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Effectiveness of polypropylene mesh coated bovine amniotic membrane with adhesion barrier (polyethylene glycol) in repair of abdominal wall hernias in rats

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ABSTRACT

This study was undertaken to investigate the effectiveness of polypropylene (PP) mesh coated bovine amniotic membrane (BAM) with 5% polyethylene glycol (PEG) 4000 as adhesion barrier in the repair of experimental 2 × 2 cm of abdominal hernias in rats. Rats (32) were divided into 4 groups. A 2 cm × 2 cm defect was created in the full thickness of abdominal muscle on the anterior abdominal wall. PP mesh was implanted in the abdominal cavity (Gr 1 to 4). BAM covered the abdominal face of the graft (Gr 3 and 4). It was given before the abdominal closure 5 ml of 5% PEG 4000 (Gr 2 and 4) and 5 ml of 0.9% NaCl (Gr 1 and 3). After 21 days following the operations, 32 rats were euthanized. Macroscopic and microscopic evaluations were performed according to the scoring systems. The differences between the groups was evaluated by Mann-Whitney U test and Kruskal Wallis analysis of variance. Highest adhesion percentage was observed in Gr 1 and lowest in Gr 4. Inflammatory reaction was observed in Gr 1 and 2. According to the results of this study, the combined use of BAM and 5% PEG 4000 was helpful to prevent the complications of PP mesh.

Key words: Adhesion, Amniotic membrane, Bovine, Hernia, Polyethylene glycol, Polypropylene mesh.

For hernia repair, PP (polypropylene) mesh is still highly preferred material as it is non-absorbing, braided monofilament mesh material produced from polypropylene. It is easily available all over the world and frequently used due to its low price (Aydin *et al.* 2017, Qiao *et al.* 2017, Ulrich *et al.* 2017, Yang 2017). In addition to trauma caused by surgical intervention, synthetic materials used in repairing abdominal wall defects due to trauma or large hernias increase the degree of adhesiveness by causing foreign body reactions (D'Amore *et al.* 2017, Qiao *et al.* 2017, Yucel *et al*. 2017). Various meshes having anti adhesive features have been produced (Wen *et al.* 2017). However, these meshes are highly expensive, the low cost PP mesh is preferred in practice (Muzio *et al.* 2017, Qiao *et al.* 2017, Ulrich *et al.* 2017, Yang 2017, Wen *et al.* 2017). Studies were conducted to prevent the PP mesh from direct contact with the abdominal organs and are still in progress (Aydin *et al.* 2017, Yucel *et al.* 2017, Gil *et al.* 2017*)*. Amniotic membrane has been used in numerous surgical conditions, either as a surface covering to encourage epithelization, prevention of hypertrophic scar formation or to prevent adhesion in the abdominal cavity (Haugh *et al.* 2017, Mohammadi *et al.* 2017, Sabater and Perez 2017). Polyethylene glycol (PEG) having both the antibacterial and anti-adhesion properties is used to prevent postoperative intra-abdominal adhesions (Buxadera-Palmero *et al.* 2017, Lin *et al.* 2017, Peng *et al.* 2017).

The aim of this study was to investigate the effectiveness of PP mesh coated BAM with 5% PEG 4000 as adhesion barrier in the repair of experimental 2 × 2 cm abdominal hernias in rats.

MATERIALS AND METHODS

The present experimental study was approved by Firat University, Chair of The Local Ethics Committee on Animal Experiments (Verification number: 2016/123/71).

The placenta of bovine obtained from the local cattle slaughterhouse was washed with sterile saline for clearance of blood clots and tissue residues. Amniotic membrane was separated from chorion by blunt dissection. Amniotic membrane patches (16) measuring 2.5 × 2.5 cm were prepared and immensed in sterile saline including penicillin 1,000,000 IU and 1 g streptomycin/liter for 24 h at 4°C (Ozcelik and Yavuz 2006).

