Structural differences and architectural features of two different polypropylene slings (TVT-O and I-STOP) have no impact on biocompatibility and tissue reactions

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Introduction To evaluate the impact of design features of the synthetic mid-urethral slings on tissue integrity and inflammatory responses.

Material and methods In total 30 female Sprague-Dawley rats were implanted with type I monofilamentous, macroporous polypropylene meshes: Gynecare TVT-Obturator tape® (Ethicon Inc., Johnson & Johnson, Somerville, NJ, USA) and I-STOP® (CL Medical Inc., Lyon, France). All animal groups were sacrificed at set time intervals – 6 weeks, 3 months, 6 months, 9 months and 12 months – and the abdominal wall was harvested with mesh strips for histological evaluation.

Results All mesh strips appeared to be well incorporated into the abdominal wall, and no signs of shrinkage was noticed. All specimens showed a thin/delicate, loose, fibrous interface between the synthetic graft plate and abdominal wall, along with mild inflammatory reactions from 6 weeks to 12 months.

Conclusions Both mesh brands induced comparable, minimal foreign body reactions and integrated well into the host tissues despite differences in architectural features. TVT-O® and I-STOP® evoked similar low-grade inflammatory responses up to 12 months in this animal model. Structural differences and architectural features of polypropylene slings used in this study have had no impact on tissue integrity and inflammatory responses.

Key Words: mid-urethral sling • TVT • I-STOP • inflammatory response • tissue reaction • histological assessment

INTRODUCTION

Female stress urinary incontinence (SUI) is defined as an involuntary leakage of urine upon effort, exertion, sneezing or coughing [1]. It is a common condition with worldwide prevalence and incidence estimated to be 5–61% and 4–11%, respectively [2, 3]. Trans-obturator mid-urethral sling procedures with synthetic tapes have proven to be efficient therapeutic options. The rate of complications, especially extrusion/vaginal erosion, is approximately 1.1 to 3% [4, 5]. The remaining post-operative complications such as voiding dysfunction, de novo urgency, chronic pain and dyspareunia, entail longer recovery affecting the patients’ quality of life and sometimes requiring reoperation and sling removal. Complications depend on the patients’ general condition, prior pelvic surgeries, placement technique, tape material and structure [6]. Whereas, clinical factors indicating success in mesh procedures have been well-investigated, the structural differences and architectural features of tapes have not been sufficiently studied. Existing variations in manufacturing have led. Loosely-woven or knitted, monofilamentous, macroporous polypropylene meshes appear to better integrate in tissues than multifilament or cadaveric materials.
However, it is known that different knitting patterns of polypropylene tapes can significantly change their biomechanical properties and further contribute to the hypothesis that histological responses can vary [7]. The literature does not provide strong evidence on this issue.

Gynecare TVT-O® slings (Ethicon Inc., Johnson & Johnson, Somerville, NJ, USA) have been associated with a low rate of extrusion/erosion (1.7%) at 4 years [8]. I-STOP® (CL Medical Inc., Lyon, France) has been reported to elicit few complications at 7 years, making them significantly effective [9]. Both slings are made of type I macroporous, monofilamentous polypropylene mesh [10], but differ according to knitting mode and edge design. TVT-O® is characterized by high elasticity, in contrast to I-STOP®, which is non-elastic [11]. Elasticity is the physical property of materials that returns them to their initial shape after suppression or deformation [12]. It has not been established whether elasticity and elastic modulus impact the long-term performance of mesh implants [13, 14]. Furthermore, studies have shown that the chemical, physical, and morphological characteristics of implanted synthetic materials play a role in modulating cellular events and tissue reactions [15]. Polypropylene mesh elicits ongoing host inflammatory responses. However, the data are scant when it comes to histological evaluation of implants with different mechanical features (especially elastic modulus) and knitting patterns made with polypropylene, the most popular artificial material integrated into pelvic reconstruction surgery. Histological assessment could provide a synthesis of cellular and humoral immunity processes linking tissue responses to possible complications.

The objective of the present study was to evaluate the impact of these design specificities and structural differences on tissue integration and inflammatory reactions. Reduction of tissue responses with effective integration will lower the risk of adverse clinical consequences [16, 17, 18].

**MATERIAL AND METHODS**

**Ethics statement**

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

**Study design**

30 Sprague-Dawley (SD) rats were randomly allocated into 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 animals initially underwent mesh implantation of the type I monofilamentous, macroporous polypropylene meshes Gynecare TVT-O® (Ethicon Inc., Johnson & Johnson) and I-STOP® (CL Medical Inc.), prepared in 20 × 10 mm strips, and were followed for pre-determined periods. Table 1 summarizes the characteristics of both slings, while Figure 1 presents both meshes before implantation. The rats were euthanized at the end of each period and the implanted meshes were retrieved for histological evaluation.

