Folding, Shrinkage and Infection Relation at Polypropylene Mesh Placed on the Rat Abdominal Wall

Sıçan Karın Duvarına Uygulanan Polipropilen Yamada, Katlanma, Büzüşme ve Enfeksiyon İlişkisi

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ABSTRACT

Background: Although mesh implementation provides better results in terms of recurrence during abdominal wall hernia repair, mesh infection is an important issue that can cause prolonged hospitalization, recurrence, and increased costs. Sometimes is not possible to treat the infection without mesh extraction. Among the various commercially available meshes, polypropylene-based non-absorbable mesh most commonly used. Mesh composition, surface properties, and textile are the mesh-related factors that contribute to infection. Bacterial properties can also contribute to infection. Additionally, inappropriate surgical technique during mesh application can be another factor facilitating infection. The aim of this study is to investigate the relationship between folding, shrinkage, and infection in polypropylene mesh that is implemented on the rat abdominal wall.

Materials and Methods: Forty rats were divided into four groups of ten rats. Non-infected and infected 20 × 20-mm meshes were applied in groups 1 and 3, respectively, and after folding the non-infected and infected 40 × 20-mm meshes, were placed onto the abdominal wall in groups 2 and 4, respectively. After 16 days, all rats were sacrificed and bacterial colonization levels and mesh shrinkage rates were measured.

Results: Statistically significant mesh shrinkage was detected in both the infected groups. A strong causal relationship between surface area and bacterial colonization was detected.

Conclusion: Folding the mesh during hernia repair increased bacterial colonization. Infection leads to mesh shrinkage, which is a reason for recurrence. Unfolded mesh resulted in less bacterial colonization in this study.

Key Words: Hernia, Infection, Recurrence

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ÖZET

Amaç: Karın duvarı fıtıklarının onarımı sırasında yama kullanımı nüks açısından daha iyi sonuçlar sağlasa da yama enfeksiyonu uzamış hastane yatışı, nüks ve artmış maliyete yol açabilir. Bazı durumlarda enfeksiyonu tedavi edebilmek için yama çıkarılması zorunlu hale gelebilir. Polipropilen bazlı emilemeyen yamalar en yaygın kullanılan yamalardır. Yama materyali, yüzey özellikleri ve dokusu enfeksiyon gelişimine katkıda bulunan faktörlerdir. Ek olarak yama yerleştirilmesi sırasında uygunsuz cerrahi teknik kullanımı enfeksiyonu arttıran bir diğer faktör olabilir. Bu çalışmanın amacı sıçan karın ön duvarına uygulanan polipropilen yamada katlanma büzüşme ve enfeksiyon ilişkisini araştırmaktır.

Yöntem: Kırk sıçan her biri on adet olmak üzere dört ayrı gruba ayrıldı. Grup 1 ve 3' deki sıçanların karın ön duvarına 20x20 mm boyutlu enfekte ve enfekte olmayan yama, grup 2 ve 4 deki sıçanların karın ön duvarına 40x20 mm boyutlu yama katlandıktan sonra enfekte ve enfekte olmayan şekilde uygulandı. Onaltıncı günde sıçanlar sakrifiye edilerek her gruptaki bakteriyel kolonizasyon ve yama büzüşme oranları ölçüldü.

Bulgular: Her iki enfekte grupta istatistiksel olarak anlamlı şekilde yama büzüşmesi tespit edildi. Yüzey alanı ile bakteriyel kolonizasyon arasında güçlü nedensellik ilişkisi tespit edildi.

Sonuç: Fıtık onarımı sırasında yama katlanması bakteriyel kolonizasyonu arttırmaktadır. Enfeksiyon nüksün nedeni olan büzüşmeye yol açmaktadır. Bu çalışmada katlanmamış yama daha az bakteriyel kolonizasyon ile sonuçlanmıştır.

Anahtar Sözcükler: Fıtık, Enfeksiyon, Nüks

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INTRODUCTION

Mesh-based hernia repair became almost a standard treatment for all types of a hernia either by laparoscopic or open technique because this approach has a lower recurrence rate (1). However, mesh repair is associated with more surgical site infection compared with direct suture repair(2). Besides being a foreign material, composition, surface properties, and the type of graft material textile may be important factors for development and progression of graft infection. (3). Multifilament texture, large filament diameter, higher mesh weight, and small pore size and the presence of suture material increase bacterial adhesion and further facilitate the occurrence of an infection (4).

