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CLINICAL RESEARCH / KLİNİK ARAŞTIRMA

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The Effect of Tissue Adhesive Coated Onlay Meshes on Wound Healing in Rabbits

Doku Yapıştırıcı ile Kaplanan Meşlerin Onlay Yerleştirilen Tavşanlarda Yara İyileşmesi Üzerine Etkisi

Ulvi Mehmet Meral[1], Nazif Zeybek[2], Nail Ersöz[2], Yaşar Subutay Peker[2], Yusuf Peker[2], Ali Fuat Çiçek[2]

[1]Kayseri State Hospital, Clinic of General Surgery, Kayseri, Türkiye
[2]University of Health Sciences Türkiye, Gülhane Training and Research Hospital, Clinic of Radiology, Ankara, Türkiye

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Abstract

Objective: The aim of our study was to evaluate the histopathological effects of coating different types of meshes with tisseel® on wound healing in rabbits operated for incisional hernia (IH).

Methods: In this study, we used 35 New Zealand type rabbits. Midline defects were created in order to simulate IH and repaired with two different types of meshes [heavyweight (HW) vs. lightweight (LW)]. Each mesh group was further subdivided into two groups depending on the mesh fixation method (suture vs. tisseel®). The rabbits were re-operated on and full thickness samples were examined histopathologically 90 days postoperatively. American Society for Testing and Materials scales were used to score the inflammatory response to these meshes. Mean overall response (MOR) scores were calculated and statistically significant differences were examined.

Results: In the suture fixation group, histopathological examination of specimens revealed a significantly higher inflammatory response to HW meshes when compared to LW meshes. However, the inflammatory response and MOR values were not significantly higher when HW meshes were fixed with tisseel®. Surprisingly; LW meshes covered with tisseel® led to a significantly higher inflammatory response and MOR values when compared to tisseel® covered HW meshes, sutured LW meshes and sutured HW meshes.

Conclusion: In the routine surgical practice HW meshes create an elevated inflammatory response when fixed with suture materials. Tisseel® leads to a higher inflammatory response when used alone and when combined with LW meshes this response is even higher than HW meshes.

Keywords: Experimental, ventral hernia repair, polypropylene, tissue adhesive material

Öz

Amaç: Çalışmamızın amacı, tavşanlarda insizyonel herni tabanına serilen farklı yamaların doku yapıştırıcı ile kaplanması veya kaplanmamasının, yara iyileşmesi üzerine etkilerini histopatolojik olarak değerlendirmektir.

Yöntem: Çalışmada 35 adet Yeni Zelanda türü tavşan kullanılmıştır. Tavşanlarda orta hat defekti oluşturulmuş ve gruplara göre bu defektler onarılarak onarım alanının üzerine [ağır siklet (AS) ve hafif ağırlık (HA)] olmak üzere farklı iki meş konulmuştur. Her bir meşin bir grubu sütürle tespit edilip operasyon

Address for Correspondence/Yazışma Adresi: Ulvi Mehmet Meral MD, Kayseri State Hospital, Clinic of General Surgery, Kayseri, Türkiye
E-mail: ulvimeral@yahoo.com
ORCID ID: orcid.org/0000-0001-7399-6353

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Öz

sonlandırılırken diğer grubun üzeri doku yapıştırıcı tisseel® ile kaplanarak işlem sonlandırılmıştır. Ameliyatın 90 gün sonrasında tavşanlar tekrar ameliyata alınarak meş ve altındaki dokudan tam kat örnek alınmış histopatolojik incelemeye tabi tutulmuştur. Histopatolojik inceleme Amerikan Test ve Malzeme Derneği değerlendirme skalasına göre yapılarak her bir denek için bir ortalama doku cevabı (ODC) sonucu alınmıştır. Gruplar istatistiksel olarak anlamlılık açısından birbiri ile kıyaslanmıştır.

Bulgular: Sonuç olarak tisseel® ile kaplanmayan AS ve HA meşler kıyaslandığında; AS meşin dokudaki enflamatuvar yanıtının anlamlı bir şekilde fazla olduğu görülmüştür. Bunun yanında HA meş tisseel® ile kaplandığında enflamatuvar yanıt ve ODC değerinde anlamlı artış olmamıştır. HA meşin kaplamasız kullanıldığında AS meşe göre ODC cevabı düşükken, HA meş tisseel® kaplandığında kaplamasız HA meşe, kaplamasız AS meşe ve kaplamalı HW meşe göre ODC skorunun anlamlı bir şekilde arttığı sonucuna ulaşılmıştır.

