

Elastic modulus for long-term evaluation of the tensile properties of polypropylene meshes in an *in vivo* rat model

MIKOLAJ PRZYDACZ¹, WALLY MAHFOUZ¹, OLEG LOUTOCHIN¹,
CHIARA E. GHEZZI², MAUDE CARMEL¹, JACQUES CORCOS^{1*}

¹ Department of Urology, Jewish General Hospital, McGill University, Montreal, Quebec, Canada.

² Department of Mining and Materials Engineering, McGill University, Montreal, Quebec, Canada.

Purpose: Mid-urethral slings have become the gold standard treatment of stress urinary incontinence in women. Their tensile properties should be evaluated in order to measure how they wear off with time. Our objective was a long-term assessment of the tensile properties of 2 synthetic tapes (TVT-O and I-STOP) after *in vivo* implantation in rats in terms of elastic modulus. *Methods:* Strips from both meshes were implanted in the abdominal wall of 30 rats, which were sacrificed at 5 time intervals. Their fibers were untangled to single components. Ultimate tensile strength (UTS), strain at UTS and the elastic modulus of each fiber type were measured. *Results:* I-STOP maintained UTS and strain over time, while TVT-O UTS and strain were significantly reduced. However, the elastic modulus of both tapes remained constant. *Conclusions:* Both meshes maintained their stiffness and elasticity with time. Elastic modulus could be an appropriate factor to predict long-term implantation outcomes. The clinical significance of such findings remains to be demonstrated by long-term analysis.

Key words: tensile properties, elastic modulus, mid-urethral slings, I-STOP, TVT-O

1. Introduction

Stress urinary incontinence (SUI) surgery has been revolutionized by the tension-free vaginal tape (TVT) technique, first described in 1995 by Ulmsten and Petros [25]. To minimize tissue trauma and complications, the outside-in trans-obturator route was developed for sub-urethral tape implantation and modified later with the inside-out trans-obturator variant (TVT-O). Both trans-obturator approaches decrease the risk of bladder and bowel injury [5].

Nowadays, mid-urethral sling procedures are considered to be first-line surgical treatment of SUI in women. In recently published European meta-analysis, the trans-obturator route showed no differences in either patient- or clinically-reported outcomes (85% and 77%, respectively), in terms of cure rates at 12 months compared to the retro-pubic variant [5].

The relatively high failure rate of these tapes was attributed to multiple factors, including tape resistance.

The Gynecare TVTTM obturator system was launched as an alternative to surgical treatment of SUI. TVT-O tape consists of the same material as original retro-pubic TVT. The safety, clinical efficacy and tensile properties of this mesh have been well-studied [5], [24], [27]. The I-STOP trans-obturator device has been developed by CL Medical (Lyon, France). Regarding its structure and current composition, the tape matches “standard” specifications currently required of monofilaments and macroporus frameworks for the best mechanical performance and tissue integration [5], [10]. However, long-term evaluation of these meshes have been insufficient.

Polypropylene, the principal biomaterial used for prosthetic repairs, seems to be the most effective [21]. It is sufficiently compliant and well-tolerated by the body after implantation, exhibiting significant reduc-

* Corresponding author: Jacques Corcos, Department of Urology, Jewish General Hospital, 3755 chemin de la Côte Sainte-Catherine, Montreal, Quebec, Canada H3T 1E2. Phone: (+1) 514 340 8222, Fax: (+1) 514 340 7559, e-mail: jcorcos@uro.jgh.mcgill.ca

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tion of the inflammatory response compared to polyester-based materials. The mechanical properties of polypropylene slings play a key role in the prosthetic performance of long-term implantation. Most studies have concentrated on the evaluation of the tensile properties of meshes based on maximum break load (MBL), ultimate tensile strength (UTS) and strain at UTS [21]. The data regarding elastic modulus as a crucial factor in the long-term assessment of polypropylene slings are rather scarce.

The objective of the present study was to characterize the mechanical properties of fibers extracted from I-STOP mesh in comparison to TVT-O mesh with clinically-proven efficacy. The 2 meshes were examined for 1 year after *in vivo* implantation in a rat model. Uniaxial tensile tests were conducted to evaluate the mechanical performance of fibers in terms of UTS, strain at UTS and elastic modulus.

