

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

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# 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



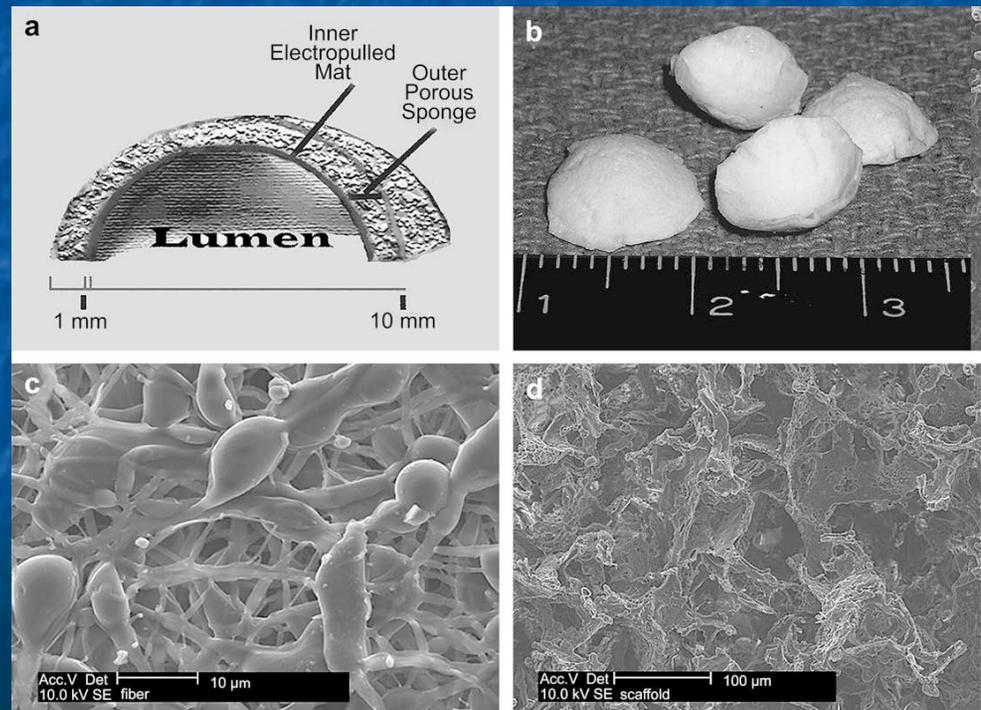
Differentiation into different  
lineages considered ASCs