Sonuç: Rutin cerrahi pratikte kullanılırken; AS meşler sütür materyalleri ile tespit edildiğinde ileri derecede enflamatuvar yanıt oluştururlar. Tisseel® ise gerek tek başına gerekse HA meş ile kombine kullanıldığında AS meşin yarattığı enflamasyondan da yüksek bir yanıta sebep olmaktadır.

Anahtar Kelimeler: Deneysel, ventral herni tamiri, polipropilen, doku yapıştırıcı

Introduction

Abdominal wall hernia is one of the most frequent health problems encountered by general surgeons[1]. Incisional hernia (IH), is defined as bulging of abdominal organs from a defect on the abdominal fascia as a late complication of previous abdominal surgical procedures[2]. The risk of IH ranges from 10-20% after midline laparotomy[3]. Recurrence rates differ following IH repair with levels of 30-50% reported after repair without prosthetic material and 0-15% with mesh repairs. Because of the high recurrence rate, IH is related to significant loss of labor, important morbidity and mortality[2].

Surgery is the only treatment for IH and involves primary repair, repairs performed with different types of materials or laparoscopic mesh repair. Mechanical failure, postoperative pain, mesh reaction, adhesion, seroma and erosion are the main reported complications from using prosthetic materials in IH repair. To reduce or avoid these complications; different types of meshes have been produced including meshes covered with various materials for the prevention of infection and excessive inflammation. These treatment options offer both advantages and disadvantages and there is currently no consensus about the ideal surgical choice[2,3]. Fibrin sealants are also alternative for fixation of meshes in hernia surgery. Tisseel® (fibrin sealant) is a two-component fibrin sealant made from pooled human plasma. When combined, the two components, Sealer Protein and Thrombin mimic the final stage of the blood coagulation cascade[4].

The purpose of our study is to evaluate the histopathological effects of tisseel® (Baxter Healthcare Corporation, Westlake Village, CA, USA) coating of lightweight (LW) [DynaMesh® pancreatic polypeptide Light 36 g/m^2 1.6x2.6 mm pore size FEG Textiltechnik mbH Aachen, Germany] and heavyweight (HW) (paha® Polypropylene Mesh, 115 g/m^2 0.75 mm. pore size. Altaylar Medical, Ankara, Türkiye) on wound healing in rabbits operated for IH.

Materials and Methods

The study was approved by the Ethics Committee of GATA Animal Experiments (date: 07/10/2011, no: 2011-10). Thirty-five (n=35) female New Zealand rabbits were utilized. Guidelines of Helsinki Declaration on the animal care and use were carefully implemented during the study.

Surgical Preparation

After one-night fasting, anesthesia was administered with ketamine (ketalar®, Parke Davis and Co. Inc., 40 mg/kg) ve xylazine (rompun®, Bayer Ag, Leverkusen, Germany; 5 mg/kg) by intramuscular injection. Before incision, cefazolin (50 mg/kg) was intramuscularly administered. The hair of the rabbits was removed with a surgical clipper (3M®, St. Paul, MN, USA) and povidone iodine solution was used for skin preparation (Figure 1).

Mesh Fixation

The rabbits were divided into five groups. In all groups; 3 cm. midline incision was made, including skin, soft tissue fascia and peritoneum. The defect was reapproximated with 2/0 polypropylene (PP) (prolene®; Ethicon, Somerville, NJ/USA) sutures, mimicking the wall closure in abdominal surgeries (Figure 2). Seven rabbits were signed as a control group and their skin defect was also sutured in the first surgical procedure. Four groups were mesh groups. Two types of meshes (DynaMesh® and paha®) 3x3 cm in size, were fixed on the sutured fascia defect with PP sutures in the first

two groups (Figure 3a-d). The same meshes were fixed to the fascia with tisseel® in the fourth and fifth groups (Figure 4a-d). Rabbits were assessed 12 hours postoperatively and started to eat normal feed.

