International Surgery Journal
Yur M et al. Int Surg J. 2018Sep;5(9):2942-2947
http://www.ijsurgery.com
pISSN2349-3305 | eISSN2349-2902

DOI: http://dx.doi.org/10.18203/2349-2902.isj20183711

Original Research Article

The effect of polypropylene mesh on different layers to the formation of collagen type I/ III ratio and total collagen density in incisional hernia repair

Mesut Yur[1]*, Ziya Çetinkaya[2], Tuncay Kuloğlu[3], Dürrin Özlem Dabak[3], Erhan Aygen[2], Osman Doğru[4]

[1]Department of Surgical Oncology, Kanuni Training and Research Hospital, Trabzon, Turkey
[2]Department of General Surgery, [3]Department of Histology and Embryology, Fırat University, Elazığ, Turkey
[4]Department of General Surgery, Konya Training and Research Hospital, Konya, Turkey

Received:18 May 2018
Accepted: 27 June 2018

***Correspondence:**
Dr. Mesut Yur,
E-mail: mesutyur@hotmail.com

ABSTRACT

Background: In incisional hernia patients, collagen type I/III ratio and total collagen density (TCD) emerges a main etiological factor. If they are low, recurrence risk can be high. The aim of this study was to determine the ratio of collagen type I/III and TCD after incisional hernia repair in different layers with polypropylene mesh.
Methods: In the study, 40 Wistar Albino rats were used. They were divided into four equal groups: control, preperitoneal, intraperitoneal and onlay. In control group, defect was repaired with prolene suture. In other groups, prolene mesh was placed to the different layers. Incisions were reopened 30 days later, and tissue samples were taken. Samples were stained by double-immunohistochemical methods and examined under light microscope.
Results: Collagen type I/III ratio and TCD was highest in preperitoneal group. Intraperitoneal group was the second highest group. There was no difference between onlay and control group for collagen type I/III ratio except TCD. There was a significant correlation between TCD and collagen type I/III ratio in all samples.
Conclusions: Mesh repair in different layers has an effect on TCD and collagen type I/III ratio in incisional hernia repair. Preperitoneal layer is better than other layers.

Keywords: Collagen, Incisional hernia, Polypropylene, Wound healing

INTRODUCTION

A hernia that occurs in the anterior abdominal wall after incision is called incisional hernia. Incisional hernia is one of the common problems that occurs after surgical initiatives of the abdomen. Because of their morbidity in daily life; they cause economic burden, important labor loss and decrease life quality.[1,2] The rate of development of incisional hernia after laparotomy vary between range of 2-20% and 2-30% in America.[3,4]

The treatment of incisional hernia is surgery. Nowadays, many surgical techniques are used in order to eliminate this problem. These are; repairing with suture, prosthetic mesh, biological mesh and anatomical reconstruction. Repairing with suture is not preferred because of the high recurrence rates. Especially, repairing with prosthetic mesh is a preferred technique because of lower recurrence rate.[5] Recently, mesh is used by placing it on the fascia of anterior abdominal wall (onlay), on the peritoneum under the fascia (preperitoneal), under the

peritoneum (intraperitoneal), behind the rectus muscle (retrorectus) or between the free ends of two fascias (inlay).

Polypropylene mesh has been the most preferred mesh yet and has been used commonly. Because it is flexible, cheap, cuttable and easy to find and use. It doesn't produce biological tissue reaction. Fibroblasts fill the pores of mesh and produced collagen is integrated to the regional tissue[6]. Due to its permeability, it is not always necessary to remove the graft when it is infected.[7]

The most important integral part which effects tissue strength in wound healing is collagen. Type I collagen, with its high tensile strength, is predominantly found in skin, bone, and fascia, whereas type III collagen is predominantly seen in blood vessels and parenchymatous organs. In a healing wound or in repair, type III collagen is the first type of collagen to be laid down by fibroblasts. Initially, it consists of firstly type III collagen, a weaker form of the structural protein that can be produced rapidly. This is later replaced by the stronger, longstranded type I collagen, as evidenced in scar tissue.[8] In healthy skin, type I and III collagen exist in a ratio of approximately 4:1.[9,10] If the ratio of type I/III collagen decreases, the durability of healing tissue decreases. Authors can see this evidence in many studies.[11,12]

The aim of this study is to determine and compare collagen type I/III ratio and total collagen density in different abdominal wall layers placed prolene mesh. By this way, authors observe whether the type I/III collagen formation and total collagen density will change or not in different layers.