The aim of this study was to investigate the effects of polypropylene mesh surface area and mesh folding on infection, bacterial load, and mesh shrinkage.

MATERIALS and METHODS

This study was approved by the Hacettepe University Institutional Animal Experimentations Ethics Board (2015/94-01, 05.05.2016).

Forty female Sprague–Dawley rats, weighing 220–290 g, were fed standard laboratory chow and water used for this study. Rats were randomly assigned to one of four groups, as follows:

Group 1: Non-infected, 20 × 20 mm mesh implemented;
Group 2: Non-infected, 40 × 20 mm folded mesh implemented;
Group 3: Infected, 20 × 20 mesh implemented; and
Group 4: Infected, 40 × 20 mm folded mesh implemented.

Before the procedure, all rats were weighed twice using a digital balance (Sartorius AG®, Goettingen, Germany). Anesthesia was administered intraperitoneally using 5 mg/kg of xylazine (Alfazyne®, Woerden, Holland) and 30 mg/kg of ketamine hydrochloride (Ketalar®; Pfizer, Istanbul, Turkey). After surgery, 200 mg/kg of paracetamol was administered subcutaneously for analgesia.

A non-absorbable monofilament polypropylene mesh with a density of 70 g/m^2, filament thickness of 0.15 mm, mesh thickness of 0.56 mm, and pore size of 1.2−1.4 mm was used for the study.

The standard surgical technique for mesh placement was used in all rats. After local shaving and sterilization of the abdominal wall, a 3-cm incision was made and subcutaneous tissue was opened 2 cm laterally. Mesh was fixed via four 4.0 polypropylene sutures (Prolen®, Ethicon, Cincinnati, USA) (Figure 1a–b).

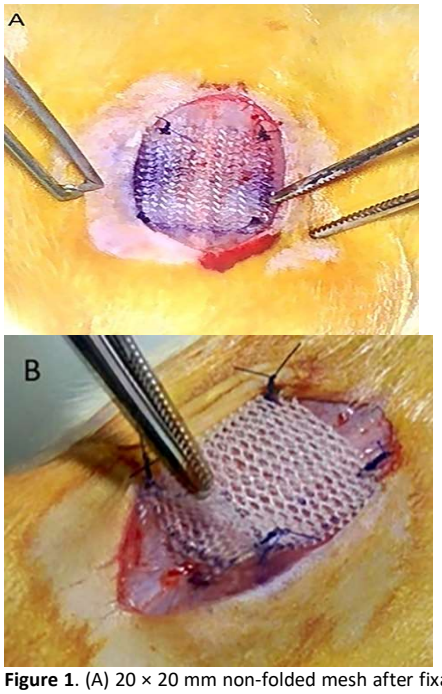

Figure 1. (A) 20 × 20 mm non-folded mesh after fixation (B) 40 × 20 mm folded mesh during fixation.

Before skin closure, 0.5 mL of saline for control groups was injected onto the mesh. To cause a mesh infection, *Staphylococcus aureus* (American Type Culture Collection® 25923 strain, Manassas, Virginia, USA)grown on blood agar was used. A suspension of *S. aureus* was prepared at a concentration of 1 × 10^9 CFU/mL and analyzed using a densitometer (Biosan® , Riga, Letonia). The solution was diluted to 3.0 McFarland density (1.1 × 10^9 CFU/ml). Then, 0.5 mL of the suspension was placed homogeneously onto the mesh surface using a micropipet just before the skin incision was closed (Figure 2).

