The Use of Autologous Skeletal Muscle Derived Cells as a Sling in the Treatment of Induced Stress Urinary Incontinence, An Experimental Study in Dogs

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Abstract

Introduction:

This is an experimental pre-clinical study, testing the applicability of autologous skeletal muscle derived cells as a treatment of SUI in canine model

Methods:

10 Mongrel dogs included. Skeletal muscle biopsy was harvested in 4. 1 month later, incontinence was induced in 8 dogs through urethrolisis. Muscle biopsy was incubated and expanded for 8 weeks. Muscle derived cells were collected and covered a Polyglycolic acid (PGA) scaffold immersed in culture medium and coated with matrigel to be used as a sling. Placed suburethral in 8 dogs; 4 had cell- seeded and 4 had scaffold only. Urethral pressure (UP) measurement was done at baseline 2 &6 weeks after sling insertion. The urethra was harvested 4 weeks after sling insertion for histopathology.

Results:

UP shows increase of maximum urethral pressure during static measurement in all dogs with a scaffold inserted. The increase ranged from 5-40 cmH₂O Histopathology shows significant periurethral proliferation of skeletal muscles in 4 dogs with cell-seeded scaffold. This was maximum in dogs # 1& 2. This was not the case in the 4 dogs that had sling only.

Conclusion: Use of skeletal muscle –seeded PGA scaffold is a practical technique with preserved integrity of histological differentiation in canine model.

Brief Summary

Autologous Skeletal muscle-derived cells could be propagated in vitro and seeded to PGA scaffolds and used as slings

Introduction

The treatment of stress incontinence is challenging and different surgical techniques evolved throughout numerous clinical trials in order to efficiently treat this condition. So far, no procedure is considered “gold standard”. However, a midurethral sling (MUS) is the most widely used procedure and is considered as the standard of care in women with stress incontinence. Synthetic MUS were used for decades but were not without risks and sometimes could be life- threatening. Many trials, both experimental and human, attempted to provide an autologous, efficacious and durable tissue-engineered sling. We conducted this experimental trial with the purpose of assessing the potential of an autologous cell- seeding of a biodegradable scaffold in treating induced incontinence in canines

It evaluates a biodegradable *seeded (PGA) scaffold* enriched with autologous skeletal muscles without the need for a harvest procedure such as that used during rectus fascia sling, being the prototype of autologous slings.

Materials And Methods

The study comprised 10 Mongrel female dogs. Approval from the local Ethics Committee was obtained (Research center Ethics panel, Urology & Nephrology center).

All methods were carried out in accordance with relevant regulations and in accordance with ARRIVE guidelines; including housing, anesthesia and surgical procedures in dogs.

Open episiotomy was carried out 2 weeks prior to the study to facilitate the vaginal approach.

Group 1 comprises 4 dogs in which a cell-seeded sling was applied while in group 2, only the PGA sling was applied.

Isolation and expansion of muscle-derived cells (MDCs):

In 4 dogs (group 1), muscle biopsy was harvested from biceps under general anesthesia. Obtained biopsy averaged 0.2 gm. and was incubated in *0.2% collagenase type 1A* in Dulbecco's Modified Eagle Medium (DMEM) medium for 20 minutes at 37 C. (Sigma-Aldrich®, St. Louis, USA) digestion. Cells were cultured on laminin-coated tissue culture flasks in SKGM-2 medium (Lonza®, Walkersville, MD). Medium was replaced every 3 days. At confluence, cells were passaged and split 1:2 in tissue culture flasks. After 8 weeks and 4 passages, MDCs were collected for transplantation.

• Characterization of MDCs:

Morphology of cells was evaluated using phase-contrast microscopy. The expression of Desmin was assessed by immunohistochemistry (IHC), using monoclonal anti-Desmin antibodies (DakoCytomation®, Glostrup, Denmark).

• Cell seeding on scaffolds:

Neoveil absorbable PGA sheet (Gunze Limited®, Kyoto, Japan) was used as (2x3 cm) segments. Seeding started by immersion in culture medium supplemented with 10 % fetal bovine serum and 1 % penicillin/streptomycin for 24 h. at 37 ° C before seeding. The scaffold was coated with matrigel and MDCs were seeded at a concentration of 1 million cells/ cm². Matrigel was being used in our study in order to increase cell adherence to the scaffold. It is one of the promising materials in this regard. After 24 h. the seeding side was flipped and the other side was treated the same way. Both surfaces were fully immersed in medium during seeding process. Seeded scaffold was cultured for further 3 days in the same culture medium

• Induction of incontinence:

One month after biopsy, incontinence was induced in 8 dogs through midline suprapubic incision and identification of the urethra and bladder. Sharp dissection of the urethra all around until disruption of the periurethral sphincter is achieved. This method is similar to that previously described by Rodriguez et al, with the addition of pubourethral ligament disruption.