**Animals and implants**

30 female SD rats, 90 days of age and weighing 250–300 g each (Charles River Laboratories, St. Constant, Quebec, Canada), were housed separately

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**Table 1. Textile properties of the meshes used (as provided by manufacturers)**

| Mesh type | TVT-O | I-STOP |
|-----------|-------|--------|
| Material  | Polypropylene | Polypropylene |
| Structure | Monofilament | Monofilament |
| Fiber size (diameter) | 0.15 mm | 0.15 mm |
| Pore size | 1379 µm | 100 µm |
| Knitting | Knitted (interlock) | Knitted |
| Mesh edges | Tanged | Not tanged, curled edges |
| Mesh thickness | 0.63 mm | 0.3 mm |
| Elasticity | Extensible | Non-extensible (low elasticity) |

**Figure 1. Appearance of mesh strips before implantation:** TVT-O® (right) and I-STOP® (left).
in regular cages. They were maintained under the same feeding, temperature, humidity and lighting conditions.

**Surgical procedure**

Meshes were implanted in all 30 rats, and the surgical procedures were performed with autoclaved instruments. The rats were anaesthetized with a mixture of isoflurane 5%-oxygen 1.5 l/min for induction and isoflurane 2.5%-oxygen 0.8 l/min for maintenance. Analgesia (buprenorphine 0.05 mg/kg of body weight) was sustained subcutaneously every 8 hours for 2 days post-operatively, via 24 G needle. The abdomen of each rat was shaved and disinfected with 2% chlorhexidine solution.

A midline abdominal incision (3 cm) was made and covered with sterile drape. Both brands of sterile mesh strip were fastened to the inner surface of the abdominal wall, one to the right and the other to the left of midline, at 2-cm distance, with 6/0 prolene sutures (Johnson & Johnson) fixed at the 4 corners of each strip. The peritoneal cavity and abdominal fascia were closed with 4/0 PDS II sutures (Johnson & Johnson) in a running manner. The skin was closed with interrupted 3/0 PDS II sutures (Johnson & Johnson).

**Euthanasia**

Animals from each group were euthanized at the designated time intervals (6 weeks, 3 months, 6 months, 9 months and 12 months) in a CO₂ chamber.

**Sample collection**

After each animal was sacrificed in an aseptic manner, a midline abdominal incision was made and skin was dissected from the abdominal fascia. The abdominal wall, including the mesh strips, was harvested from each side. Final implant size in width and length was measured and analyzed. The explanted tissues were then placed in 10% phosphate-buffered formalin and subsequently embedded in paraffin.

**Histological evaluation**

Paraffin blocks were cut into 5-µm slices by microtome and stained with hematoxylin-eosin (H&E) and Masson’s trichrome. They were cut at a distance from the original sutures to avoid inappropriate tissue reactions. Histopathological analysis was performed by an uropathologist (LRB) blinded to the mesh types. The degree of inflammation was recorded on the basis of a 4-point scale, along with the presence of giant cell reactions, and graded as 0 (absent), 1 (mild), 2 (moderate) and 3 (severe) in terms of acute and chronic inflammation (Table 2) to specifically compare histological differences [19]. The samples were also analyzed for fibrosis, necrosis and neovascularization and each feature was scored as absent (less than 5% of the analyzed sample), mild (5–25% of the analyzed sample), moderate (25–50% of the analyzed sample) or severe (more than 50% of the analyzed sample).

The sample size of a long-term evaluation, lasting at least 1 year, of tissue integration and inflammatory responses to polypropylene meshes in an in vivo rat model has never been undertaken before, so that sample size could not be calculated from previous studies. Therefore, the number of included rats was decided in consultation with the McGill University Animal Care Committee, according to the principles of replacement, reduction and refinement.