# Scaffold Construction

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

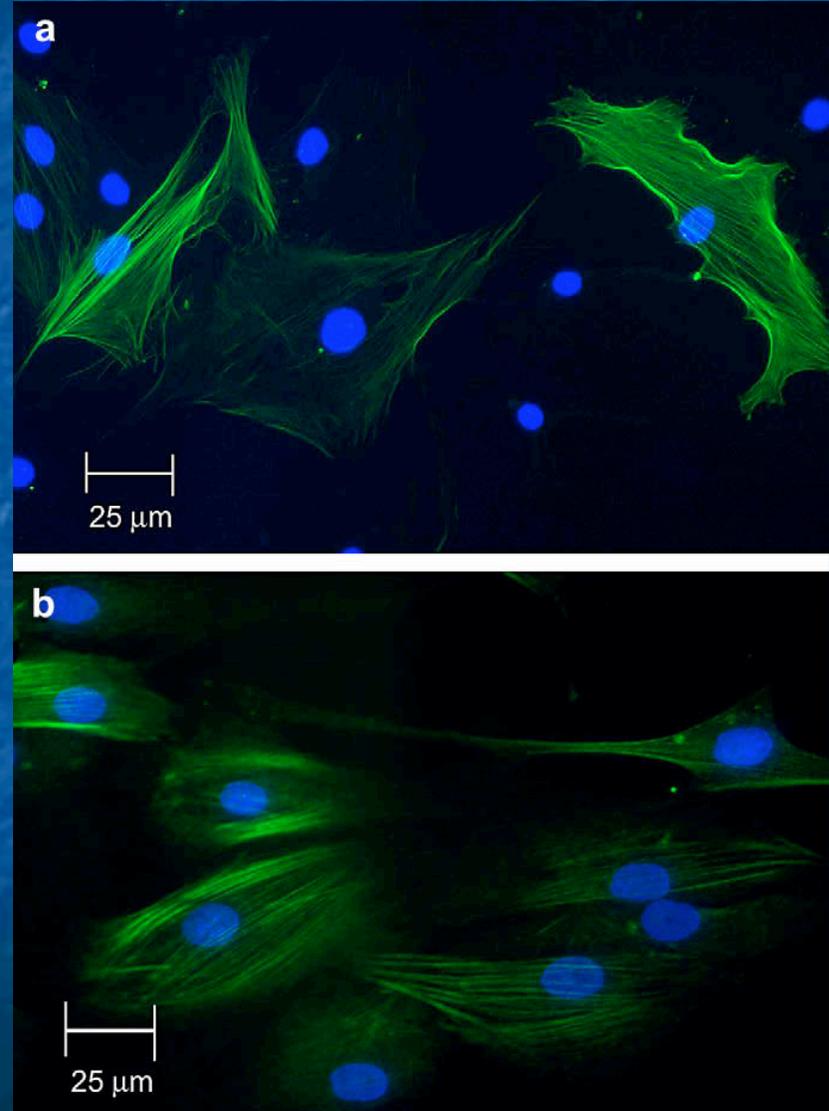
## Scaffold Construction

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

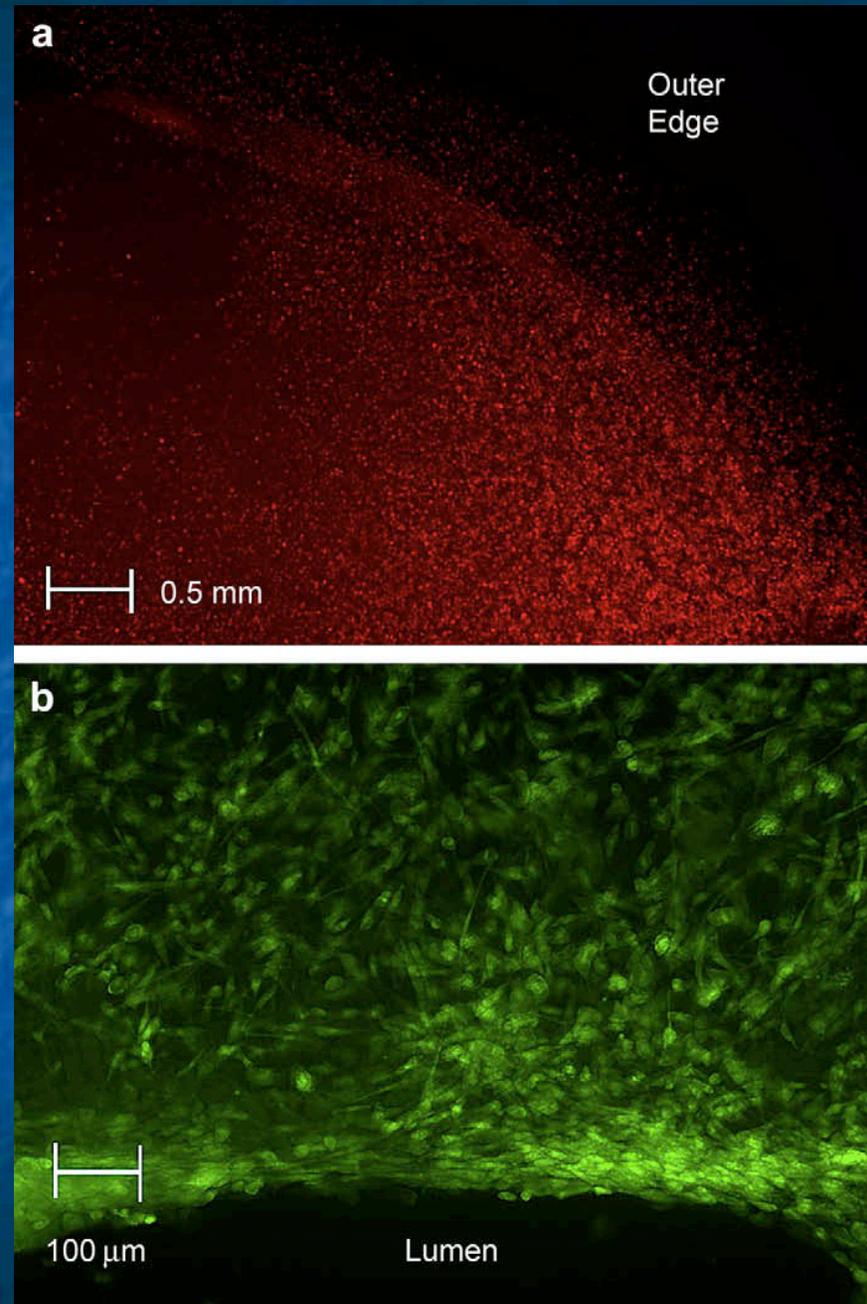


# Smooth Muscle Tissue Engineering

- 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

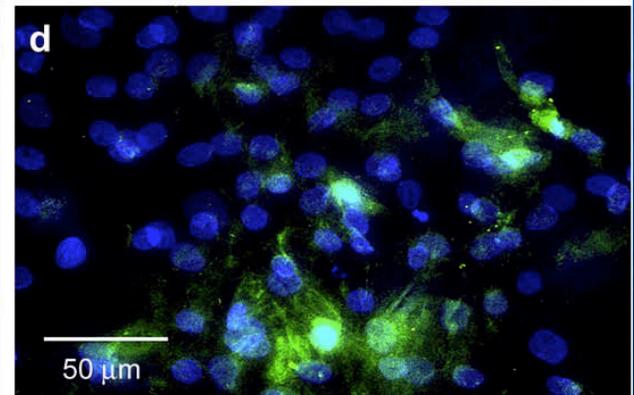
