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Comparison of Biograft (Bovine Small Intestinal Submucosa) and Synthetic Graft (Polypropylene Mesh) Healing Effects when Repairing Abdominal Muscle Defects in Rats

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ABSTRACT

Background: Abdominal wall injuries are commonly encountered in veterinary medicine and may lead to significant complications if not appropriately treated. Different types of grafts, including autologous, xenogeneic, biologic or non-biologic and composite grafts, are widely used to ensure adequate tissue repair and prevent recurrence in cases of hernia. However, identifying the most suitable graft for each case is particularly challenging.

Methods: In the present study, a commercial graft derived from bovine small intestinal submucosa (bSIS) was compared with the polypropylene mesh (PPM) in terms of their repair efficacy and tissue compatibility. These grafts were used to repair artificially induced abdominal wall defects in 24 rats categorised into two groups. The morphologic and histologic analyses were used to compare and assess the grafts.

Result: The results showed that bSIS has several merits over PPM in terms of biological activity and tissue compatibility.

Key words: Biograft, Bovine, Hernia, Polypropylene mersilene mesh, Rat, Submucosa.

INTRODUCTION

Defects in the skin, fascia and muscle tissues forming the abdominal wall caused by congenital or acquired causes are common in domestic animals and these lesions are classified as open or closed depending on skin integrity (Chai *et al*., 2020; Wang *et al*., 2018). If not repaired in a timely and in effective manner, these defects may cause pain and discomfort and other complication; herniation, bowel incarceration, strangulation, necrosis, obstruction, perforation, and even potentially fatal peritonitis (Radlinsky, 2013; Slatter, 2003). Surgical management of hernias caused by muscle tissue defects remains a major problem (Chai *et al*., 2020; Wang *et al*., 2018). The use of autologous, allogeneic, xenogeneic, non-biologic, biologic, or composite grafts to prevent repeated operations and to provide a satisfactory and tension-free repair has become increasingly common (Chai *et al*., 2020; Wang *et al*., 2018; Parmaksiz *et al*., 2018; Pu *et al*., 2005; Steurer *et al*., 2011; Suckow *et al*., 2017).

The main advantage of using autologous tissues for repair is that it produces minimal immune reaction, however its use is limited owing to disadvantages such as the creation of a new wound in the donor site and the need to acquire a limited amount of tissue compared to the size of the defect (Wang *et al*., 2018; Zhang *et al*., 2019). Polypropylene mesh (PPM), polyethylene and polytetrafluoroethylene meshes are the most widely used non-biological materials (Wang *et al*., 2018). Although they have some advantages over closure with suture techniques such as having a high tensile strength, most of these patches have low *in vivo* biodegradability and can induce a severe immune response. Their use can lead to serious complications including risk of infection, local irritation, formation of a fistula, skin erosion and adhesion (Costa *et al*., 2016; Greca *et al*., 2001; Welty *et al*., 2001). An ideal mesh should not only provide strength and flexibility but also facilitate tissue integration and be resistant to infection. Therefore, in recent years, there has been an increase in the use of acellular extracellular matrix derived from tissues such as small intestinal submucosa (SIS), pericardium and dermis using autogenic, allogenic or xenogeneic techniques and provided in the form of biologic patches (Chai *et al*., 2020; Parmaksiz *et al*., 2018; Ayubi *et al*., 2008; Parmaksiz *et al*., 2017; Parmaksiz *et al*., 2019). The biological materials are reported to rapidly degrade after implantation and consequently lose a considerable

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proportion of their biomechanical strength, leading to complications such as rupture, stretching and hernia recurrence (Wang *et al*., 2018).

In the present study, full-thickness abdominal muscle defects were repaired in different groups of rats using a commercial biologic graft obtained from bovine SIS (bSIS) (Matrisis™, Biovalda Health Technologies Inc., Ankara University Technology Development Zone, Golbası, Ankara, Turkey) and PPM, a synthetic graft material. Furthermore, macroscopic and histologic analysis were used to determine the advantages and disadvantages of these two grafts based on different criteria including biocompatibility, resorption ability (biodegradability), ability to mimic host tissues in new tissue formation, interaction with abdominal muscles, and infection in the recipient site.