Wistar albino rats (32; adult, female, average 250 g) were divided into 4 groups. General anesthesia was performed via ketamine hydrochloride (Ketalar, Parke-Davis) @ 80 mg/kg IM. After general anesthesia, the abdominal region was prepared for operation, the rats were identified on the operation table in the supine position and the region was disinfected and preapered for operation with sterile coverings. Following the median incision (4 cm), defect with 2 cm × 2 cm was created in the full thickness of abdominal muscle on the anterior abdominal wall at a

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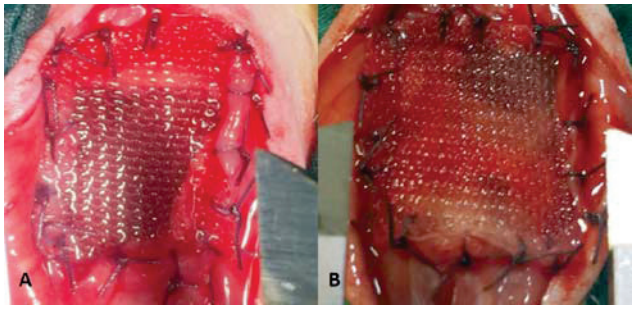

Fig. 1(A-B). **A**. Appearance of implanted PP mesh in groups 1, 2. **B**. Appearance of implanted PP mesh covered with BAM in groups 3, 4.

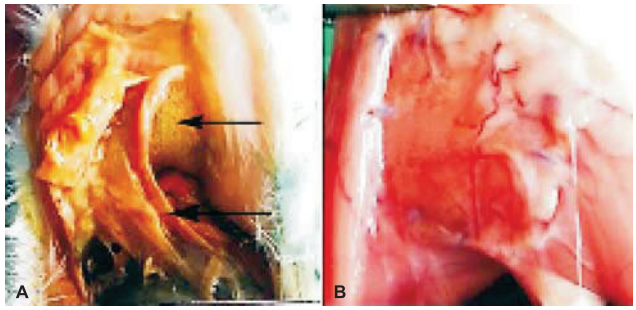

Fig. 2(A-B). Appearance of non-dissectible adhesions (arrows, Grade 4) in Gr 1 (**A**) and no adhesion formation (Grade 0) in Gr 4 (**B**).

distance of 1 cm from the xiphoid process.

PP mesh (2.5 × 2.5 cm, Bard mesh, Davol Inc., USA) was implanted in the abdominal cavity with 0/2 vicryl as inlay simple interrupted sutures (Gr 1 to 4). BAM covered the abdominal face of the mesh (Gr 3, 4). It was given before the abdominal closure 5 ml of 5% PEG 4000 (Gr 2, 4) and 5 ml of 0.9% NaCl (Gr 1, 3). The skin was routinely closed with simple interrupted sutures. Gr 1, PP mesh and 5 ml I.P. 0.9% NaCl; Gr 2, PP mesh and 5 ml I.P. 5% PEG 4000 as adhesion barrier (Fig. 1A); Gr 3, PP mesh covered with BAM and 5 ml I.P. 0.9% NaCl; Gr 4, PP mesh covered with BAM and 5 ml I.P. 5% PEG 4000 as adhesion barrier (Fig. 1B).

Antibiotics including penicillin (30,000 U/kg 1× 1) and flunixin meglumin (2.5 mg/kg 2× 1) (Fundamin, Baver) were administered intramuscularly for 5 days postoperatively in all rats. Water and feed restrictions were not made. After 21 days following the operations, 32 rats were euthanized by carbon dioxide inhalation. The abdominal wall was opened in the form of a "U" and the condition of the grafts was examined macroscopically.

Adhesion formation was evaluated macroscopically and microscopically. Macroscopic evaluation was performed according to the scoring system; grade 0, no adhesion; grade 1, blunt dissectible, easily separable filmy adhesions; grade 2, freely dissectible mild to moderate adhesions; grade 3, difficult dissectible moderate to dense adhesions; grade 4, non-dissectible adhesions (Majuzi *et al*. 1964). Grafts were excised along with abdominal wall and fixed in 10% formalin solution. Five micrometer sections in thickness from the tissues embedded into paraffin were prepared. Sections were stained with hematoxylin-eosin (H&E) and were evaluated under light microscope (Olympus BX43, DP72) with respect to fibrosis (grade 0, no fibrosis; grade 1, minimal, loose fibrosis; grade 2, moderate fibrosis; grade 3, florid, massive fibrosis) and inflammation (grade 0, no inflammation; grade 1, large cells, rare, dispersed lymphocytes and plasma cells; grade 2, large cells together with increased number of lymphocytes, neutrophils, eosinophils and plasma cells; grade 3, multiple mixed inflammatory cells and presence of micro-abscess) (Hooker *et al*. 1999).