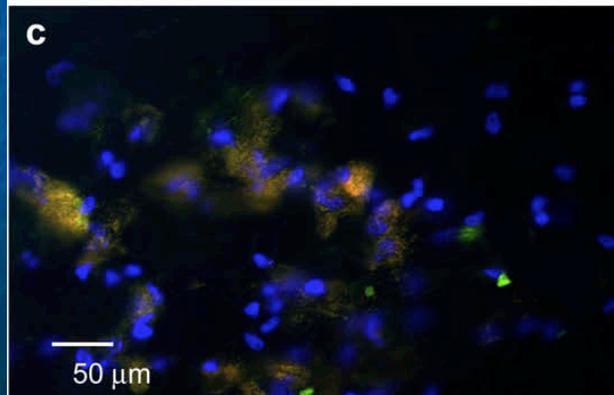
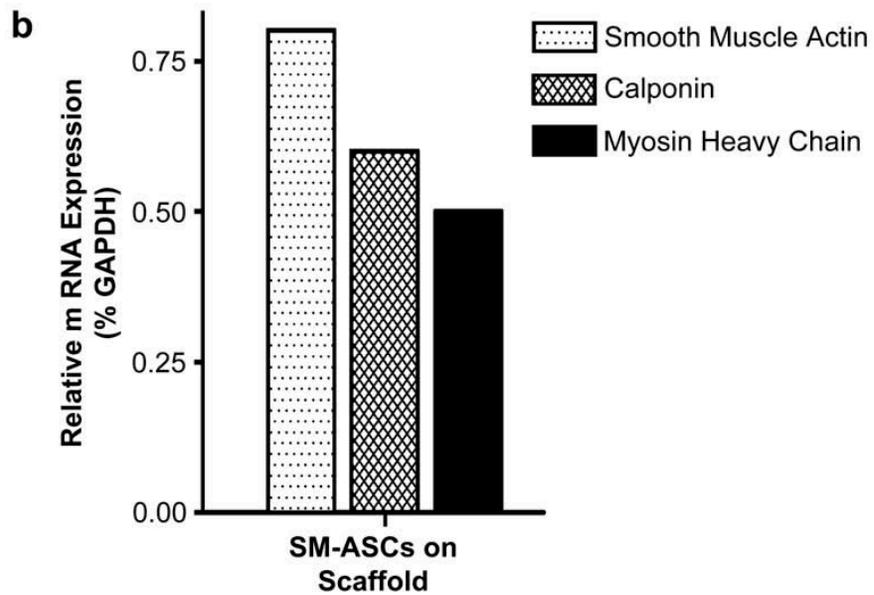
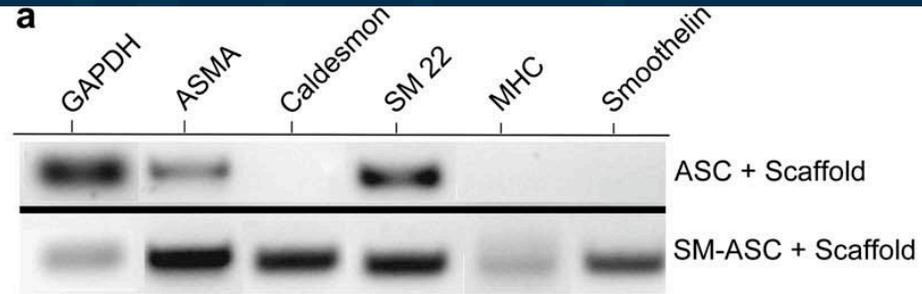
- SM-ASC differentiation and viability prior to implantation
- Histologic exam of grafts ex vivo at weeks 2, 4, 8, 12
- Urodynamics under anesthesia at weeks 2, 4, 8, 12 postop
  - Capacity
  - Compliance (dv/dp)
- Isometric evaluation of harvested grafts

