

Silk Fibroin Scaffolds for Urethral Tissue Regeneration

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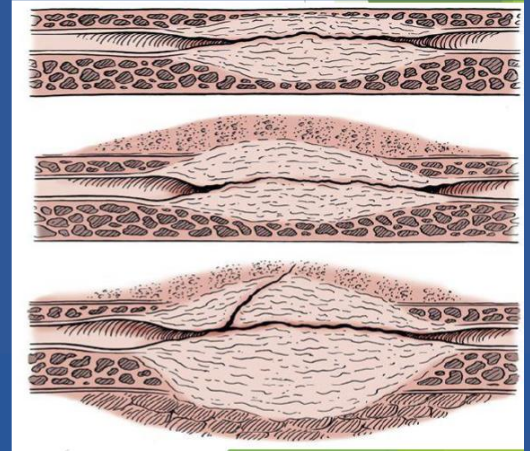
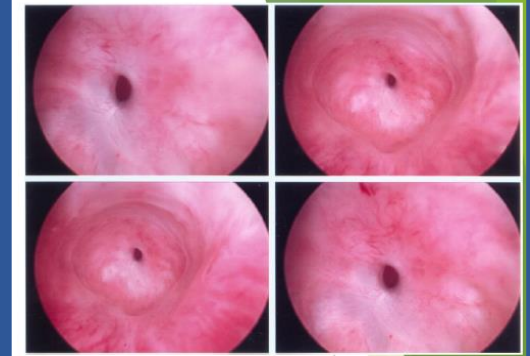
UCI Health

Outline

- Adult and Pediatric Disorders of the Urethra
- Current Approaches in Urethral Reconstruction
- Tissue Engineered Strategies for Urethral Repair: Success and Failures
- Silk Biomaterials: Overview and Preclinical Studies
- Looking Ahead...Innovations in Urethral Health

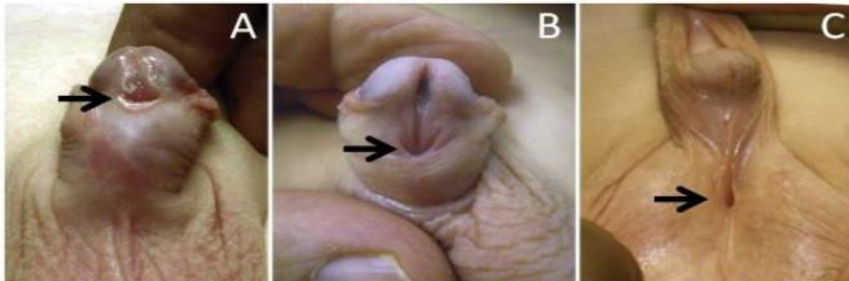
Urethral Stricture Disease

- A urethral stricture is scarring in or around the urethra that narrows or blocks urine flow.
- Urethral strictures can result from trauma (straddle injury), infection (STD), and chronic inflammation (lichen sclerosus).
- Relatively common disease in males (~400 per 100,000) with increased incidence after 55 years of age.
- 1.5 million outpatient visits per year. Complications including stones, incontinence, infertility, and renal damage.



Hypospadias

- Hypospadias is a congenital defect resulting in malpositioning of the urethra opening which can result in voiding difficulty and infertility.
- Frequency of the disease is 1 per 200 male births. 10,000-15,000 new cases in US per year.
- Approximately 30% of cases are severe (proximal) and require extensive surgical repair with tissue flaps. Surgical complication rate is 5-50% with staged operations needed in many cases. Repair performed 6-12 months of life.

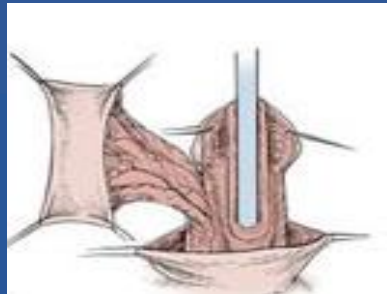


van der Horst HJR & de Wall LL. Hypospadias, all there is to know. *Eur J Pediatr* 2017, 176:435.

Carmichael SL, et al. for the National Birth Defects Prevention Network. Brief report, population-based birth defects data in the United States, 2008 to 2012: Presentation of state-specific data and descriptive brief on variability of prevalence. *Birth Def Res (Part A)* 2015, 103:972.

Surgical Strategies for Urethral Repair

- Endoscopic approaches for urethral stricture repair
 - Dilation or incision (cold knife internal urethrotomy)
 - 100,000-235,000 procedures performed annually in the US*
 - Success rate of dilation is low and typically unsuccessful for >1 cm strictures
 - Repeat internal urethrotomy offers no chance of cure after 3rd treatment or restricture in 3 months.
- Open Urethroplasty
 - Approach based on length and severity of the defect
 - End to end anastomosis (stricture) versus onlay urethroplasty with autologous tissue grafts (stricture and hypospadias)
 - Treatment limited to highly specialized centers, ~2500 procedures in the US annually



*Extrapolated from: Blaschko SD, et al. Trends, utilization, and immediate perioperative complications of urethroplasty in the United States: Data from the national inpatient sample 2000-2010. *Urology* 2015, 85:1190.

Onlay Urethroplasty with Autologous Tissues

- High success rates (85-95%), but complex, 4% of urologists practice. Buccal mucosa and prepubital skin flaps are common.
- State-of-the-art therapy for urethral defects >2cm where end-to-end approach is not feasible (i.e. penile shortening, etc.)
- Limitations include harvest site morbidity and scarce tissue supply. Penile complications including pain, erectile dysfunction, loss of sensation.



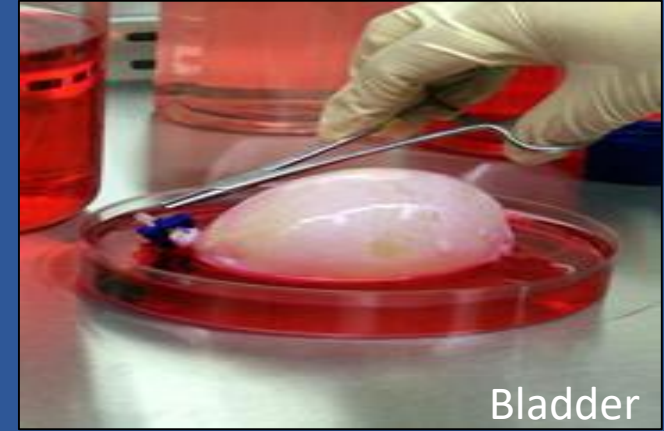
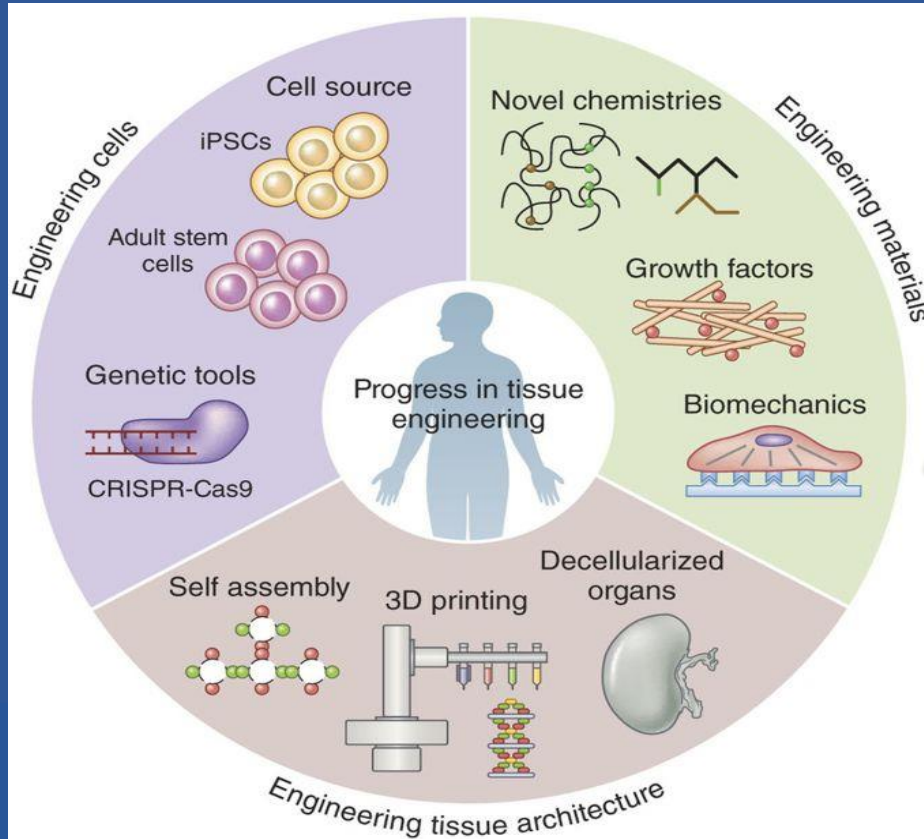
Table 4 – Buccal mucosal onlay urethroplasty complications, n = 19.