SPPS 22 program was used for statistical analysis. The differences between the groups was evaluated by Mann-Whitney U test and Kruskal Wallis analysis of variance.

RESULTS AND DISCUSSION

Macroscopic examination: Highest adhesion percentage was observed in Gr 1, and the lowest in Gr 4 (Fig. 2A,B). There was no abscess formation between the PP mesh and visceral organs in any group. Inflammatory reaction containing lymphocytes and macrophages was present in Gr 1 (2 cases, Fig. 2A) and Gr 2 (1 case). Subcutaneous inflammatory exudate was present in Gr 1 (3 cases) and Gr 3 (1 case). There were adhesions between the intestines and the mesh in Gr 1 (2 cases), Gr 2 (1 case), Gr 3 (2 cases) and Gr 4 (3 cases). There were adhesions between the stomach and the mesh in Gr 1 (1 case). Other adhesions were formed between the omentum and the mesh in all groups. Suture dehiscence, small bowel obstruction, shrinkage and dislocation of PP mesh were not observed in any group. Comparison of the groups in terms of macroscopic adhesion severity grade are presented in Table 1.

Microscopic findings: In histopathological examinations, fibrous adhesions and giant cell infiltration due to foreign body reaction were observed in majority of cases of Gr 1 (Fig. 3A). Common inflammatory cell infiltration and fibrosis was observed in cases of Gr 2 (Fig. 3B). Medium inflammatory cell infiltration and fibrosis was observed in cases of Gr 3 (Fig. 3C). Large number of small blood vessels, a small number of inflammatory cells infiltration, and fibrosis was observed in cases of Gr 4 (Fig. 3D).

Comparison of the groups with regard to fibrosis and inflammation according to the scoring system is presented in Tables 2,3.

Table 1. Comparison of the groups in terms of macroscopic adhesion severity grade.

Group	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4	(n)
Group 1	–	1(12.5%)	1(12.5%)	2 (25%)	4 (50%)	8
Group 2	1 (12.5%)	1 (12.5%)	3 (37.5%)	2 (25%)	1(12.5%)	8
Group 3	2 (25%)	2 (25%)	3 (37.5%)	1 (12.5%)	–	8
Group 4	4 (50%)	3 (37.5%)	1 (12.5%)	–	–	8

Figures in parenthesis indicate percentage

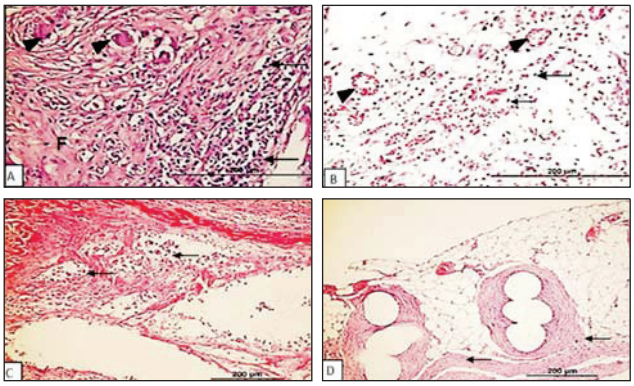

Fig. 3(A-D). **A**. Fibrous adhesions (arrows) and foreign body gian cell (arrow heads) and fibrosis (F) in Gr 1. **B**. Normal lymphohistiocytic infiltration (arrows) and vascularisations (arrow head) in Gr 2. **C**. Medium inflammatory cell infiltration (arrows) and fibrosis in Gr 3. **D**. A large number of small blood vessels in Gr 4, a very small number of inflammatory cell infiltration and fibrosis (arrows) (H&E, 200×).