2. Materials and methods

This study protocol complied with Canadian Council on Animal Care guidelines, and was approved by the McGill University Animal Care Committee.

2.1. Study design

30 Sprague-Dawley (SD) rats were chosen for the present study and randomly allocated to 5 different groups:

- Group 1: 6 rats euthanized after 6 weeks,
- Group 2: 6 rats euthanized after 3 months,
- Group 3: 6 rats euthanized after 6 months,
- Group 4: 6 rats euthanized after 9 months,
- Group 5: 6 rats euthanized after 12 months.

All rats received mesh implants at the beginning of the study and were observed for pre-determined time periods. The animals were euthanized at the end of the period accordingly to the group they were put in, and implanted meshes were retrieved for tensile testing. After retrieval, the fibers were untangled to single components. Single fibers underwent tensile measurements within 3 hours of sample collection.

2.2. Experimental animals and housing

30 female SD rats weighing 300–350 g were obtained from Charles River Laboratories (St. Constant, QC, Canada) and housed separately in regular cages.

They were maintained under the same dietary, temperature, humidity and lighting conditions.

2.3. Surgical implantation procedure

The rats were anesthetized with isoflurane 5%/oxygen 1 L/min for induction, and isoflurane 2%/oxygen 1 L/min for maintenance. Their abdomens were shaved and prepared for aseptic surgery with povidone/iodine solution. After mid-line laparotomy incision (3 cm in length) the anterior abdominal wall was exposed, avoiding traumatic manipulation. Then, 1 cm × 2 cm strips of TVT-O and I-STOP were attached to the inner surface of the anterior abdominal wall with 6/0 Prolene sutures at the 4 corners of the strips (Fig. 1). The peritoneal cavity and abdominal fascia were closed with 3/0 Vicryl sutures. The skin was sealed with buried 4/0 catgut sutures. The animals received post-operative analgesia subcutaneously with 24 G needle, in the form of bupramorphine 0.002 mg/100 g once a day.

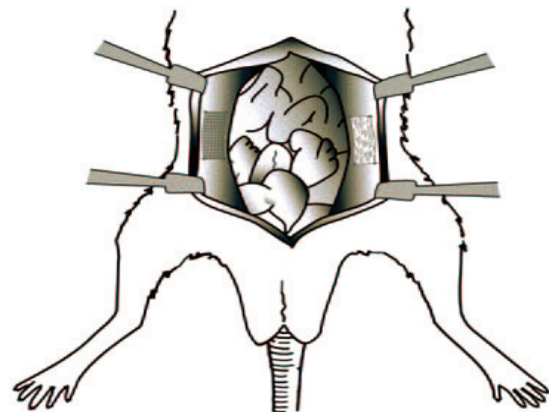


Fig. 1. Mesh Implantation in a rat model. TVT-O mesh (dark mesh) was implanted on the inner surface of the right anterior abdominal wall, while I-STOP mesh (white mesh) was implanted on the left side

2.4. Euthanasia

The animals were euthanized in a CO₂ chamber at timed intervals: 6 weeks, 3, 6, 9 and 12 months after implantation, 6 rats per time period (5 time intervals).

2.5. Sample collection

After sacrifice, the animals' abdomens were opened, and the TVT and I-STOP strips were retrieved for variable analysis. Meshes were extracted through a mid-

line laparotomy incision, by cutting part of the abdominal wall with the mesh sutured to it. They were then carefully dissected from the abdominal wall and immediately placed in saline. After the procedure, the fibers were untangled, and if any force was exerted during untangling, they were excluded from the study to avoid altering their mechanical behavior. Multiple fibers were obtained from each sling (4 minimum). The strips were maintained in normal saline until their testing in the tensiometer. Control meshes were treated in the same manner.

2.6. Uniaxial tensile testing

The tensile properties of fibers from TVT and I-STOP strips were evaluated after each euthanasia and sample-collecting procedure. Individual fibers from the strips underwent uniaxial-mechanical characterization.

