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## Introduction:

Development of a Neo-Kidney Augment (NKA) combination product requires evaluation of selected regenerative cell/biomaterial NKA Product Prototypes for regenerative potential in mammalian kidney. Previous work evaluated *in vivo* responses to cell-free hydrogel-based biomaterial NKA prototypes<sup>1</sup> and the bioactivity of selected regenerative renal cell populations<sup>2</sup>. This report provides evidence that intra-parenchymal delivery of renal cell/hydrogel NKA Product Prototypes triggered induction of neo-kidney tissue in healthy Lewis rat kidneys within 4 weeks post-implant suggesting that NKA Product Prototypes 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.<sup>1</sup> are the first *in vivo* and intra-renal investigations of the biological response of mammalian kidney to implantation of a selected regenerative renal cell/biomaterial NKA Product Prototype.

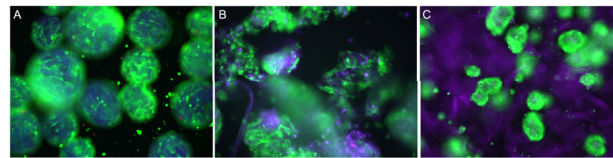
## Materials and Methods:

Therapeutically-relevant renal cell populations were isolated from rodent, canine, and human kidneys<sup>3</sup> for *in vitro* assays and to produce NKA Product Prototypes. Cell phenotype of the NKA Product Prototypes was evaluated *in vitro* by Live/Dead staining, confocal imaging, and analysis of proteomic and secretomic profiles. NKA Product Prototypes were produced with selected regenerative renal cells and one of three biomaterials: gelatin beads (Gel), hyaluronic acid (HA) particles, or HA/gelatin particles (HA/Gel). To evaluate the *in vivo* response of renal tissue to NKA Product Prototype implantation, 35µl of loosely packed NKA Product Prototype 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, 4, and 8 weeks post-injection were evaluated for fibrosis, foreign body reaction, neo-vascularization, biomaterials degradation, necrosis, and tissue infiltration.

## Results:

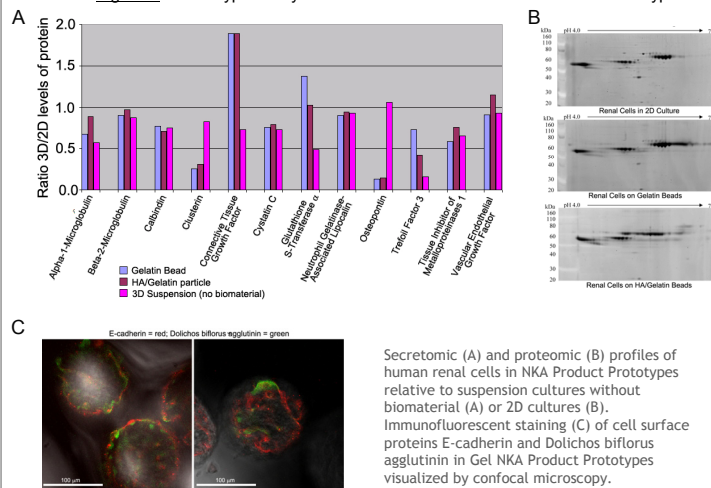
Renal cells derived from canine, rat, or human kidneys in NKA Product Prototypes produced with Gel, HA, or HA/Gel were viable (Figure 1) and retained a comparable phenotype relative to cells from 2D cultures as assayed by proteomic and secretomic profiling (Figure 2) and immunofluorescence (Figures 2 and 3). To evaluate *in vivo* response, rat NKA Product Prototypes were produced with Gel biomaterials and implanted with 100% survival to scheduled necropsy. Tissues were harvested at 1, 4 and 8 weeks post-injection and sections stained with Masson's Trichrome (TRI) or Periodic Acid Schiff (PAS) (Figure 4). The temporal response to NKA Product Prototype implantation was initially fibrovascular and phagocytic multinucleated macrophages and giant cells were observed at 1 week. Biomaterial degradation occurred within 4 weeks with progressive development of renal tissue structures (glomerular and tubular) replacing the space formerly occupied by the implanted biomaterial. By 8 weeks, minimal fibrosis was observed with continued development of glomerular and tubular renal tissue structures.