- Two weeks after induction of incontinence, sling was applied through vaginal incision in suburethral position in all 8 dogs: Cell- seeded scaffold in the first 4 and scaffold – only in the other 4. The sling is fixed to the periurethral tissue. Urethral pressure (UP) measurement was done before (baseline) and 2 weeks after insertion of the sling.
- The urethra with its surrounding was harvested and dogs were sacrificed 4 weeks after sling insertion. Figure 1 shows the scaffold and steps of surgical procedure
- The urethra was fixed in 10 % buffered formalin and processed in paraffin blocks, sectioned at 5 μ m and stained with hematoxylin and eosin (H&E), methenamine silver (for reticuline fibers detection) and Masson trichrome stain (for fibrosis detection and muscle bundles delineation).
- 2 dogs were considered as control, in whom no urethrolisis or insertion of slings were carried out. UP was carried out in these dogs before being sacrificed and their urethra was taken as control.
- For IHC, Desmin antibodies were used (Abcam, Cambridge, UK) at dilution of 1:100. Nerve fibers were identified through staining with polyclonal anti-S100 (DAKO, Carpinteria, CA) at a dilution of 1:200. Immunolabeling was performed using an avidin–biotin detection kit (Vectastain Elite ABC, Vector, Burlingame, CA). All sections were counterstained with Gill's hematoxylin.

A flowchart for the research procedures was supplemented (Supplement)

Results

- UP shows increase of maximum urethral pressure in all dogs with a scaffold inserted. Table 1 demonstrates change in UP over time. Median maximum urethral closure pressure (MUCP) at baseline in the first group; where the scaffold was seeded with skeletal muscle cells, was 28.5 cmH₂O that increased to 67.5 at 2 weeks and 65.5 at 6 weeks. While in group 2 (scaffold -only group), the median MUCP increased from a basal value of 19 to 21 cmH₂O and stayed at this level at 6- week. The increase in (MUCP) ranged from 13-60 cmH₂O (Median 39 cmH₂O) in the first group. In the second one, the increase of MUCP ranged from -3 to 18 (median 5.5 cmH₂O)
- Histopathology:

Table 1
Maximum urethral closure pressure (MUCP) over time

Dog#	Baseline (cmH2O)	2- week post sling (cmH2O)	6-week post sling (cmH2O)
1	25	40	42
2	32	45	43
3	30	90	98
4	27	90	88
Median	28.5	67.5	65.5
5	20	38	37
6	21	18	20
7	17	20	19
8	18	22	22
Median	19	21	21
9	40	NA	
10	44	NA	

Luminal diameter, lining epithelium, lamina propria thickness with its elastic and collagenous fibers content (normally about 70% of whole sphincter thickness), fibrous tissue deposition and thick walled veins condition (stratum cavernosum), smooth and striated muscle(both constituting about 30% of total urethral thickness) were assessed.

In group 1: urethral lumen was slightly dilated in comparison to the control group; mucosal lining is of near normal thickness. Lamina propria is slightly thicker and no evidence of fibrous tissue deposition as evidenced with Masson trichrome. Reticular fibres are the same as in control group. Thick walled veins are mildly increased in number and caliber compared to control group. The overall muscle layer appears as lavender red bundles in Masson trichrome are normal in thickness with some degree of crowding and disorganization. Areas of newly formed muscle bundles with central nuclei were confirmed.

In group 2:The damaged sphincter shows mildly dilated luminal area, with atrophic urothelial lining. The lamina propria thickness is increased with moderate fibrous tissue deposition in addition to lower reticular fiber content and mild inflammatory lymphocytic infiltrate. The vascular component is unremarkable. There is substantial loss of muscle layer with an overall decreased wall thickness.

IHC for anti-desmin shows significantly higher sphincter muscle mass in seeded scaffold specimens as compared to the other group. Newly formed muscle bundles are wider and more randomly aligned,

compared to normal sphincter. Staining for S100 shows the presence of nerve fibers between the regenerated sphincter muscles. Figure 2 and 3 shows histopathological changes in the two groups.