The average fiber length was set as 15–18 mm to limit a potential stretching effect caused by grips placed at the end of mesh fibers. Cross-sectional area was 0.0154 mm^2 and it was measured constantly in all samples over time. Load cells were chosen on a scale of 220 N, as the breaking load of fibers was around

8–10 N. ElectroForce Biodynamic[®] Test Instrument 5160 (Bose Corporation, Framingham, MN, USA) with 220 N load cells was deployed with specially-designed, modified grips (Fig. 2A) to secure fiber ends and to achieve breaking point at the central zone. The system was implemented in displacement control, with fibers shifted at the rate of 0.1 mm/s. Applied stress was calculated as the ratio of force to resistance area of fibers, measured by microscopy. Strain was computed as percentage of initial length. Moreover, UTS, defined as maximum stress reached during sample rupture, and ultimate strain, corresponding to ultimate stress, were measured. Elastic modulus was calculated as the slope of the linear phase of stress/strain curves (Fig. 2B). Modulus slope was gauged in the 8–10% range. Slopes with values greater than 10% were not included.

2.7. Sample size

Long-term evaluation of the tensile properties of polypropylene meshes in an *in vivo* rat model in terms of elasticity has never been reported before, rendering sample size calculation difficult from previous studies. Therefore, rat numbers were decided in consultation with the McGill University Animal Care Committee, according to the principles of replacement, reduction and refinement.

2.8. Statistical analysis

The data were assessed for statistical significance by 2-way analysis of variance with significance level $p = 0.05$. Means were compared by Tukey–Kramer and Holm–Bonferroni methods (Origin Pro v. 8 software, OriginLab, Northampton, MA, USA).

3. Results

After harvesting, all synthetic strips retained most of their morphologic and geometric properties presented before implantation. Both meshes exhibited constant width over time in comparison to control samples, and held their morphologic configuration, without an unraveled appearance or distorted organization (Fig. 3).

In terms of uniaxial tensile characterization, fibers extracted from I-STOP were compared to untreated control samples. Table 1 summarizes average values of

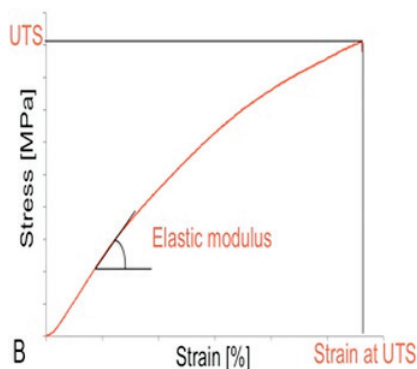
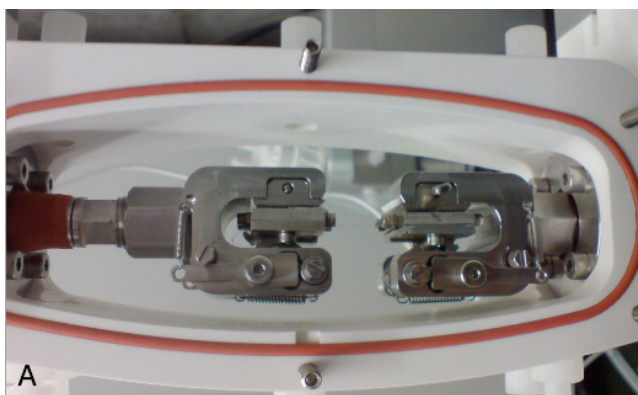


Fig. 2. Uniaxial tensile test set-up. A. Samples tested by ElectroForce Biodynamic[®] Test Instrument 5160 with *ad hoc* modified grips. B. Representative stress versus strain curves displaying main parameters of interest

UTS, strain at UTS and elastic modulus. Each parameter was consistent over time without any statistical difference ($p > 0.05$). Figure 4A depicts representative stress/strain relationship curves at each time interval, with all samples exhibiting similar mechanical behavior over time equivalent to control values.

