Mammalian Kidney Nephrogenic Response to Primary Renal Cell/Biomaterial-based Neo-Kidney Augment™ Prototype

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Introduction: Development of a tissue engineered Neo-Kidney Augment (NKA) requires evaluation of defined, therapeutically relevant cell and cell/biomaterial composites for regenerative potential in mammalian kidney. Previous work evaluated *in vivo* responses to cell-free hydrogel-based biomaterial NKA prototypes[1] and the bioactivity of defined renal cell populations[2]. This report provides evidence that intra-parenchymal delivery of a cell/hydrogel NKA prototype triggered induction of neo-kidney tissue in healthy Lewis rat kidneys within 4 weeks post-implant suggesting that this NKA prototype could potentially modulate renal regeneration in a mammalian model of chronic kidney disease. To the best of our knowledge, the current study and Basu et al.[1] are the first *in vivo* and intra-renal investigations of the biological response of mammalian kidney to implantation of a therapeutically-relevant primary renal cell/biomaterial composite.

Materials and Methods: Primary renal cells and defined subpopulations used for *in vitro* assays and/or to produce NKA Constructs (cells+biomaterial) were isolated from rodent, canine, and human kidneys[3]. Cell phenotype of the NKA Constructs was evaluated *in vitro* by Live/Dead staining, confocal imaging, quantitative RT-PCR, and analysis of proteomic and secretomic profiles. For the illustrative data presented in Figure 2, Cultispher-S gelatin beads (Sigma) were seeded overnight with 2.5 X 10^6 cells. To evaluate the *in vivo* response to NKA Construct implantation, 35µl of loosely packed NKA Construct was microinjected into the left kidney parenchyma of healthy 3-month old female Lewis rats. Injections were directed (i) from the pole in parallel to the cortex and/or (ii) from the cortex to the pelvis. Fixed kidney sections prepared at 1 and 4 weeks post-injection were evaluated for inflammation, fibrosis, foreign body reaction, neo-vascularization, biomaterials degradation, necrosis, and tissue infiltration.

Results: Cells in NKA Constructs were viable (Figure 1) and predominantly exhibited a tubular, epithelial phenotype *in vitro*. All NKA Construct-implanted animals survived the study. Trichrome and Periodic Acid Schiff (PAS) staining of tissues harvested at 1 and 4 weeks post-injection (Figure 2) showed that, at 1 week post injection, gelatin beads were present as focal aggregates (left panel, circled area) of spherical and porous material staining basophilic and surrounded by marked fibrovascular tissue and phagocytic multinucleated macrophages and giant cells. The fibrovascular tissue surrounded the bead's surface and pores with tubular epithelial components indicative of neo-kidney tissue formation. Additionally, tubular (t) and vasculo-glomerular (g) structures were identified by morphology (PAS panels). By 4 weeks post-injection, the hydrogel was completely resorbed and the space replaced by progressive renal regeneration and repair with minimal fibrosis (note the numerous functional tubules within circled area of 4 week Trichrome panel).

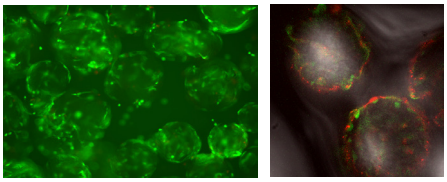
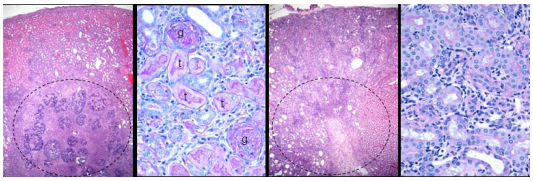


Figure 1. Live/Dead staining (left panel) and confocal imaging (right panel, Ecadherin-red / Dolichos biflorus agglutinin-green) analysis of NKA Constructs.



--1 week post-implant-- --4 week post-implant--

Figure 2. NKA Construct injection site showing regeneration of early tubules and glomeruli.

Discussion and Conclusions: NKA Construct implantation into healthy renal tissue was well-tolerated and associated with minimal fibrosis and inflammation and moderate cellular in-growth and neo-vascularization at 4 weeks post-implantation, providing preliminary evidence that a regenerative response was induced *in vivo*. Implantation of NKA Construct into the context of the host organ tissue may facilitate regeneration of neo-kidney tissue and attenuate non-regenerative (e.g., reparative healing) responses. Ongoing studies will extend these results by investigating the regenerative effect of NKA Constructs in established animal models of chronic renal disease.

References:

1. Basu J, et al. *In vivo* evaluation of biomaterials in mammalian kidney. TERMIS-NA, submitted, 2010.
2. Kelley R, et al. A tubular cell-enriched subpopulation of primary renal cells improves survival and augments kidney function in a rodent model of chronic kidney disease. *Am J Physiol Renal Physiol*, submitted, 2010.
3. Presnell SC, et al. Isolation, characterization, and expansion (ICE) methods for defined primary renal cell populations from rodent, canine, and human normal and diseased kidneys. *Tissue Eng Part C*, submitted, 2010.

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