MATERIALS AND METHODS

Ethical statement and animals

Approval for this study was received from the Local Ethics Committee of the Near East University Experimental Animal Research Centre in 2021 (Approval No: 2020/125).

The study was conducted with 24 Wistar Albino rats (age, 6-8 weeks) with a mean weight of 250-300 g. Rats were housed at an ambient temperature of 21°C-25°C with 6 rats in each cage and fed with rat chow. The rats were randomly and equally divided into the bSIS and PPM groups, and 6 rats from each group were euthanised at 7 days (G1M1 and G2M1) and 28 days (G1M2 and G2M2), respectively. The tissue samples were subsequently collected from the graft site and examined histologically. The control groups were labelled as G1C1 and G1C2 for the bSIS group and G2C1 and G2C2 for the PPM group.

Anaesthesia and preparation of animals for the surgical procedure

Animals were anaesthetised using 100 mg/kg Ketamine HCL (Ketamine 10%, 25 ml vial, Dutch Farm, Holland) and 10 mg/kg intraperitoneal Xylazine HCL (2% VETAXYL® 50 ml vial, VET-AGRO, Lublin). Bavet Meloxicam 1 mg/kg dose (Meloxicam, Bavet İlaç San. ve Tic A.Ş, Tuzla-Istanbul) was used subcutaneously to ensure perioperative and postoperative pain control and depth of anaesthesia. The abdominal region was then prepared for the surgical procedure under aseptic conditions.

Preparation of graft material

The biograft (Matrisis™) used in G1 was cut to the desired dimensions and placed in isotonic solution for at least 30 min to soften before use (Fig 1A). PPM used in G2 was prepared for use after being cut to the desired dimensions (Fig 2D).

Surgical method

In all groups, the animals were immobilised in the right lateral recumbent position and a full-thickness defect of 1 cm^2 was created in the abdominal muscles on the left fossa paralumbalis.

Biografts (Fig 1C) and PPM grafts (Fig 1F) were sutured to the defect area with 4-5/0 non-absorbable monofilament suture material using interrupted suturing technique without tension on the wound line. The skin was closed with the same material.

The skin and muscle incisions made in the right fossa paralumbalis of all rats for control purposes were closed using the same suture materials and techniques.

Macroscopic results and evaluation of adhesions

In all subjects, the implant site was checked for subcutaneous seroma, haematoma and infection before euthanising the rats. Adhesions formed between the graft material and intra-abdominal organs were evaluated during removal of the implant sites after euthanising the rats. In this procedure, the degree of adhesion was evaluated using a numerical score of 0-4 according to the following criteria reported by Wang *et al*. (2018): (0) no adhesion; (1) thin adhesions that can be easily separated by blunt dissection; (2) definite localised adhesions; (3) definite multiple

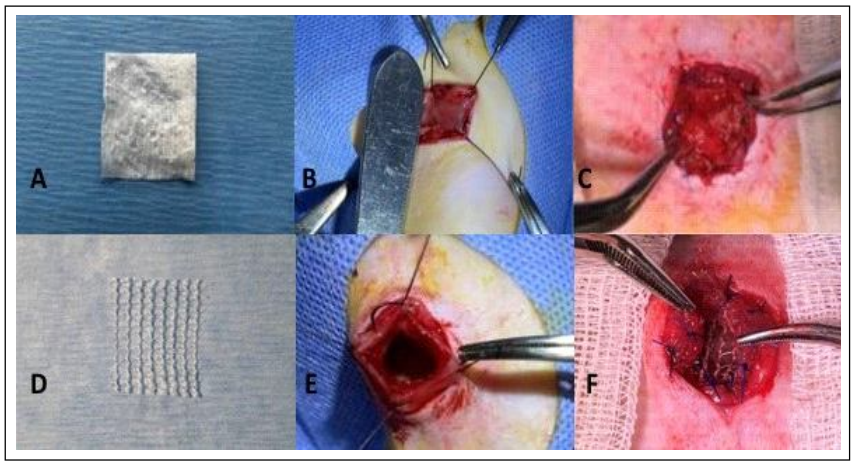


Fig 1: A. Matrisis™, B. and E: Creation of an abdominal muscle defect, C: Suturing the biograft to the defect, D: PPM, F: Suturing the PPM to the defect.

visceral adhesions and (4) dense adhesions extending to the abdominal wall (Table 1).