Histopathological Evaluation

Ninety days after the first surgical procedure; rabbits were administered with the same preoperative management. The midline incision was repeated and the mesh region was dissected. For histologic assessment; a 1x1 cm sample was excised including mesh and fascial tissues. The samples were immersed in paraffin, 5 micron sections taken and stained with hematoxylin and eosin. Histopathological examination was performed with a light microscope (Nikon E 200 Tokyo/Japan) at 40x, 100x and 200x magnification by an experienced pathologist. The American Society for Testing and Materials (ASTM) scale was used for the histopathological assessment[5]. Inflammation score was calculated by the evaluation of polymorphonuclear leukocyte, lymphocytes, plasma cells, macrophages, giant cells and necrosis. For every sample, the inflammation subtotal was calculated by the sum of inflammatory cell scores multiplied by two (Table 1). Mean inflammation score (MI) was calculated by average of all inflammation subtotals. MI was calculated for each group. Mean overall response (MOR) was calculated by MI + fibrosis score (Table 2) + fatty infiltration score. Overall response was correlated with the extension of inflammation and fibrosis[6].

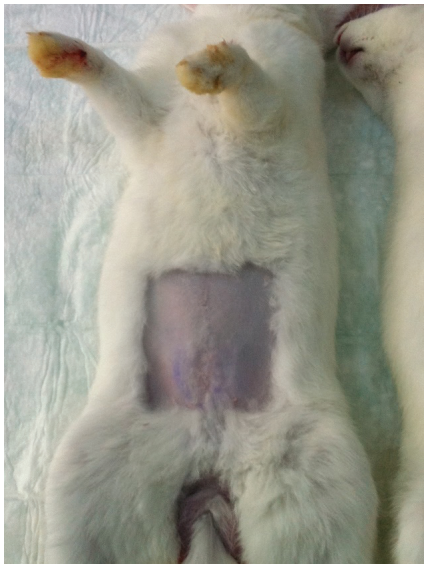

Figure 1. Preoperative preparation

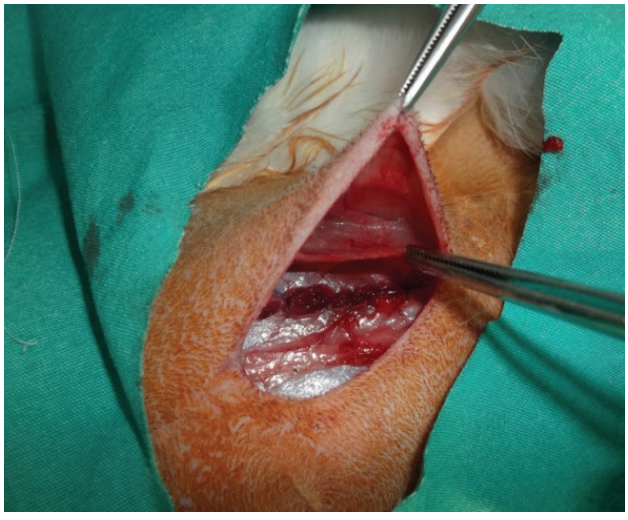

Figure 2. Reapproximating fascia defect

Statistical Analysis

DATA were analyzed with Statistical Package for Social Sciences software (SPSS version 15.0, SPSS Inc., Chicago, IL, USA) using Mann-Whitney U test and a p-value of less than 0.05 was considered significant.

Results

At 90 days; no mortality and morbidity was seen in all subjects. Samples were assessed for MI and MOR scores. The control

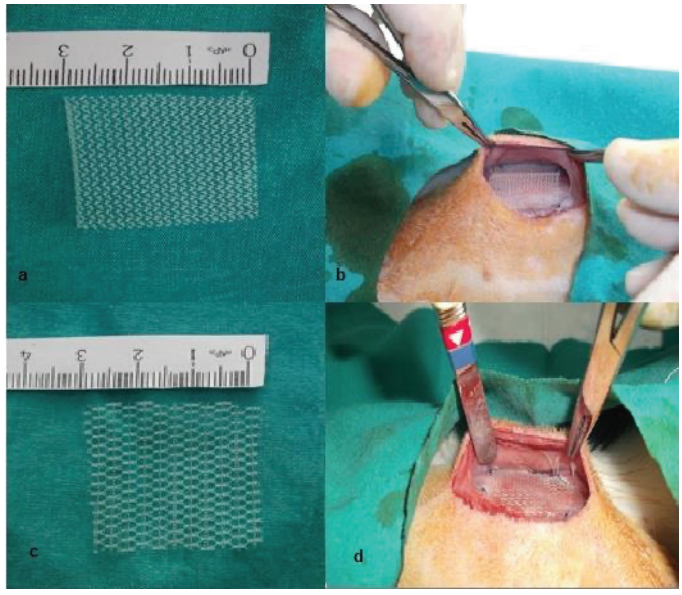

Figure 3. Macroscopic structure and application of meshes, **a)** Hight weight (HW) mesh **b)** Onlay fixation of HW mesh with pancreatic polypeptide suture **c)** Low weight (LW) mesh **d)** Onlay fixation of LW mesh with PP suture