Discussion

Induction of incontinence by urethrolisis with pubourethral ligament injury was our approach of choice. It resulted in disruption of the native support of the urethra and durable loss of urethral resistance. Incontinence was confirmed by lower maximum urethral closure pressure as compared to control dogs, in which no urethrolisis was carried out

Myoblasts have been used as a treatment of stress incontinence in animal model by means of intra-urethral injection. In 2014, a similar approach was used in adult 35 women. Gras et al described a technique performed largely under local anesthesia/intravenous analgesia. An open muscle biopsy was obtained from vastus lateralis muscle and was “minced” and injected as a suspension in the same session via periurethral route in 35 women; under vaginal US. Cure/improvement was noted in 7–63% of the patients.

Other human trials entailed intraurethral injection of muscle-derived stem cells were published, both in adult women and in children. Although results were promising, some reports turned out to be unfounded.

Another research strategy involved making a structured skeletal muscle tissue in vitro, with the potential of making an all- autologous sling. Many studies evolved to reconstruct skeletal muscle tissue. Some focused on developing a self-organizing tissues, without artificial scaffolds; others preferred seeding cells on a natural or a synthetic biodegradable substrates e.g. collagen matrices

We preferred Polyglycolic acid as a biodegradable scaffold as it was used by Saxena et al ⁸ where myoblasts derived from neonatal rats, Fisher CDF-F344, were seeded onto Polyglycolic acid meshes and implanted into the omentum of syngeneic adult Fisher CDF-F344 rats with promising results and comprehensive description of the approach. The advantage of the sling approach is that it applies potentially successful technique with an added effect of autologous skeletal muscle fibers to the mid-urethra

To our knowledge, no human study involving biodegradable sling seeded with autologous muscle-derived cells was yet reported. Such a study will be of paramount importance, considering that the current treatment options of women with SUI are far from optimum

The question we tried to answer is whether muscle-seeded biodegradable scaffold is any different from a plain one? So, we compared absorbable sling to sling seeded with autologous muscle cells.

Cell -seeded sling proved to be more efficacious than plain PGA. Urethral pressure measurement 6 weeks after sling insertion showed that the median increase of urethral closure pressure in the cell-seeded sling was over 40 cm H₂O while in the other group where only PGA sling was applied, it was 5.5 cmH₂O

Histopathological study of harvested urethral segments 4 weeks after insertion of slings proved the persistence of viable skeletal muscle and nerve fibres.

This sling design avoids polypropylene-related adverse events. Application of this technique in humans is easy and promising, based on functional and urodynamic outcome.

One of the shortcomings of our study is reliance on the measurement of maximum urethral pressure in female dogs; that might not be very reproducible. This is probably true according to one study; yet, others have adopted the same parameter we used

Conclusion

A biodegradable PGA sling seeded with autologous myoblasts shows evidence of survival 6 weeks after insertion in dogs. The use of skeletal muscle –seeded PGA scaffold is a practical technique with preserved histological differentiation in canine model at short term.

Abbreviations

DMEM: Dulbecco's Modified Eagle Medium

IHC: immunohistochemistry

PGA: Polyglycolic acid

MDCS: muscle –derived cells

UPP: Urethral pressure profile

Declarations

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Conflicts of interest/Competing interests: None for any of the authors

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Figures

Figure 1

A) Double-faced seeded scaffold in sterile medium. B) Midline vaginal incision with distal 2/3 of the dog urethra completely exposed, c) PGA sling is attached to the periurethral tissue using 5/0 Vicryl, D) Whole urethra is dissected from the surrounding, and harvested

Figure 2

A) Diffuse increase in thickness of skeletal muscle bundles with mild disorganization in PGA seeded with MDC using IHC for desmin stain (brown), B) Minimal increase in thickness of skeletal muscle bundles in PGA only using IHC for desmin stain (brown), C) Abundant nerve fibers in between muscle bundles in PGA seeded with MDC using IHC for S100 stain (brown), D) Minimal increase in nerve fibers in between muscle bundles in PGA only using IHC for S100 stain (brown)

Figure 3

A} Venous plexus is increased in number with marked congestion and mild edema in PGA seeded with MDC, D} Venous plexus is near normal in number with mild congestion with mild inflammatory infiltrate in PGA only, B} Silver stain shows near normal amount of reticular fibers (black) in PGA seeded with MDC, E} Silver stain shows lower amount of reticular fibers (black) in PGA only, C} The lamina propria

expressed absent fibrous tissue (blue) in PGA seeded with MDC using Masson trichrome stain, F} The lamina propria showed diffuse moderate fibrous tissue (blue) in PGA only using Masson trichrome stain

Supplementary Files

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