Bioengineered Urethral Augmentation: Preliminary Results

Donna Haworth; Douglas Chew; Naoki Yoshimura, MD, PhD; Michael Chancellor, MD; David Vorp, PhD.
University of Pittsburgh, Departments of Bioengineering, Surgery, Urology, and
the McGowan Institute for Regenerative Medicine, Pittsburgh, PA

Statement of Purpose: Urethral dysfunction is a common complication of diabetes mellitus, spinal cord injury, vaginal childbirth, and pelvic trauma. Stress urinary incontinence (SUI) is the involuntary loss of urine due to the inability of the urethral sphincter to maintain a tight seal during the storage phase. SUI is a disease that physically and emotionally affects more than 25 million American women annually.[1] There are currently several treatments for SUI including surgery, Kegel exercises, and electrical stimulation. However, each of these is accompanied by limited effectiveness and/or complications.[2-4] We believe that cellular therapies applied to the native urethra will aid in the function and support of the diseased urethra. Toward this end, we have begun the development of a tissue engineered urethral wrap (TEUW) that can be placed as a cuff around the native urethra and become integrated with the host tissue. The goal of this preliminary work was to fabricate and evaluate tubular construcst from fibrin and either bone marrow progenitor cells (BMPCs) or native urethral smooth muscle cells (uSMCs). Specifically, this work aimed to develop procedures for the uSMC isolation and to determine an optimization of cell density in the TEUW constructs.

Methods: *uSMC Isolation:* uSMCs were isolated from female Sprague-Dawley rats by enzymatic digestion essentially as described elsewhere[5]. The bladder and urethra were dissected from the surrounding tissue and placed in oxygenated Hank's Balanced Salt Solution (HBSS)[6]. All excess tissue was removed from the urethra, which was then sliced longitudinally and pinned. The urothelium was subsequently removed via sharp dissection, and the urethra was washed in HBSS for 30min at 4^{o}C. The remaining urethral tissue was minced and placed into dispersal medium (15mg collagenase, 0.5mg protease type XXIV, 10mg bovine serum albumin, and 10mg trypsin inhibitor in 5mL HBSS). Digestion was performed at 37^{o}C for time points of 10, 15, or 20 min with gentle agitation[5]. Digestion was stopped by centrifugation, and the cells were resuspended in 1mL Dulbecco's Modified Eagles medium supplemented with 10% bovine serum and 1% penicillin/streptomycin. Cells obtained from each digestion were plated in one well of a 6 well plate. Cells were grown to confluence and passaged at 5000 cells/cm^2. At passage three, cells were passaged onto coverslips and grown to confluence. Coverslips were then fixed and stained for the smooth muscle cell markers α-smooth muscle actin, calponin, and myosin heavy chain to confirm the phenotype.
Construct Fabrication and Analysis: Tubular constructs were fabricated by first positioning a Teflon mandrel concentrically within a glass sheath. A mixture containing 3mg/mL bovine fibrinogen with 0.5 unit/mg thrombin and either 7.5x10^5 cells/mL, 1.0x10^6 cells/mL, or 1.25x10^6 cells/mL in serum-free media was placed around the mandrel and incubated. Constructs were removed from the mandrel and maintained in spinner flask culture for either 3 or 5 days in media supplemented with 0.3mM ascorbic acid and 0.1g/mL aminohexanoic acid. At each time point, the construct length was measured, a small ring was cut, and cell content measured using the MTT assay. A burst test was performed by mounting the remainder of the construct in a bathing chamber and increasing its intraluminal pressure until construct failure. The pressure and outer diameter of the constructs were recorded during the burst test, from which the compliance was calculated. Following burst, a ring was cut for viability assessment using the Live-Dead assay and the remaining portions of the constructs were fixed in 4% paraformaldehyde, sectioned, and stained for F-actin, TUNEL, and nuclear content.

Results/Discussion: Early observation of the isolated uSMCs revealed that they are viable and proliferating, with a digestion time of 20 min yielding the best proliferative results. The ratio of initial and final length revealed that construct compaction increased with cell density and culture duration. MTT assays indicated a increase in cell number compared to the original seeding density across all seeding concentrations and culture durations except 1.25x10^6 cells/mL at 3 and 5 days. The constructs seeded with 1.25x10^6 cells/mL had the greatest ratio of compaction, leaving a smaller volume for cells to proliferate, therefore impeding further growth. The burst pressure increased with cell concentration and culture duration, while compliance values appeared to decrease with time in culture, but appeared to be independent of cell number. The increased burst pressures and decreased compliance over the culture duration indicate that a longer culture time increases strength and stiffness. The burst pressures of all constructs, not including 7.5x10^5 cells/mL at 3 days, compare well to pressures sustained by the native urethra (20-60 mmHg), while compliance values are slightly lower (0.004 and 0.01 compared to the native 0.022 mmHg^{-1}).[5,7]

Conclusions: This preliminary study demonstrates our ability to construct tubular-shaped constructs for potential use as a TEUW and establishes preliminary data with regards to the optimization of the construct vis-à-vis culture duration and cell density.

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