Table 2. Comparison of the groups with regard to fibrosis

Group	Grade 0	Grade 1	Grade 2	Grade 3	(n)
Group 1	–	2 (25%)	2 (25%)	4 (50%)	8
Group 2	1 (12.5%)	1 (12.5%)	4 (50%)	2 (25%)	8
Group 3	2 (25%)	2 (25%)	3 (37.5%)	1 (12.5%)	8
Group 4	3 (37.5%)	3 (37.5%)	2 (25%)	–	8

Figures in parenthesis indicate percentage

Table 3. Comparison of the groups with regard to inflammatory changes

Group	Grade 0	Grade 1	Grade 2	Grade 3	(n)
Group 1	–	2 (25%)	2 (37.5%)	4 (37.5%)	8
Group 2	1 (12.5%)	1 (12.5%)	4 (50%)	2 (25%)	8
Group 3	2 (25%)	2 (25%)	3 (37.5%)	1 (12.5%)	8
Group 4	3 (37.5%)	3 (37.5%)	2 (25%)	–	8

Figures in parenthesis indicate percentage

Comparison of the groups with regard to macroscopic adhesion severity grade, fibrosis and inflammation revealed that Gr 4 was significantly different from Gr 1 (control group) ($P<0.05$). Gr 2 was not significantly different from Gr 3 ($P>0.05$); Gr 2 and 3 were not significantly different from Gr 1 (control group) ($P>0.05$).

Intra-abdominal adhesions can cause small bowel obstruction, female infertility, chronic abdominal and pelvic pain. The formation of adhesions additionally causes secondary problems like prolongation and risking future intra-abdominal operation (Makarchian *et al.* 2017, Perko *et al*. 2011). Ischemia and foreign body enhance the improvement of adhesions. The most vital factors to reduce adhesions are introduction of minimum surgical trauma, reducing trauma to the peritoneum, minimizing preliminary damage, medical interventions within the fibrin formation/degradation balance, avoiding coagulation of exudate, reducing contact times of tissues and organs with each other through barriers, stopping or slowing fibroblast proliferation (Perko *et al*. 2011, Leblebici *et al.* 2014, Karabulut and Canpolat 2016, Kocaay *et al*. 2015). Fibrinolytic agents used to prevent adhesions during surgical interventions should not be preferred as they may increase bleeding. It has been advocated that solid barriers should be preferred because liquid barriers cannot be effective as they are absorbed within 1–2 days (Ergin and Demirel 2017).

In general, adhesions rise up from any tissue damaged in the first week after injury, and consist of omental fat and formed mainly at the edges of the mesh and at the fixating sutures (Schreinemacher *et al*. 2013, Barbuto *et al*. 2015). Prosthetic mesh edge exposure is a main source of adhesions, specifically when the mesh edge is adjacent to the peritoneal cavity (Butler and Prieto 2004). It was observed that Grade 1 and Grade 2 adhesions formed at the edges of the mesh and at the fixation sutures in this study. Suture material additionally performs an important role in infection, and for this cause monofilament materials have been widely recommended because they have fewer tendencies to harbour microorganisms (Vilar *et al*. 2009). In this study, absorbable, with antibacterial protection, braided Vicryl was used. Vicryl have tension durable for 2–3 weeks and absorbed in 55–70 days. Mesh shrinkage and dislocation were not observed in this study.

It was reported that running suture technique, which provides more balanced tension around the mesh until its integration into adjacent healthy tissue, to be more useful for decreasing of mesh contraction (Sekmen *et al*. 2009). The continuous suture pattern was used within the inlay technique, in which the breakdown of one stitch results in the dehiscence of the whole suture line. It was observed that interrupted sutures used for fixation of the implant in the interlay method provided multiple factors of no tension fixation which helped divide stress evenly over the mesh and reduced mesh folding and bulging (Abouelnasr *et al*. 2014). In this study we used simple interrupted suture to reduce dehiscence of the whole suture line.

Absorbable meshes used for hernia repair only provide a temporary solution. Therefore, a mesh used for hernia repair should be non-absorbable (Xu *et al*. 2014). Hence non-absorbable PP mesh was used in this study. It was reported that complications related to double application of mesh because of technical difficulties or accelerated mesh rejection and infection (Vilar *et al*. 2009). Therefore, one layer of PP mesh was used in this study to reduce infection and rejection of mesh. Dislocation of PP mesh was not observed and inflammation was detected in only 3 cases.

