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## ABSTRACT

A treatment modality for bladder cancer patients is a cystectomy or surgical removal of the bladder, requiring the formation of a channel to remove urine from the body by using a gastrointestinal segment. However, complications arise when using this tissue, including gastrointestinal and metabolic abnormalities. Tengion is developing autologous regenerative products such as the Neo-Urinary Conduit™ (NUC) for urinary diversion and other urologic applications as alternative to the use of gastrointestinal tract segments that consists of adipose-derived smooth muscle cells (SMC) seeded onto a tubular scaffold<sup>1</sup>. To manufacture this product efficiently, we have designed a multi-functional bioreactor system that provides: (i) placement and positioning of the NUC scaffold; (ii) a closed system for sterilizing the NUC scaffold; (iii) a closed system for cell seeding, medium exchanges, and in-process sample collection; (iv) a container for shipping the NUC to the surgical site; (v) user-friendly handling of the NUC in the surgical suite.

## MATERIALS AND METHODS

The NUC bioreactor system is made primarily from USP Class VI grade polycarbonate, with Tygon® tubing (Class VI) connections for cell seeding and media exchange (Figure 1). A polycarbonate frame suspends and centers the NUC scaffold in the bioreactor. A polycarbonate shroud on one end of the bioreactor contains and protects Tygon tubing during transport. Access to the product at the surgical site occurs on the end opposite to the shroud. Bioreactor and scaffold are sterilized prior to cell seeding via ethylene oxide. Cells are seeded dynamically on the scaffold and the NUC construct is matured for 5 days in the bioreactor with one culture medium exchange before transport. Glucose and lactate are measured using a Nova Bioprofile 400 and MCP-1 (a cytokine expressed by seeded cells) by ELISA. To characterize some of the bioreactor's characteristics, destructive testing of human NUC Constructs was performed by harvesting punch biopsies at multiple locations along length of cell seeded Constructs and assessing viability and relevant biomarker expression with Live/Dead assay (Molecular Probes) and immunohistochemistry for smooth muscle  $\alpha$ -actin (SMAA), respectively.

## RESULTS

Table 1. NUC bioreactor design features

Biocompatible Materials (Figure 1)	
Bioreactor Body, Lids and Scaffold Frame	Class VI Polycarbonate
O-rings	Medical grade silicone
Tubing for Media Exchange	Medical Grade Tygon®
Container Closure	
Microbial Incurion Challenge	Passed
Closed seeding and media exchange	Multiple runs and engineering runs established aseptic process
Sterilization	
Testing with BI strips and identification of Ethylene Oxide Cycle	Sterilization Validation Established
Cell Seeding and Construct Maturation (Figures 2, 3, 4, and 5; Table 2)	
Cell seeding	Demonstrate cell attachment on scaffold
Maturation	Measurement of metabolites and MCP-1 Immunohistochemical Staining
Shipping	
Shipping Conditions	Cold to ambient conditions
Shelf Life of final Construct	Five days at ambient temperature

Figure 1. NUC Bioreactor design features:

(A) schematic; (B) NUC scaffold mounted in polycarbonate frame and Tygon tubing protected in shroud; (C) easy removal of NUC at the surgical site

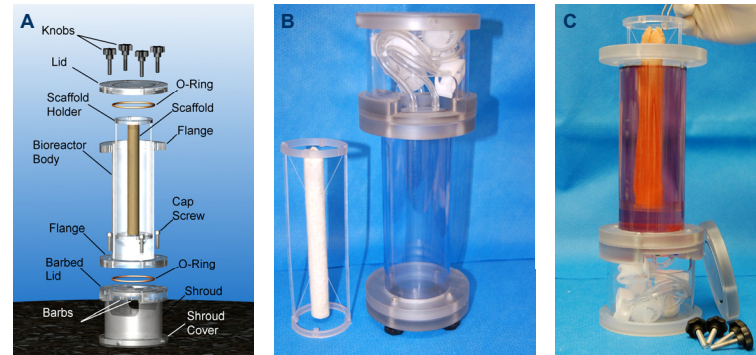


Table 2. Dynamic seeding results in high cell attachment

% cells attached measured at 2 days post-seeding

Bioreactor	% Cell Attachment
1	91.2%
2	92.8%
3	90.9%
4	96.0%
5	97.7%
6	95.4%
7	85.3%
Average $\pm$ SD	92.7% $\pm$ 4.1%

Figure 2. Seeded cells retain biomarker expression at 5 days maturation: SMAA (green fluorescence), nuclei (DAPI) at 10X magnification

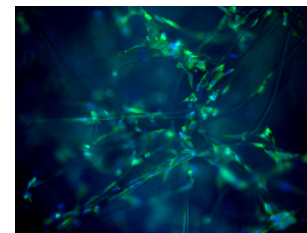


Figure 3: Dynamic seeding results in even distribution of cells that remain viable after 2 days post-seeding:

Representative samples of Live / Dead staining (green fluorescence) of punch biopsies harvested along the length of a single NUC construct. The images show viable cells distributed throughout the scaffold. Magnification at 50X.

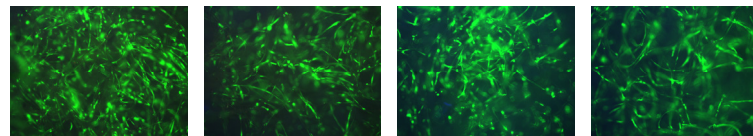


Figure 4. Seeded cells metabolize glucose and produce lactate during maturation

Glucose decreases and lactate increases during Days 3-5 of maturation (n=3 NUC Constructs)

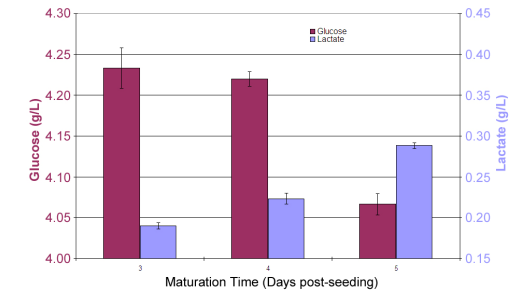
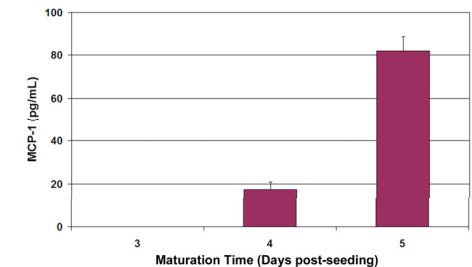


Figure 5. Seeded cells exhibit cell-type specific metabolic activity during maturation:

MCP-1 (cytokine secreted by seeded smooth muscle cells) progressively accumulates in media (n=3 NUC Constructs)



## CONCLUSIONS

Tengion has developed a bioreactor system for preparation of tubular organs that:

- Maintains container closure
- Is sterilized using ethylene oxide gas
- Supports reproducible cell seeding on a tubular three-dimensional scaffold
- Allows closed system operation
- Allows monitoring of product maturation by nutrient utilization and MCP-1 production
- Tengion's bioreactor system is designed to manufacture tubular organs in a closed system while allowing for shipping and delivery of product to clinical site

## References:

1. Basu, J. and Ludlow, J. W. (2010). Platform technologies for tubular organ regeneration. Trends Biotechnol 28, 526-533.