Early complications	N (%)	Late Complications	N (%)
Minor Complications		Minor Complications	
Hematuria	1 (5)	Hand numbness	1 (5)
Retrograde urethrography leak	3 (16)	Perineal hypoesthesia	1 (5)
Scrotal hematoma	1 (5)	Post void leak	2 (5)
Small wound dehiscence	1 (5)	Scrotal hyperesthesia	1 (5)
Wound tightness	1 (5)	Stensen's duct squirting	
All	7 (37)	Saliva out of mouth when eating	1 (5)
		UTI	1 (5)
		All	7 (37)

Bullock RL & Brandes SB. Adult anterior urethral strictures: A national practice patterns survey of board certified urologists in the United States. *J Urol* 2007, 177:685.

Al-Qudah HS & Santucci RA. Extended complications of urethroplasty. *Int Braz J Urol* 2005., 31:315.

Urologic Tissue Engineering



Summary of Preclinical Studies

Examples

Synthetic biomaterial scaffolds

Polyethylene terephthalate

Poly (L-lactic acid)-co-poly-(ε-caprolactone) (PLLCL)

Poly(lactic acid)/PLLCL composite

Natural biomaterial scaffolds

Collagen type I and III

Silk fibroin

Small intestine submucosa (SIS)

Decellularized human amniotic membrane

Decellularized urinary bladder

Decellularized porcine dermis

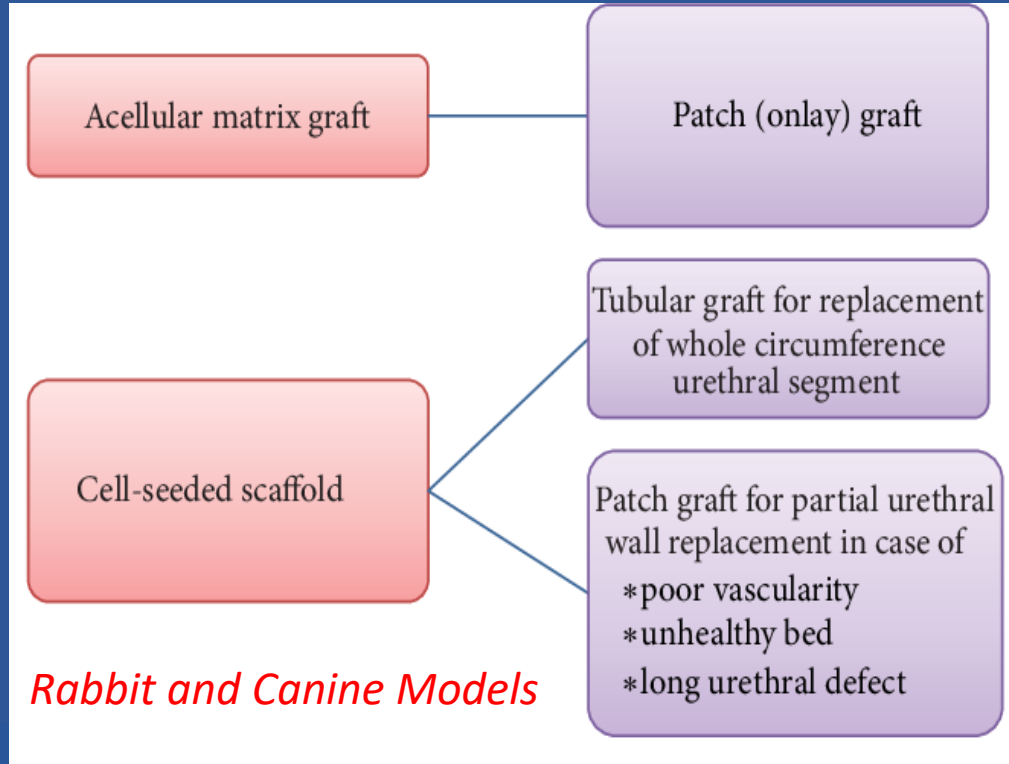
Cells

Urine-derived stem cells

Urothelial cells derived from bladder washes

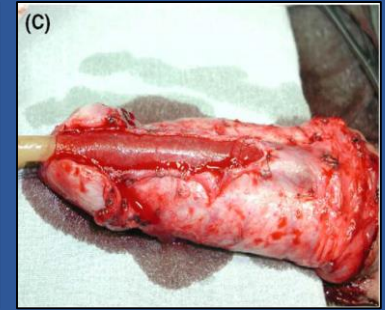
Adipose-derived stromal cells

Oral keratinocytes



Acellular SIS Grafts for Urethroplasty

- Onlay urethroplasty using SIS grafts (50 patients)
- Mean follow-up of 31.2 months
- Urethral Function restored in 40 (80%) patients.
- Re-strictures developed in 10 patients (<6 months).



Fiala et al., 2007

Patient	Age (yr)	Stricture			Urethroplasty		Time to recurrence (mo)	Recurrence	Therapy	Follow-up (mo)
		Etiology	Original therapy	Location	Matrix (mm)	Surgeon				
1	62	iatrogenic	UP	bulbar	50	B	3	P	BMG	24
2	45	iatrogenic	dilation	bulbopenile	80	A	4	P, D	dilation	18
3	75	iatrogenic	UT	bulbopenile	150	B	3	P	BMG	17
4	78	idiopathic	UT	bulbopenile	55	B	2	P	BMG	18
5	56	idiopathic	UT	bulbopenile	60	B	3	P, D	dilation	24
6	67	trauma	UP	bulbopenile	65	A	3	P	BMG	24
7	63	iatrogenic	UT	penile	80	B	2	entire	UP	24
8	71	inflammation	UP	penile	30	B	6	D	dilation	18
9	52	inflammation	dilation	penile	35	B	3	entire	UP	24
10	73	inflammation	UT	penile	40	B	6	D	UT	18
p value	ns	ns	0.01	ns	0.01	ns	—	—	—	—

Abbreviations: UP, urethroplasty; UT, endoscopic urethrotomy; P, proximal; D, distal; ns, no significant difference within column; —, not tested.

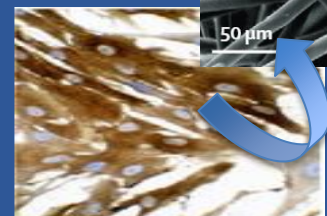
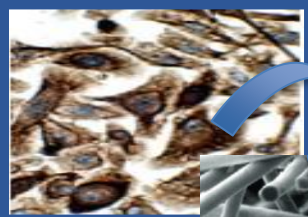
Cell-Seeded Therapies for Urethroplasty

Age (years)	Primary diagnosis	Previous urethroplasty	Defect site	Defect length (cm)	Follow-up (months)
1	Motor vehicle accident	No	Membranous urethra	5	76
2	Straddle trauma	Buccal mucosa	Membranous urethra	6	73
3	Motor vehicle accident	No	Membranous urethra	4	71
4	Motor vehicle accident	Foreskin	Membranous urethra	4	65
5	Straddle trauma	No	Membranous urethra	5	36*

*Patient followed up for 36 months because he was the last patient to enter the study.

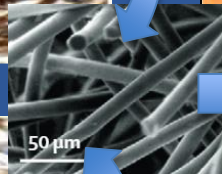
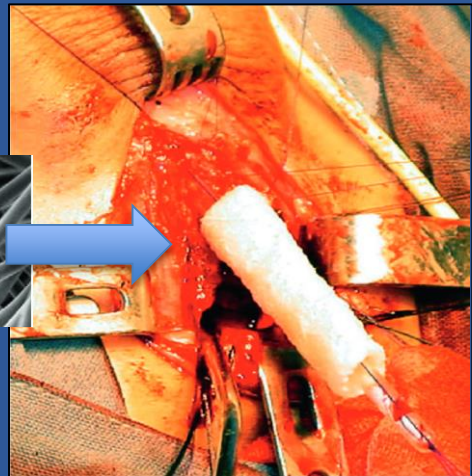
Bladder Biopsy

Urothelium

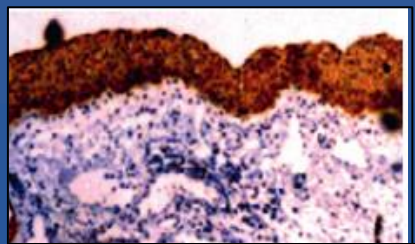


Smooth Muscle

Tubular PGLA Graft

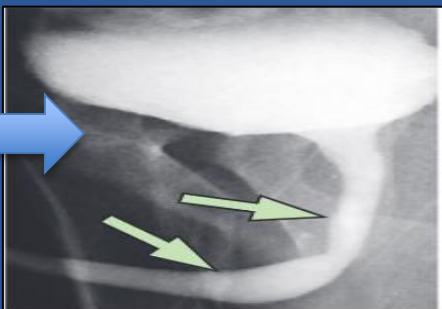
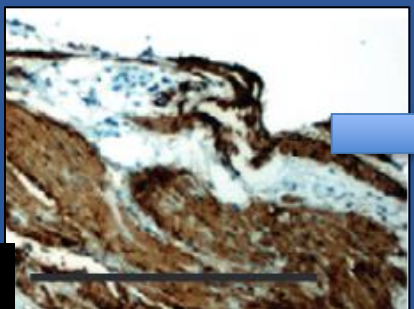