METHODS

This experimental study was carried out in Fırat University School of Medicine Experimental Medicine Research Laboratory (FUTDAM). The study was started when ethics committee approval (approval number:10523) was received. 40 Wistar Albino rats with body weights between 210-240 gm, taken from the same laboratory were used. Standard pellet feed and urban drinking water were used for the care of the animals. The rats are protected under constant temperature and humidity. They were divided into 4 groups and kept in cages consisting of five rats per a cage during the experiment.

The animals were fasted for 24 hours before surgery and only water drinking was allowed. Operations were carried out under sterile conditions in all groups. All of the instruments and patches were sterilized. The rats were anesthetized for a prolonged time with intraperitoneal 25 mg/kg Ketamine + intramuscular 10 mg/kg Xylasine Hydrochloride. After abdominal shaving of the rats, abdominal skins were sterilized by Povidone Iodine. The patches were prepared in standard sizes of 3 x 1.5 centimeters(cm).

When all of these preoperative preparations were finished, 4 cm skin incision was made starting from 1 cm below the midline of the sternum on the abdomen. After skin and subcutaneous tissue incision, linea alba and peritoneum were incised and a 3.5 cm defect was created in the abdomen (standard laparotomy-SL) and this defect was accepted as an incisional hernia.

Control Group (Group I): After SL, fascia of linea alba with peritoneum was closed with continuous suture (3/0 polypropylene) and skin incisions were closed (4/0 silk suture) primarily.

Preperitoneal Group (Group II): After SL, midline of peritoneum was closed solely with continuous suture (4/0 90% glycolide and 10% L-lactide suture) and flap between deeper rectus fascia and peritoneum was created for the prolene patch by the blunt dissection of preperitoneal space. After placing standardized prolene patch on preperitoneal surface, it was fixed to the anterior abdominal wall with 3/0 prolene suture and the fascia was closed continuous sutures (3/0 prolene suture). Skin incisions were closed primarily with 4/0 silk.

Intraperitoneal Group (Group III): After SL, prolene patch was placed into the abdominal cavity behind the peritoneum. Suspensory sutures were placed with 3/0 prolene suture to avoid bowel injury. Fascia of linea alba with peritoneum was closed with continuous suture (3/0 prolene) and suspensory sutures were attached. Skin was closed primarily with 4/0 silk.

Onlay Group (Group IV): After SL, linea alba and peritoneum was closed together with a continuous suture (3/0 polypropylene). Flap between superficial rectus fascia and subcutaneous tissue was created for the prolene patch. Standardized patches were placed onlay and fixed with 3/0 prolene sutures. Skin was closed primarily with 4/0 silk.

Rats were immediately were taken to their cages postoperatively and the cages were put under spot light in order to avoid hypothermia and related mortality. They were fed with standard pellet feed starting from postoperative first day. Urban drinking water was given to rats. No drug was given rather than feed and water. The rats were followed for 30 days and no complications observed as enterocutaneous fistulas, wound infection or others.

The rats were anesthetized for a prolonged time with intraperitoneal 25 mg/kg Ketamine + intramuscular 10 mg/kg Xylasine Hydrochloride for tissue sampling. Starting from the previous incision scar, same layers were incised with a new incision and reached the patches. Samples were taken from linea alba in control group, deeper fascia of rectus muscle in group II, peritoneal surface in group III and superficial rectus fascia in group IV. All subjects were sacrificed with extreme ether anesthesia after sampling.

