

Development of a Neo-Kidney Augment Product to Prevent or Delay Need for Dialysis or Transplantation in CKD



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Introduction

Chronic Kidney Disease (CKD) affects over 26 million in the US alone. Disease progression typically leads to dialysis and eventually a kidney transplant. Current standard of care is to treat the underlying conditions of CKD to slow down progression of disease. Tengion is developing regenerative medicine solutions to CKD with a Neo-Kidney Augment (NKA) product intended to catalyze kidney tissue regeneration to prevent or delay the need for dialysis or transplantation in this growing patient population. NKA is composed of autologous, homologous Selected Renal Cells (SRC) that are primarily of tubular epithelial phenotype, and a gelatin-based hydrogel. Renal cells are obtained from a biopsy of the patient's kidney, isolated using enzyme digestion and expanded in culture. SRC are obtained by density gradient separation of harvested cells after a brief exposure to hypoxia. Cells are characterized with multiple markers to ensure tubular phenotype. SRC are formulated with the hydrogel to produce NKA. Biomaterial addition to SRC provides cell stability, enhanced shelf life and targeted delivery. Cell viability and function in NKA product was evaluated by testing for ability to metabolize PrestoBlue™, uptake albumin and presence of LAP and GGT enzyme activity. *In vivo* response to NKA implantation was evaluated by direct injection into the kidney cortex in animal models of CKD. NKA treatment was well tolerated with no morphological alterations observed in the tubular or glomerular compartments and histology confirmed reduction of kidney disease. NKA is packaged for implantation in the clinic via a product delivery system. The delivery system utilizes a syringe and needle compatible for NKA delivery. The system is designed to enable distribution of the product at multiple sites in the patient's kidney. The surgical procedure is anticipated to be laparoscopic with direct injection of the product into the diseased kidney cortex. Taken together, these data suggest that NKA implantation into the kidney has the potential to catalyze regeneration of kidney tissue and provide a much needed treatment of CKD.

NKA Preparation

The Neo-Kidney Augment (NKA) prototypes were made up of the following components:

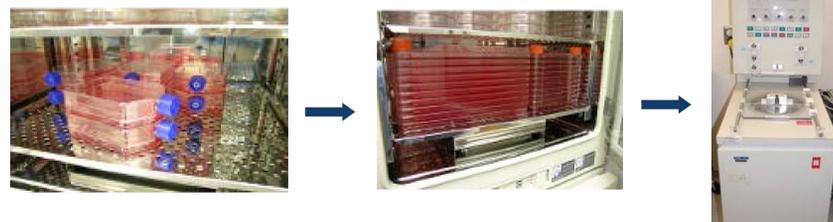
1. Cells: Selected Renal Cells
2. Biomaterial: Gelatin-based hydrogel

Kidney biopsies are obtained using a kidney biopsy tool commonly used in clinical practice (Figure 1). SRC are obtained from enzymatic digestion of the kidney biopsy and density gradient separation of expanded renal cells (Figure 2).

Figure 1 Kidney biopsy obtained using a Kidney Biopsy Tool



Figure 2 Illustration of cell expansion and SRC separation by density gradient centrifugation



NKA Prototype Characterization

SRC were characterized by phenotypic analysis (Figure 3). Cell viability and function in NKA prototypes were demonstrated by presence of leucine aminopeptidase (LAP) and gamma glutamyltransferase (GGT) enzyme activity (Figure 4), albumin uptake (Figure 5) and ability to metabolize PrestoBlue™ (Figure 6).

Figure 3 Phenotypic analysis of SRC used in preparation of NKA prototypes

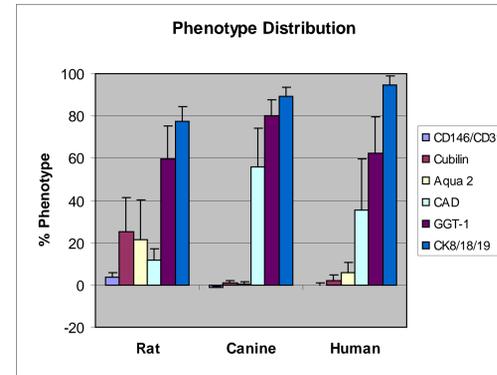


Figure 5 Functional activity of canine SRC in NKA prototype demonstrated by albumin uptake

