Regenerating Kidney Tissue with a Neo-Kidney Augment Implant Containing Selected Renal Cells and Natural Biomaterial

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Introduction

Chronic kidney disease (CKD) is a global public health concern involving progressive loss in renal function. New treatments to restore renal function thereby delaying or eliminating dialysis and transplant are needed. We have identified a population of selected renal cells (SRC) that positively affect several aspects of the CKD condition. Biomaterial addition to SRC provides cell stability, enhanced shelf life and targeted delivery. This study reports on the development of a Neo-Kidney Augment (NKA) product containing SRC and natural biomaterials that, upon implantation into rat or canine kidney, catalyzes kidney tissue regeneration. SRC are obtained from a kidney biopsy and density gradient separation of cells. NKA product prototypes use SRC and gelatin-based hydrogel biomaterials. SRC have been shown to provide a significant regenerative stimulus in the rodent models of CKD, delaying disease progression and reduced disease-related mortality. NKA was evaluated in the large animal model of CKD (canine reduced kidney mass model). Treatment with SRC results in a statistically significant increase in uromodulin (THP) and a decrease of vitamin D binding protein in the urine, indicative of restoration of tubular cell function. Histological evaluation in the dogs revealed that NKA product prototypes were well tolerated. These observations provide evidence that selected renal cells and natural biomaterial may be effective for neo-kidney tissue regeneration in chronic kidney disease.

NKA Prototypes

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 Illustration of a commonly used Kidney Biopsy Tool

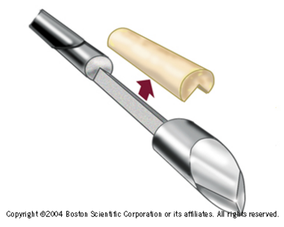
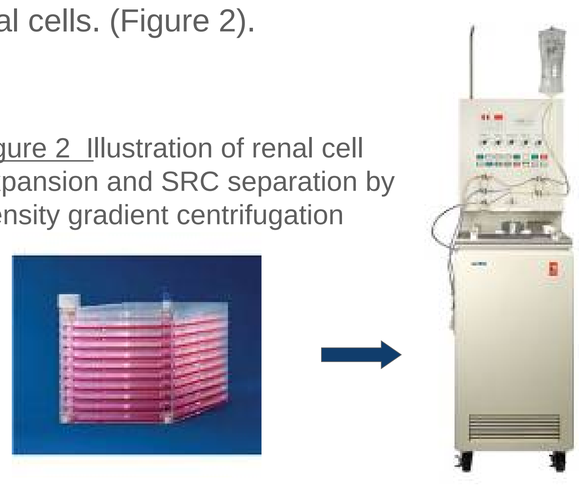


Figure 2 Illustration of renal cell expansion and SRC separation by density gradient centrifugation



The NKA prototypes are prepared by formulating the autologous SRC obtained from the patient's kidney with a gelatin-based biomaterial into an injectable product (Figure 3).

Figure 3 NKA product prototype package

NKA was evaluated by microinjection into the kidney cortex using a syringe and needle suitable for cell delivery (Figure 4).

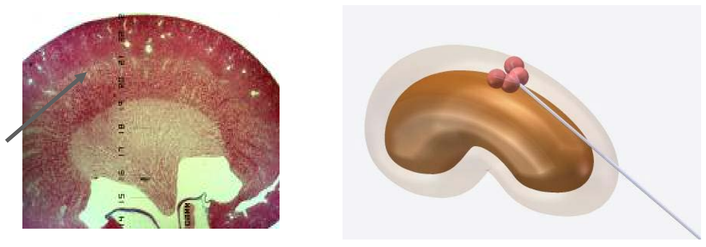


Figure 4 NKA product delivery into the kidney cortex

NKA Prototype Characterization

SRC were characterized by gene expression (Figure 5) and protein markers (Figure 6) of tubular kidney cells. Cellular function in NKA prototypes was demonstrated by leucine aminopeptidase (LAP) and gamma glutamyltransferase (GGT) activity (Figure 7).

