

A Regenerative Neo-Bladder Construct in Trigone-Sparing Cystectomized Dogs: Long-term Safety, Continence, Voiding, and Urodynamics

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Abstract:

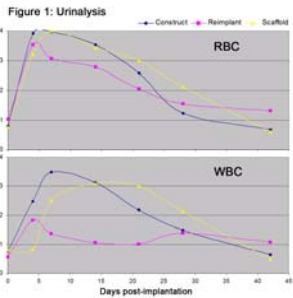
Purpose: Trigone-sparing cystectomy was used to study the structural and functional aspects of bladder regeneration in a canine model of an augmentation cystoplasty.

Methods: An autologous neo-bladder augmentation construct composed of a PLGA-based biodegradable mesh scaffold and autologous urothelial and smooth muscle cells (Construct) (n=32) was compared to re-implanted native bladder (Reimplant) (n=32), or PLGA-based biodegradable mesh scaffold alone (Scaffold) (n=8) at 1, 3, 6 and 9 months (mo) post-implantation.

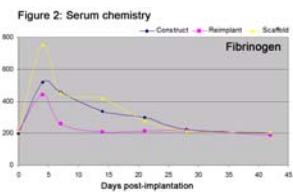
Results: Within 14 days, all 72 dogs were continent. Within 1 mo, acute phase responses, hematological and urinalysis parameters returned to baseline. Treatment-related morbidity was only observed in Scaffold and Reimplant dogs. Only Construct dogs achieved functional recovery (i.e., urodynamics) and a regenerative tissue response. Construct dogs regained baseline bladder capacity by 4 mo and compliance by 6 mo and were sustained throughout the study. Urodynamic parameters in Reimplant animals were initially comparable to Construct dogs but were unstable and significantly lower than baseline by 9 mo (60-75% decrease from baseline). Histologically, decreased compliance in Reimplant and Scaffold groups at 9 mo correlated with limited healing and incomplete bladder wall regeneration.

Conclusions: Construct implants were safe and able to restore urodynamic, continence, and voiding functions by 6 mo and retained these functions to study termination. Bladder wall regeneration was obtained only in the Construct implanted animals. Scaffolds lacking cells (Scaffold) elicit repair of bladder wall with incompletely developed components reduced organ capacity and restricted compliance.

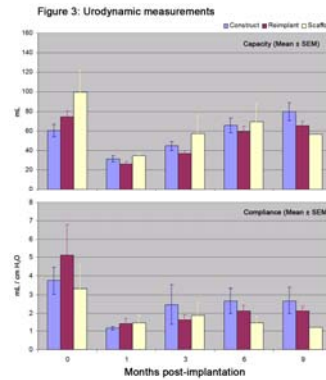
Results:



- In general, red/brown/amber-colored urine with ≥ 300 mg/dL of protein, increased pH and large-to-moderate amounts of blood was observed in all animals until ~30 days after definitive surgery
- From 1 month onward, sporadic increases in urinalysis parameters were observed in all animals with no remarkable trends and no biologically significant differences among the three study groups

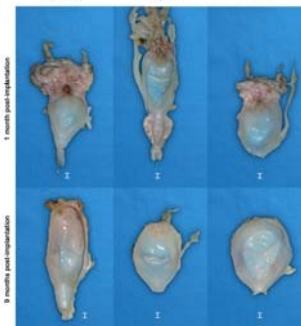


- For 5 days post-surgery in all animals, expected inflammatory responses were reflected in leukograms showing elevated WBC, neutrophil, and monocyte counts consistent with an acute phase response
- The average inflammatory response in animals receiving SC and S implants was somewhat higher than that seen in RIB animals between 5 and 21 days post-implantation but were still consistent with an acute phase response



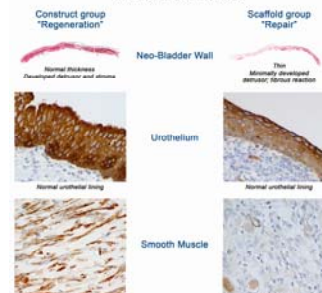
- By 6 months post-implantation, all Construct animals achieved bladder capacities (top panel) that equaled or exceeded mean native bladder capacity (baseline)
- Post-implantation bladder capacities in Reimplant and Scaffold animals increased over time, but did not return to or exceed that of native bladder (baseline)
- Mean compliance (bottom panel) in Construct animals increased to values not significantly different from native bladder (baseline) at 3 months and was stable throughout the rest of the study
- Mean compliance in the Reimplant group reached a plateau at 6 months, yet remained significantly lower than baseline ($p < 0.05$)
- Mean compliance in Scaffold group reached the best compliance at 3 months and declined steadily thereafter
- At 9 months post-implantation, both Construct and Reimplant groups had significantly higher compliance than Scaffold group animals ($p < 0.05$)