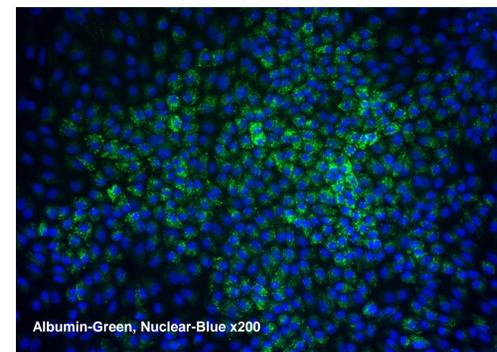


Figure 4 LAP and GGT enzyme activity of rodent and canine SRC in NKA prototypes

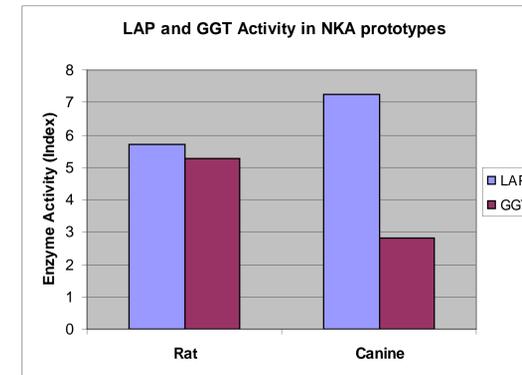
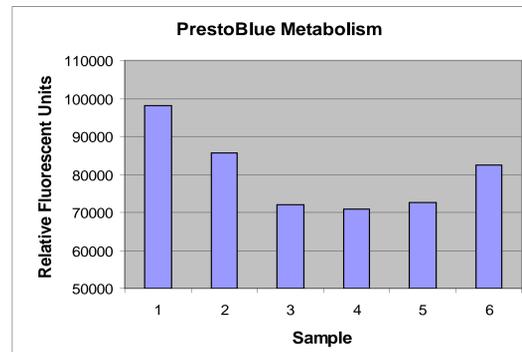


Figure 6 Functional activity of canine SRC in NKA prototype demonstrated by PrestoBlue metabolism



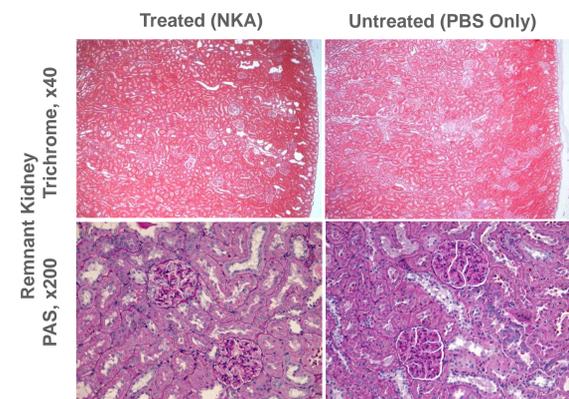
Results

NKA prototypes were tested in rat and canine animal models of CKD.

Rat 5/6 Nephrectomy Model:

NKA prototypes were tested in a 5/6 Nephrectomy rat model using 3-month old female Lewis rats. NKA prototypes (2x35µl) were microinjected into the remnant kidney parenchyma. NKA is biocompatible with rat kidney tissue in the 5/6 Nephrectomy model producing minimal inflammatory response (Figure 7).

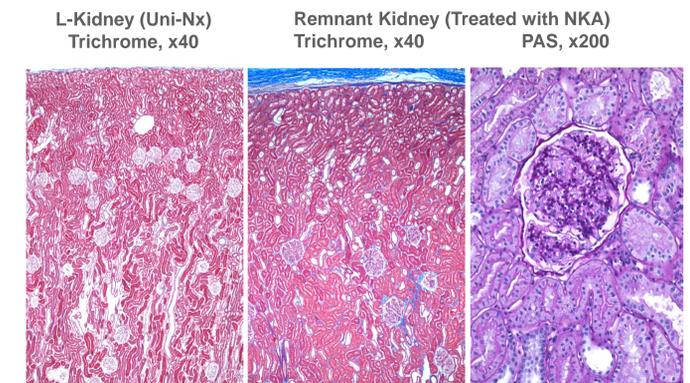
Figure 7 Representative image of rat kidney 12 weeks post NKA implantation



Canine Reduced Kidney Mass Model:

A reduced kidney mass model of CKD in dogs, where approximately seventy percent of kidney mass was surgically removed, was used to evaluate NKA prototypes. Uninephrectomized (Uni-Nx) animals were used as controls. NKA treatment appears to be well tolerated in the 5/6 canine Nephrectomy model - no morphological alterations were observed in the tubular or glomerular compartments other than expected renal compensatory hypertrophy (Figure 8).

Figure 8 Representative microphotographs of dog kidney cortex (47-weeks post treatment) showing histological outcomes of remnant kidney treatment with NKA prototypes



NKA Delivery and Implantation in the Clinic

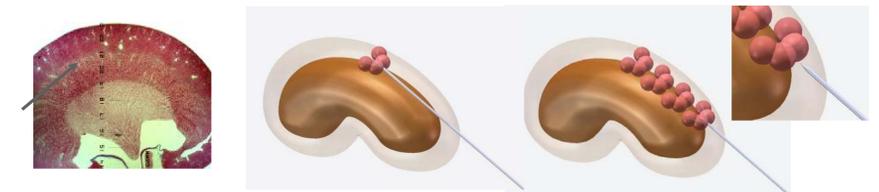
The NKA prototypes are prepared by formulating the autologous SRC with a gelatin-based biomaterial into an injectable product (Figure 9).

Figure 9 NKA clinical product



NKA is intended to be implanted into the kidney cortex using a product delivery system consisting of a syringe and needle compatible with cell delivery (Figure 10). NKA will be deposited at multiple sites in the kidney along the needle track.

Figure 10 NKA product delivery into the kidney cortex



Conclusions

- Neo-Kidney Augment prototypes composed of Selected Renal Cells and Biomaterials demonstrate functional characteristics of renal tubular cells.
- Implantation into a rat 5/6 Nephrectomy model of CKD was biocompatible and elicited minimal inflammatory response.
- Treatment with NKA implantation appears to be well tolerated in the canine Reduced Kidney Mass model - no morphological alterations were observed.
- An NKA delivery system was designed for laparoscopic implantation of NKA in the clinic.
- Implantable regenerative medicine products developed using tissue engineering principles can be used in reconstructing solid organs.