Urothelium



1 year Follow-up

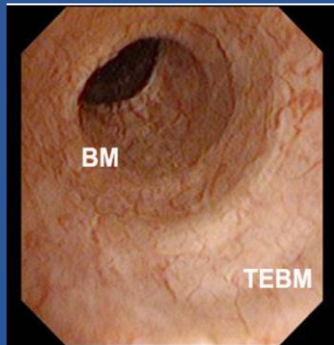
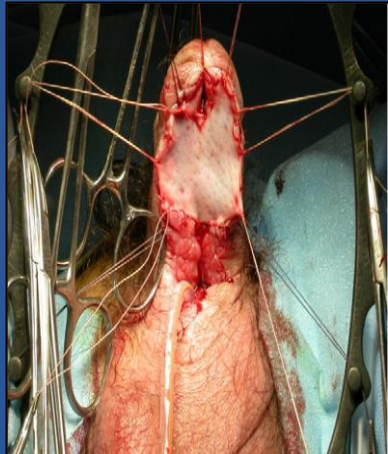
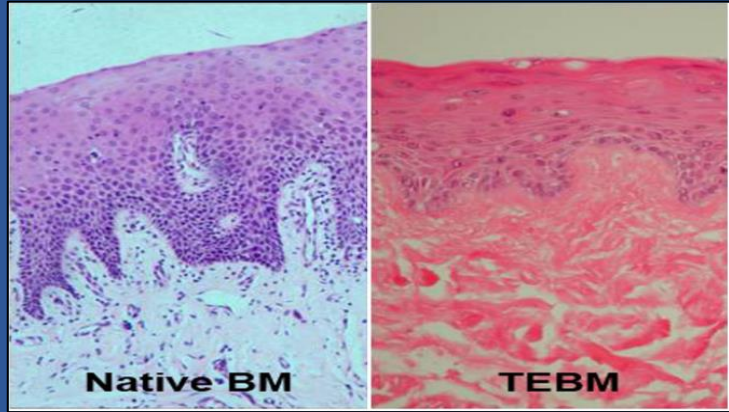
Smooth Muscle



Raya-Rivera et al., 2011

UCI Health

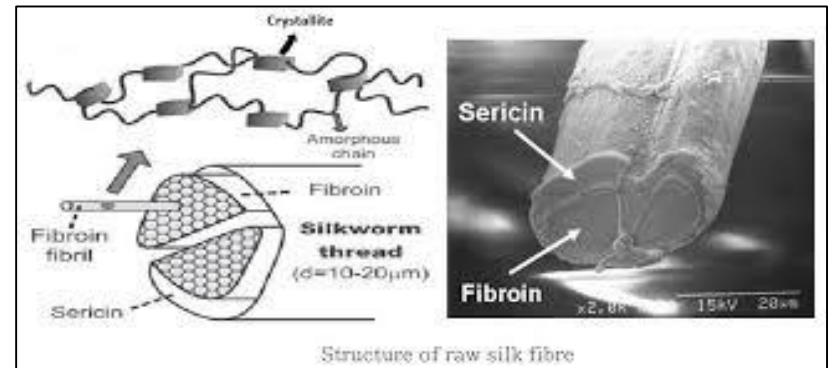
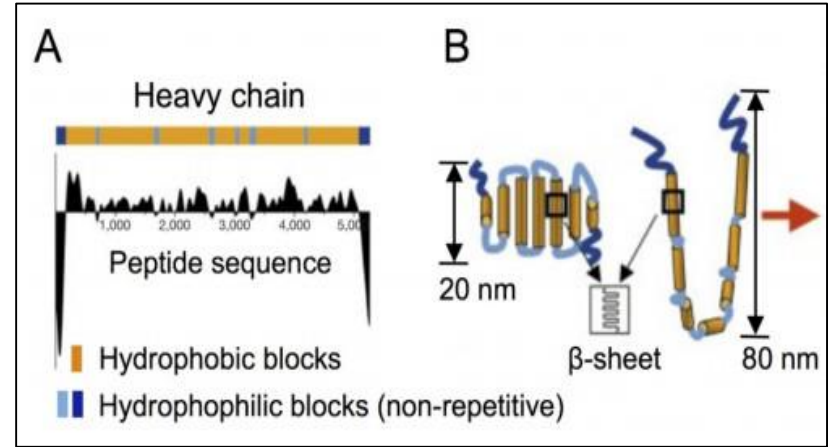
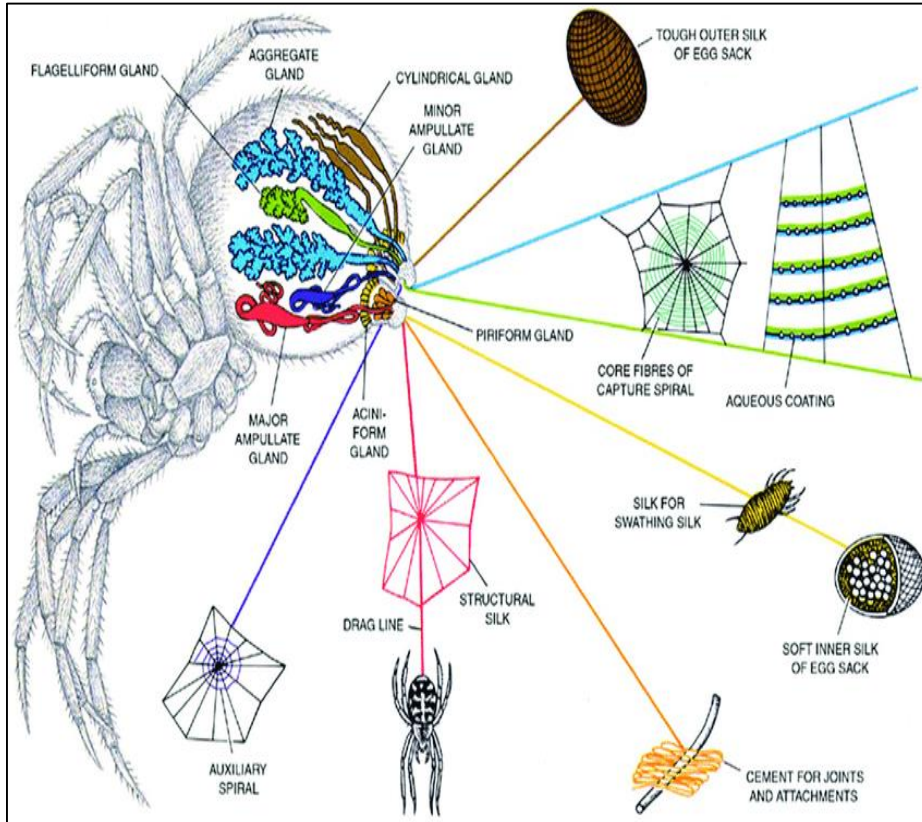
Tissue Engineered Buccal Mucosa for Urethroplasty



	Site of stricture	Previous surgery	Procedure	Follow-up (mo)	Outcome
P1	Full length	Urethroplasty × 2	2-stage	37	Second stage at 9 mo. Submeatal stenosis managed by intermittent meatal dilatation from 12-36 mo; now discontinued and asymptomatic. No other recurrence.
P2	Penobulbar	Urethrotomy × 2	2-stage	33	Complete excision of TEBM due to fibrosis 8 mo after first stage. Repeat of two-stage procedure with native BM successful without recurrence.
P3	Bulbar	Urethrotomy × 2 Urethroplasty × 1	1-stage	33	DIVU for diaphragmatic distal anastomotic stricture at 11 mo. Performed ISD. No further recurrence.
P4	Bulbar	None	1-stage	33	DIVU for diaphragmatic distal anastomotic stricture at 7 mo. Performed ISD for 24 mo; now discontinued and asymptomatic. No further recurrence.
P5	Penobulbar	Urethrotomy × 3 Urethroplasty × 2	2-stage	32	Partial excision of TEBM. Second stage at 9 mo after repeated first-stage. No recurrence at 14 mo.

TEBM, tissue-engineered buccal mucosa; DIVU, direct in-line visual urethrotomy; ISD, intermittent self-dilatation.

