DEVELOPMENT OF TISSUE ENGINEERED REGENERATIVE MEDICINE PRODUCTS USING SELECTED AUTOLOGOUS CELLS AND BIOMATERIALS

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Introduction

Regenerative medicine holds the promise of fulfilling unmet medical needs, especially in the area of restoration of organ function and organ replacement (transplantation). Development of tissue engineered regenerative medicine products requires a multi-disciplinary effort involving cell biology, biomaterials, bioprocess development, engineering, preclinical development, clinical translation, and manufacturing. Strategies should be evaluated early in development with input from Clinical, Regulatory, and Marketing to improve the probability of success. For example, use of autologous cells can provide early entry into the clinic but may present challenges in manufacturing scale-up and not be commercially acceptable. Biomaterials used to formulate biologically active components may involve a more complicated regulatory approval pathway. Tengion has created a unique integrated technology platform to develop regenerative medicine products from research to commercialization.

Applications

Tengion developed its first regenerative medicine product, the Neo-Bladder Augment (NBA), using bladder-derived autologous smooth muscle cells (SMC) and urothelial cells (UC) in a synthetic scaffold. Advancements in understanding the regenerative biology of the NBA allowed the next generation Neo-Bladder Replacement product to be composed of only SMC (no UC) on the scaffold, thereby simplifying manufacturing and reducing cost of goods. Further optimization of the bioprocess enabled development of the Neo-Urinary Conduit (NUC) using SMC derived from autologous adipose tissue, avoiding use of bladder cancer tissue as a cell source. The technology platform has been applied to other tubular organs including the Gastro-intestinal system. SMC-seeded tubular scaffolds (NGI) have shown promise in augmenting the small intestine in rats. The technology platform has also been successfully extended to solid organ regeneration in the Neo-Kidney Augment (NKA). Selected regenerative renal cells (SRC) provided a significant regenerative stimulus in rodent models of chronic Kidney disease, resulting in delayed disease progression, preservation of functional renal mass, and reduced disease-related mortality.

Neo-Urinary Conduit (NUC)

A tissue engineered Neo-Urinary Conduit provides an alternative to using gastrointestinal tract in patients who require a urinary diversion post-cystectomy. Use of GI tissue requires GI tissue resection and exposes gut mucosa to urine, leading to multiple acute and chronic complications, including GI and metabolic derangements, complications that a NUC capable of regeneration would be expected to reduce.

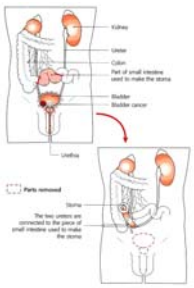


Figure 1 Diagram of cystectomy and construction of an ileal loop urinary diversion (Taken from *Cystectomy and Ileal Loop Diversion (Urostomy), Patient Information Book.* (n.d.). Dublin, Ireland: The Adelaide and Meathe Hospital)

The NUC is composed of:

1. Scaffold: Polyglycolic acid (PGA) polymer mesh scaffold fashioned into a tubular shape and coated with a 50:50 poly-DL-lactide-*co*-glycolide (PLGA) copolymer
2. Cellular: Autologous SMC sourced from adipose tissue

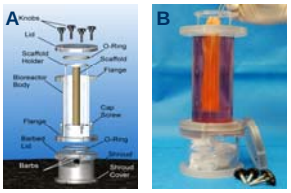
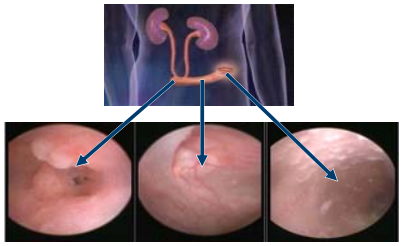


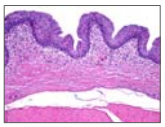
Figure 2 NUC Bioreactor design: (A) schematic of NUC scaffold in bioreactor; (B) easy removal of NUC at the surgical site

Figure 3 Endoscopic cystograms of NUC at 6 weeks post-implantation in a porcine animal model

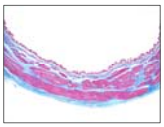


Junction with ureters | Conduit | Junction with skin

Figure 4 Histological examination of adipose-derived SMC seeded NUC implants at 3 months post-implantation in a porcine animal model



