Bio-response of a rodent hemi-nephrectomy model to implantation of Neo-Kidney Augment prototypes composed of selected renal cells and biomaterials

Joydeep Basu, Richard Payne, Elias Rivera, Neil Robbins, Darrell McCoy, Toyin Knight, Namrata Sangha, Kelly Guthrie, Thomas Spencer, Christopher Genheimer, Deepak Jain, John W. Ludlow and Craig Halberstadt

Bioprocess Research & Assay Development, Tengion Inc., Winston-Salem, North Carolina, USA

Introduction:

Continual loss of renal function over a time span of months or years is the operational definition of chronic kidney disease. Current renal function replacement therapy includes dialysis and eventual kidney transplant. An unmet need exists for new treatments to restore renal function thereby delaying or eliminating dialysis and transplant. Towards addressing this need, Tengion has developed a unique integrated regenerative medicine technology platform capable of catalyzing regeneration of tissues and organs. In the current study, we report on the development of a Neo-Kidney Augment (NKA) product prototype, comprised of biomaterials and selected regenerative renal cells (SRC), which facilitate regeneration of kidney tissue. SRC are obtained from enzymatic digestion of a kidney biopsy and density gradient separation of cells. Gelatin based hydrogels were used as biomaterial. Bio-response of mammalian kidney towards implantation of NKA prototypes has previously been evaluated in healthy adult rodents (Basu et al., 2011, *Cell Transplantation*). However, removal of single kidney from rodents (hemi-nephrectomy) increases sensitivity of the model, permitting detection of systemically acting toxicological effects. In this study, 15 hemi-nephrectomized rodents were injected with NKA prototypes within the renal parenchyma of the remnant kidney. Physiological indices derived from whole blood, serum and urine chemistries were evaluated either prior to implantation or at 4 week time points post-implantation. Animals were sacrificed at 4 weeks post-injection and remnant kidneys were examined histologically for evidence of inflammatory or fibrotic bio-response. Implantation of NKA prototypes did not significantly affect key renal physiological indices, and presented minimal evidence of inflammatory, necrotic or fibrotic bio-response. Therefore, NKA prototypes based on SRC in gelatin based hydrogels are well tolerated by remnant kidney in the rodent hemi-nephrectomy model.

Materials and Methods:

Neo-Kidney Augment prototypes were made by combining selected renal cells with biomaterials as shown in Table 1. Cell/biomaterial constructs (Figure 1,2) were delivered to remnant kidney of hemi-nephrectomized Lewis rats (2 months old) through 18 gauge needle (Figure 3). Blood and urine samples were collected at 4 weeks post-implantation and key indices of renal physiological function measured (Figure 4). Animals were then euthanized for histological analysis of remnant kidney (Figure 5).

Animal ID	Group ID	Description
HN07	A	PBS + Cells
HN11	A	PBS + Cells
HN15	A	PBS + Cells
HN21	A	PBS + Cells
HN16	B	Gelatin in PBS
HN23	B	Gelatin in PBS
HN08	B	Gelatin in PBS
HN12	B	Gelatin in PBS
HN18	C	Gelatin + Tng Beads +
HN24	C	Gelatin + Tng Beads +
HN25	C	Gelatin + Tng Beads +
HN09	C	Gelatin + Tng Beads +
HN10	C	Gelatin + Tng Beads +
HN13	C	Gelatin + Tng Beads +
HN14	C	Gelatin + Tng Beads +

Table 1: Summary of biomaterials delivery to hemi-nephrectomized rodent groups.

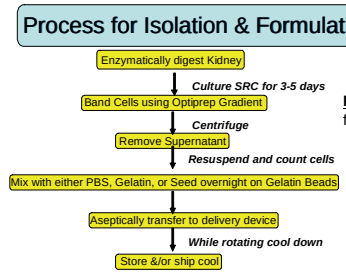
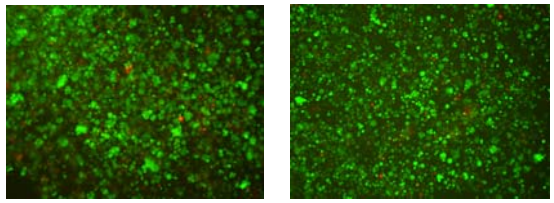


