

## **INTRAURETHRAL MUSCLE DERIVED CELL INJECTIONS INCREASE LEAK POINT PRESSURE IN A RAT MODEL OF INTRINSIC SPHINCTERIC DEFICIENCY**

### **Aims of Study**

Intrinsic sphincteric deficiency (ISD) denotes a malfunction of the striated urethral sphincter. One approach to treating ISD has been the use of injectable, intraurethral, bulking agents. The goal of these injectables is to restore mucosal coaptation and its “seal effect” contribution to the continence mechanism (1). Despite the short-term success achieved with injection therapy, the technique is hindered by reabsorption, the need for multiple injections, allergic reactions, and migration. Also, these bulking agents work to produce a functional obstruction. As such, they compensate for rather than correct the underlying pathophysiology of ISD. Research into newer ISD treatments has focused on striated sphincter reconstruction. One such approach under investigation involves using skeletal muscle derived cells (MDC) to regenerate damaged urethra. The preplate technique is used to isolate cells that, through six serial passes, adhere last onto collagen-coated flasks. Some of these cells have stem cell like properties and are termed MDC. Our objective was to determine if allogenic MDC could restore sphincteric function in rats with ISD.

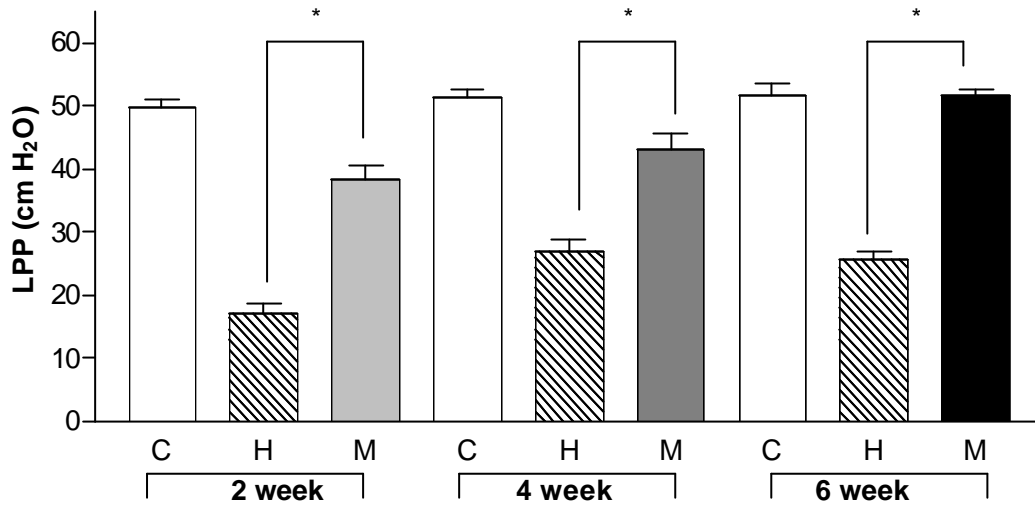
### **Methods**

Under halothane anesthesia (2%), 25 adult female Sprague-Dawley rats underwent cauterization of tissues lateral to the mid-urethra to produce ISD. One week after cauterization,  $1.5 \times 10^6$  MDC, transfected with Lac Z, were injected into the mid-urethra of 16 rats. These rats were divided into 3 groups, which were evaluated 2 weeks (n=8), 4 weeks (n=5), or 6-weeks (n=3) after MDC injection. In another group, 9 rats were injected with Hanks' Balanced Salt Solution (HBSS) after cauterization. These rats, divided into 3 groups of 3 rats each, were evaluated 2, 4, or 6 weeks after injection of HBSS. A 3/10 cc insulin syringe was used to inject either 10  $\mu$ l of MDC suspended in HBSS (approximately 750,000 cells) or HBSS only into each lateral wall of the mid-urethra. A total of 20  $\mu$ l ( $1.5 \times 10^6$  cells) of MDC were injected into the urethra of each MDC rat. As a control, 9 normal rats, divided into 3 groups of 3 rats each, underwent a sham operation during which the urethra was exposed but not cauterized. Subsequent evaluation was performed in these rats at 2, 4, or 6 weeks. Under urethane anesthesia, all rats underwent cystometry and leak point pressure (LPP) testing to evaluate bladder and sphincteric function, respectively. LPP testing was performed with the use of the vertical tilt table/intravesical pressure clamp technique. The location of the MDC was assessed using Lac Z staining. Differences in striated muscle layer and innervation between the 3 groups were noted with fast myosin heavy chain and anti-protein gene product (PGP) 9.5 staining, respectively.

### **Results**

The injection of MDC into the mid-urethra increased LPP without affecting bladder function. The mean LPPs of control rats 2, 4, and 6 weeks after a sham operation were  $49.8 \pm 1.3$  cm H<sub>2</sub>O,  $51.2 \pm 1.5$  cm H<sub>2</sub>O, and  $51.6 \pm 2.0$  cm H<sub>2</sub>O, respectively. The mean LPPs of the rats 2, 4, 6-weeks after cauterization and HBSS injection were  $17.2 \pm 1.4$  cm H<sub>2</sub>O,  $26.9 \pm 1.9$  cm H<sub>2</sub>O, and  $25.5 \pm 1.3$  cm H<sub>2</sub>O, respectively. The mean LPPs of the rats 2, 4, and 6 weeks after cauterization and MDC injection were  $38.2 \pm 2.2$  cm H<sub>2</sub>O,  $43.1 \pm 2.6$  cm H<sub>2</sub>O, and  $51.5 \pm 0.9$  cm H<sub>2</sub>O, respectively. When compared to cauterized rats injected with HBSS and matched respective to time, the increased LPPs in each MDC injected group were significantly higher ( $p < 0.001$ ). Histological analysis showed that the MDC contributed to striated muscle regeneration. Also, the MDC injected urethra had an intact striated muscle layer and more nerves in comparison to the HBSS injected and control urethras, which had disrupted muscle and few nerves.

**Figure:** The effect of MDC injection on LPP. When compared to cauterized rats injected with HBSS and matched respective to time, the increased LPPs seen in each MDC injected group were significantly higher (\* denotes  $p < 0.001$  for each of the 3 pairs of groups). C denotes control, H denotes HBSS injected, and M denotes MDC injected.



### **Conclusions**

The injection of intraurethral MDC improved sphincteric function in rats with ISD. There was no alteration in bladder function with either cauterization or MDC injection. Allogenic MDC persisted and achieved functional improvement for 6 weeks without significant inflammatory response. Histology showed that the MDC had integrated well within the striated muscle layer of the cauterized mid-urethra. In addition, the striated muscle layer of the MDC injected urethra was intact and had more nerves than the cauterized urethra injected with only HBSS. The periurethral injection of MDC may provide an attractive alternative to current therapies for ISD.

### **Reference**

1. Appell R: Injection Therapy for Urinary Incontinence. In: Campbell's Urology, 8<sup>th</sup> ed. Edited by PC Walsh, AB Retik, ED Vaughn Jr., and AJ Wein. Philadelphia: WB Saunders Co., vol. 2, part V, 1172-1186, 2002.