**Statistical analysis**

Data analysis was conducted with IBM SPSS Statistics, version 23.0 (IBM Corporation, Armonk, NY, USA) to ascertain differences between groups. The 5 distinct groups (plus 1 control) were compared 2 by 2 according to the 2-sided Mann-Whitney U test in terms of strip width and length. Categorical variables of inflammation degree and histological responses were compared by Chi-square Statistic and the Kruskal-Wallis test. Statistical significance was set at p <0.05, and Dunn’s test was applied to identify differences between the groups.

| Grade of inflammation | Description |
|-----------------------|-------------|
| 0 (absent)            | No inflammation or <5% of the analyzed sample |
| 1 (mild)              | Inflammatory reaction 5-25% of analyzed sample; scarce inflammatory infiltrate, confined to areas of giant cell reaction if present |
| 2 (moderate)          | Inflammatory reaction 25-70% of analyzed sample; moderate inflammatory infiltrate in areas of giant cell reaction area and involving adjacent connective tissue |
| 3 (severe)            | >70% of the analyzed sample; marked inflammatory infiltrate in areas of giant cell reaction and prominently involving connective tissue |
revealed that minimal to small numbers of lymphocytes and plasmocytes were noted in 2 specimens with TVT-O® strips at 6 months and in 3 specimens at 9 months (Figure 4), while the presented abnormalities were observed in one I-STOP® strip at 9 months. At 12 months, specimens of both strip types showed a small number of plasmocytes and lymphocytes. When groups of each sling type were pooled, data on both types of meshes in terms of acute and chronic inflammation were not statistically different (p >0.05).

RESULTS

Macroscopic evaluation

No post-operative complications were observed in all 5 experimental groups. All wound sites healed without signs of infection, wound dehiscence or skin-mesh protrusion. At the harvest times indicated, all mesh strips appeared to be well incorporated into the abdominal wall. There was no adhesion to the intraperitoneal organs. All strips maintained their original dimensions and morphological appearance up to 12 months without any statistical difference (p >0.05) (Figures 1, 2).

Histological evaluation

All specimens showed a thin/delicate, loose, fibrous interface between the synthetic graft plate and abdominal wall, starting from the 6-week evaluation and maintained for up to 12 months (Figures 2, 3), usually with modest foreign body giant cell reactions (mono- and multinucleated histiocytes) centered on the synthetic constituent, sometimes associated with rare lymphocytes, plasma cells, eosinophils or siderophages.

Inflammatory reactions

Mild foreign body giant cell reactions (graded 1) centered on the synthetic constituents were apparent in all specimens (p >0.05) with both strip types, from 6 weeks up to 12 months (Figure 3). Outcomes of histological assessment are presented in Table 3. Only at 3 months both strips induced the occurrence of rare eosinophils as signs of acute inflammation. Assessment of chronic inflammation

Figure 2. Appearance of mesh strips upon explantation at 12 months: TVT-O® (A) and I-STOP® (B).

Figure 3. Histological evaluation of I-STOP® mesh at 3 months. (A) Thin, uniform layer of fibrous tissue (arrows) interposed between the abdominal wall (left half) and apposed graft (right half). No associated intrinsically-active fibrosis or acute/chronic inflammation is apparent (H&E, 100x original magnification). (B) Mild foreign body reactions of mono- and multinucleated histiocytes surround the synthetic constituent without concomitant acute or chronic lymphoplasmacytic inflammation (arrows) (H&E, 200x original magnification).

Figure 4. Histological evaluation of TVT-O® mesh at 6 months. In addition to foreign body reactions (arrowheads) surrounding the synthetic constituent, which appears as empty lacunae, there is evidence of mild to moderately-active fibrosis, including a small number of admixed lymphocytes and plasma cells (arrows) (H&E, 200x magnification).
Active fibrosis

At 6 weeks, all specimens with I-STOP® strips presented mild to moderate, active fibrosis centered on the synthetic material, whereas none of the TVT-O® specimens manifested fibrosis (p < 0.05). Active fibrosis disappeared from 3 months up to 12 months, except in 1 specimen at 9 months with mild intensification of fibrosis. No fibrosis was seen with TVT-O® strips at the different evaluation intervals, except in 2 specimens at 9 and 12 months where focal, mild, active fibrosis was centered on the synthetic material (Figures 3, 4). However, when groups of each sling type were pooled, data on both types of meshes were not statistically different (p > 0.05).
Neovascularisation

No differences were evident at the various evaluation periods between all specimens, with both sling types displaying focal neovascularisation (p >0.05).

Necrosis

Both strip types manifested no signs of necrosis at the different evaluation intervals (p >0.05).

DISCUSSION

Polypropylene mid-urethral slings are widely adopted by urologists and gynaecologists for SUI treatment. Complications of these procedures, although rare, could be deleterious to a patient’s condition and possibly a source of legal conflicts. Among potential complications, vaginal extrusion is most likely associated with mesh material [6].