Figure 5 Gene Expression by RTPCR of SRC used to prepare NKA prototypes

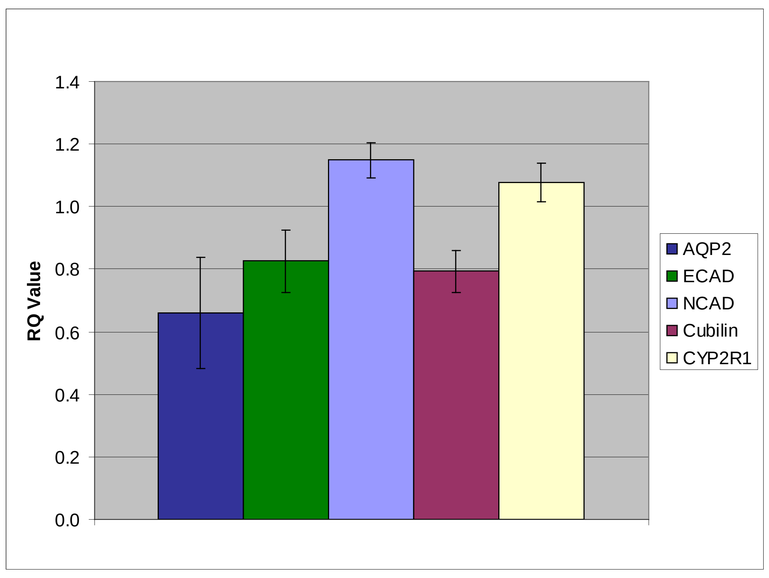


Figure 6 Protein marker (Cubilin-green) by IHC of canine SRC in NKA prototypes.

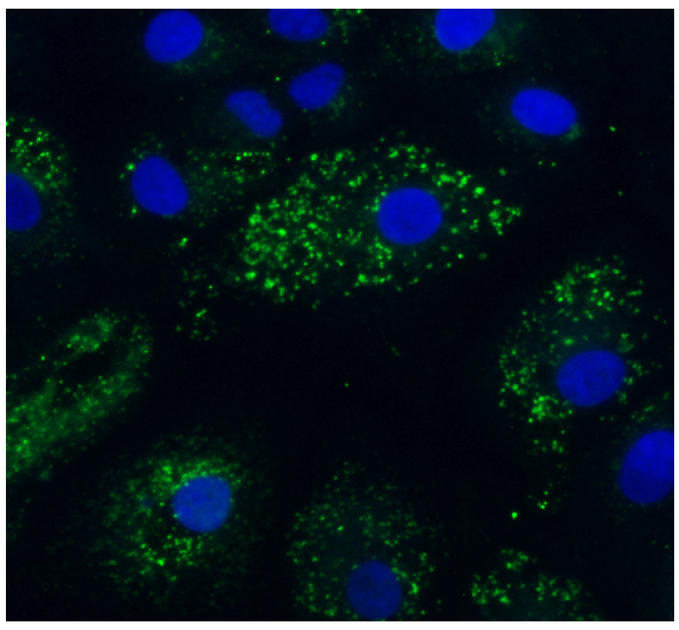
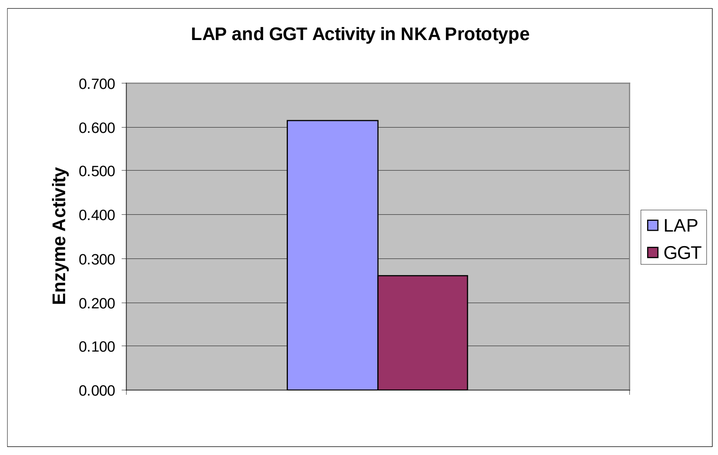


Figure 7 Functional analysis of canine SRC in NKA prototype demonstrated by presence of leucine aminopeptidase (LAP) and gamma glutamyltransferase (GGT) activity



Results

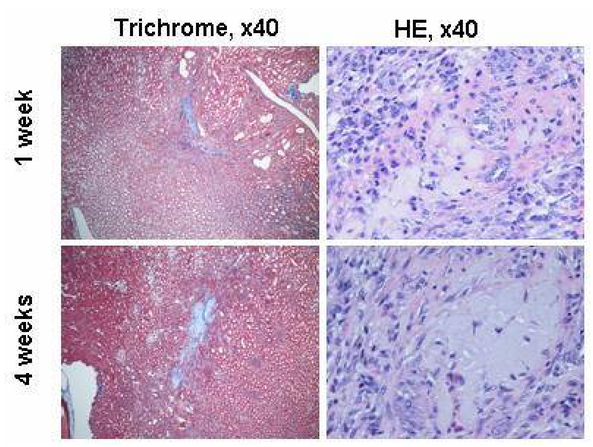
NKA prototypes were tested in rat and canine animal models of CKD.

Rat 5/6 Nephrectomy Model

NKA prototypes were evaluated in healthy rats, hemi-nephrectomized rats and a 5/6 nephrectomy model of CKD. NKA prototypes were microinjected into the kidney parenchyma.

NKA is biocompatible with healthy rat kidney tissue, producing minimal inflammatory and fibrotic responses, and facilitate neo-vascularization when delivered into the kidney (Figure 8).