In all groups, samples were 1x1 cm diameters and 1-millimeter thickness. These samples were fixed in 10% Formaldehyde solutions for 24 hours and washed under urban water for 24 hours after fixation. Routine histologic series and double immunohistochemistry series (Table-1) were applied (Sant-cruz;sc-25974® for collagen type 1 and Sigma-Aldrich;c7805® for collagen type 3). Prepared specimens were examined under an investigation microscope (Olympus BH-2) and photographed thereafter.

Table 1: Double immunohistochemistry procedure with applied times.

Operation	Time
Xylol I	10 min
Xylol II	10 min
Xylol	10 min
%100 Alcohol	10 min
%96 Alcohol	10 min
%80 Alcohol	10 min
Distilled Water	5 min
Microwave	7+5 min
Cooling in room temperature	20 min
PBS (Phosphate Buffered Saline)	3x5 min
H_2O_2	10 min
PBS	3x5 min
First normal block solution	5 min
Primary antibody (Type I Collagen)	60 min
PBS	3x5 min
Secondary antibody	30 min
PBS	3x5 min
Strepavidin HRP (Horse RadishPeroxidase)	20 min
PBS	3x5 min
DAB (3,3'-Diaminobenzidine)	5 min
PBS	3x5 min
Second normal bock solution	5 min
Primary antibody(Type III Collagen)	60 min
PBS	3x5 min
Secondary antibody	30 min
PBS	3x5 min
Streptavidin ALP(Alkaline Phosphatase)	20 min
PBS	3x5 min
Fast Red	5 min
Distilled Water	5 min
Mayer's Hematoxylin as an adverse stain	10 seconds
Running water	5 min
Covering	…

Measurements were semi-quantitative, and collagen density was measured by giving (+) values numbered from one to five according to the densities. Collagen Type I/III ratios were recorded in rate of their densities (density of type I/ density of type III).

- Collagen (+): Single-fiber collagen
- Collagen (++): Several fibers of collagen
- Collagen (+++): Denser but loose collagen
- Collagen (++++): A microscopic area filled with but scattered collagen
- Collagen (+++++): A microscopic area filled with collagen and in a form quite dense

Afterwards, parameters were tested by Mann Whitney u test. Significance was considered as $p<0.05$.

RESULTS

There were statistical differences between the groups when compared to each other. Total collagen density was higher in Preperitoneal group compared to other groups and there was a significant difference ($p<0,05$) (Table-2).

Table 2: The statistical evaluation of the collagen type I/III ratios and the total collagen density between groups.

Groups	Total collagen density	Collagen type I/III ratio
GroupI-II	0.022*	0.000*
GroupI-III	0.018*	0.000*
GroupI-IV	0.010*	0,615*
GroupII-III	0.000*	0.000*
GroupII-IV	0.000*	0.000*
GroupIII-IV	0.015*	0.000*

*: Mann Whitney U test, $p<0.05$ value statistically significant

Moreover, the collagen type I/III ratio was significantly higher in Preperitoneal group than the rest of the groups ($p<0.05$) (Figure-1).