group and the other groups were comparable. MOR was higher in the HW and suture group hereditary neuropathy with liability to pressure palsies (HNPP) (p=0.011), lower in LW and suture group lipoprotein metabolism profile (LMPP) and higher in both HW with tisseel® histone methyltransferases, (HMTS) and LW with tisseel® laterally moving tactile stimuli (LMTS) (p=0.01 and 0.026), compared with the control group (Table 3). The inflammatory response was significantly lower in the LMPP group compared to HNPP (Figure 5a). The MOR score did not differ in the heavy mesh groups (p=0.383) (Figure 5b). Although the LMPP groups overall response was even lower than the control group; it showed excessive reaction when coated with tisseel®, compared to LMPP and HMTS groups (Figure 5c, d). 100x magnified microscopic photographs of histopathological examples for ASTM scales are shown in Figure 6.

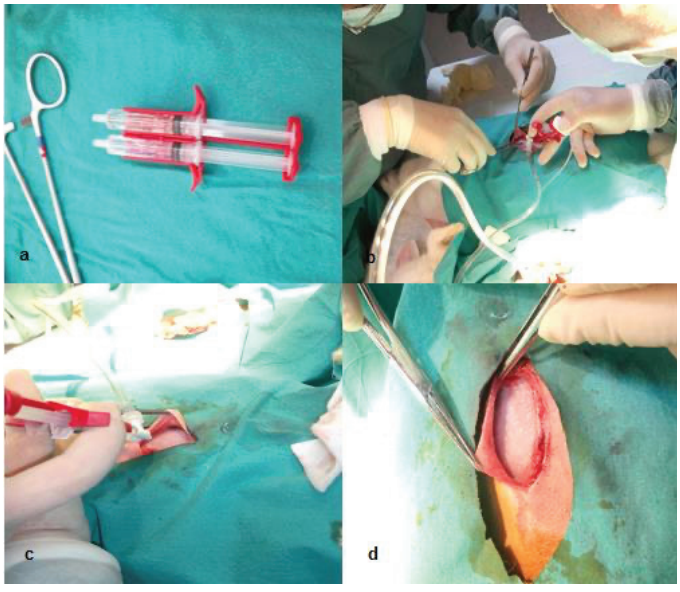

Figure 4. **a)** Two component tissue adhesive material tisseel®, **b,c)** Applying tisseel®, **d)** tisseel® coated mesh material

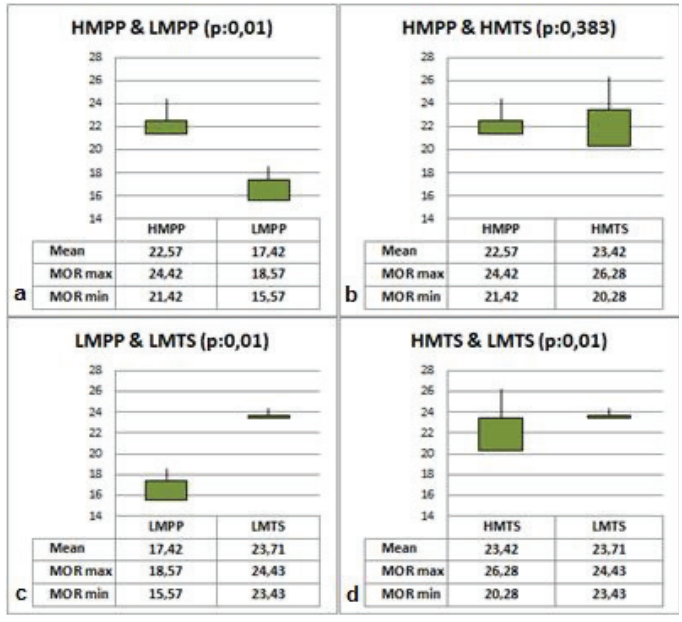

Figure 5. Comparison of groups according to mean overall response (MOR) results. **a)** Hereditary neuropathy with liability to pressure palsies (HNPP) vs. lipoprotein metabolism profile (LMPP), **b)** HNPP vs. histone methyltransferases (HMTS), **c)** LMPP vs. laterally moving tactile stimuli (LMTS), **d)** HMTS vs. LMTS