Figure 1: Outline for strategy for creation of NKA prototypes

(A) SRC/PBS

(B) SRC/gelatin



(C) SRC/ Tng beads

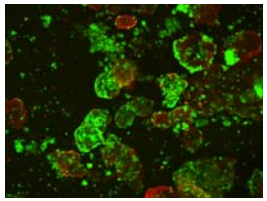
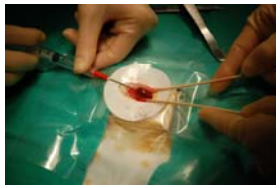


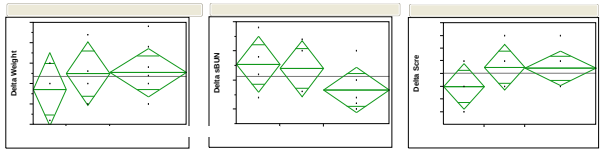
Figure 2: Representative live/dead staining of selected rodent regenerative renal cell biomaterial constructs

Figure 3: Delivery of NKA prototypes (cell/biomaterial constructs) to rodent kidneys



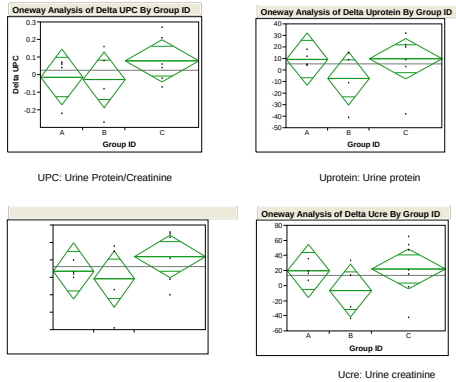
Results:

Figure 4: Summary of key renal physiological indices 4 weeks post-implantation (ANOVA analysis)



BUN: Blood Urea Nitrogen

Scre: Serum Creatinine



UPC: Urine Protein/Creatinine

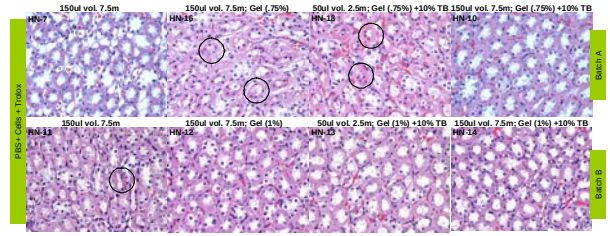
Uprotein: Urine protein

Ucre: Urine creatinine

Overall, introduction of cell/biomaterial constructs within hemi-nephrectomy rodent model did ***not*** impact key indicators of renal physiology over a one month period of time as compared to SRC.

Figure 5: Representative histological outcomes associated with implantation of cell/biomaterial constructs within rodent kidney in hemi-nephrectomy model

4-Wks Post hemi-nephrectomized Rats; kidney Outer Medulla (inner stripe); HE, x400



Batch A (top row), tubular necrosis characterized by piknotic nuclei (described in batch 1) were also observed in rat nos. (HN-16 and HN-18) but not in HN-7 and HN-10, which showed no significant lesions within the kidney parenchyma.

Batch B (bottom row); minimal , focal tubular necrosis showing piknotic nuclei in the inner stripe of outer medulla were observed one rat (HN11) but not observed in the remaining animals (HN-12, HN-13 and HN-14), and thus considered within normal limits.

Overall, introduction of cell/biomaterial constructs within hemi-nephrectomy rodent model did ***not*** significantly impact histology of remnant kidney

Conclusions:

Implantation of selected renal cell/biomaterial Neo-Kidney Augment prototypes into rodent hemi-nephrectomy model does not impact remnant kidney physiology or histology

REFERENCES: (2) **Basu** et al., (2011). Functional evaluation of primary renal cell/biomaterial Neo-Kidney Augment prototypes for renal tissue engineering. *Cell Transplant*. In Press (3) **Presnell** et al., (2011). Isolation, characterization and expansion methods for defined primary renal cell populations from rodent, canine and human normal and diseased kidneys. *Tissue Eng. Part C* 17, 261

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