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AUTOLOGOUS SKELETAL MUSCLE- DERIVED CELLS IN THE TREATMENT OF INCONTINENCE IN RABBITS

Hypothesis / aims of study
Studying the propagation and expansion of skeletal muscle derived myoblasts in rabbits and evaluating changes in the morphology of injection site in the urethral wall with time

Study design, materials and methods
This is an experimental study involving 10female white New Zealand rabbits. Mean body weight is 2.3 Kg (range 2-2.5 Kg). Rabbits were housed in the animal research facility, Urology & Nephrology Center. Proper veterinary care were provided while food and water were offered ad libitum. Rabbits were divided into 3 groups: One rabbit was subjected to sham procedure, 2 underwent urethrolysis to induce incontinence followed by injection of saline (control) while 6 were subjected to open muscle biopsy (Biceps Femoris) followed by expansion of skeletal muscle fibres. Rabbits were anaesthetized using a combination of Ketamine (35mg/Kg) and Xlazine (20mg/kg) by IM injection.
Cells were isolated through submersion in collagenase type 1A (Sigma-Aldrich, St.Louis MO, USA) for digestion. Cells were later cultured on laminin-coated tissue culture flasks in SKGM-2 medium (Lonza, Walkersville, MD, USA). The medium was changed every 3 days and unattached cells were seeded onto new plate and grown for 1 week. At confluence, cells were passaged and split1:2 culture flasks. After 8 weeks of expansion, obtained muscle-derived myoblasts (MDMs) were collected for transplantation. Injection of muscle suspension was performed via 8.5 cystoscopy at 3 and 9 o'clock position near to the bladder neck. Control rabbits were sacrificed at 1 week while active treatment group were sacrificed at 2, 4 and 6 weeks post-injection. The whole urethra was harvested and sent for histopathology (figure 1)

Results
One rabbit succumbed for unknown reason few days after urethrolysis. 8 were alive at time of sacrifice. 2 were sacrificed at 2, 4 and 6 weeks respectively after injection of myoblast suspension
Histopathological examination of section from harvested urethras was carried out using H& E, Masson trichrome stain as well as immunohistochemistry (Desmin).
Slides from sham as well as control group showed no evidence of skeletal muscle deposition. While section from active treatment arm showed progressively deposited skeletal muscle cells (myoblast)
Figure shows Masson trichrome (2) and Desmin immunohistochemistry stain (3) of rabbits with muscles injection at 4 weeks

Interpretation of results
The injection of autologous skeletal myoblasts in rabbits is associated with muscle growth and expansion after at least 4 weeks in our hands.

Concluding message
Expansion of Isolated autologous skeletal muscle myoblast in rabbits is easy to perform. The resulting muscle suspension after proper culture for 2 weeks results in definite muscle bulk at injected sites. This takes preferably 4 weeks to function. Application in human is anticipated