Table 1. ASTM inflammation scale

Inflammation	0	1	2	3	4
PMNL	0/hpf	1-5/hpf	6-10/hpf	>10/hpf	Abundant
Lymphocytes	0/hpf	1-5/hpf	6-10/hpf	>10/hpf	Abundant
Plasma cells	0/hpf	1-5/hpf	6-10/hpf	>10/hpf	Abundant
Macrophages	0/hpf	1-5/hpf	6-10/hpf	>10/hpf	Sheets
Giant cells	0/hpf	1-2/hpf	3-5/hpf	>5/hpf	Sheets
Necrosis	None	Limited	Minimal	Mild	Moderate

ASTM: American Society for Testing and Materials, PMNL: Polymorphonuclear leukocyte

Table 2. ASTM fibrosis scale

	0	1	2	3	4
Fibrosis	None	Minimal	Moderate	Extensive	X
Fatty infiltration	None	Limited	Minimal	Moderate	Extensive

ASTM: American Society for Testing and Materials

Discussion

Healing of surgical wounds includes an inflammation phase for the first 5 days, proliferation phase in 4-14 days, and a maturation phase at 11-16th days[7]. The maturation phase may extend in prosthetic hernia repairs. In recent years, low recurrence hernia repairs have been accomplished with the use of newly developed tension free meshes that provide high postoperative comfort. However, mesh-related complications have still been reported in one out of five patients[8,9].

The repair of the hernia defect with support materials started with mid-18th century, although the pancreatic polypeptide mesh was used for the first time by Usher in 1958[9]. In later years, different materials have been used for hernia repair. However, life-threatening complications including infection, migration and erosion of meshes, seroma, abscess, and fistula (entero-cutaneous, colo-cutaneous) have been reported in the short and long term, with materials applied using a tension-free technique[10-13]. There are an overwhelming number of different treatment options available including non-mesh repair for young people, wide-pore light mesh usage instead of conventional heavy meshes, coating the meshes with different materials like antibiotics, chitosan or silicone, usage of various methods of mesh fixation like progrip, tacker, suture or adhesive materials[14,15]. By covering the HW meshes with tisseel®, so reducing its direct contact with the tissue and using the tissue adhesive instead of excessive prosthetic material per unit area, we evaluated delaying the early inflammatory response and reducing complications. Weyhe et al.[16] reported in their study that; reduction of the amount of foreign materials used in ventral hernia repair were associated with postoperative comfort of the patients. In this study, we evaluated the complications and histopathologic/immunohistochemical evaluation of foreign body reactions in the tissue, when LW, large-pore mesh with lesser amounts of foreign body and HW, and small-pore mesh carrying larger amounts of foreign bodies were covered with tissue adhesive. Tissue evaluation was undertaken on the 90th postoperative day, when intensity of the immune response is complete. Junge et al.[17] analyzed the effects of the material weight, the filament structure, and the type of polymer on biocompatibility. As a result, they found that mesh biomaterial is of significant importance in causing foreign body reactions and also noted that all of these features contribute to the foreign body reaction, which is a main predictor of mesh biocompatibility, in our study, we have found that LW, large-pore mesh causes statistically (p=0.001) significantly less inflammatory tissue response

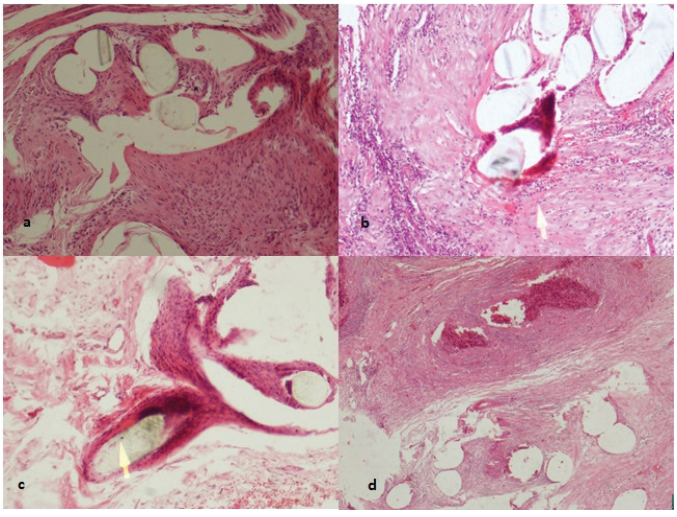

Figure 6. Examples for American Society for Testing and Materials (ASTM) scaling haematoxylin & eosin 100x, **a)** fibrosis, **b)** inflammation around the mesh fibres (arrow), **c)** giant cell around the mesh fibre (arrow) (ASTM score 4), **d)** necrosis (ASTM score 4)

