Urinary bladder smooth muscle engineered from adipose stem cells and a three dimensional synthetic composite

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K30 Journal Article
Introduction

- Bladder reconstruction needed after
  - Congenital malformation
  - Decompensation of neurogenic bladder
  - Trauma
  - Malignancy and cystectomy
  - Radiation injury
Introduction

- Traditional solution is reconstruction with intestinal segment
  - Ileal loop incontinent urinary diversion
  - Continent catheterizable pouch
  - Orthotopic neobladder
Introduction

- Complications of reconstruction with intestinal segments
  - Bowel obstruction
  - Metabolic disturbances
  - Secondary malignancies
  - Urinary calculi
  - Urinary tract infections
  - Urinary incontinence or retention
Introduction

- Alternative material needed and attention turned toward tissue engineering
- Tissue harvested from patient bladder
  - Invasive harvest method
  - Pathological bladder
- Prolonged, expensive cell expansion times
Introduction

- Adipose derived stem cells (ASC)
  - Embryonic mesodermal origin like muscle and bone marrow
  - Pluripotent progenitor cells
  - Differentiate into...
    - Myogenic
    - Adipogenic
    - Neurogenic
  - Abundant, accessible
Introduction

- Created novel 3-dimensional bladder mold of poly-lactic-glycolic acid (PLGA)
- Impregnated with smooth muscle cells derived from ASC
- Evaluated construct
  - En vitro
  - En vivo (human ASCs in nude rat surgical model)
  - Ex vivo (isometric tissue bath)
Isolation and Culture of ASCs

- Liposuction
  - Cells washed, collagenase, centrifuged
  - Resuspended, cultured in FBS, DMEM, abx
  - Differentiation into different lineages considered ASCs
Scaffold Construction

- PLGA
  - established cell affinity
  - Biocompatibility
  - can be mass-produced
  - conformed to organ shape
  - engineered for desired elasticity and strength
Scaffold Construction

- Two layers of PLGA construct
  - Inner Layer
    - Thin, microfiber scaffold
    - Malleable, soft, non-porous, water tight
  
- Outer Layer
  - Thick
  - 95% porous PLGA sponge
Smooth Muscle Tissue Engineering

- ASC treated with Smooth Muscle Inductive Medium (SMIM)

- Smooth Muscle differentiated ASC (SM-ASC) labeled for MHC and Caldesmon expression
14 day in vitro incubation on scaffold

SM-ASC labeled with dialkylcarbocyanine (top)

Stained with calcein for viability (bottom)
Bladder Augmentation

- 200-250g athymic rats
- 50% bladder defect (supratrigonal partial cystectomy)
- Augmentation cystoplasty with acellular graft control (n=15), SM-ASC seeded graft (n=15), non-grafted control (n=15)
- Repair with interrupted and running closure
Outcome Parameters

- SM-ASC differentiation and viability prior to implantation
- Histologic exam of grafts ex vivo at weeks 2, 4, 8, 12
- Urodynamics under anesthesia at weeks 2, 4, 8, 12 postop
  - Capacity
  - Compliance (dv/dp)
- Isometric evaluation of harvested grafts
Successful differentiation and scaffold-seeding of SM-ASC

(a) RT-PCR

(b) Relative Expression of SM-ASC components v non-differentiated ASC on scaffold

SM-ASC on scaffold stained for (c) caldesmon, and (d) MHC
## Histology

<table>
<thead>
<tr>
<th>Time</th>
<th>Acellular construct</th>
<th>SM-ASC seeded construct</th>
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<tbody>
<tr>
<td>2 weeks</td>
<td>+urothelium</td>
<td>+urothelium +SM-ASC</td>
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<tr>
<td>4 weeks</td>
<td>++urothelium SM ingrowth</td>
<td>++urothelium SM ingrowth</td>
</tr>
<tr>
<td>8 weeks</td>
<td>+SM, +collagen +capillaries</td>
<td>++SM, +capillaries, rare collagen, ASC oriented</td>
</tr>
<tr>
<td>12 weeks</td>
<td>++collagen</td>
<td>+SM-ASC oriented, SM bundles</td>
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</tbody>
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Histology

3 days, 4 and 12 weeks

SM-ASC implant 8 wks

Anastomosis at 4 weeks

SM-ASC red, urothelium blue at 8 weeks

Urothelium at 2 weeks
SM-ASC emitting Dil, nuclei with DAPI

Cellular and Colalgen deposition (Mason’s Trichrome)

SM and collagen above urothelium
- Suture only regained capacity by 12 weeks
- Acellular graft tending to lose capacity
- SM-ASC graft maintained capacity
Urodynamics

- Suture only improved compliance to near baseline
- Acellular graft decreased compliance
- SM-ASC graft improved compliance
Isometrics

- Acellular graft no significant contraction
- SM-ASC significant contraction at 12 weeks (no response at earlier harvest)
- Carbachol-induced contraction reversible with atropine
Conclusions

- ASC abundant, affordable, amenable to SM differentiation in vitro, maintained in vivo
- Takes approx. 12 weeks for PLGA dissolution to balance SM-ASC contractility
- Maintain capacity and compliance
- Anti-fibrotic properties of SM-ASC
- Host urothelium covers graft, but not before urinary calculi develop (20-47% rats)
Problems

- Regenerative capacity of rat bladder
- PLGA as nidus for stone formation
- Properties of SM bundles seen in acellular graft
- Rat bladder significantly smaller than required by human