For surgical repair of abdominal hernias, usage of prosthesis, appropriate surgical repairing approach, strength of the material, tissue compatibility, case of suturing, protection method of material and cost are important factors for attention to select a material which has less probability of being rejected, less tissue reactions and no damaging results in other organs (Abass 2008, FitzFerald and Kumar 2014). PP mesh is widely used in medical practice, because it has a highly affordable value, approximates the standards of an ideal material, has less tissue response, sterilized and

easy to handle (Ricciardi *et al*. 2012, Gil *et al.* 2017). A large peritoneal disorder with direct contact between the mesh and intra-abdominal organs might result in adhesion formation, mechanical bowel obstruction and fistula. To prevent this negative situation, underlay/sublay placement may be preferred (Karrouf *et al.* 2016). The foreign body reaction to PP mesh is much less pronounced than that to many different mesh materials (Malazgirt *et al*. 2000). PP mesh covered by fibrous tissue has caused less intraperitoneal adhesions and postoperative complications (Ricciardi *et al*. 2012). PP mesh is to be in direct touch with intra-abdominal contents, application of the bioresorbable membrane over the viscera may also reduce the severity of adhesion formation and subsequent complications (Baptista *et al*. 2000). To prevent adhesions, the parietal peritoneum must be preserved during incisional hernia repair because it forms a barrier. When the parietal peritoneum cannot be saved intact, the surgeon may also attempt to place the greater omentum between the abdominal contents and the prosthetic material (Aydinli *et al*. 2007). In situations where the PP mesh can not be placed sublay/underlay, although it is a suitable material, direct contact of the PP mesh with the intra abdominal organs must be prevented. In this study, direct contact of PP mesh with abdominal organs has caused intensive adhesion formation and subcutaneous seroma in Gr 1.

In various studies, to prevent direct contact with abdominal organs of PP mesh; the part of the mesh that looks inside the abdomen has been covered with human amniotic membrane, stretch film, thymoquinone, omega-3 fatty acid, α-tocopherol, antiadherential film, polyethylene glycol hydrogel and seprafilm (Szabo *et al*. 2000, Bozkurt *et al.* 2007, Petter-Puchner *et al*. 2011, Yasar *et al*. 2012, Quinino *et al.* 2013, Aydin *et al.* 2017, Gil *et al.* 2017, Muzio *et al.* 2017, Yucel *et al.* 2017). In another two studies, PEG has been sprayed underneath PP mesh (Altinli *et al*. 2011) and PP mesh seeded with fibroblasts (Mohsina *et al.* 2017). In this study, amniotic membrane was used to reduce adhesion formation and postoperative complications of PP mesh. However, the use of BAM alone (Gr 3) was not enough to prevent complications of the PP mesh ($P>0.05$). Gr 4 (PP mesh covered with BAM and 5% PEG 4000 as adhesion barrier) was significantly different when compared to Gr 1 (control group). In terms of preventing complications of PP mesh, the combined use of 5% PEG 4000 and BAM was observed to be better than all other groups according to macroscopic and microscopic evaluations.

It has been reported that PEG provides good results in prevention of intra-abdominal adhesions (Altinli *et al*. 2011, Karabulut and Canpolat 2016). In present study, 5% PEG 4000 was used in Gr 2, which used PP mesh only, and Gr 4, which used PP mesh covered with BAM. Especially in Gr 4, good results were obtained in terms of prevention of adhesions ($P<0.05$). It is concluded that PEG was more effective in Gr 4 than Gr 2, because BAM allows PEG to stay in the abdominal cavity longer and prevents the PP mesh from directly contacting the internal organs.

The conclusion is that though PP mesh is widely used in hernia repair because it is low cost and easy to find, it causes many complications such as postoperative adhesions inflammation, seroma and abscess. Various drug and adhesion barriers have been used to prevent these complications. But the desired result has not been achieved. In this study, the combined use of BAM and 5% PEG 4000 was helpful to prevent the complications of PP mesh.

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