Silk Fibroin Diversity and Structure



Silk Fibroin Biomaterials



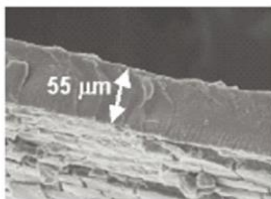
High Structural Strength and Elasticity

Low Immunogenicity

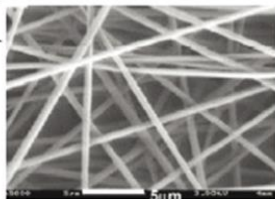
Controllable Degradability

Diverse Processing Plasticity

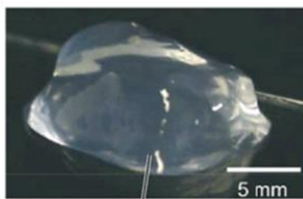
Films



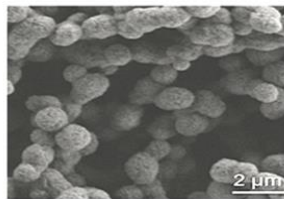
Nanofibers



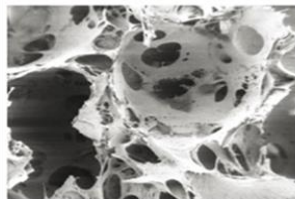
Hydrogels



Microspheres



Foams

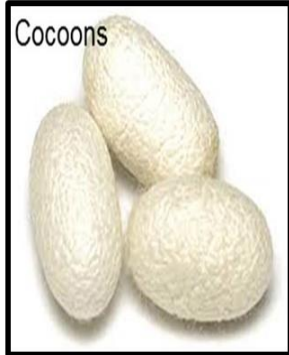
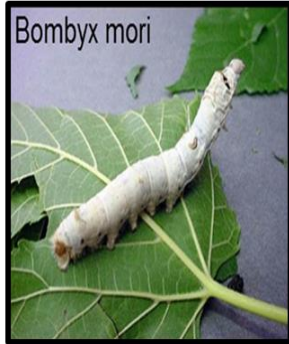


Tubes



Bi-Layer Silk Fibroin (BLSF) Grafts for Hollow Organ Reconstruction

Silkworm Cocoons



Silk Processing and Graft Fabrication



Silk Fibroin Isolation and Sericin Removal



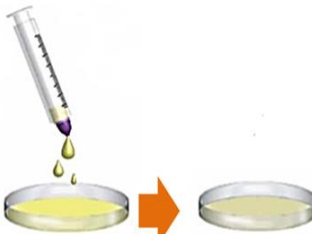
Silk Fibroin Dissolution in LiBr



Aqueous Silk Fibroin Solutions

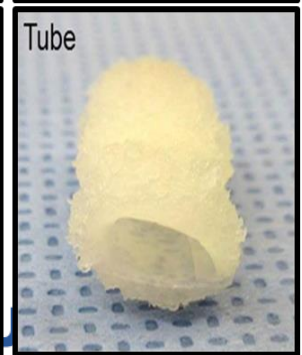
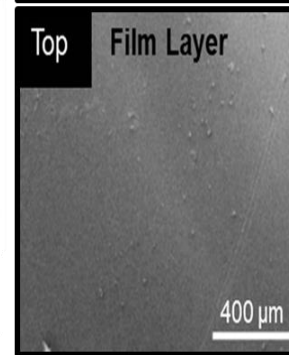
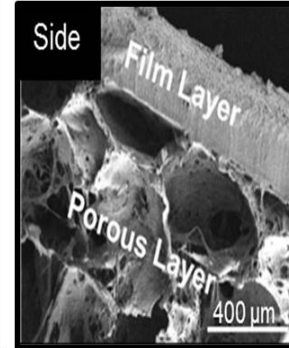


Solvent Casting/NaCl Leaching to Create Silk Fibroin Foams



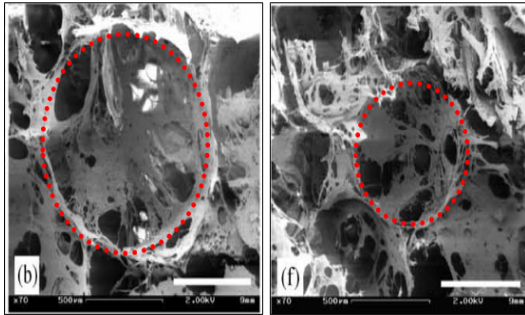
Film Casting of Silk Fibroin

Bi-Layer Silk Fibroin Graft



Versatile Graft Platform Allows for Optimization of Material Properties

Structure

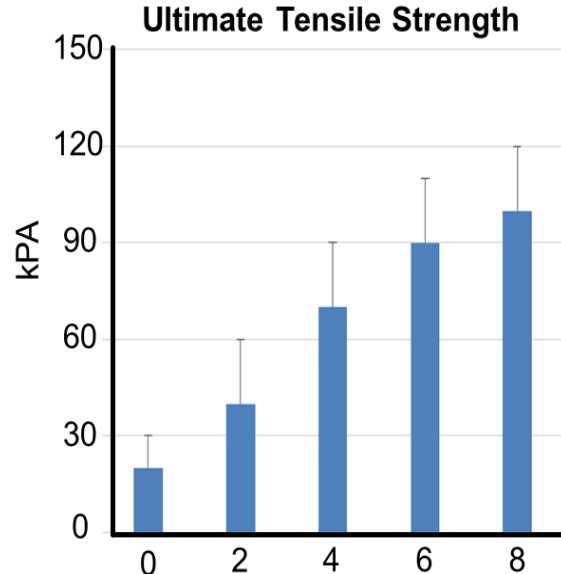


1000-1180 μm NaCl

710-850 μm NaCl

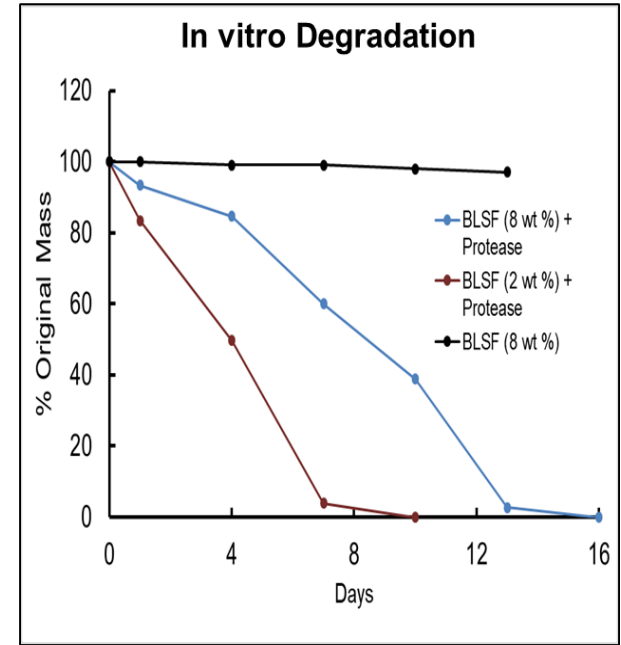
NaCl Particle Size (μm)	SF Concentration	
	4 wt %	6 wt %
1000-1180	940 \pm 50	930 \pm 40
850-1000	760 \pm 30	750 \pm 50
710-850	650 \pm 30	650 \pm 50
600-700	570 \pm 30	550 \pm 30
500-600	470 \pm 30	420 \pm 20

Mechanics



Film SF Concentration (wt %)

Degradation

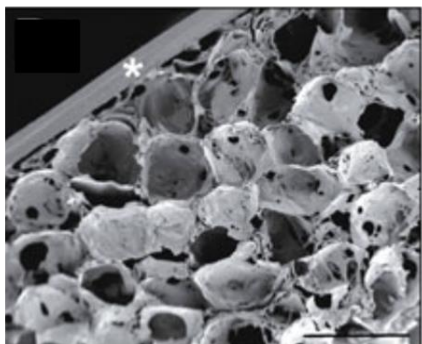


Unique Properties of BLSF Grafts for Clinical Translation

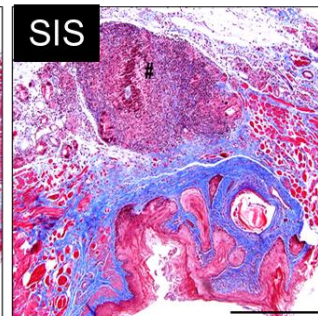
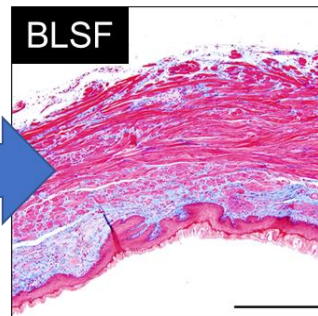
Restores Hollow Organ Integrity



BLSF Graft

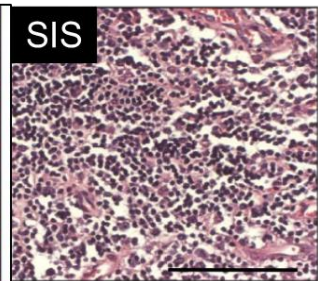
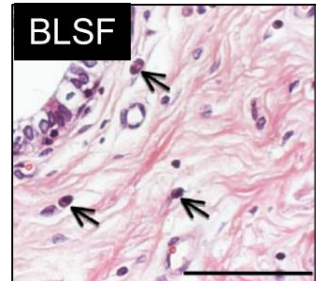
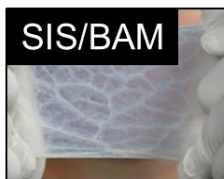