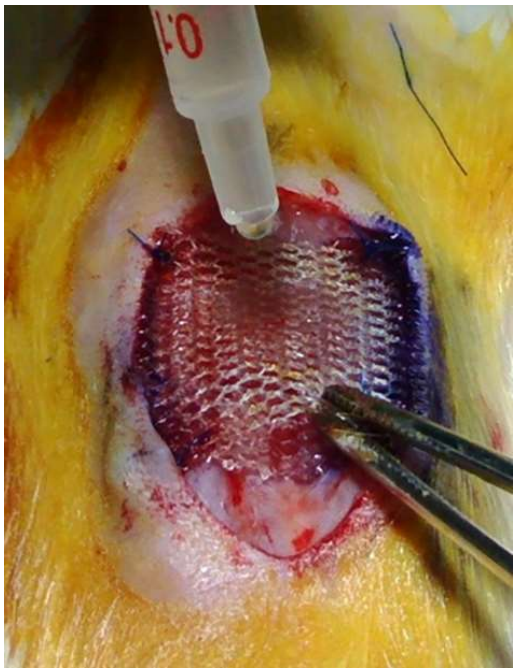

Figure 2. Bacterial seeding.

The skin incision was closed using continuous 3.0 polypropylene sutures (Prolen®, Ethicon, Cincinnati, USA). Rats were sacrificed using intra-cardiac high-dose ketamine hydrochloride (Ketalar®; Pfizer, Istanbul, Turkey) injection after 16 days. After sterilization of the abdominal area, the wound was opened and a standardized ruler that was 20 mm in diameter was placed onto the wound at the same level in each rat. Photographs were taken and the surface area was calculated twice using the Image J computer program designed by the National Institute of Health (Bethesda, Maryland,USA)(Figure 3).

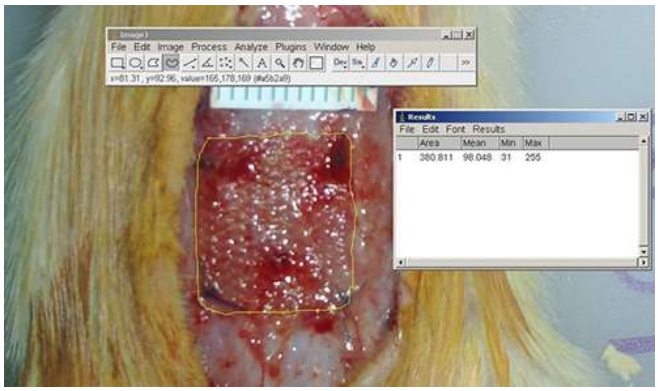

Figure 3. Loading of the photograph into the Image J program and calculating the surface area based on a standard reference measurement.

One measurement was performed by the study authors and the other was performed by an independent observer.

Each mesh was placed into a sterile tube containing 10 mL isotonic saline and sonicated five times for 30 seconds using a sonication device (Bandelin Sonopuls®, Berlin, Germany). Between sonication periods, the tubes were placed on ice and after sonication, 50-µL samples were taken and the quantitative seeding method was used to seed the bacteria onto blood agar. The plates were incubated overnight under normal atmospheric conditions. After incubation, colonies were counted and multiplied 20 times to count the bacteria per milliliter.

Statistical Analysis

All numeric data were entered into the Excel Office version 2007 (Microsoft, Redmond, Washigton ,USA)program and transferred to SPSS v. 22 (IBM Inc., Armonk, New York, USA) for statistical analysis. The Mann-Whitney U test was used to determine the differences among groups. The Kruskal-Wallis variance analysis was used for multiple comparisons between different groups. The Spearman correlation analysis was performed to determine the causality relationship between mesh surface area and bacterial colonization. A p-value < 0.05 was considered to be statistically significant.

RESULTS

All of the rats survived the 16-day follow-up period after mesh placement surgery. Three rats from group 4 had incisional dehiscence and during sacrification, an abscess formation was detected on the mesh (Figure 4).

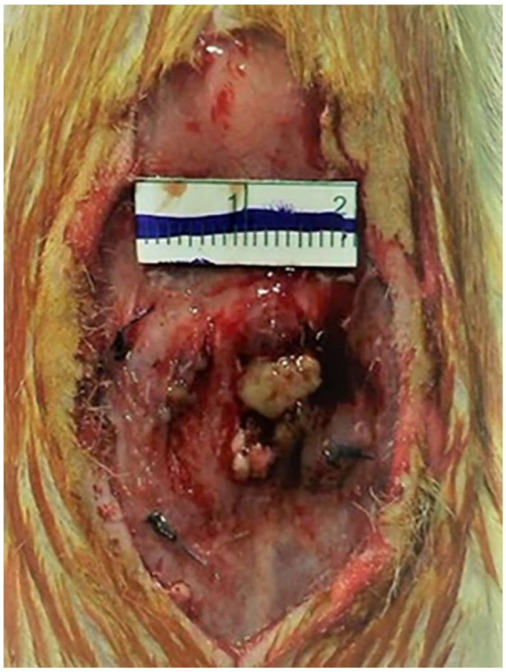

Figure 4. Incisional dehiscence and abscess formation.