Table 3. Comparison of control & mesh groups according to MOR values

Group	MOR/SD	p	n
Control	21.57/0.487	0.011	7
HMPP	22.57/0.899		7
Control	21.57/0.487	0.01	7
LMPP	17.42/1.214		7
Control	21.57/0.487	0.026	7
HMTS	23.42/1.864		7
Control	21.57/0.487	0.01	7
LMTS	23.71/0.487		7

MOR: Mean overall response, SD: Standard deviation, n: Samples, LMTS: Laterally moving tactile stimuli, HMTS: Histone methyltransferases

when compared to higher density prosthetic material including HW mesh. These results were similar to the study of Klosterhalfen et al.[18], which reported low-weight meshes cause a lesser inflammatory response, because low-weight meshes contain lesser prosthetic material per unit. There was no significant difference in terms of inflammatory response between HW mesh covered with tissue adhesive and HW mesh without tissue adhesive (p=0.383). This was histopathologically confirmed and the authors assume this response was associated with delayed or diminished immune response secondary to covering. Additionally, when LMTS and long-term postoperative pneumonia groups were compared, a significant increase in the inflamatory response in the covered group was detected (p=0.01). In the case of comparing tissue adhesive-covered heavy and light meshes, although uncovered LW meshes initiate a reduced tissue response, covered LW meshes cause higher rates of tissue response (p=0.01). The ideal mesh for hernia repair is still unclear but should be tissue compatible, not alter abdominal wall compliance, support the defect entirely, not cause tissue tightness, be affordable, easily applicable, available, and resistant. Research about prosthetic materials are based on these principles. Melman et al.[6], compared LW and HW, PP mesh and large pore polytetrafluoroethylene mesh in a porcine model of ventral IH repair. They evaluated inflammation and tissue fibrosis at 1, 3, and 5 months in tissue samples and found no significant differences. On the other hand, they determined that the tissue response was decreased in the following months. In a randomised controlled study by Ladurner et al.[19], patient defects were prepared with HW and LW meshes and no differences were found in terms of life quality between the two patient groups. Bellon et al.[20] categorized the meshes into three categories, with a weight 35 g/m² as LW, with a weight 35-80 g/m² as medium weight, and meshes with a weight >80 g/m² as HW. In a review of mesh biocompatibility written by Weyhe et al.[21], two studies conducted by Junge et al.[22] were evaluated and authors claimed that foreign body reactions were lesser in lighter meshes. On the other hand, in an experimental study by Weyhe et al.[16], they reported that there was a greater inflammatory response in subjects with LW meshes when compared to subjects with HW meshes. In our study, there was a lesser inflammatory response in subjects with large-pore meshes. Carboxymethylcellulose coated PP mesh and pure mesh was compared in terms of developing fibrosis and inflammation in a study by Yelimlieş et al.[23] , and the authors found coated mesh reduced adhesions but there was no significant differences in terms of inflammation and fibrosis. Junge et al.[22] studied PP meshes modified by titanium coating and non-coated tissue samples taken from animals. Formation of granulomas and immunohistochemically detected macrophage counts were compared and no significant advantages of titanium coated PP meshes were found in terms of biocompatibility. Lehle et al.[24] presented results in contrast to this study in their *in vitro* study however an *in vitro* evaluation might not represent the real tissue response. Scheidbach et al.[25] compared conventional two different PP meshes with a titanium coated very low molecular weight mesh (16 g/m²) in terms of inflammatory response and claimed that LW mesh showed a lesser response[25]. Although the response to the very LW mesh in this study is low and compatible with its weight, we found low levels of inflammation to the LW mesh and an increase in inflammatory response when the LW mesh was coated with tissue adhesive. This might be secondary to narrowing of mesh pores when coated with tisseel®.

