which did not significantly affect key renal physiological indices, and presented minimal evidence of inflammatory, necrotic or fibrotic bio-response. Similarly, when scaled up to a reduced kidney mass model of CKD in canines, treatment with NKA appears to have been well tolerated. This methodology of leveraging an iterative series of animal models presenting progressively more pronounced CKD symptomatology identified SRC/gelatin-based hydrogel construct as an appropriate NKA product prototype that may promote regenerative outcomes. Additionally, this approach may serve as a model platform system for the rapid screening and identification of cell/biomaterial construct candidates for application in organ-level tissue engineering and regenerative medicine.

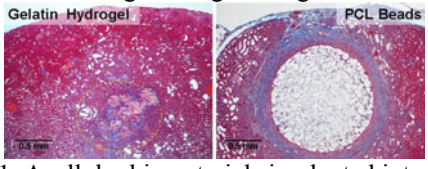

Figure 1. Acellular biomaterials implanted into healthy rodent kidneys at 1 week.

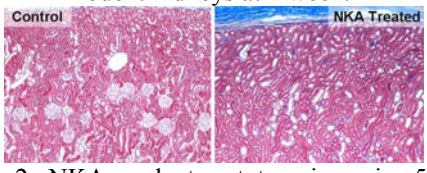

Figure 2. NKA product prototype in canine 5/6-Nx kidneys at 47-weeks