There was a statistically significant difference between the surface area of the non-folded group and the infected control and between the folded group and its control (group 1 vs. 3 and group 2 vs. 4, p=0.001 and p=0.002, respectively). The shrinkage percentage for the infected groups was 5.29% and 4.74% for groups 3 and 4, respectively, which was statistically significant (p = 0.001; Table 1).

Table 1. Mean surface area calculation and shrinkage percentage at day 16

	16. day surface area (mm^2) ± SD	% Shrinkage
Group 1	**414.54 ± 14.92**	**NA**
Group 2	**416.34 ± 14.65**	**NA**
Group 3	**378.84 ± 14.27**	**5.29**
Group 4	**380.83 ± 5.1**	**4.74**

Bacterial colonization was not detected in groups 1 and 2. Group 4 had the highest level of colonization (Table 2).

Table 2. Colony measurements (Cfu/mm^2)

	16. day Colony measurement (Cfu/ mm^2)
Group 1	**0**
Group 2	**0**
Group 3	**4300.30 ± 7557**
Group 4	**61660.00 ± 49553**

When bacterial colonization levels were analyzed among groups 3 and 4, four rats (40%) from Group 4 and nine rats (90%) from Group 3 showed a similar level of bacterial colonization. However, six (60%) rats from Group 4 had significantly higher bacterial colonization and the frequency of the increased bacterial colonization was also higher in Group 4 compared to group 3. The Spearmen correlation analysis showed a strong causal relationship between surface area and bacterial colonization (Figure 5).

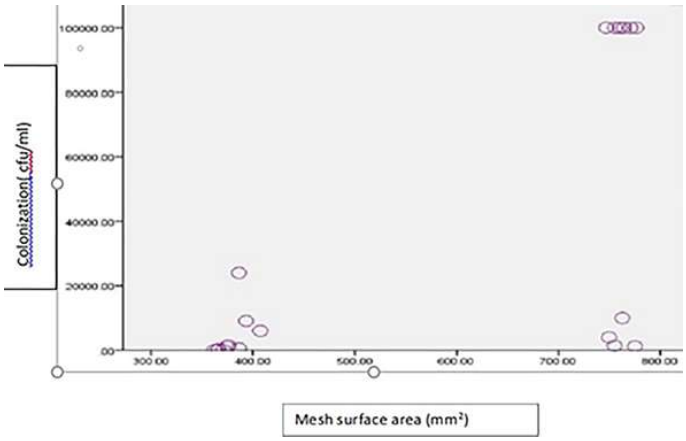

Figure 5. Bacterial colonization per mm^2.

DISCUSSION

Hernia repair is one of the most common surgical interventions throughout the world, and 20 million hernia repairs, mostly for inguinal hernias, are performed each year worldwide. The high recurrence rates for primary repairs showed that prosthetic materials are required for the treatment. A lower recurrence rate and the relatively technically simple mesh-based repairs make them almost standard for many types of hernia repairs. Along with abdominal wall hernia repairs, there are many other surgical procedures that require the use of mesh. Some pelvic prolapse and urinary incontinence procedures are performed using mesh. However, using prosthetic materials for any reason can increase infection-related problems.

Mesh, host, and bacterial properties are the main determinants of infection, and changing mesh properties is an option to decrease infection rates. Over 100 different types of mesh are available on the market and new types are being developed(5). Absorbable synthetic, non-absorbable synthetic, or organic materials can be used for mesh production. Polyglactin (PG) 910 or polyglycolic acid is mainly used to produce absorbable meshes. Polypropylene (PP, polytetrafluoroethylene (PTFE), expanded (ePTFE) polytetrafluoroethylene, and polyester (POL) are non-absorbable materials that are used for mesh production. Organic material-based meshes are the newest kind that are produced from human, porcine, or bovine collagen-rich tissues(6, 7). These meshes are especially useful if the field is contaminated. Although different coating materials are used to provide antibacterial activity and resistance to infection in experimental studies, these kinds of mesh have not yet gained acceptance.