# 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

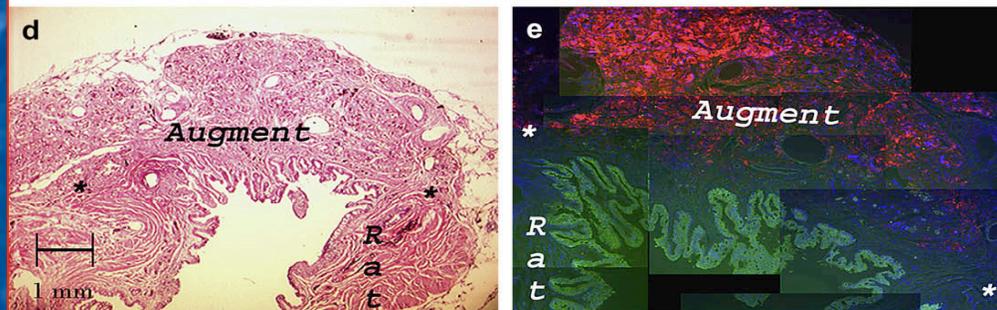
	Acellular construct	SM-ASC seeded construct
2 weeks	+urothelium	+urothelium +SM-ASC
4 weeks	++urothelium SM ingrowth	++urothelium SM ingrowth
8 weeks	+SM, +collagen +capillaries	++SM, +capillaries, rare collagen, ASC oriented
12 weeks	++collagen	+SM-ASC oriented, SM bundles