Superior Tissue Regeneration

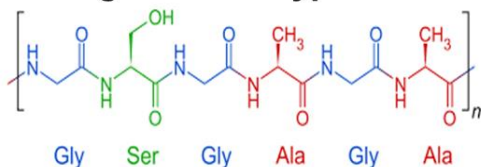


*Versus Conventional
Acellular Grafts*

Lack of Chronic Inflammation



Natural Amino Acid
Degradation Byproducts



Rabbit Onlay Urethroplasty-Hypospadias Model



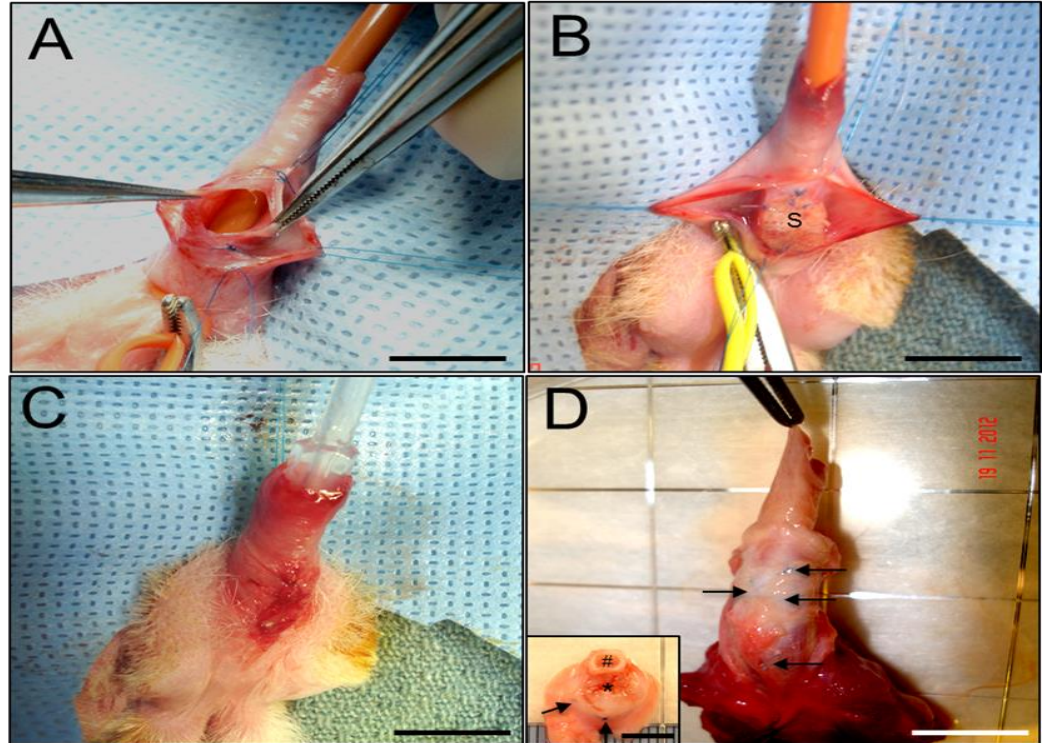
4 Silk Grafts (2 cm²)
4 SIS Implants
3 Urethrotomy



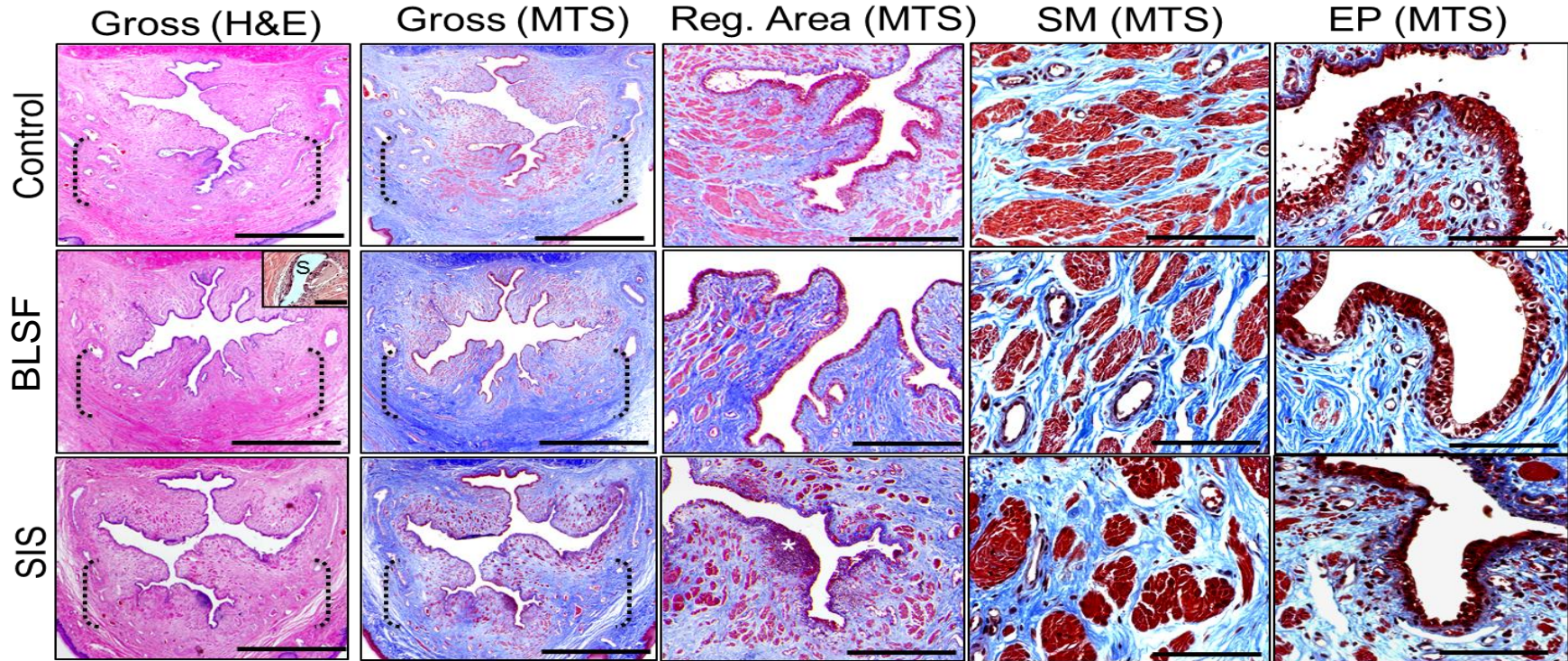
3 m Implantation



Outcome Analyses:
Histology
Histomorphometry
RUG



Regenerated Urethra Tissue Properties

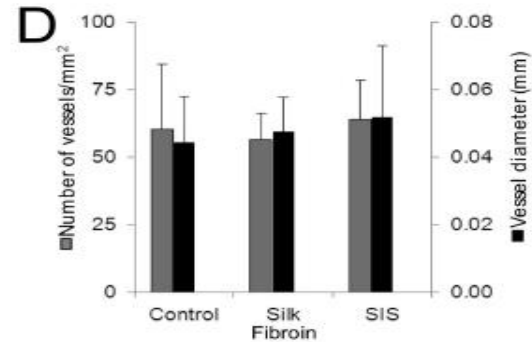
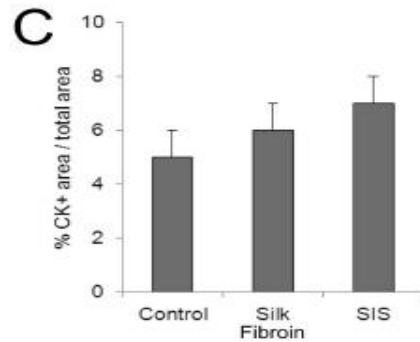
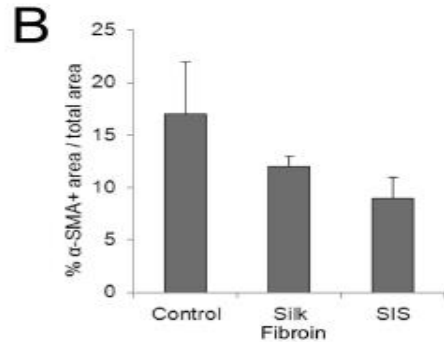
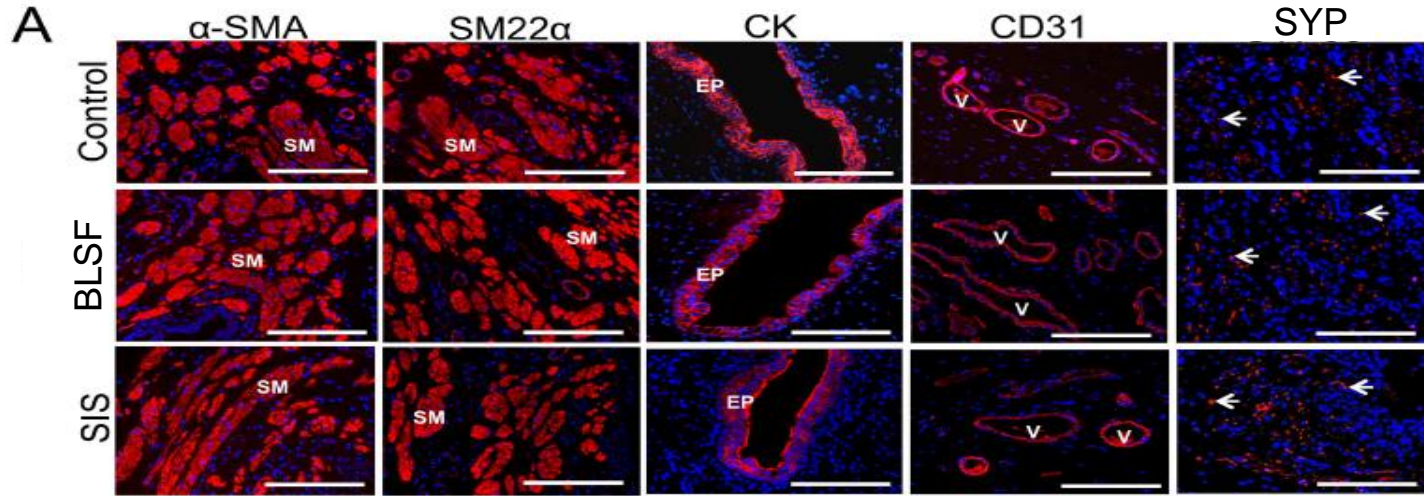