According to Hamer-Hodges and Scott's description, the ideal mesh must be non-carcinogenic, chemically inert, durable against mechanical strains, resistant to body fluids, hypo-allergic, and cause limited foreign body reaction(6).

Unfortunately, no mesh meets all of these properties. Historically, different metals such as silver, tantalum, and stainless steel were used for mesh production, but after Usher and colleagues described and used Marlex mesh to replace tissue defects, polypropylene became the most commonly used material(10).Polypropylene is a hydrophobic polymer that is resistant to biodegradation and tissue enzymes and it also has a high tensile strength. A woven or knitted structure with different densities is available. Additionally, yarns can have a monofilament, dual filament, or multifilament nature(11). Meshes greater than 140 g/m^2 are classified as heavy, 70−140 g/m^2 are standard, and 35−70 g/m^2 are light-weight meshes. Mesh properties affect the host's reaction to the mesh and incorporation. The degree of inflammation differs depending on the pore size, textile structure, and density of the mesh, and this inflammation causes fibrosis and scar formation. As a result of scar formation, mesh shrinkage occurs and this shrinkage can cause pain, recurrence, or even erosion into other organs and fistulae formation(12).

Mesh infection is a serious complication of hernia repair and sometimes mesh removal is required(13).Bacterial contamination and adhesion are the initial steps in the development of an infection. Additionally, all bacterial adhesion mechanisms have not clarified whether microorganisms can affect mesh integration, which is a predisposing factor for recurrence. Microorganism contamination of the mesh or surgical site is the first step in the development of an infection and *S. aureus*, which was used for inoculation in our study, is the microorganism that is most frequently responsible for mesh infection(14). As mentioned above, mesh properties are crucial for development of an infection.

Comparison of experimental animal models in which mesh infection was created showed that a multifilament texture has a higher infection rate and more bacterial colonization than a monofilament texture (15, 16). Pore size is another important factor for mesh infections, and materials with large pore sizes have lower infection rates. Biomaterials that have pores less than 10 µm in diameter do not permit the passage of macrophages and neutrophilic granulocytes to eliminate bacteria, thus resulting in more infections(12, 17).

Mesh shrinkage occurs because of fibrous tissue contraction during wound healing (18). Healing of an infected wound can cause increased fibrosis formation and this formation can cause more mesh shrinkage. Another study that investigated mesh shrinkage during infection showed that there was more shrinkage in the infected polypropylene group, which is consistent with the results of our study(19).

We used standard density polypropylene mesh, which is the most frequent mesh that is used for hernia repairs, and *S. aureus,* which is the microorganism most frequently responsible for mesh infections, to simulate the most common mesh infection scenario. Our hypothesis was that increasing the surface area by folding the mesh can cause more infections and once infected, increased fibrosis during healing can cause more shrinkage. This study showed a strong positive correlation between colony density and increased surfaced area and there was also 5.29% and 4.74% shrinkage in the infected groups.

Main limitation of this study is short follow up time. As chronophysiology of healing process considered, remodeling phase can take a few months which can influence the results. Second limitation of this study is using only S.aureus for bacterial contamination. Although, S.aureus is the most often microorganism cause surgical site infections effect of other forms like coagulase negative staphylococci, enterococcus species, E.Coli and effect of polimicrobial infection on mesh shrinkage did not investigated in this study. Our mesh shrinkage rates were lower than some other published studies and this can be explained by the early termination and unimicrobial contamination simulated at our study (20, 21).

CONCLUSION

This study investigated the effects of mesh folding and surface area on the development of S*. aureus*-induced infection. Bacterial load and mesh shrinkage showed that folding the mesh increased bacterial colonization, shrinkage rates were significantly higher in infected groups compared to non-infected groups, and that there is a strong correlation between the surface area and bacterial colonization. During hernia repairs, using the smallest size of mesh that does not compromise the repair and avoiding mesh folding can decrease bacterial colonization on the mesh and help to avoid a possible mesh infection. The presence of infection can cause more shrinkage, which is a possible reason for recurrence.

Conflict of interest

No conflict of interest was declared by the authors.

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