# Histology

3 days, 4  
and 12  
weeks

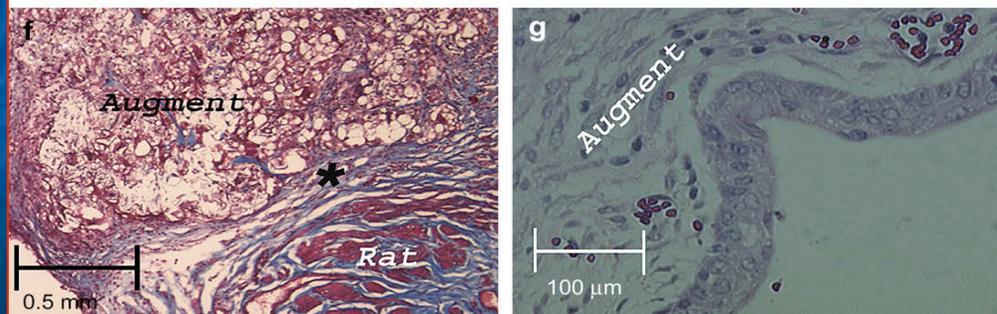


SM-ASC  
implant 8 wks



SM-ASC red,  
urothelium blue  
at 8 weeks

Anastomosis  
at 4 weeks

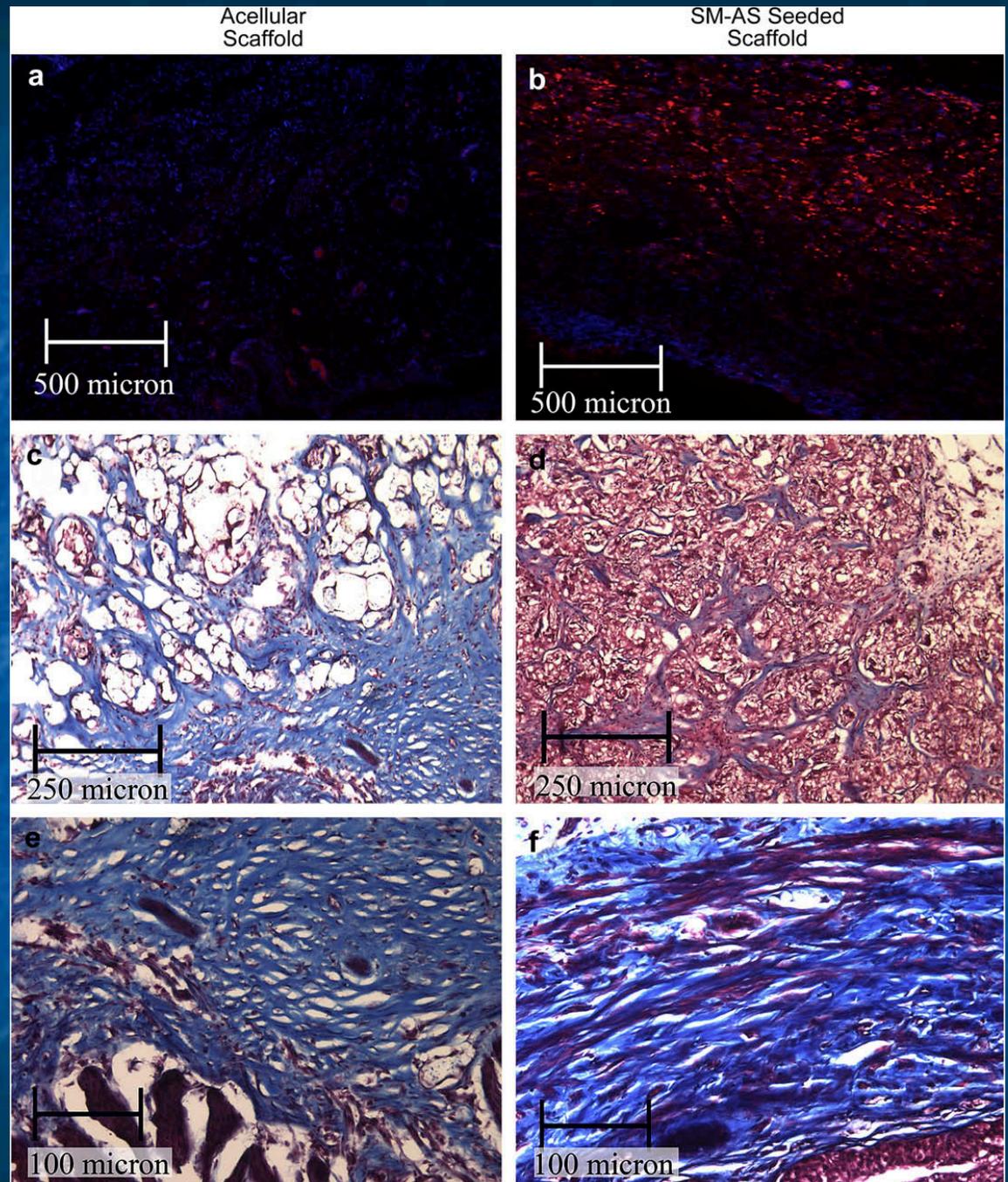


Urothelium at  
2 weeks

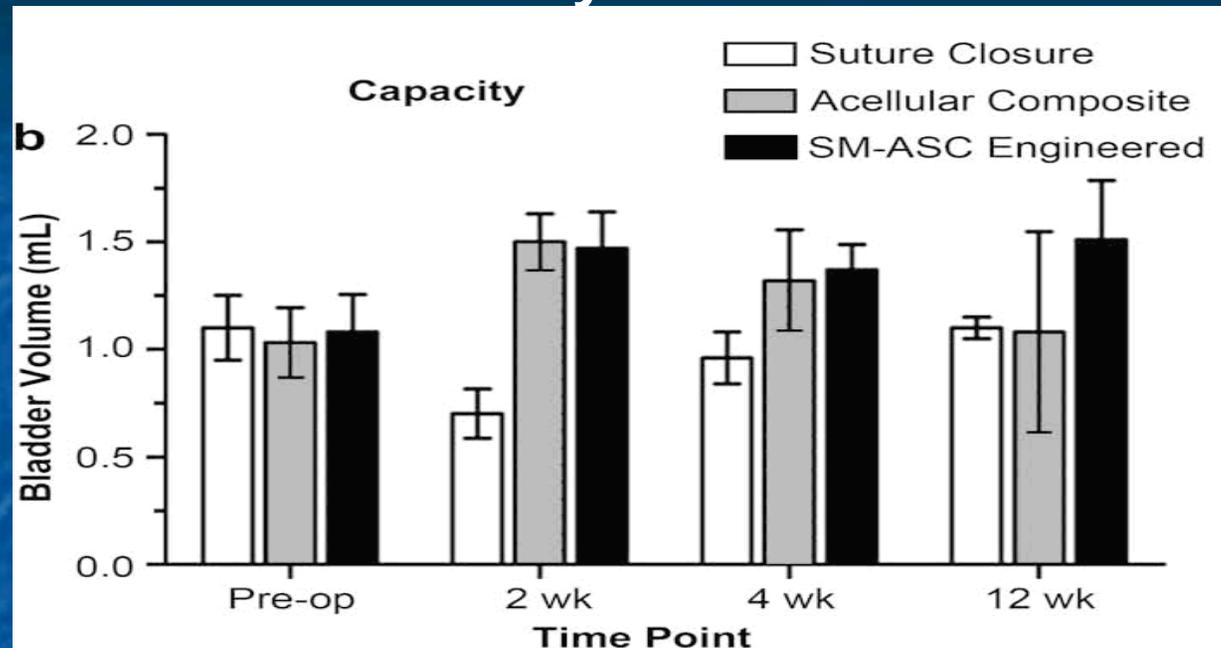
SM-ASC emitting Dil,  