Control = Urethrotomy
BLSF = Bilayer silk fibroin
SIS = Small intestinal submucosa

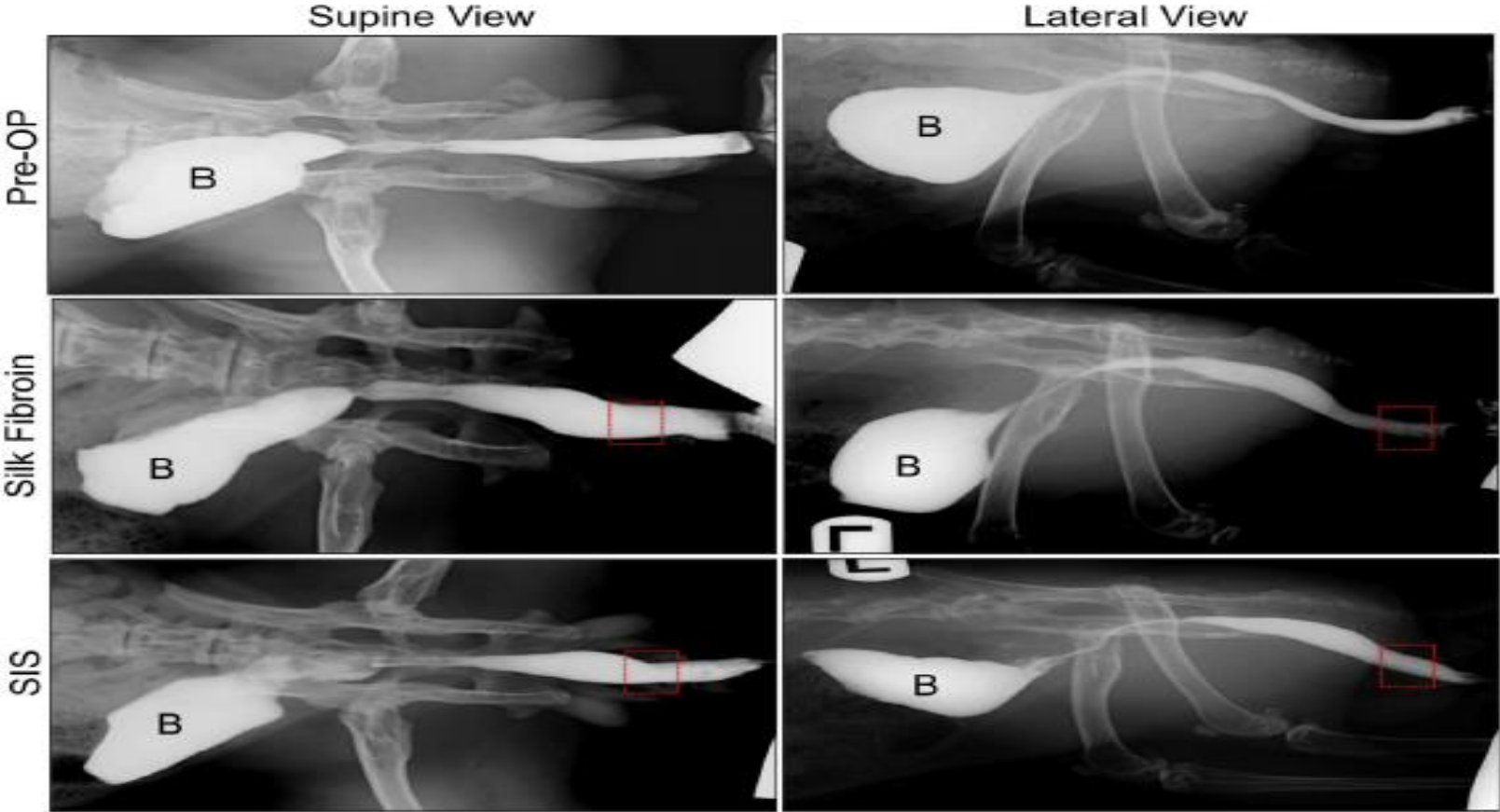
H&E= Hematoxylin and eosin
MTS = Masson's trichrome
Reg. Area = Regenerated Area

