Attenuation of Disease Progression with Selected Renal Cell Populations in Animal Models of Chronic Kidney Disease

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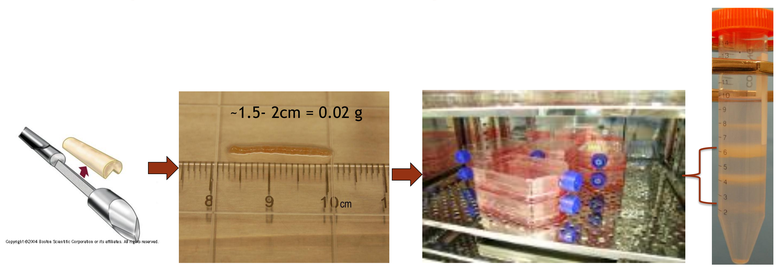
Introduction

Regenerative biology and tissue engineering offer potential solutions for the repair and augmentation of diseased tissues and organs. Tengion's proprietary Organ Regeneration Platform™ is targeted to function in part by recapitulating key mechanistic and signaling pathways associated with embryonic organogenesis. For chronic kidney disease (CKD), we have demonstrated in multiple animal models that a selected population of therapeutically bioactive renal cells (SRC) can be effectively delivered to the kidney through intra-parenchymal injection and can attenuate the progression of the disease within rodent models of renal insufficiency (Table1).

Study Model			
Canine Nephrectomy	Lewis Nephrectomy	ZSF-1 Diabetic	Measures
	✓	✓	Anemia (ie: Hct, Hgb, RBC)
✓	✓	✓	Tubular Injury
✓	✓	✓	Blood Pressure (ie: MAP, Renin)
		✓	Metabolic (ie: Bicarb)
✓	✓	✓	Protein Handling (ie: uPro, UPC, sAlb)
✓	✓	✓	Glomerular Injury
✓	✓	✓	eGFR (calculated, iohexol)
	✓	✓	sCr, BUN
		✓	Urine Conc (ie: spGrav, uOsm)
	✓	✓	Survival
	✓	✓	Anemia

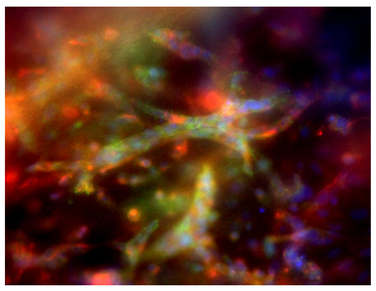
We have previously described the isolation and characterization of a population of therapeutically bioactive primary renal cells (Selected Renal Cells, SRC) from normal and diseased kidneys. We show that direct injection of SRC can reduce chronic infiltration by monocytes/ macrophages and T-lymphocytes, typically observed during pathogenesis of fibrotic tissue in multiple animal models of CKD. We show SRC can attenuate the NFκB response known to drive tissue inflammation, while simultaneously promoting host tubular cell expansion through trophic cues. We have demonstrated that SRC were significantly retained in the kidney, up to six months, following renal implantation in rodent models of CKD. In addition, we leverage a combination of *in vivo* and *in vitro* functional bioassays to investigate mechanistic pathways for SRC therapeutic bioactivity in these models. These data suggest that SRC may provide immuno-modulatory and trophic cues to host renal tissues, thereby catalyzing long-term functional benefits *in vivo*. For the clinic, SRC are formulated in a gelatin-based hydrogel biomaterial as Neo-Kidney Augment (NKA), an injectable product, to provide cell stability and improve the targeted delivery of SRC to the patient's kidney.

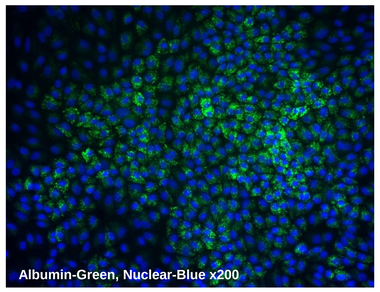
1. Isolation procedures of SRC from kidney tissue



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

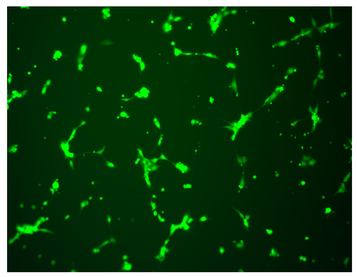
2. SRC form *de novo* tubules in 3D hydrogel cultures and are functionally active

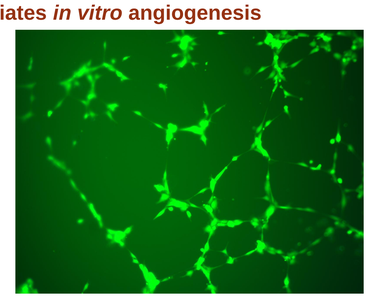




SRC are composed principally of tubular epithelial cells. A key functional characteristic of this cell population is self-organization into three-dimensional cellular aggregates such as spheroids, organoids, tubules and tubular networks. These self-organized structures present robust expression of key functional markers diagnostic of tubular epithelial cells, including (panel A) aquaporin1 (green) and cytokeratins (red). DNA in blue. B. Functional activity of canine SRC in NKA prototype demonstrated by albumin uptake.