nuclei with DAPI

Cellular and Colalgen  
deposition (Mason's  
Trichrome)

SM and collagen above  
urothelium

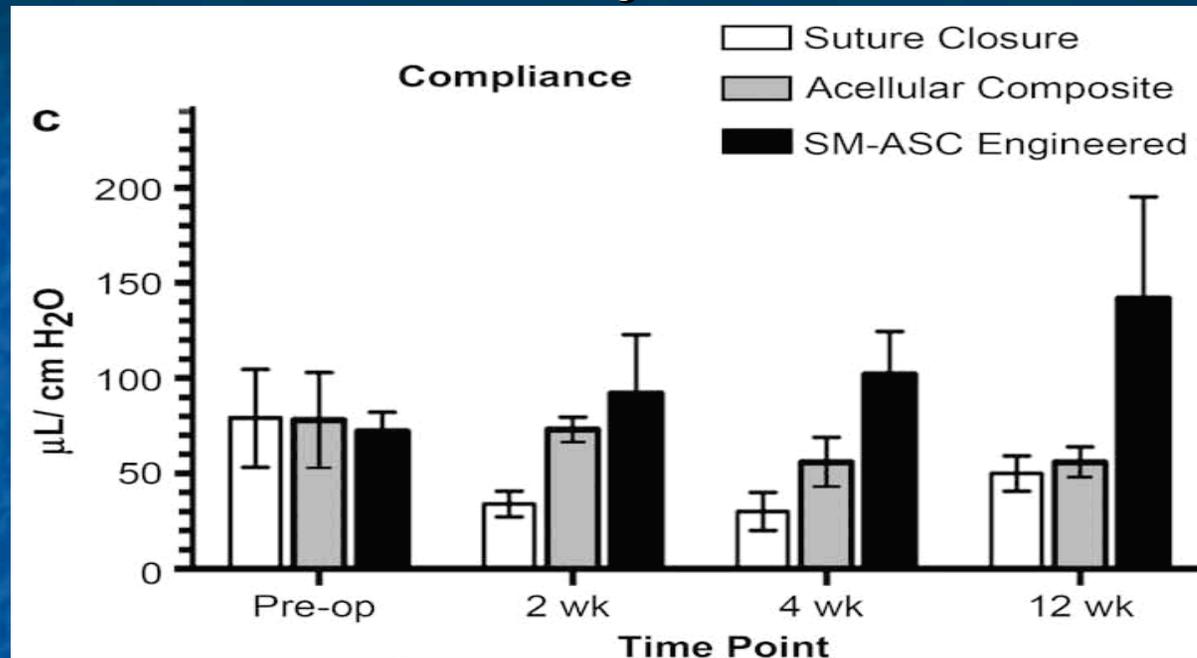


# Urodynamics



- Suture only regained capacity by 12 weeks
- Acellular graft tending to lose capacity
- SM-ASC graft maintained capacity

