PRIZE AWARD: Best Basic Science Abstract

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ESTABLISHING A RODENT MODEL FOR CHARACTERIZATION OF NATURAL IN VIVO BLADDER REGENERATION

Hypothesis / aims of study

Regeneration of the urinary bladder has been studied for over a century including studies by Daniel Liang in the 1960's demonstrating that both humans and rats have the ability to regenerate the bladder following subtotal cystectomy (STC).[1,2] Despite this knowledge, most preclinical studies have employed the use of scaffolds to guide regeneration of the bladder. In fact, the first autologous tissue-engineered neo-bladder has recently been successfully implanted in patients.[3] This seminal study documents that the body is a sufficient incubator for successful bladder regeneration. Improvements in the utility, efficacy and clinical applicability of tissue engineering/regenerative medicine, including therapies for urological disease(s) will undoubtedly benefit from increased understanding of the normal regenerative process *in vivo.* As a first step in this direction, we have begun to more completely characterize bladder regeneration *in vivo* in a rodent model. The overall goal of this study is to elucidate the cellular, molecular and physiological differences between regenerating and native tissues.

Study design, materials and methods

12 week old female F344 rats underwent trigone-sparing, subtotal cystectomy (STC; removal of ≈70% of the bladder), and the regenerative process that followed was monitored in several ways. *In vivo* filling cystometry studies were performed on conscious, freely-moving animals at 2, 4, and 8 wks post-STC. High-resolution Micro CT scans were also performed at 0, 1, 2, 4, and 8 weeks post-STC, and linked to *in vivo* filling cystometry studies on **the same animal**. After euthanasia, contractility of bladder detrusor muscle was characterized *in vitro*. Carbachol response-curves were examined, as well as the contractility to electrical field stimulation. Immunostaining was also performed in order to examine the differentiation status of the urothelium (Uroplakin3); demonstrate innervation of the bladder (PGP9.5); and to begin to identify cell populations involved in the regenerative process (CD117).

Results

Filling cystometry analysis showed that cystometric capacity in control animals was 0.96 ± .05 ml which decreased to 0.46 ± 0.03 ml at 2 weeks post-STC, then gradually increased to 0.73 ± 0.16 ml at 4 weeks post-STC and reached control values at 8 weeks (0.85 ± 0.08 ml,). Normal detrusor function was seen at all timepoints post-STC, with little or no non-voiding contractions, and the animals were continent. Bladder capacity calculated from Micro CT scans confirmed the cystometric findings of a gradual increase in volume following STC. Maximum (micturition) pressure was also reduced following STC (30.5 ± 5.87 cmH_2O 2 weeks post-STC) compared to controls (49.24 ± 3.72 cm H_2O), but did show some functional recovery at 4 weeks (38.15 ± 4.95 cmH_2O) and 8 weeks (37.31 ± 3.33 cmH_2O) after STC. Bladder circumference form the anterior view as calculated by microCT analysis also positively correlated with micturition pressure, r=.634, p<0.05. Maximal steady state contraction of isolated detrusor tissue strips from regenerating bladders also decreased following STC, but did displayed some functional recovery (Figure1). Electrical field stimulation induced responses from tissue at all timepoints post-STC, indicating the presence of nerves in the newly formed bladders. This latter observation was supported by positive PGP9.5 immunostaining in the regenerating bladder wall at 4 weeks post-STC. A mature urothelium (i.e., terminally differentiated umbrella cells) was shown as early as 1-week after surgery with positive immunostaining to uroplakin 3. Immunohistochemistry staining was positive for c-kit (CD117) in the detrusor denoting a possible role for progenitor cells in regeneration of the muscle layer. Finally, bladder wall thickness, as measured on hematoxylin and eosin staining was not different for control bladders compared to those examined 8 weeks post-STC.

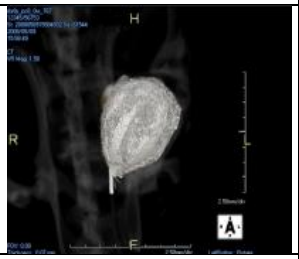
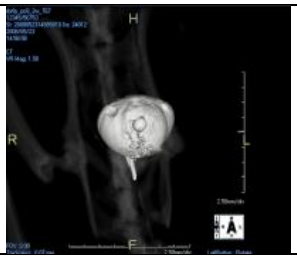
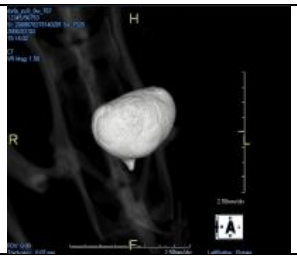
Time points	Control (pre-STC) (n=16)	2 weeks (post-STCl) (n=10)	8 weeks (post-STC) (n=8)
Siemens microCAT scans via TeraRecon analysis			
Maximum Pressure	49.24 ± 3.72 cmH_2O	30.5 ± 5.87 cmH_2O *	37.05 ± 3.36 cmH_2O
Bladder Capacity	0.96 ± 0.05 mL	0.46 ± 0.03 mL *	0.85 ± 0.08 mL
Residual Volume	0.05 ± 0.01 mL	0.06 ± 0.02 mL	0.09 ± 0.04 mL
E_{max}	148.0 ± 10.23 g/ g tissue	30.91 ± 4.49 g/ g tissue **	54.03 ± 5.36 g/ g tissue **#

Fig. 1. Top row: Representative longitudinal CT images taken of bladder regeneration in the **same animal.** Bottom 4 rows depict mean values for all animals, where: maximum (micturition) pressure, cystometric capacity and residual volume are parameters evaluated via filling cystometry, and E_{max} reflects maximal bladder contractility *in vitro.* (g=grams) Asterisks represent values that are significantly lower than age-matched control values determined by 1-Way ANOVA (*-P<0.05), (**-P<0.001). #- Significantly higher than 2-week post-STC values (P<0.05).

Interpretation of results

Importantly, despite the observed differences, the regenerating bladders emptied normally and the animals were continent. The long-term anatomy/physiology of the regenerating bladder remains to be determined. The combination of high resolution imaging modalities with direct measures of bladder function *in vivo* and tissue function and histology *in vitro* will help establish the baseline characteristics of bladder regeneration as well as noninvasive markers for important physiological milestones associated with normal bladder regeneration. Further investigations to identify the specific cell types involved and key molecular events associated with bladder regeneration will provide further mechanistic into this process. The power of this approach stems from the **longitudinal multidisciplinary comparison** of the regenerated bladder with the native bladder **from the same animal**.

Concluding message

These initial investigations establish the utility of an important preclinical animal model using the bladder as an appropriate multidisciplinary test system for studying and elucidating the characteristics of tissue regeneration in mammals. In addition to their potential direct impact on basic and clinical urological research, these findings should point toward more general features of tissue regeneration that would be helpful to advancing the field of regenerative medicine in multiple tissue and organ systems.

References

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Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**
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