Figure 4: Gross morphology of neo-bladders at 1 and 9 months post-implantation



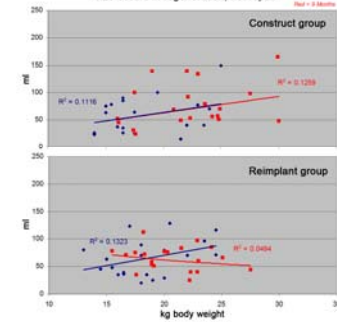
- At 1 month post-implantation (top panel), remnant native urinary bladders appeared normal and anastomotic sites were distinct in all groups
- Extensive vascular responses were seen in Construct neo-bladders, but were limited in Reimplant and Scaffold neo-bladders retrieved at 1 month
- In Construct and Scaffold neo-bladders retrieved at 1 month, residual PLGA material was limited to the dome portion cranial to the anastomotic site and represented $\leq 25\%$ of the neo-bladder wall
- Reimplant neo-bladders retrieved at 1 month had 25-50% implant tissue
- At 9 months post-implantation (bottom panel), Construct neo-bladder walls were macroscopically developed with both mucosal and serosal lining surfaces
- Walls of Scaffold neo-bladders retrieved at 9 months were thin, translucent, and shrunken

Figure 5: Histological comparison of regeneration and repair at 9 months



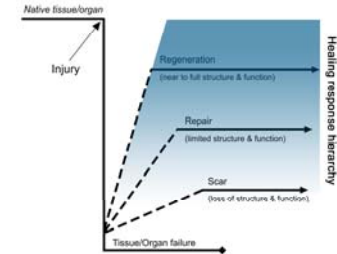
- Key events occur in the smooth muscle layer of neo-bladder tissue healing (e.g., myofibroblastic response; Jayo, 2007) that leads to a full thickness detrusor only in Construct-implanted animals
- At 9 months, neo-bladder walls from Construct animals (left panel) were fully developed with mature muscle bundles, formation of a full thickness detrusor, and limited fibrous connective tissue deposition
- At 9 months, neo-bladder walls from Scaffold animals were thin with incompletely developed detrusor muscle bundles and elevated levels of fibrous tissue deposition
- Structural differences are consistent with the functional (Figure 3) and gross morphological differences (Figure 4) between Construct and Scaffold neo-bladders

Figure 6: Biofeedback between animal growth and neo-bladder size occurs in regeneration, not repair



- The correlation (R^2) between neo-bladder capacity and body weight at 9 months was constant with that of native bladder capacity and body weight (baseline R^2) only in animals receiving a Construct
- In the Reimplant group, the correlation (R^2) between neo-bladder capacity and body weight at 9 months was flattened and significantly lower than that of native bladder capacity to body weight (baseline R^2)
- Neo-bladder capacity in animals receiving a Scaffold did not return to baseline so were not evaluated for evidence of biofeedback

Figure 7: Healing response hierarchy



- Only in Construct-implanted animals did myofibroblasts continue to form into layers and bundles to restore a complete detrusor muscle (Regeneration)
- In Reimplant and Scaffold group animals, healing was characterized by fibroblastic deposition of dense, collagen-rich connective tissue and associated inflammatory cells (fibroplasia), without an integrated vasculature (i.e., fibrovascular tissue), and a minimally developed detrusor muscle layer (particularly in animals receiving a Scaffold) (Repair)
- Regeneration and repair represent two distinct healing responses to injury

Conclusions:

- In preclinical evaluation, the autologous neo-bladder augmentation construct (Construct) was uniquely associated with a regenerative outcome that emulated the native bladder's structure, function, and biofeedback
- An outcome of regenerative vs. reparative healing may be established as early as 1 month post-implantation based on histological evidence of early cellular and stromal responses following Construct vs. Scaffold implantation (Jayo, 2007)
- Serum chemistry and hematology assessments during the first 30 post-surgical days in all animals were indicative of an acute phase response related to the implantation surgery, regardless of group and implant type
- Activity and in-life findings for animals receiving a Construct were consistent with common post-operative findings of a major abdominal surgery with no evidence of bladder tearing/rupture, abdominal adhesions, abscess formation, or post-operative morbidity/mortality
- In the present study, progenitor cells were added to a PLGA mesh biopolymer and, following implantation into Construct animals, the resulting neo-bladders not only achieved sizes consistent with each recipient's body but grew at a rate consistent with that of a native bladder, suggesting that it was the addition of the progenitor cells that influenced the structural and functional development of a neo-bladder capable of responding to feedback mechanisms responsible for native bladder homeostasis
- The safety and functionality of the neo-bladder augment used in these studies supported the application to the U.S. Food & Drug Administration seeking permission to begin Phase II trials which are now currently active in pediatric and adult patients