Saygun et al.[26] compared gold, gold and palladium coated PP meshes and pure meshes in an infectious model of research, examining prevention of material infection. At first, the three different meshes used were washed with saline, following contamination with *S. epidermidis* washed with saline again and in the 3rd day implanted in the hernia areas of rats. After this procedure, culture samples were collected from the wounds at the 8th postoperative day and wound infection rates were 0% in the gold-palladium coated mesh implanted rats, 30% in the gold coated mesh group and 100% of infection rates in the PP used sample but further research is required to verify this theory. In another study; Çakmak et al.[11] used Chitosan, a polymer obtained by alkaline deacetylation of chitin to coat PP meshes in a model of hernia graft infection. There were no reported graft infections in the hernia repair with Chitosan coated meshes and no need for antibiotic prophylaxis. Sucullu et al.[27] conducted research into reusing the meshes after sterilizing them, and concluded that there were no significant differences between resterilized meshes and new meshes in tissue resilience, inflammatory response and development of an infection. Brandt et al.[28], reported lower rates of inflammatory response in pure polyvinyl difluoride (PVDF) meshes compared with hydrocortisone and spironolactone coated PVDF meshes in an experimental study. We observed higher rates of inflammatory response in the samples from tisseel coated LW meshes than in the samples from non-coated LW meshes. Lobato et al.[29] conducted a randomised prospective study in IH patients.

They found 20% postoperative complication rates including abscess, hematoma formation, and cellulitis in the group in which tisseel/tissucol was used with sutures for mesh fixation, and infection rates were 46.6% in the group without tisseel/tissucol. Mean hospitalization time was 7.1 days in the first group and 12.6 days in the second group. Eriksen et al.[30] compared tisseel/tissucol laparoscopic use with a titanium stapler for hernia repair. They reported significant differences in postoperative pain, time to returning to daily activity and hospitalization time in the tissue adhesive-used group. Stergios et al.[31] reported the results of their study on tisseel usage for colorectal anastomosis in diabetic rats. According to the study, tisseel was not only a positive factor for wound healing, but also a positive promoter for inflammatory response and fibroblast accumulation.

Research on tisseel® in the literature is commonly based on clinical symptoms and there have been no studies designed for gauging reactions against mesh. This topic is addressed in our study and according to the results, tisseel increases the inflammatory response, which is an important factor in wound healing around prosthetic material, but further studies are needed to determine appropriate coating of commercial meshes.

Study Limitations

Due to pre-study analyses, we used the smallest number of subjects possible statistically. For this reason, we believe that studies with more subjects or on humans for clinical use would be more meaningful. When the study was planned, MMP-2 antibody examination and sirrius red dye were planned for the pathological evaluation of wound healing. Due to problems during the supply phase, these materials could not be used and the evaluation was made with hematoxylene eosine dye and inflammation and fibrosis scoring. We believe that the pathological evaluation performed with the planned dye will yield more meaningful results.

Conclusion

The only treatment option for IH that develop as a complication after abdominal surgery is surgery. The most commonly used surgical treatment option is mesh repair. Many mesh materials have been developed that vary in terms of their weight, materials they contain, mesh structure and pore width. Although there are opposing views in the studies, it is argued that low-weight and large-pore meshes have less inflammatory response in the tissue and have a higher quality wound healing. In our study, we found that the LW large-pore mesh we used showed less inflammatory response than the HW small-pore mesh. We concluded that tisseel® coating is not an effective repair method in mesh IH repair because tisseel® which is biocompatible when used in other surgical areas, did not suppress/increase the inflammatory response of the meshes we used in our study. Experimental studies are needed with new parameters, in larger numbers of subjects and for longer periods, using different mesh materials.

Ethics

Ethics Committee Approval: The study was approved by the Ethics Committee of GATA Animal Experiments (date: 07/10/2011, no: 2011-10). This study was conducted in Gülhane Military Medical Academy Command.

Informed Consent: Since it is an experimental animal study, patient consent information is not required.

Footnotes

Authorship Contributions

Concept: N.Z., N.E., Y.P., Design: N.Z., N.E., Data Collection or Processing: U.M.M., Analysis or Interpretation: U.M.M., N.E., A.F.Ç., Y.P., Literature Search: U.M.M., N.Z., Y.S.P., Writing: U.M.M., Y.S.P.

Conflict of Interest: No conflict of interest was declared by the authors.

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