**Figure 1:** Viability of Canine Renal Cells in NKA Product Prototypes

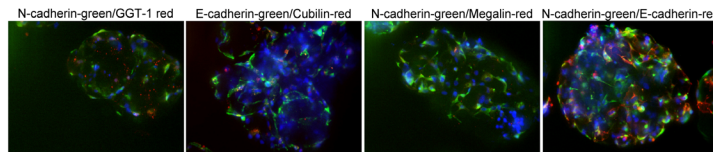


Live/Dead staining of canine renal cells in NKA Product Prototypes produced with Gelatin beads (A), HA/Gelatin particles (B), and HA particles (C).

**Figure 2:** Phenotypic analysis of human renal cells in NKA Product Prototypes



**Figure 3:** Confocal analysis of rat renal cells in NKA Product Prototypes used for *in vivo* studies



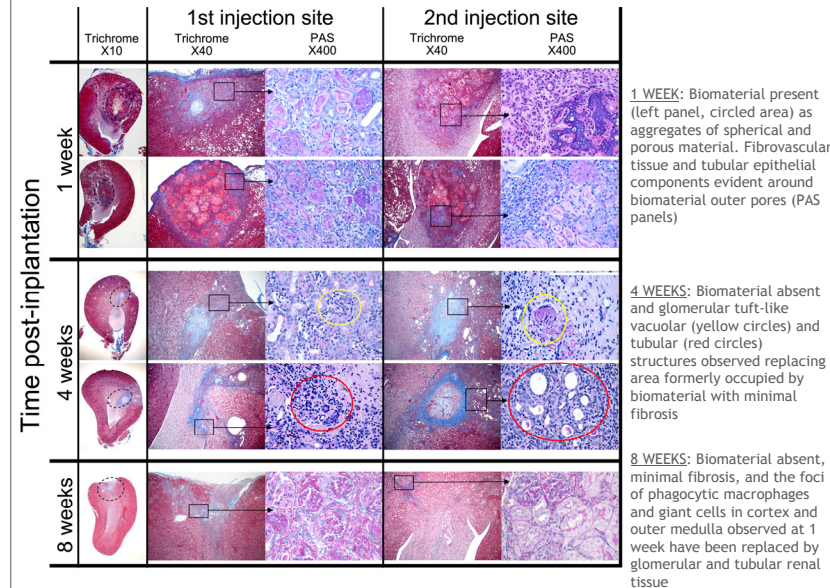
## References:

- Basu J, et al. (2010) *In vivo* evaluation of biomaterials in mammalian kidney. TERMIS-NA 2010.
- Kelley R, et al. (2010) 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* 299, F1026-1039.
- Presnell SC, et al. (2010) 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*, in press.

## Acknowledgements:

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

**Figure 4:** *In vivo* response of healthy rat kidney to NKA Product Prototype implantation



## Conclusions:

- Selected regenerative renal cells prepared from multiple species, including human, remained viable in NKA Product Prototypes, secreted proteins characteristic of tubular epithelial cells, and expressed a proteomic profile almost identical to cells in 2D cultures
- NKA Product Prototype implantation into healthy renal tissue was well-tolerated
- During the 8-week *in vivo* study:
  - Implant location in renal parenchyma could be identified at all time points
  - Histological disappearance of the biomaterial occurred by 4 weeks by a combination of biomaterial degradation and infiltration by neo-renal tissue nephron components (e.g., glomerular and tubular structures)
  - Implant location was gradually replaced by renal tissue expansion, tubular regeneration, and formation of glomerular-like nephron components
  - Fibrovascular stroma formation was minimal to mild and the numbers of phagocytic multinucleated macrophages and giant cells decreased over time resulting in neo-tissue formation with minimal fibrosis
- Taken together, these data suggest that NKA Product Prototype implantation into healthy kidneys was well-tolerated and elicited neo-kidney tissue regeneration at site of NKA Product Prototype implantation
- Ongoing studies will extend these results by investigating the regenerative effect of NKA Product Prototypes in established animal models of chronic renal disease.