SM = Smooth Muscle
EP = Epithelium

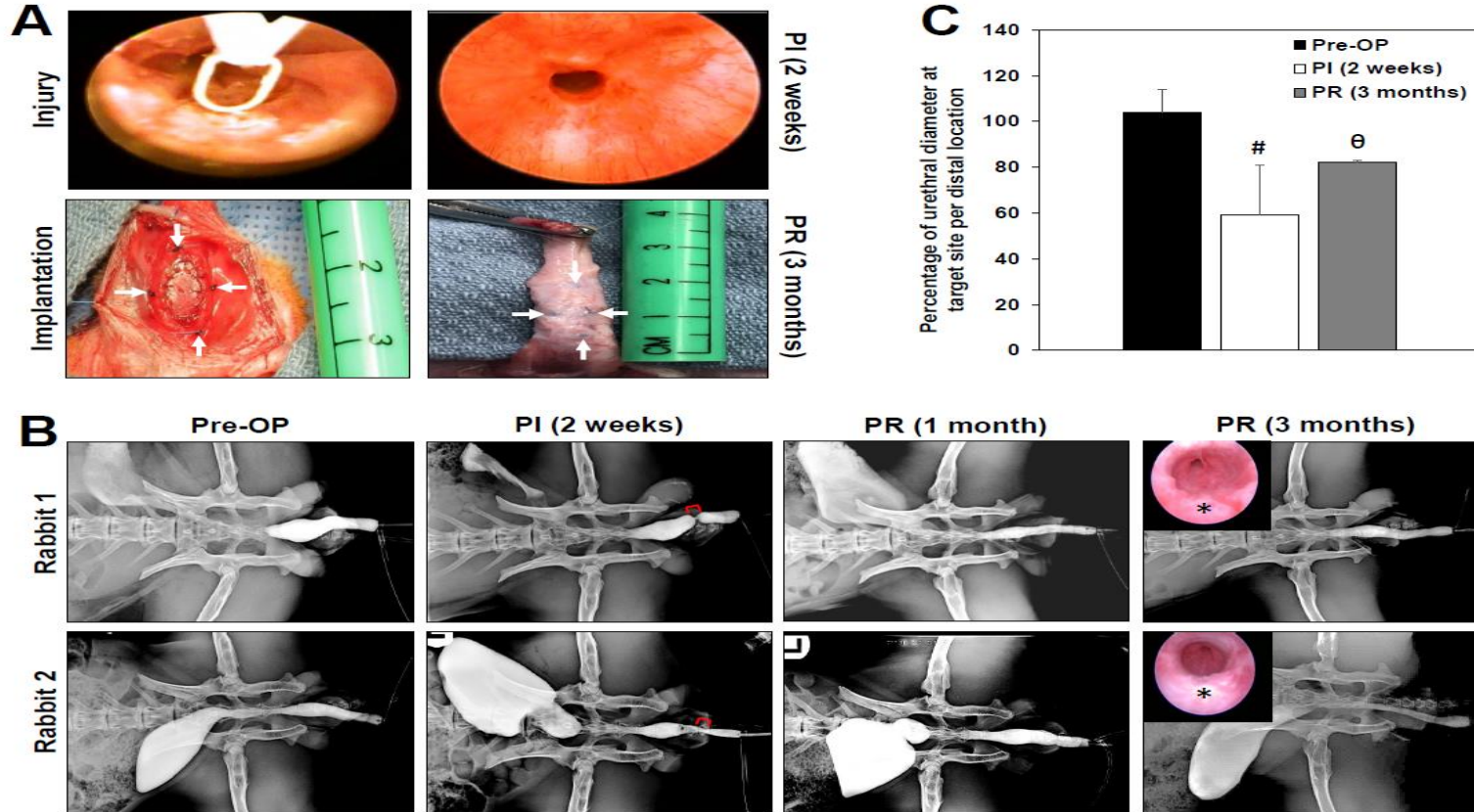
Regenerated Urethra Tissue Properties



Regenerated Urethra Tissue Properties

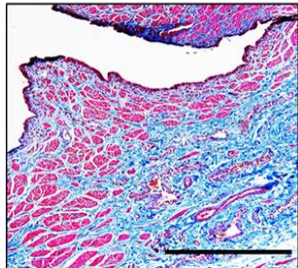
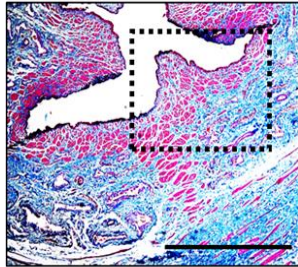
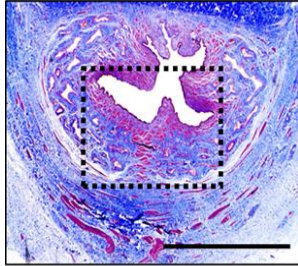


Rabbit Onlay Urethroplasty-Urethral Stricture Model

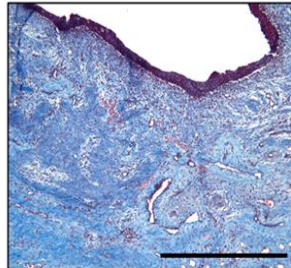
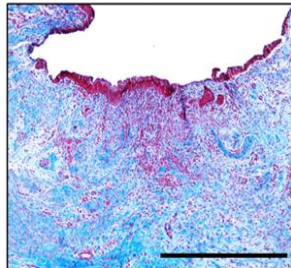
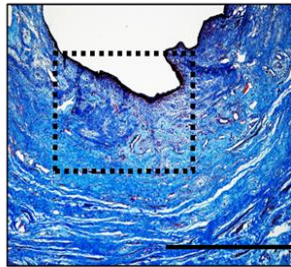
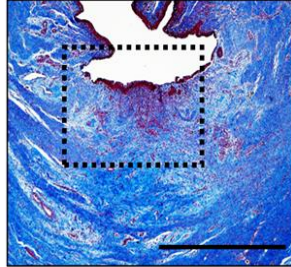
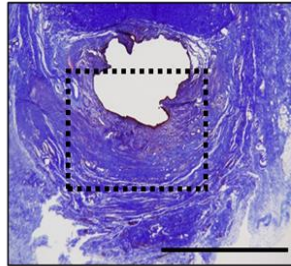
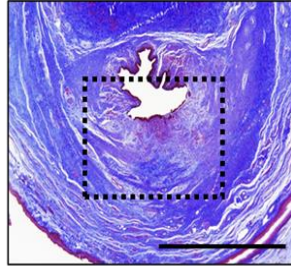


Regenerated Urethra Tissue Properties

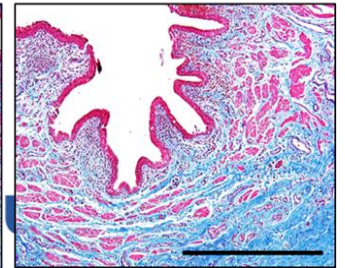
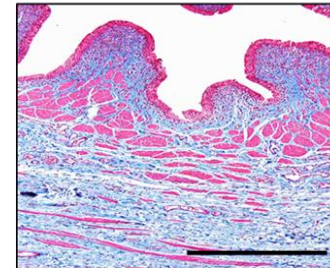
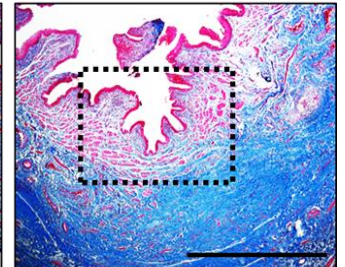
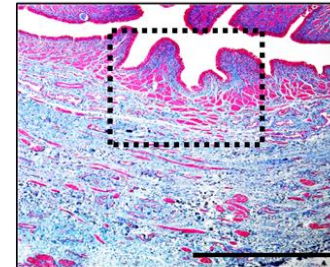
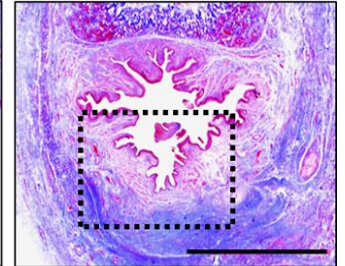
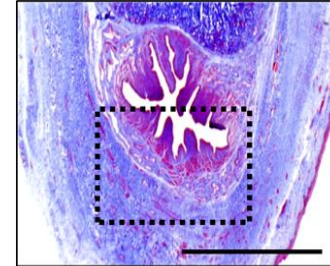
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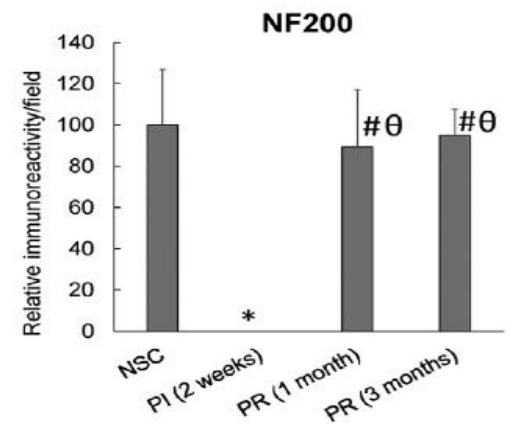
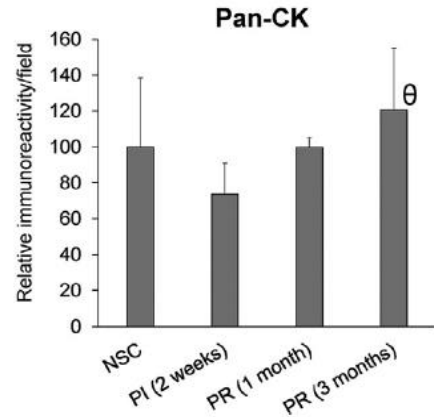
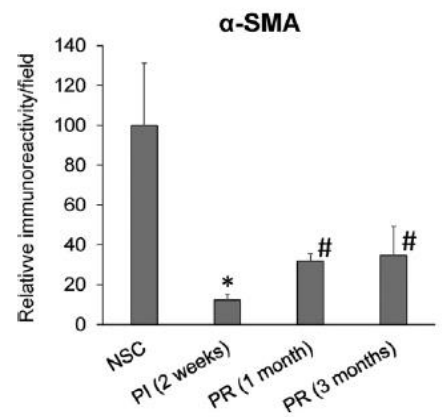
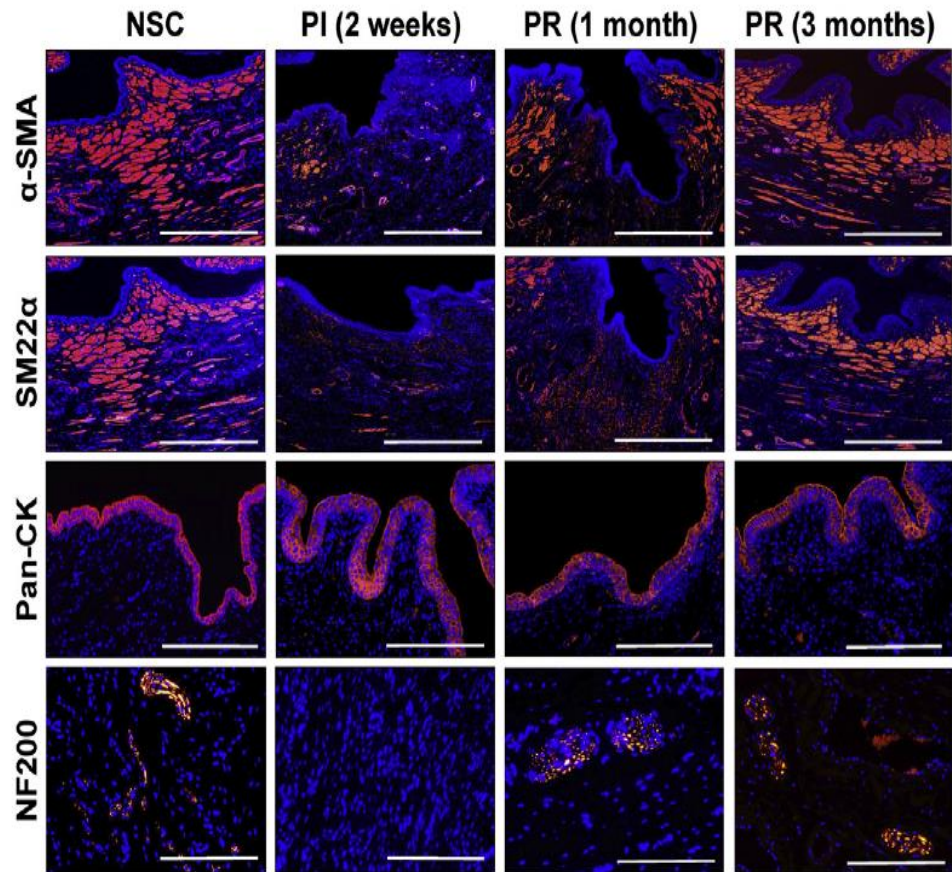
Stricture (2 wk)