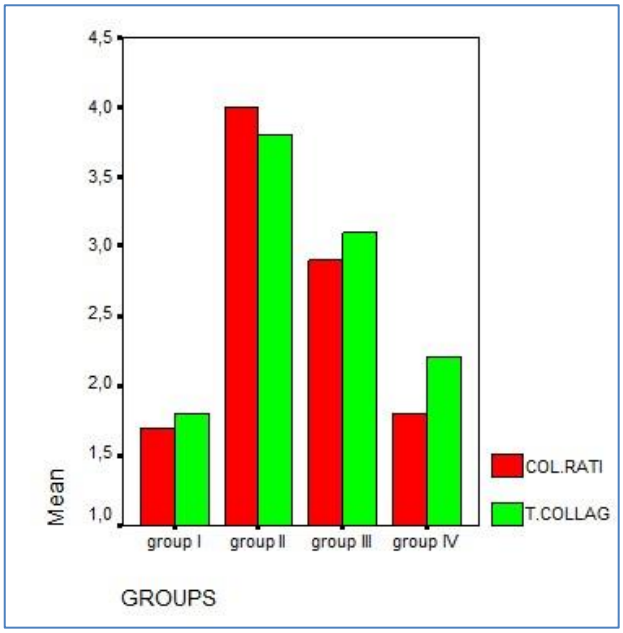
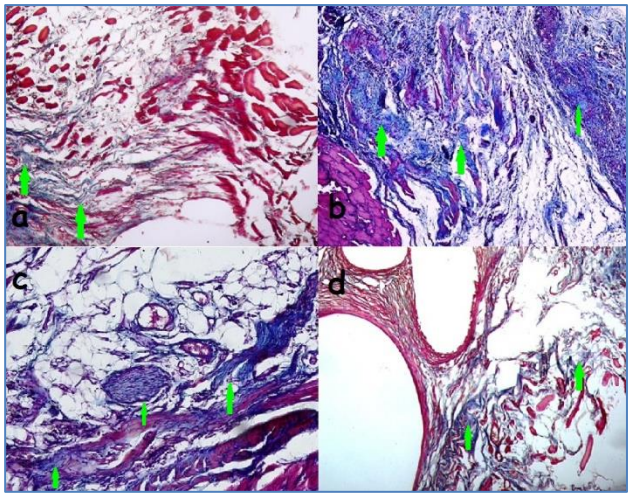


Figure 1: Distribution of collagen type I/III ratio and total collagen amount in all groups.

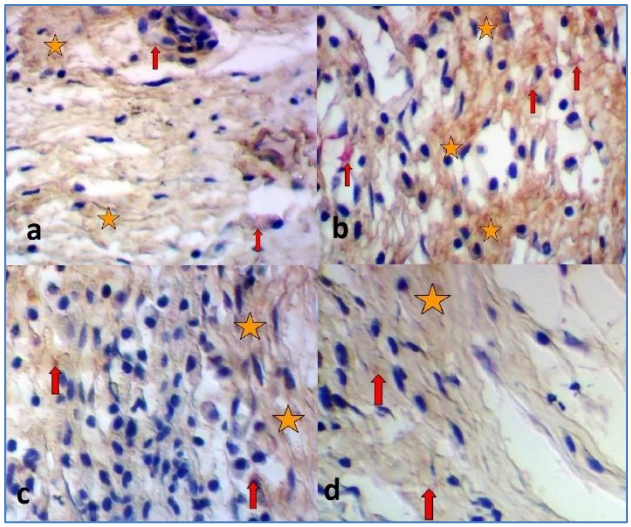
Beside these, in Intraperitoneal group, there was higher levels and significant difference for total collagen density and collagen type I/III ratio compared to Onlay and Control group ($p<0,05$). In Onlay group, there was significant difference for only total collagen density ($p<0,05$) and no difference for collagen type I/III ratios ($p>0,05$) compared to Control group (Figure2 and 3).



↑: shows the collagen tissue in blue.

Figure 2: Distribution of the total collagen amount in groups (magnification x200); a:control, b:preperitoneal, c:intraperitoneal, d:onlay groups.

Another important finding was correlation of collagen type I/III ratio and total collagen density. There was a significant correlation between them (r:+0,832). As total collagen density increased, the collagen type I/III ratio also increased.



↑: shows the collagen type I in pink and purple; *: shows the collagen type III in brown.

Figure 3: Distribution of collagen type I/III ratio in groups (magnification x400); a:control, b:preperitoneal, c:intraperitoneal, d:onlay groups.

DISCUSSION

Incisional hernia is an important problem after laparotomies. It can occur up to 30% of all laparotomies and can cause economic burden, important labor loss and low quality of life.[4] There are a lot of repair types for incisional hernia. Recurrence rate of primary repair is higher than other procedures due to suture tension on incision.[4-13] After Lichtenstein described tensionfree surgery on incisional hernia repair, the number of operations with prosthetic materials were increased rapidly.[14] Beside the studies on incisional hernia repair procedures, many studies performed for the causes and predisposition factors of incisional hernia. Total collagen density and ratio of collagen type I/III are conspicuous of them.