3. SRC-derived conditioned media mediates *in vitro* angiogenesis



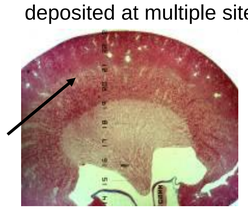


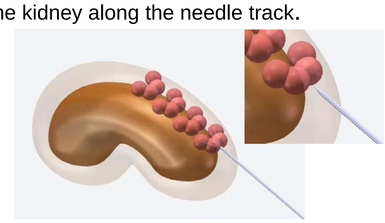
SRC may function in part by promoting the assembly of vasculature through secretion of pro-angiogenic factors including VEGF. Although absolute amounts of VEGF in the cellular milieu may be measured through ELISA, such assays do not discriminate between functional and non-functional variants of secreted VEGF. inRegen has leveraged a Human Umbilical Vein Endothelial Cell (HUVEC) based assay to evaluate expression of functional VEGF secreted by SRC as an indicator of SRC cell function, bioactivity and potency. In this assay, assembly of tubular networks from HUVEC in 2D culture in the presence of SRC-derived conditioned media is used as a cell-based readout for functional VEGF secreted by SRC as shown (A) control media at 4hrs (B) SRC-derived conditioned media at 4hrs post-incubation

4. NKA Delivery and Implantation into the kidney

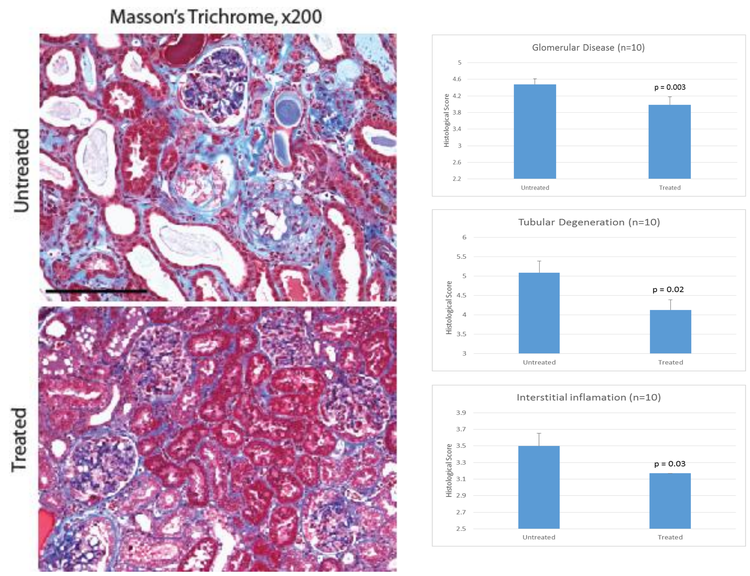
The NKA prototypes are prepared by formulating the autologous SRC with a gelatin-based biomaterial into an injectable product .

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. NKA will be deposited at multiple sites in the kidney along the needle track.



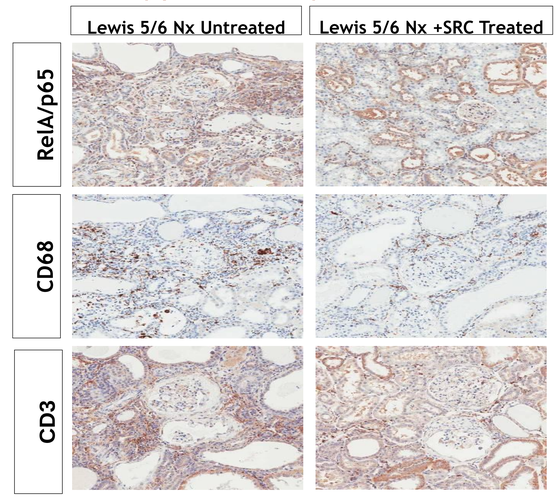


5. Remodeling and regeneration in tubular and glomerular compartment



Structural changes support functional outcomes. Modulation of fibrotic and degenerative pathways in CKD seen 5/6 Nx rat treated with SRC.

6. Transplantation of SRC into 5/6 Nx model attenuates NFκB activity and alters inflammatory profile of kidney



Kidney tissue was harvested 6 months post transplantation or at time of death, in rats with or with out treatment with SRC. Immuno-histochemistry for the NFκB p65 subunit, CD68, CD3 was performed to assess the inflammatory state of tissues

Conclusions

- Neo-Kidney Augment prototypes composed of Selected Renal Cells and Biomaterials demonstrate functional characteristics of renal tubular cells
- Histological assessment showed decreased levels of NFκB, monocytes and macrophages and T-lymphocytes at 6 months post-transplantation
- At 6 months transplanted SRC were observed to attenuate NFκB activation and reduce macrophage and T-cell infiltration.
- These data suggest that SRC may provide immuno-modulatory and trophic cues to host renal tissues, thereby catalyzing long-term functional benefits *in vivo.*

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