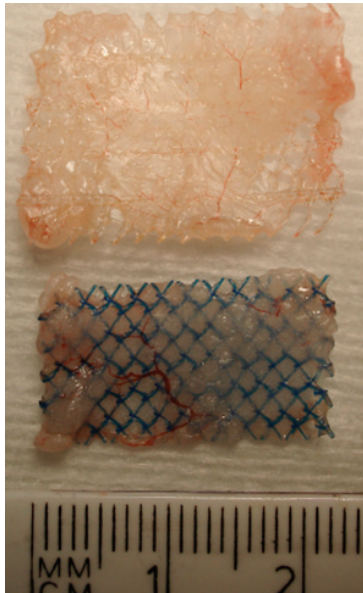


Fig. 3. Morphological characterization of both tapes (TVT-O and I-STOP) after 12 months

Table 1. Ultimate tensile strength (UTS), strain at UTS and elastic modulus of I-STOP fibers measured at each time interval

I-STOP	UTS [MPa]	Strain at UTS [%]	Modulus [MPa]
Control	441.56 ± 45.33	54.97 ± 5.60	717 ± 54.96
6 weeks	399.35 ± 41.70	53.52 ± 2.33	796.24 ± 91.47
3 months	442.64 ± 48.96	53.48 ± 6.62	786.36 ± 26.34
6 months	428.90 ± 55.56	54.67 ± 11.50	815.57 ± 71.86
9 months	409.96 ± 25.79	50.18 ± 1.32	691.65 ± 25.16
12 months	419.70 ± 14.48	50.32 ± 4.73	740.68 ± 55.21

TVT-O mesh is made up of 2 fiber types: TVTW (white) and TVTB (blue). Tables 2 and 3 summarize the mechanical characteristics of TVTW and TVTB, respectively, in terms of UTS, strain at UTS and elastic modulus. Both TVTW and TVTB fibers demonstrated significant decay of UTS and strain at UTS after 12 months ($p < 0.05$), while the elastic modulus was constant over the 1-year follow-up period ($p > 0.05$). Figures 4B (TVTW) and 4C (TVTB), respectively, report representative stress/strain relationship curves at each time interval.

Elastic modulus remained constant over time in all fiber types, as the slopes of the curves in the linear region were comparable between all samples. Representative stress/strain curves of I-STOP, TVTW and

