

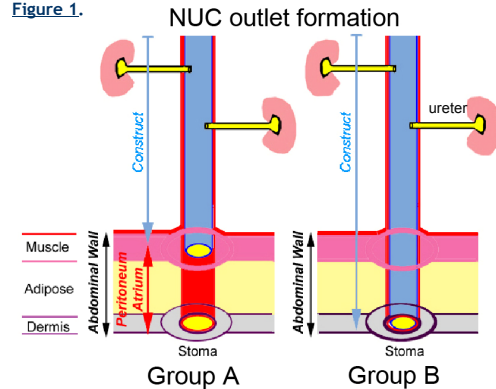
Introduction

Urine exits the body via the urethral meatus, a distinct structure incorporating features that defend the opening against local and/or ascending infections, and emptying in the vaginal vestibule in females and fossa navicularis in males. Specifically, the mucocutaneous region is a non-keratinized stratified squamous epithelium composed of glycogen-rich cells that provide substrate for a protective endogenous lactobacteria flora. Also, as the epithelium nears the skin it is associated with acid-phosphatase activity and lysozyme-like immunoreactivity indicative of the presence of macrophages that secrete bactericidal compounds.¹ Regenerative medicine strategies for channeling urine from ureters to skin have been tested in large mammals and one option, Tengion's Neo-Urinary Conduit™ (NUC), is currently in Phase I clinical trials. This study reports on the ability of the NUC to initiate the formation of a native-like transition between urinary mucosa and skin epithelium that has the structural features of mucocutaneous regions observed in native urethras within 3 months post-implantation in cystectomized pigs.

Materials and Methods

NUC implants were produced by seeding autologous porcine smooth muscle cells (SMC) on a biodegradable tubular scaffold to form a NUC Construct. The 3-month study included two groups of 8 Yorkshire cystectomized swine (4M and 4F per group) implanted with NUC. All implants were wrapped with peritoneum to provide a vascular source and connected in the same manner to the ureters; however, the outflow end of the NUC (NUC-O) was attached differently in each group (Figure 1). In Group A, the NUC-O terminated at the wall of the abdominal cavity and an "atrium" connector comprised of 2-3 cm of tubularized peritoneum was used to connect the NUC-O through the abdominal muscle to the skin surface opening (stoma). In Group B, the NUC-O was transabdominal and connected directly to the subcutaneous layer of the skin stoma (percutaneous). At necropsy, the entire length of neo-conduit tissue (ureters to stoma) was harvested and analyzed histologically.

Figure 1.



References

- Holstein AF et al. (1991) *Cell Tissue Res* 264: 23.
- Knabe C et al. (1999) *Biomaterials* 20: 503.
- Isenhardt SN et al. (2007) *J Biomed Mater Res A* 83: 915.
- von Recum AF and Park JB. (1981) *Crit Rev Bioeng* 5: 37

Results

All animals experienced intermittent obstruction of urine flow that was attributed to a combination of the quadrupedal stance (placing intestinal weight on the NUC) and rapid growth of the animals during the 3-month study (body elongation). Obstruction led to safety findings including hydroureter & hydronephrosis (left side most frequent), and chronic active inflammation; however, these safety findings were markedly reduced in both incidence and severity in Group B animals. One unscheduled death occurred in Group A. The procedure by which the NUC outlet was formed influenced the barrier function of the neo-tissue as measured by Serum:Urine Creatinine ratio (Figure 2). Histologically, epithelium covered mucosa and muscle layers characteristic of native urinary tissue wall regenerated along the length of the conduit within the body cavity in both Groups. In contrast, the distal portion of the neo-conduit at the abdominal wall - skin interface differed markedly between the two Groups. In Group A animals (peritoneal atrium connector to skin), the distal neo-conduit mucosa was typically denuded of epithelium, lined by chronic-active inflammation, and often filled with detritus (Figure 3, top panels). In contrast, in Group B animals (NUC transmural percutaneous connection), a full mucosa with urothelium-to-epithelium transition characteristic of an inborn urethral meatus was observed (Figure 3, bottom panels).