De novo Tissue (3 months)



Regenerated Urethra Tissue Properties



Next Steps in Urethral Tissue Engineering

Validation of BLSF Grafts in a Porcine Model of Long Urethral Strictures (3-6 cm)

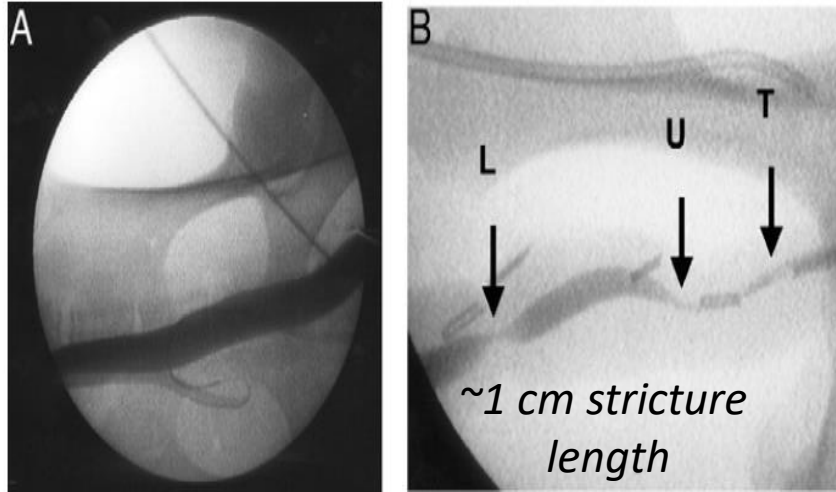
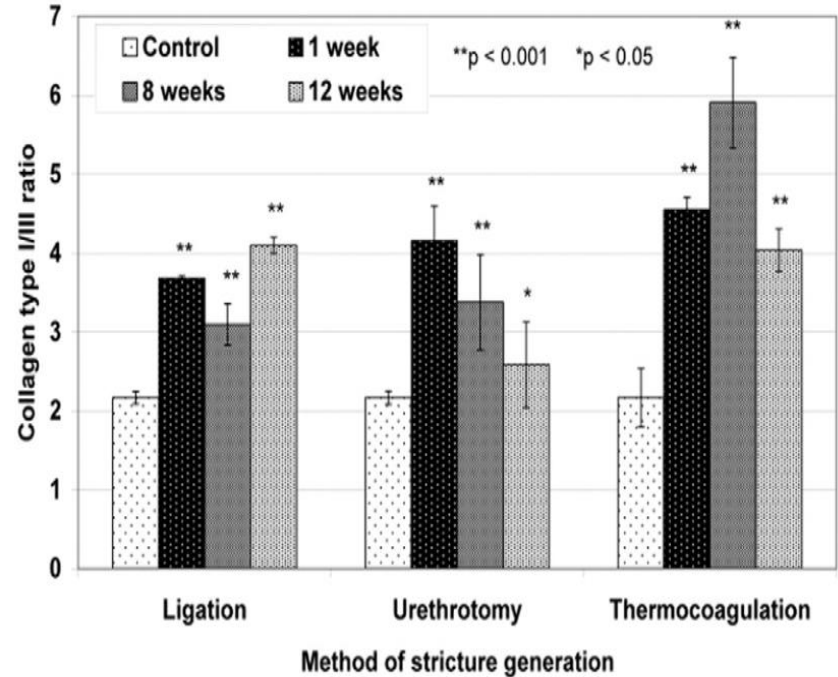


Figure 1. Urethrogram shows male minipig urethra. A, normal. B, after injury (arrows). L, ligation. U, internal urethrotomy. T, thermocoagulation.



Silk Fibroin Slings for Stress Urinary Incontinence

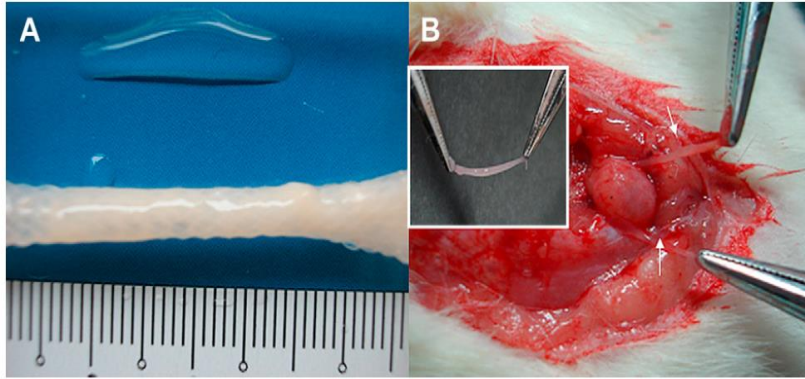
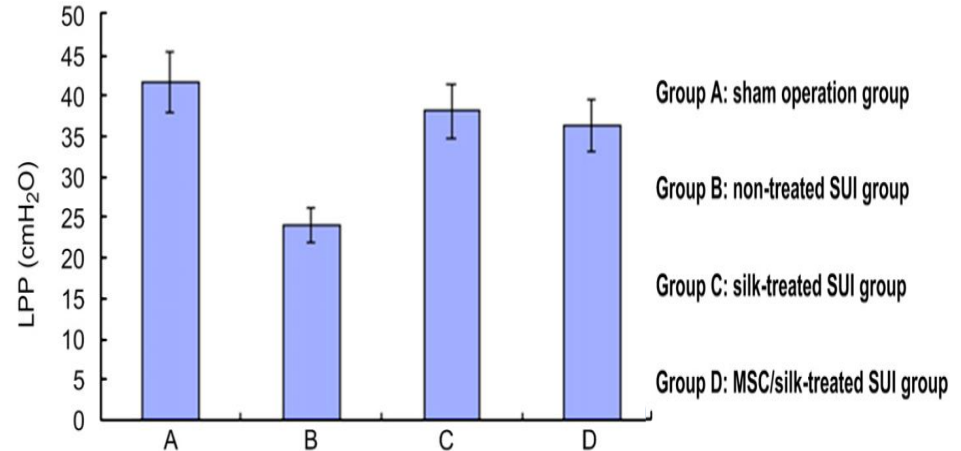
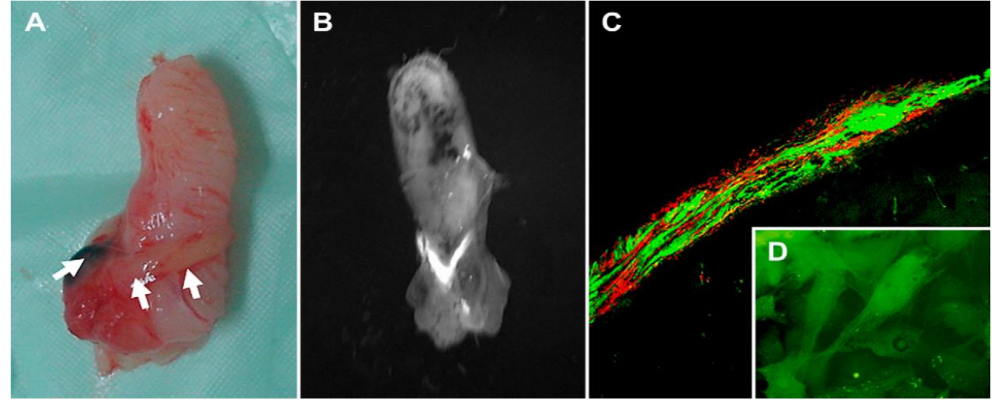


Fig. 3. Gross image of (A) bMSC/silk composite and (B) the surgery of placing suburethral sling (arrow).

Female Rats
Bilateral Sciatic Nerve Transection
12 week sling implantation period

Zou et al., 2010





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**Thank you,
Questions?**

UCI Health