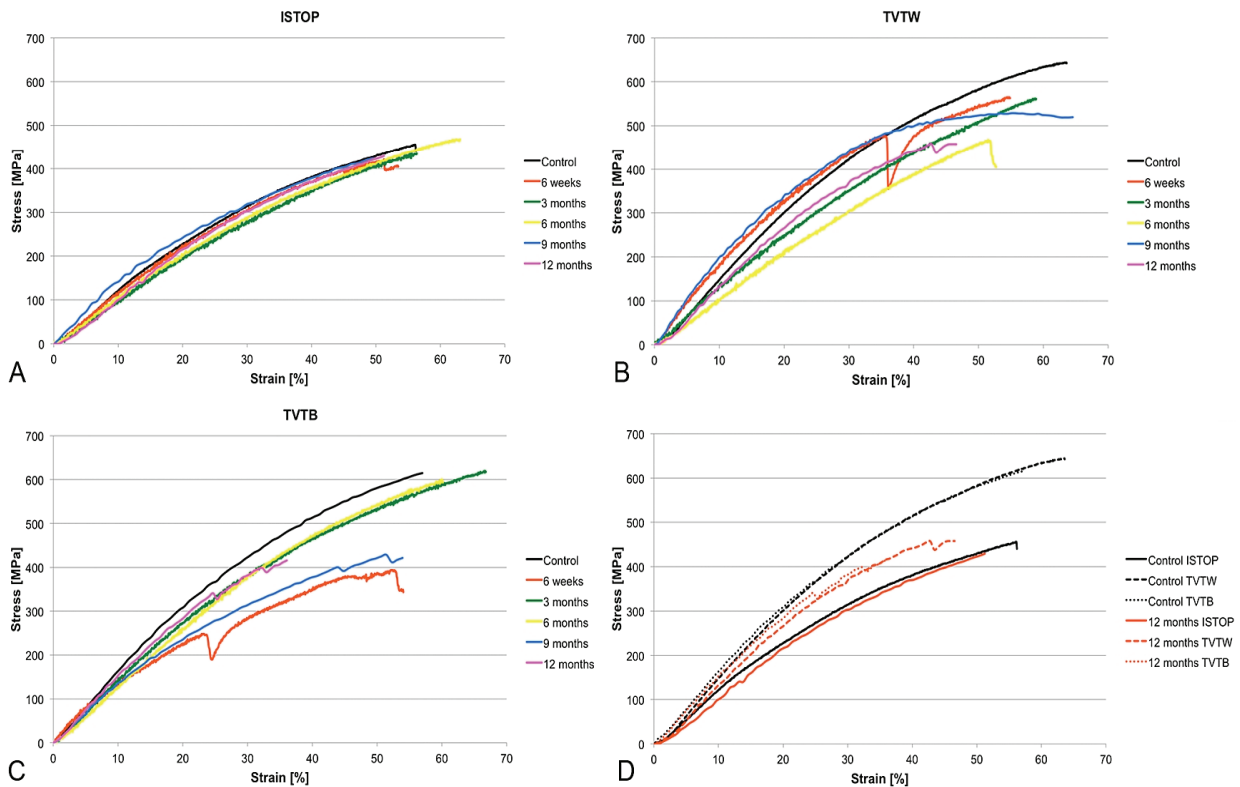


Fig. 4. Uniaxial mechanical characterization. Representative stress/strain relationships reported for samples extracted from I-STOP (A), TVTW (B) and TVTB (C) at 6 weeks, 3, 6, 9 and 12 months. Each sample at the latest time point was compared to untreated relative controls (D)

TVT-O are charted in Figure 4D for direct comparison between the latest time point (at 1 year) and untreated controls.

Table 2. Ultimate tensile strength (UTS), strain at UTS and elastic modulus of TVT-O white fibers measured at each time interval

TVT-O white	UTS [MPa]	Strain at UTS [%]	Modulus [MPa]
Control	565.91 ± 107.90	59.03 ± 6.46	1,109.50 ± 46.39
6 weeks	543.29 ± 46.85	57.81 ± 5.95	1,040.08 ± 55.53
3 months	560.61 ± 55.57	58.64 ± 7.09	938.41 ± 18.37
6 months	517.53 ± 48.46	57.66 ± 6.34	935.19 ± 15.72
9 months	521.43 ± 33.18	59.47 ± 3.52	906.08 ± 167.31
12 months	405.52 ± 47.09	45.20 ± 4.51	974.54 ± 152.42

Table 3. Ultimate tensile strength (UTS), strain at UTS and elastic modulus of TVT-O blue fibers measured at each time interval

TVT-O blue	UTS [MPa]	Strain at UTS [%]	Modulus [MPa]
Control	497.19 ± 103.70	53.47 ± 3.79	984.98 ± 24.28
6 weeks	408.44 ± 12.97	51.62 ± 2.28	814.97 ± 156.09
3 months	590.91 ± 11.49	57.82 ± 1.40	982.10 ± 31.44
6 months	586.80 ± 52.34	58.46 ± 5.51	1,042.78 ± 59.36
9 months	512.01 ± 38.32	58.84 ± 6.32	994.06 ± 148.70
12 months	412.77 ± 14.81	36.88 ± 4.48	1,177.30 ± 105.80

4. Discussion

This project had 2 goals. First one was a long-term evaluation of the tensile properties of commonly-used polypropylene meshes and the second was an assessment of elastic modulus as a biomedical factor in urogynaecological research. 2 polypropylene tape types (TVT-O[®] and I-STOP[®]) were examined for up to 1 year after implantation *in vivo*. Both types retained their elastic modulus over time, i.e., their elastic modulus remained constant. I-STOP maintained UTS and strain, which were significantly reduced with TVT-O.

The present study was the first to evaluate the long-term tensile properties of individual polypropylene tape fibers with elastic modulus. To analyze tensile properties more precisely, new biomechanical grips were designed. Assessment of single mesh fiber may be translated properly into quality of entire meshes placed in live organisms. This method has been established from the bioengineering point of view as it avoids bias that could be caused by different knitting

patterns and pore sizes [4]. The properties of entire meshes are additive combinations of many individual fibers and mesh constructions. Interesting is the fact that small differences in single fibers can have great impact on entire meshes [12]. These factors may be critical in minimizing patients' suffering and maximizing implant success.