Based on the classification of synthetic meshes, first developed for hernia surgery [10], type I macroporous, monofilamentous polypropylene meshes have been shown to induce mild histological foreign body reactions, in contrast to other mesh types used for SUI procedures [20]. Our project went one step further and proved that the architectural features of polypropylene meshes have no impact on histological tissue responses in a 1-year period. The nature and intensity of inflammatory reactions may be critical for acceptance or rejection, and could subsequently influence mechanical properties as well as sling efficacy [21, 22].

Various marketed mid-urethral sling brands differ in the knitting and weaving properties of polypropylene material. Aside from pore size, surface features may impact tissue integration [23, 24]. Thus, each material should be evaluated individually. TVT-O® is commonly used worldwide in clinical practice and reported to evoke a low incidence of erosion and vaginal extrusion. I-STOP® is more recent, confined mainly to Europe for the time being, but is known to elicit a low incidence of complications as well. Both are made from polypropylene. However, TVT-O® is extensible and thicker, characterized by tangled edges and interlocking knitting patterns [25]. On the other hand, I-STOP® is a non-extensible tape with low elasticity, curled edges and smaller pore sizes [26].

Recently published non-systematic review confirmed that polypropylene evokes a less inflammatory or similar host response when compared with other materials used in SUI mesh devices, with large-pore and light-weight meshes being favoured [27].

The conclusion of the pore size impact was based on the study conducted by Klinge et al. who implanted polypropylene mesh in the abdomen of rats for 90 days [28]. A small-pore mesh (with a mean pore size of 480 µm) was associated with intense chronic inflammation accompanied by extensive scar bridging. Conversely, the large-pore mesh (with a mean pore size of 2800 µm) exhibited scarring similar to the control group. The authors that pore size is a significant factor in the tissue response and the overall biocompatibility of polypropylene mesh. However, our study showed that the difference in pore sizes was of little importance. More importantly, their observation lasted only 3 months, whereas results presented by us were collected during a 1-year period. Moreover, in the study of Klinge, the small-pore mesh was of monofilament design, whereas the large-pore was of multifilament design. Since multiple studies have showed that tissue integrity and histological response vary between monofilament and multifilament meshes, conclusions of pore size impact presented in this study are rather overestimated. Both TVT-O® and I-STOP® meshes, investigated in our study, are characterized by the monofilament structure. Recently, Sindhwani et al. studied in vivo changes in pore dimensions of a textile implant and demonstrated the dynamic nature of the material during the tissue integration process [29]. They stated that this phenomenon depends more on mesh-produced material than architectural features, but more research is warranted. Nevertheless, researchers of hernia implants stress that reliable analysis based on mesh materials is not currently possible to perform as recordings of them are restricted to brand names [30]. Thus, system classifications should be based on biological response. Studies investigating meshes enriched by coating bio components seem to support this idea [31].

Cobb et al. suggested that reducing the weight of polypropylene mesh beyond that currently considered as light-weight may lower the host response and inflammation, thereby reducing potential morbidity [32]. However, given the multifactorial nature of complications, the overall impact of reducing mesh volume and weight is unclear. Data obtained from our study presents that architectural and structural differences, resulted in different mesh weights, have no impact on histological response indicating mesh-produced material to be the crucial factor for tissue incorporation. This finding is concurrent with results presented by Roman et al. [33]. Moreover, Ozog et al. demonstrated that reduction of mesh weight may have a negative impact on procedure’s process [34]. They implanted an ultra-lightweight polypropylene mesh into the abdomen of rabbits and deemed the handling characteristics of this mesh inappropriate.
because of folding upon insertion, which was not observed with the heavier mesh. Most previous studies have evaluated tissue reactions to synthetic slings for up to 3 months. In current literature, Gerullis et al. reported the longest time interval for the analysis of synthetic mesh integration [35]. In their experiment, lasting up to 24 months, they analyzed three different hernia meshes implanted in 14 sheep, euthanized at 4 different time points. However, investigated meshes were made from different materials (polypropylene, reinforced polypropylene and polyvinylidene fluoride). Our experiment investigated the longest interval between implantation and retrieval of urethral slings made from exactly the same material (polypropylene), but with different architectural features, allowing sufficient time for host-graft interaction stability [36]. In this work, both meshes induced comparable, minimal foreign body reactions and integrated well into host tissues up to 12 months with no signs of infection, extrusion or erosion, even though one would expect that these mesh types with different architectural features, could provoke different inflammatory reactions once implanted in the host. Also, since the 2 meshes have different elastic properties, the absence of significant differences in tissue reactions indicates that this parameter is not influential, at least for mesh integration and tissue reactions. Riccetto et al. [37] evaluated tissue reactions, inflammation, and collagen fiber density elicited by monofilament and multifilament polypropylene meshes in subcutaneous tissues of female rats. Although tissue reactions and inflammatory responses were not statistically different, stereological analysis disclosed significant variations between mono- and multifilament meshes. Monofilament materials induced more collagen deposition and greater density of collagen fibers, indicating fibrosis as a possible, more favourable biocompatibility factor of implantation mesh procedures on short-term evaluation. These authors stated that collagen fibers developing among mesh filaments could evoke its complete integration and minimal or absent inflammatory responses. Complete integration would reduce the risk of mechanical damage. The present study obtained similar results with I-STOP® in terms of fibrosis at 6 weeks. However, their analysis lasted only 120 days, whereas the current work showed that monofilament meshes do not induce excessive fibrosis over a 1-year period, representing appropriate long-term follow-up in an animal implantation model [36]. This outcome corresponds with clinical data, which have argued that excessive and prolonged fibrosis could predispose to mesh complications [16].