Figure 8 Healthy rat kidney 4 weeks post-NKA implantation



NKA implantation into hemi-nephrectomized rats showed evidence of nephrogenesis characterized by presence of stage 1 nephrons (Figure 9). Implantation of NKA into remnant 5/6 nephrectomy rat kidney showed minimal inflammation and reduced tubular ectasia (Figure 10).

Figure 9 Hemi-nephrectomized rat kidney at 4 weeks post-implantation

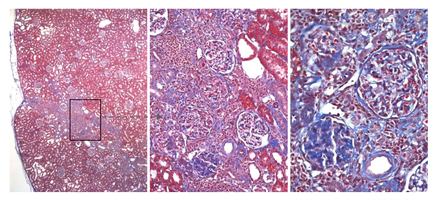
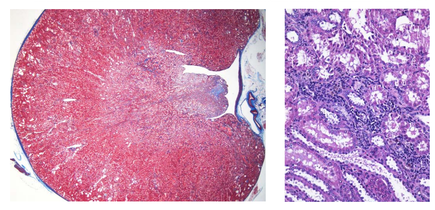


Figure 10 5/6 nephrectomized rat kidney at 4 weeks post-implantation

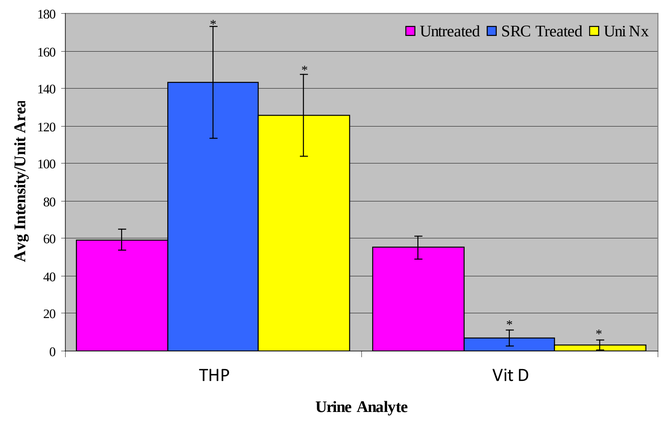


Canine Reduced Kidney Mass Model

A reduced kidney mass (RKM) model of CKD in dogs was where approximately seventy percent (5/6 Nephrectomy) of kidney mass was surgically removed was used to evaluate SRC and NKA. Uninephrectomized (Uni-Nx) animals were used as controls.

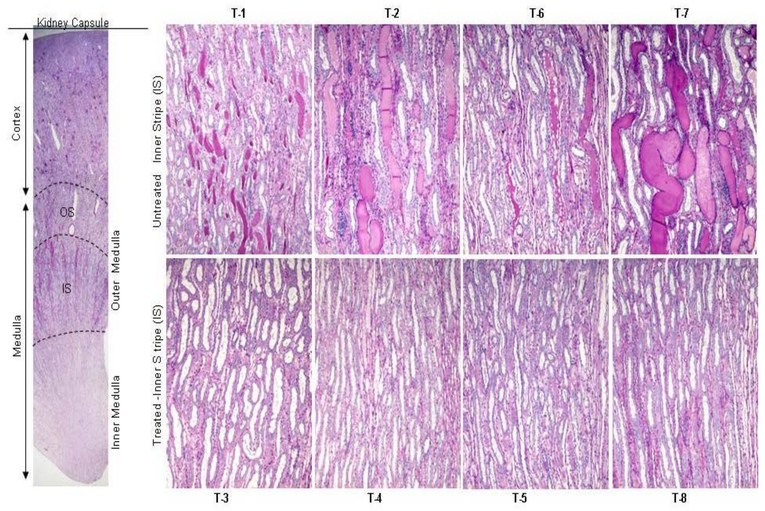
Treatment with SRC results in a statistically significant increase in uromodulin (THP) and a decrease of vitamin D binding protein in the urine, indicative of restoration of tubular cell function (Figure 11).

Figure 11 Significant differences in THP and Vitamin D Binding Protein with SRC treatment



Representative images of kidney tubular compartment post treatment with NKA are shown in Figure 12. Panel representative of tubules within the inner stripe of medulla shows marked protein casts in untreated animals. Histology confirms induction of tubular disease in canine model by RKM and significant reduction of disease with SRC-treatment.

Figure 12 Representative microphotographs of dog kidney cortex showing histological outcomes of treatment with NKA prototypes



Conclusions:

- Neo-Kidney Augment prototype implantation into healthy, hemi-nephrectomized and 5/6 nephrectomy rat kidneys was well-tolerated and elicited neo-kidney tissue regeneration.
- Reduced Kidney Mass canine model of CKD induces tubular and glomerular disease and SRC treatment results in significant reduction of disease.
- NKA implantation appears to be well tolerated in the 5/6 canine and rat models of CKD.
- Implantable regenerative medicine products developed using tissue engineering principles can be used in reconstructing solid organs.

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