Figure 1: Harvested urethra at 4 weeks

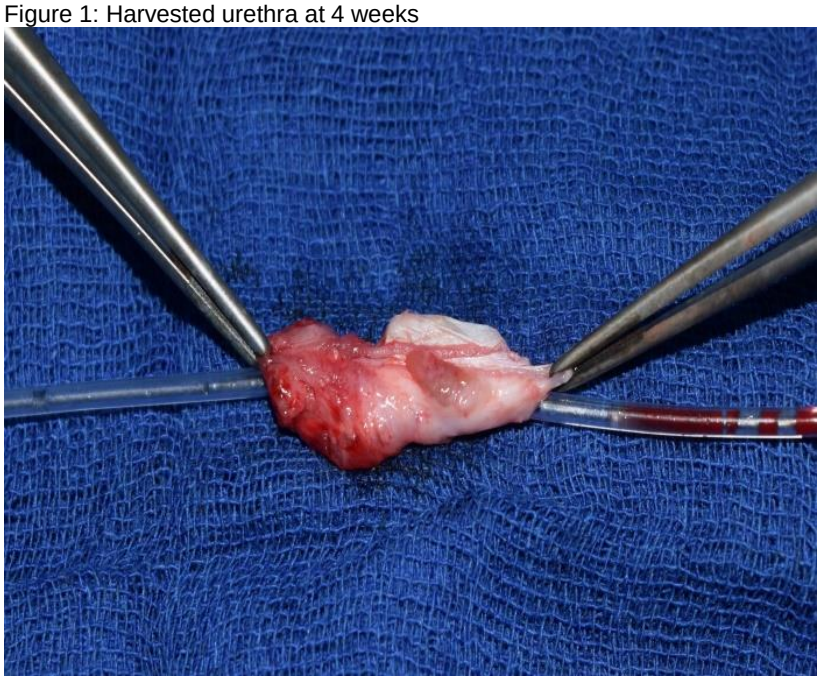


Figure 2

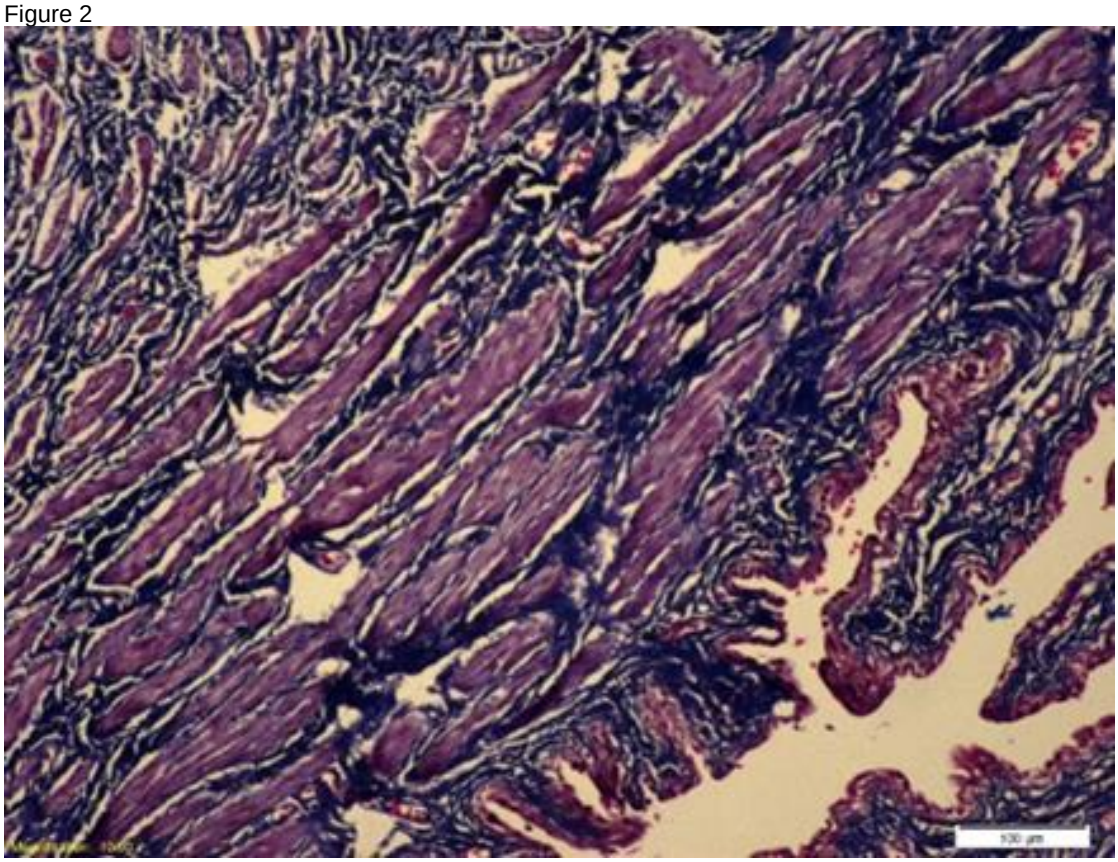
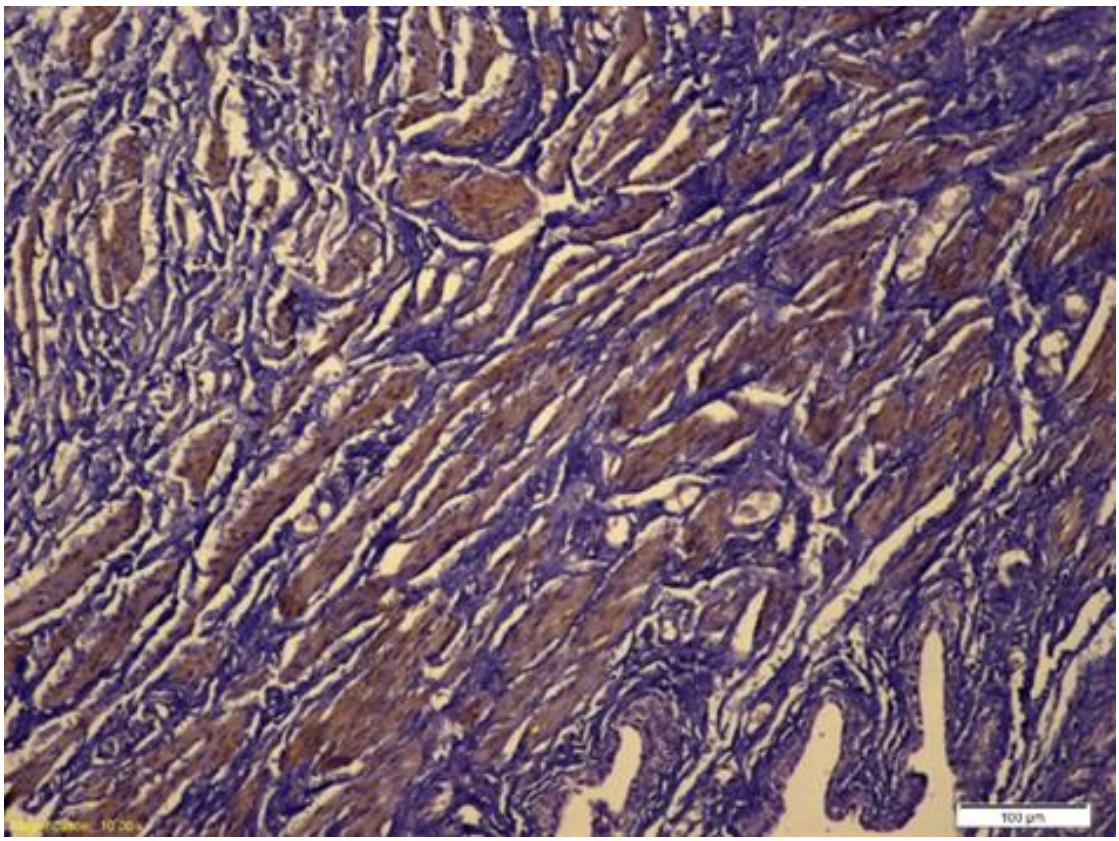


Image shows the increased muscle bundles stained red by Masson trichrome 4 weeks after injection)



The same specimen with skeletal nature of the muscle bundles being confirmed with IHC staining for Desmin (in beige)

Disclosures
Funding: Institutional **Clinical Trial:** No **Subjects:** ANIMAL **Species:** Rabbits **Ethics Committee:** Internal review board