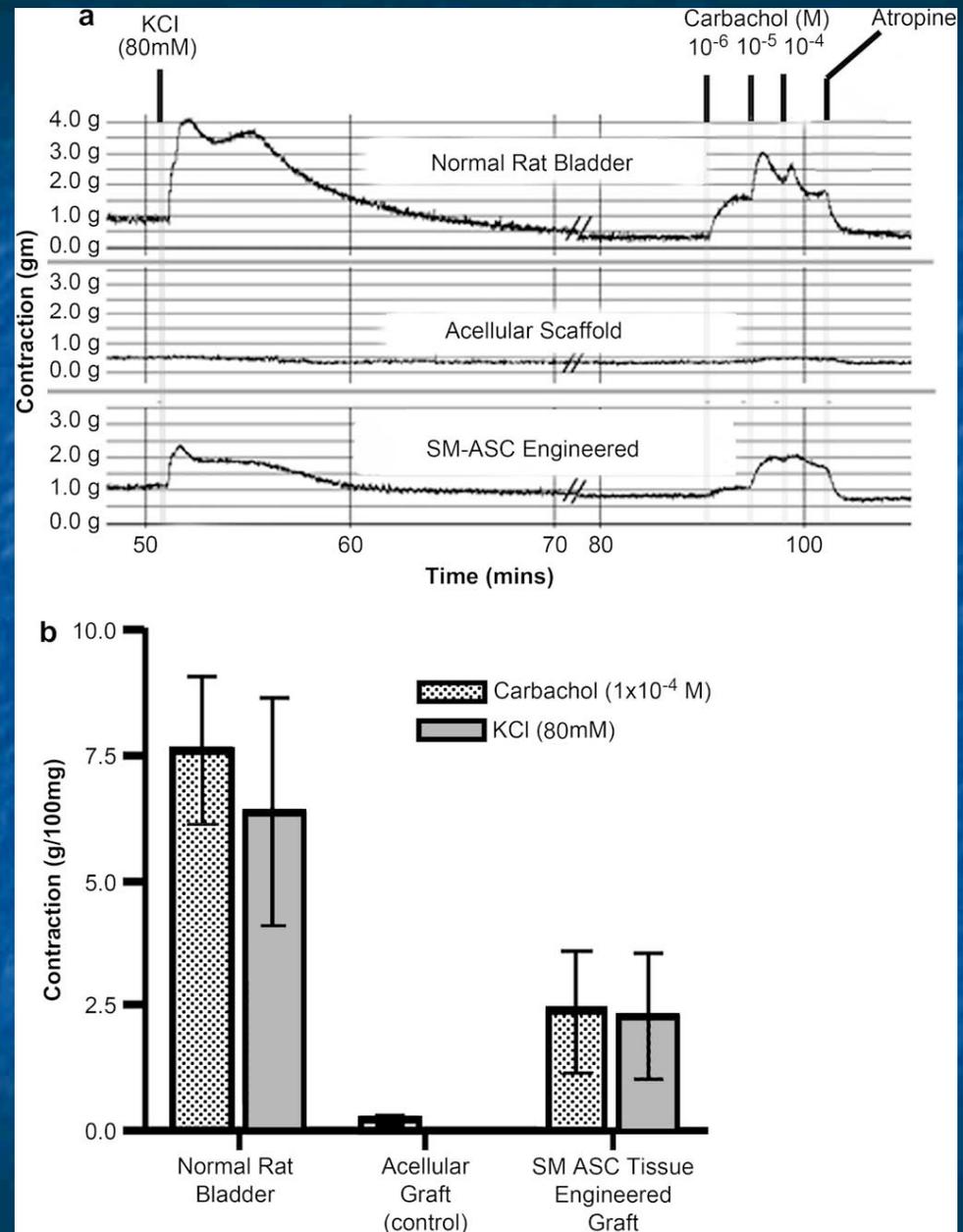
## Urodynamics



- 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