Polypropylene meshes, nowadays the most frequently-used synthetic biomaterials for pelvic floor reconstruction, possess mechanical properties of durability and compliance. Recent data suggest that parameters, such as mesh size, regrowth inside fabrics, mechanical, chemical, and physical features as well as anchoring technique are important for the success of prosthetic implants and dictate host responses after implantation [11], [14], [22]. Their mechanical properties should be comparable to those of natural tissues, and they should be stable for a long time, showing resistance to damage.

The tensile properties of different synthetic meshes have been evaluated in previous studies through MBL estimation [9], [11], [24], [27]. MBL is defined as maximum load that materials can withstand while being stretched or pulled up to break point [7]. This is also referred to as UTS. These ultimate values do not have clinical significance since slings never break when implanted in patients. Instead, they may become loose or redundant, which may be the probable theoretical cause of their occasional failure. Another theory holds that surrounding native tissues may fail to attach [5].

Abdominal pressure, which normally ranges around 20 mmHg (2.6 kPa) is another important factor. Polypropylene fibers break or fracture at around 300 MPa, which means that these tapes are functioning at very low pressures. It led to consideration of the elastic modulus, another variable suitable for measuring mesh tensile properties. Elasticity is the physical capacity of materials that returns them to their initial shape after suppression or deformation [7]. Materials are perfectly elastic if they completely find their original form after suppression of traction. Elastic modulus (also known as modulus of elasticity) is the measure of elastic material stiffness, i.e., the mathematical description of a substance's tendency to be deformed elastically (non-permanently) when force is applied to it. As a mathematical equation, elastic modulus is stress-divided by strain. Stress is load or force per unit cross-sectional area, and strain or deformation is change of original length. It is calculated as the slope of the straight-line proportion of stress-strain curves (Fig. 2B). Thus, the strain-stress curve is steeper with higher elastic modulus. Therefore, a ma-

terial whose modulus is high is “stiff”, and a material whose modulus is low is “supple”.

The erosion rate of polypropylene meshes in surgery for stress incontinence management is around 1–3% [19]. Synthetic meshes made of other polymers usually have low elasticity and may predispose to erosion and pain in up to 20% of patients [1], [23]. These differences in complication rates indicate the importance of elastic modulus. Moreover, the role of structural elasticity has been established in terms of tissue erosion, mesh exposure and pain from vaginally-implanted meshes for repairing pelvic organ prolapse (POP), and underscoring the significance of this factor [22]. A recent *ex vivo* study of the mechanical properties of Prolene mesh showed that anisotropy and time-dependent visco-elasticity of meshes under relevant physiological loading conditions may be related to post-operative complications, including chronic pain, hernia/prolapse recurrence, and infections [13]. These authors concluded that the viscoplastic-elastic behavior of meshes may significantly impact the long-term performance of such implants. From this viewpoint, elastic modulus may be the best factor to assess the long-term outcomes of sling treatment in urinary incontinence.

In another study, a British group attempted to find the best candidate scaffolds for tissue engineering to manage SUI and repair POP [17]. All materials were tested regarding their ability to support fibroblast attachment and the formation of new extracellular matrix – to acquire biomechanical properties with cells close to those of native tissues. They conducted *ex vivo* analysis of UTS, strain at UTS and Young’s modulus, implicating them as the most appropriate factors for bioengineering assessment of meshes. These authors concluded that the biomechanical properties of materials can change after implantation. Further studies of this phenomenon are warranted.

Lei et al. explored the relationship between biomechanical properties and POP occurrence through elastic modulus measurement [16]. Biomechanical analysis of vaginal tissue in pre- and post-menopausal women in their study demonstrated that connective tissue is less elastic, and stiffness is increased in POP, meaning that POP vaginal tissues have higher elastic modulus. This time elasticity was a key factor in bioengineering assessment.

Bellows et al. investigated the mechanical properties of biological meshes (acellular human dermis and porcine small intestine submucosa) in response to bacterial encounter in a rat model [3]. After 10 or 20 days of mesh implantation inoculated with various concentra-

tions of methicillin-resistant *Staphylococcus aureus* (MRSA), the materials were explanted and cultured for bacteria. Histological changes and bacterial recovery were assessed together with biomechanical properties. Control samples of both meshes retained elastic modulus, whereas those inoculated with MRSA had significantly decreased elasticity. This study highlights elastic modulus as one of the most sensitive factors for bioengineering of meshes.

