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AUTOLOGOUS ADULT ADIPOSE DERIVED STEM CELLS (ADSC) REMAINS VIABLE AFTER TRANSPLANTATION INTO RABBIT'S URETHRAL WALL: A NEW PERSPECTIVE FOR STRESS URINARY INCONTINENCE TREATMENT

Hypothesis / aims of study
The impairment on anatomic urethral support and intrinsic sphincteric deficiency (ISD) are the two major components related to SUI, which can be associated in different degrees. Surgical techniques are effective repairing the anatomic support defect. However, the treatment of ISD component is more complex. It has been previously shown that human adipose derived stem cells (ADSCs) injected in the urethra and bladder wall of immunosuppressed mice and rats could be tracked after 3 months with complete integration to the receptor's tissue. ADSCs have an enormous potential for intrinsic urethral regeneration and reconstruction, because they are easy to obtain, provide a large number of cells and can differentiate into different cell lines including skeletal and smooth muscle, and neuron. In the last year at the ICS meeting we showed that the ADSC could be seeded on scaffolds as a possible source of cells for tissue engineering. In the present paper, we evaluate the feasibility of ADSCs autologous transplantation into female rabbits' urethra wall as a possible alternative for intrinsic urethral regeneration.

Study design, materials and methods
This work was performed in our institution by collaboration between urology and nephrology basic research laboratories. Inguinal fat pad of 9 New Zealand adult female rabbits was harvested under aseptic conditions. The fat was washed extensively with phosphate buffer saline (PBS), minced and enzymatically digested using 0.075% collagenase. The digested tissue was strained to obtain a stromal vascular fraction. The pellet was re-suspended in Dubelco's modified Eagle's media containing 10% fetal bovine serum (CM). The cells were plated in 100mm dishes at the concentration of $1X10^5$ and cultured in CM until reaching confluence. Before urethral injection, the cells were labeled with Vybrant CM-DiI (Molecular Probes). The autologous transplantation was done by the injection of 30 µl of Hank's Balance Salt Solution, containing $1 X 10^7$ labeled cells into the urethra wall of the donor rabbit. The urethra was harvested at 2, 4 and 8 weeks. The harvested urethra was paraffin embedded, H&E stained or processed to identify the fluorescent DiI marked cells, following the manufacturer's recommendations.

Results
The paraffin embedded urethra was cross sectioned in its whole extension to track DiI positive cells. At the first 2 and 4 weeks, we were able to identify a viable ADSCs nodule localized in the urethral sub-mucosa. At 8 weeks, the ADSCs presented a tendency to spread and integrate with the urethra wall from the initial injection site. Figure 1 and 2 shows the urethra nodules at 2 and 4 weeks (magnification 200x).

Interpretation of results
This is the first study to demonstrate a successful autologous ADSCs transplantation into rabbit's urethra wall. ADSCs survived and integrated to the urethral tissue after 8 weeks. This study opens a new frontier for SUI treatment by creating the possibility to repair urethral damaged tissue, not only bulk it. Several studies must be done before it can be applied in clinical practice, but the successful autologous transplantation was the first step to achieve this goal.

Concluding message
The results confirm that ADSCs can survive and integrate within the urethral wall 8 weeks post-transplantation. The observed integration between ADSCs and urethra may suggest adjustment of these cells to urethra microenvironment, which could further lead to an *in vivo* differentiation following smooth muscle pathways, as observed *in vitro.*

Figure 1. DiI labelled ADSC forming a bulking nodule in the urethral wall 2 weeks after implantation.

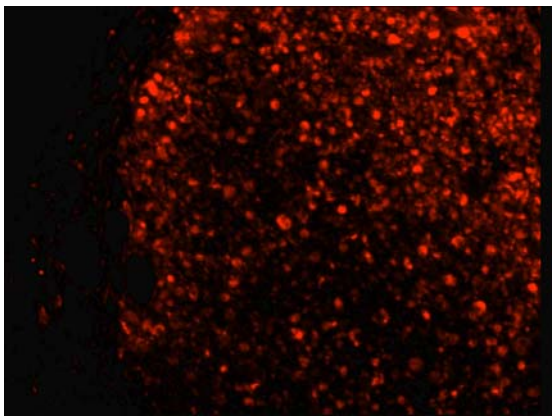

Figure 2. DiI labelled ADSC forming a more spread bulking nodule in the urethral wall, 4 weeks after implantation.