The Utility of *in vivo* Imaging to Monitor *de novo* Bladder Regeneration

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Introduction

Most models of *de novo* organ regeneration occur in lower vertebrates. We have established a model of organ regeneration in adult mammals, using the rodent bladder. Specifically, we have shown that after subtotal cystectomy (STC; removal of ~70% of the bladder), the bladder will regenerate to its original volume, while maintaining overall function, and improving smooth muscle contractility over time. Herein, we investigate the potential for *in vivo* Computed Tomography (CT) and Magnetic Resonance Imaging (MRI) scans to noninvasively track the regeneration process.

Materials and Methods

Twelve week old female F344 rats underwent subtotal cystectomy (STC) and the regenerative process that followed was examined. At 0, 1, 2, 4, and 8 weeks post-STC microCT scans were performed after transurethrally infusing the bladder with Iothalamate Meglumine (94 mg/mL). In a separate cohort of animals *in vivo* MRI scans were performed in a 7T MicroMR after manual emptying of the bladders at these same timepoints. *In vivo* cystometric studies were performed at 2, 4, and 8 weeks post-STC. After euthanasia, bladders were either excised for pharmacologic studies, or examined histologically via H&E staining and immunohistochemical studies to ensure the presence of proliferating cells (PCNA).

<table>
<thead>
<tr>
<th>Time points</th>
<th>Control (pre-surgical) (n=16)</th>
<th>2 weeks (post-surgical) (n=10)</th>
<th>8 weeks (post-surgical) (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Siemens microCATII scans</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
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<tr>
<td>Micturition Pressure</td>
<td>49.24 ± 3.72 cmH2O</td>
<td>30.5 ± 5.87 cmH2O</td>
<td>37.05 ± 3.36 cmH2O</td>
</tr>
<tr>
<td>Bladder Capacity</td>
<td>0.96 ± 0.05 mL</td>
<td>0.46 ± 0.03 mL</td>
<td>0.85 ± 0.08 mL</td>
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</tbody>
</table>

Table. 1. Top row: Representative CT images of bladder regeneration in the *same animal*. Bottom rows depict mean cystometric values for all animals.

Results

In agreement to cystometric bladder capacity estimates, microCT imaging demonstrated a rapid increase in bladder volume seen after STC (Table 1). Micturition pressures found via cystometry were significantly lower than control values, however these pressures showed functional improvement, and correlated with bladder circumference measurements as determined by microCT scans (r=0.63, P<0.05). MRI scans revealed a thinner bladder wall 1 week post-STC (281.96µM) compared to controls (493.53 µM) which improved greatly by 8 weeks (427.76 µM). (Figure 1; within 10% of original wall thickness) This time-dependent thickening of the bladder wall was associated with hyperplasia, as demonstrated by positive staining to Proliferating Cell Nuclear Antigen (PCNA).

Discussion and Conclusions

This study demonstrates the potential of noninvasive imaging to track the progress of *de novo* tissue regeneration. Information obtained with this model has obvious implications to urologic tissue engineering, but may also have broader impact on our understanding of regeneration in other tissues/organ systems, leading to additional clinical applications.

Acknowledgments

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Fig. 1. Sagittal view of bladder pre-STC (A) and the same animal 8 weeks post-STC (B). (C) Quantification of *in vivo* bladder wall thickness for 5 animals with 5 measurements taken per animal at each timepoint. Each timepoint is significantly smaller than control (***, P<0.001, *P<0.01).