Pariente et al. evaluated the biomechanical properties of different commercially-available sub-urethral slings *in vitro*, according to the elastic modulus concept [18]. They concluded that those with high elastic modulus should be proposed for trans-obturator procedures to provide real perineal support while tapes with lower elastic modulus should be married with retro-pubic techniques.

A recently-published study indicated that changes in the elasticity of mesh filaments could improve prostheses benefits [6]. The authors compared the short- and long-term behavior of Ciberlastic and Optilene elastic commercial meshes in the repair of partially-herniated abdomens in New Zealand White rabbits. Based on the results obtained, they came to the conclusion that elastic properties should be taken into account in the preclinical evaluation of new materials.

The clinical significance of the present results is rather difficult to assess. However, the well-preserved morphological appearance of TVT-O and I-STOP tapes was noted upon harvesting graft tissues. The width of both meshes was constant compared to control specimens. Both tape types retained their pre-implantation morphological configuration, without a shriveled appearance or distorted architecture. This is consistent with our previous work, where TVT and SPARC retained their morphological characteristics, compared to the indistinct appearance of cadaveric fascia lata grafts [24], [27]. Although UTS and strain of meshes examined over time were significantly different, both showed no changes in elasticity until study end. It may correspond to results from studies which evaluated the clinical efficacy of these slings over a 1-year period [2], [15]. Both slings had good clinical outcomes in long-term patient assessment. Consequently, it could be stated that elastic modulus better corresponds to clinical outcomes than MBL, UTS and strain at UTS in long-term follow-up. Rigid materials can develop excessive stress at the interface, inducing prosthesis erosion and tissue exposure [26]. Assessment of mesh elasticity could help physicians to foresee the success of mesh implants. Mechanical features/environment may impact host responses and mesh degradation.

Site of mesh implantation was a study limitation in terms of mechanical degradation. Although TVT-O and I-STOP were not implanted sub-urethrally, as it is usually the flashpoint of sling procedures, the fact, that the extensive dissection needed to place the tapes sub-urethrally might have affected the outcomes, as a result of local reactions to surgery and prolonged anesthesia, was taken into consideration. Furthermore, mesh implantation into the rat peritoneal cavity provides an environment akin to that in clinical practice, and might have similar impact on physical properties because of inflammatory responses. The urethro-pelvic ligament, which supports the bladder neck/urethra and is assisted by slings in humans, arises from endopelvic fascia, which is a continuation of the parietal peritoneum. Moreover, implantation techniques are more challenging in animals and could expose them to unnecessary complications and contamination due to any local surgical procedures. However, to partially mimic 'real' sling loads, the tapes were connected to the abdominal wall under tension with sutures at the 4 corners. Another limitation refers to obtained results. As the stress-strain curves are nonlinear, it would be beneficial to assess tangent modulus for long-term evaluation of the tensile properties of commonly-used polypropylene mid-urethral slings. Researchers of hernia implants seem to support this idea. Recently published data indicate that tangent modulus of synthetic meshes may better correspond with the physiological state in the human abdominal wall [8]. However, until now no real *in vivo* studies confirming that idea have been conducted [20].

Summarizing, meshes should be evaluated in terms of success and complications. Meshes considered to be highly compliant would be associated with low incidence of erosion. On the other hand, meshes considered to be less compliant would be more durable and less liable to failure. The dilemma remains as the ideal meshes should respect both aspects.

5. Conclusions

The mechanical behavior of the mesh is defined by its flexibility or compliance (related to elastic modulus) and by the breaking strength (related to UTS). Elastic modulus consistency could be a principal factor in evaluating the tensile properties of synthetic meshes. Therefore, any new mesh launched in the marketplace should undergo assessment of its tensile properties, most importantly elastic modulus. This knowledge

should be tapped to improve the design of synthetic mesh implants.

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The present protocol was approved by the McGill University Animal Care Committee and complied with Canadian Council on Animal Care guidelines.

Disclosure

The authors declare no conflict of interest.

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