In another analysis, a Brazilian group [38] compared inflammation and collagen production induced by 4 sling materials in female rats. They stated that polypropylene caused more intense and longer-lasting inflammatory reactions with greater visceral penetration than autologous fascia or swine intestinal submucosa. The present results do not correspond with these conclusions. The major bias in their findings was the short analysis time: 90 days. This time period does not allow evaluation of appropriate histological responses to artificial materials [36]. Previous in vitro studies have revealed that TVT-O® and, to a lesser degree, I-STOP® both release material particles after elongation [24]. This issue, based on the present results, does not seem to impact inflammatory responses in the host and could be attributed to mesh integration that causes minimal friction and avoids the risk of mechanical damage [24]. However, it has not been investigated whether these released particles have an effect on the ease of removal secondary to complications. Further research has to look at this phenomenon.

In addition, data extrapolated from the treatment of pelvic organ prolapse and hernia surgery indicated that mesh-induced chronic inflammation correlated with chronic pain [16] and reduced mesh flexibility [39], which are significant issues in SUI surgery. In the present study, the absence of significant chronic inflammatory reactions with up to 12 months follow-up would explain the good tolerance and functional results obtained with these meshes. The limitation of the present experiments, as in all studies of animal models, is that data extrapolation to humans could be hazardous, since it is not possible to exactly replicate implantation, and the human body could adjust the local environment in times of stress and wound-healing. The impact of anatomical location is crucial and current evidence suggests the pelvic region exhibits a more exaggerated host response than the abdominal region [27]. Although both tapes were not implanted sub-urethrally, as it is usually the flashpoint of sling procedures, we considered 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 have significant contribution to final results. Furthermore, mesh implantation into the rat peritoneal cavity provides an environment akin to that in clinical practice for SUI incontinence procedures, 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 the 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. Rat animal model allows to make our results comparable to previously published studies [27]. Furthermore, anatomical location is a factor that cannot be adjusted when correcting SUI which may in turn lead to a higher level of complications in some patients. Also, additional factors, such as incision length, menopausal status and previous pelvic surgery or radiation, cannot be overlooked as they could affect tissue reactions to sling materials. Interspecies differences between animals and humans are considerable and an ideal animal model is yet to be determined [27]. Synthetic sling length in the present model may have had an impact as well, since the magnitude of inflammatory responses may grow with increased sling surface. Recently published study emphasizes the fact that the surface area of implanted material is proportional to the host-produced response [27]. The cause of this is multifactorial and includes patient and material factors. However, they clearly added that the presented issue is very difficult to investigate with various animal models. Grading inflammatory responses is one of the major drawbacks of pathology studies, as intra-observer variations are well-known. Readers should be aware that other researchers have not yet validated the present histological evaluation. However, the same pathologist who was blinded to the mesh type analyzed all pathology specimens. In addition, the constructed histological grading system allowed systematic evaluation of individual specimens to compare outcomes with both meshes.

CONCLUSIONS

Despite different structural features, both TVT-O® and I-STOP® similarly induced minimal inflammatory responses that were maintained for up to 12 months in the present animal model. The architectural features of polypropylene meshes had no impact on histological responses in this experiment.

CONFLICTS OF INTEREST

Drs. Przydacz, Adli, Mahfouz, Loutochin and Bégin have nothing to disclose. Dr. Corcos is a consultant for Astellas, Pfizer, Allergan, outside the submitted work.

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