In Peeters' study, collagen type I/III ratio was decreased in incisional and inguinal hernia group.[11] Morover, Si et al presented decreased collagen type I/III ratio by procollagen mRNA in incisional and recurrent incisional hernias.[15] Junge et al presented decreased collagen type I/III ratio in recurring hernia group compared to pain or infection groups.[12] All these studies are about incisional hernias and authors think that decreased collagen type I/III ratio might be an etiological reason for incisional hernia.

In the early stages of wound healing, type III collagen is produced by fibroblasts actively.[16] Type I collagen becomes the principal collagen by week 2 of healing. Type I collagen is the main structural collagen and is found in fibrous supporting tissue, skin (dermis), tendons, fascias, ligaments and bone. It is responsible for mechanical tissue resistance.[17-18] Therefore, the more increase of type I/III collagen ratio, the more stability of tissue.

Baktır et al performed collagen type I/III ratio and total collagen density in an experimental study for incisional hernia. They used different types of meshes in retrorectus plane for incisional hernia repair. They found out the prolene mesh has more collagen density and type I/III ratio than other mesh types (mersilene, parietex, ePTFE).[19]

Biondo-Simões et all performed a similar study like Baktır et al. But in this study, they use only two different meshes in a single layer and they observe similar results for both polypropilene and polypropylene/poliglecaprone group. Type I and type III collagen densities were increased in both groups however there were no significant differences in polypropilene and polypropylene/poliglecaprone group.[20]

However collagen type I/III ratio and total collagen density were decreased in most incisional hernia patients, Meyer et al didn't report any differences for the average percent area of collagen (type I + type III) and the density of it.[21] This study contradict the other studies.The other important issue of incisional hernia repair is the layer for repair. Authors can place the mesh to the inlay, onlay, sublay and retrorectus spaces.[4] Holihan et al, in their meta-analysis, reported that sublay mesh location may result in fewer recurrences.[22]

Intraperitoneal space is not safe for placing prolen mesh. However it is not recommended, intraperitoneal mesh placement can be safe with prolen mesh reported by Brandi et al.[23] Neither acute nor chronic enterocutaneus fistulas were encountered during follow-up in any of the patients in their study. This study can encourage for intaperitoneal prolen mesh usage. In present study, there were no enterocutaneus fistulas in intraperitoneal group. However it looks like safe, authors observe collagen type I/III ratio and total collagen density lower than preperitoneal group.

On the basis of these information, authors performed the collagen type I/III ratio and total collagen density in different layers of abdominal wall. Authors use only prolen mesh due to its features. Polypropylene mesh is a hydrophobic macroporous mesh that allows for the ingrowth of native fibroblasts and incorporation into the surrounding fascia. It is semirigid, somewhat flexible, and porous.[4] Authors observed the highest level of collagen type I/III ratio and total collagen density in preperitoneal (sublay) group however intraperitoneal group is higher than only onlay and control groups.

Collagen type I/III ratio and total collagen density can be an etiological factor. Beside this, it is important to aim increased rates of collagen type I/III ratio and total collagen density in hernia repair. This study has pointed out that especially preperitoneal repair is more durable than the others and this can be the reason why preperitoneal repair has lower recurrences than the others. Beside these, authors found a high positive correlation between total collagen density and collagen type I/III ratio in this study. There is no study about this but authors think that it is a result of normal wound healing.

The limitation for this study is the differences of tissue sampling locations. Authors couldn't take tissue samples from the same locations. Because authors placed the mesh different layers of abdominal wall and sampling from same location would be pointless.

CONCLUSION

In conclusion, the result of present study suggest that the use of prolene mesh in preperitoneal layer for incisional hernia repair might be a safe and durable procedure compared with the other layers. Authors think that its reason is increased collagen type I/III ratio and total collagen density.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee (approval number: 10523)

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Cite this article as: Yur M, Çetinkaya Z, Kuloğlu T, Dabak DO, Aygen E, Doğru O. The effect of polypropylene mesh on different layers to the formation of collagen type I/ III ratio and total collagen density in incisional hernia repair. Int Surg J 2018;5:2942-7.