Regenerated tissue has normal lining



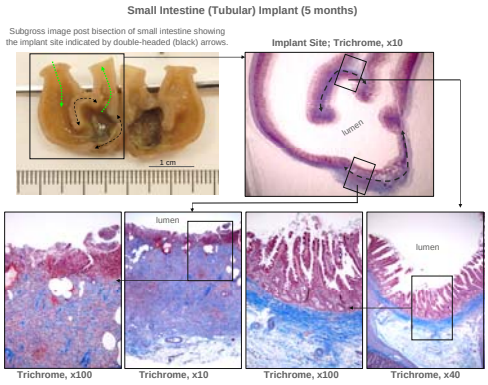
Regenerated tissue has normal thickness and muscle structure

Neo-Gastrointestinal Augment (NGI)

A tissue engineered Neo-Gastrointestinal augment provides patients suffering from small bowel syndrome and related diseases an alternative to lengthening of small bowel or transplantation with their associated complications and poor quality of life including lifelong immunosuppression. An NGI augment capable of regeneration presents a viable option that would be expected to provide a functional organ and reduce complications.

Figure 5 Surgical implantation of tubular NGI constructs by anastamosis to resected native small intestine of adult Lewis rat
Top: start of surgery
Bottom: completed anastamosis of construct

Figure 6 Regeneration of small intestine at 20 weeks post-implantation

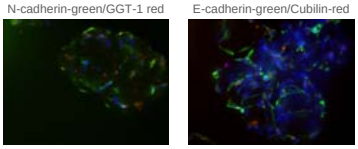


Longitudinal sectioning of an NGI implantation site at 20 weeks post-treatment showed nearly complete regeneration of the intestinal mucosa and submucosa. Smooth muscle cell bundle regeneration was most apparent at the implant margins. There is no evidence of remnant scaffold fibers

Neo-Kidney Augment (NKA)

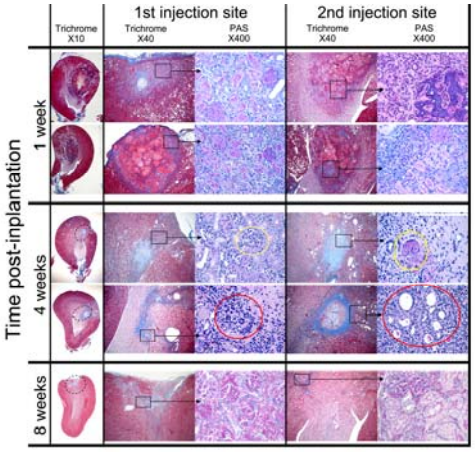
A Neo-Kidney Augment product using selected regenerative renal cells and biomaterials can be used for catalyzing kidney tissue regeneration to restore renal function thereby delaying or eliminating the need for dialysis and transplant.

Figure 7 Confocal analysis of rat renal cells in NKA prototypes used for *in vivo* studies



NKA Product Prototypes were produced with SRC and one of three biomaterials: gelatin beads (Gel), hyaluronic acid (HA) particles, or HA/gelatin particles (HA/Gel). Loosely packed NKA Product Prototype (2x35µl) were microinjected into the left kidney parenchyma of healthy 3-month old female Lewis rats.

Figure 8 *In vivo* response of healthy rat kidney to NKA Product Prototype implantation



1 Week: Biomaterial present (left panel, circled area), fibrovascular tissue and tubular epithelial components evident around biomaterial outer pores (PAS panels)
4 Weeks: Biomaterial absent and glomerular tuft-like vacuolar (yellow circles) and tubular (red circles) structures observed replacing area formerly occupied by biomaterial with minimal fibrosis
8 weeks: Biomaterial absent, minimal fibrosis, and the foci of phagocytic macrophages and giant cells and outer medulla observed at 1 week have been replaced by glomerular and tubular renal tissue

Conclusions

- Neo-Urinary Conduit seeded with autologous SMC sourced from adipose tissue was capable of establishing a patent incontinent urinary diversion for post-cystectomy management of urine elimination in pigs and is currently being evaluated in Phase I clinical trials.
- Neo-Gastrointestinal Augment product prototypes implanted into small intestines of Lewis rats elicited a functional regenerative response.
- Neo-Kidney Augment product prototype implantation into healthy rat kidneys was well-tolerated and elicited neo-kidney tissue regeneration at site of implantation.
- Implantable regenerative medicine products developed using tissue engineering principles can be used in reconstructing tubular organs (*e.g.*, bladder, GI) and solid organs (*e.g.*, kidney).