Figure 2. Regeneration of urinary tissue capable of barrier function is influenced by implantation procedure

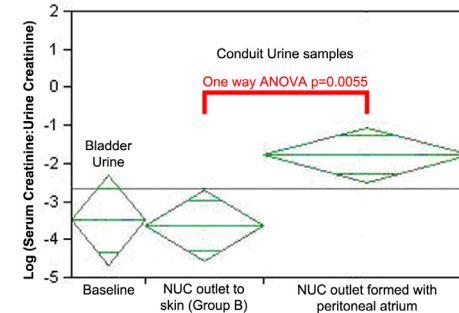
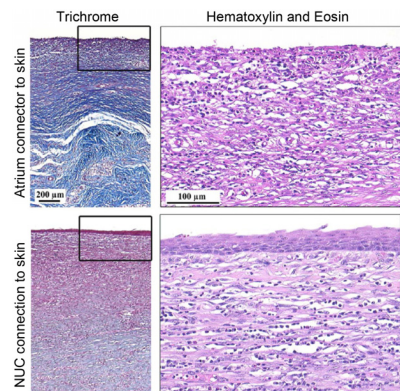


Figure 3. Implantation procedure influences the tissue structure of regenerated neo-tissue - view at 3 months post-implantation



Group A: Peritoneal atrium connector is composed of muscle fibers and collagen with scattered inflammatory cells and no mucosal lining

Group B: Transmural NUC with percutaneous connection to skin resulted in a fully-epithelialized mucosal lumen composed of non-keratinized squamous epithelium

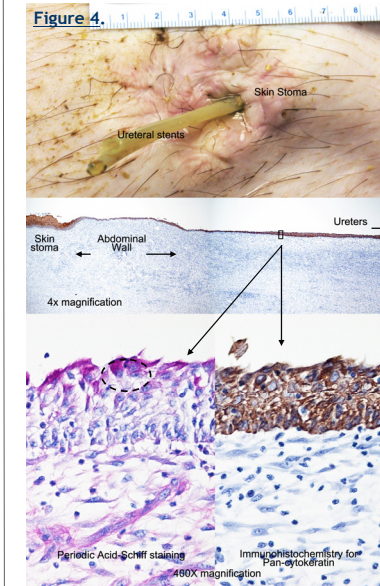
(The boxed regions in left panels are shown enlarged in right panels from serial sections stained with hematoxylin and eosin)

Discussion

Achieving urinary diversion via an abdominal stoma represents a major challenge. It is accepted that the longevity of percutaneous devices is often hampered by exit-site infection.² Percutaneous devices such as catheters, cannulas, prosthetic attachments, and glucose sensors, regardless of their intended medical goal, penetrate the skin, disrupt its protective barrier, and create a sinus tract for bacterial invasion.³ Breakdown of the product-skin interface due to improper epidermal healing, lack of biocompatibility, or mechanical stresses can cause additional failure risks.⁴

The NUC - host tissue interaction regenerated a tubular organoid (Figure 4) that transferred urine from the ureters to outside the animals while maintaining native-like functional properties found in bladders, urethras, and stomas (i.e., a meatus or opening). These observations are consistent with a recapitulation of the complex, but natural, organization of tissues and organs via developmental signals elicited after NUC implantation.

The transabdominal-percutaneous placement of NUC led to the formation of a mucocutaneous junction very similar to that found at the anterior urethra's opening; at the vaginal vestibule and fossa navicularis, of the human female and male, respectively.



These natural junctions are covered by mucosal zones critical to wet-dry surfaces that may provide protection against ascending infections. The squamous epithelium of these mucosal zones is 1) glycogen-rich, 2) secretory (able to release enzymes and bactericidal agents), and 3) phagocytic; and can rapidly migrate to injured surfaces.

Figure 4: Urothelial-epithelial transition zone elicited when NUC outlet was formed by transabdominal percutaneous connection to skin stoma (3 months post-implantation)

Top panel: external view of skin stoma with ureteral stents

Middle panel: Section of Neo-Conduit tissue stained for Pan-cytokeratin by immunohistochemistry (4x magnification)

Bottom panels: PAS staining (left) and immunohistochemistry for Pan-cytokeratin at 400x magnification. Box indicates area of view. Dashed circle indicates glycogen detected by PAS.

Conclusions

- Transabdominal percutaneous connection of the outflow end of the peritoneum-wrapped NUC implant in Group B animals triggered the formation of a mucocutaneous junction very similar in structure and function to native anterior urethra
- The formation of a native-like mucocutaneous junction was correlated with a marked reduction in the incidence and severity of the sequelae of intermittent obstruction of urine flow that occurred in both Groups
- Surgical implantation of regenerative templates procedures are contextual and impact the regenerative process, tissue structure, and functional outcome of the regenerated neo-tissue.