Postoperative care

The rats health status (overall, feeding status, urination and defecation) and wound sites (signs of local infection) were checked daily.

Sacrifice of rats

Six animals from each group were euthanised at 7 and 28 days by injecting high-dose anaesthetic agents. Full-thickness tissue samples were collected from the left and right abdominal muscles and stored in 10% formaldehyde for histological examination.

Histological examination

The tissues fixed in 10% formaldehyde were placed in an automatic processor (Leica TP1020) and subjected to a graded series of alcohol. Paraffin blocks of the tissue samples were prepared, and 4-5 µm thick sections were collected from these blocks. The sections were stained with haematoxylin eosin (H&E; Sigma-Aldrich) to perform general examination and histological scoring and with Masson trichrome (Merck) to elucidate the collagen fibre organisation and evaluate the muscle fibres.

Slides that underwent H&E staining were examined under light microscope (Leica DM500) at ×10 and ×40 objective magnification. The slides were semi-quantitatively scored for neovascularisation, inflammation, fibroblastic activity-fibrosis and muscle regeneration. Scoring was performed using a modified version of the method reported by Cianforlini *et al.* (2020) (Table 2).

Statistical analysis

The Mann-Whitney U test was used to compare G1M1 and G1M2 with G2M1 and G2M2 groups and G1C1 and G1C2 with G2C1 and G2C2 groups in terms of measurements recorded on days 7 and 28. In addition, the Wilcoxon Sign Rank test was used to compare the G1M1-G1C1; G1M2-G1C2; G2M1-G2C1 and G2M2-G2C2 groups and G1C1-G1C2; G2C1-G2C2; G1M1-G1M2; G2M1-G2M2 and G2M1-G2M2 groups. Statistical significance was set at $p \leq 0.05$ and analysis was performed using SPSS (ver. 25) software.

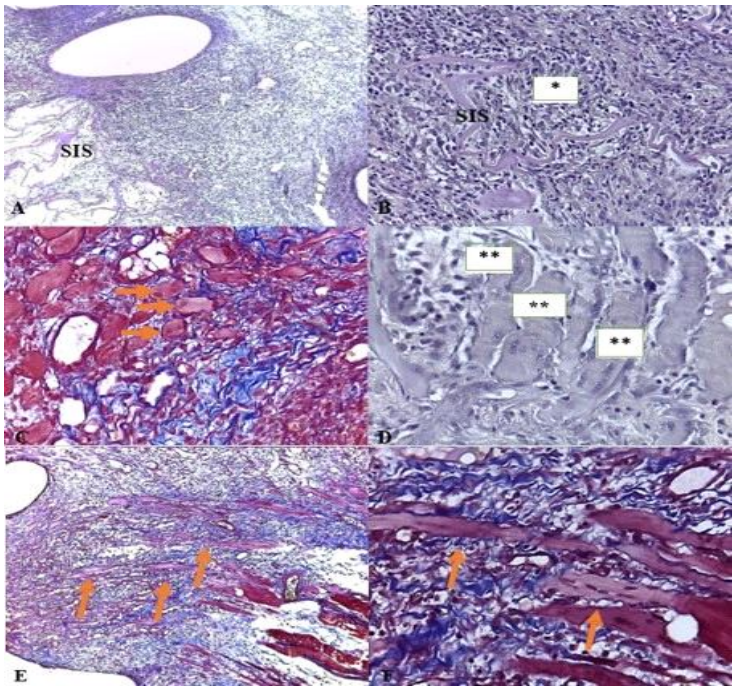
RESULTS AND DISCUSSION

Macroscopic results

None of subjects had any seroma, haematoma or infection at the surgical site. Additionally, herniation or bulging of the implant sites beyond the abdominal wall borders was not noted (Pu *et al.*, 2005; Ayubi *et al.*, 2008; Buell *et al.*, 2021).

Histological findings

The rats in G1M1 had inflammatory cells (Fig 2A) and increased fibroblastic activity in the graft site (Fig 2B) compared to those in the control group. The rats in this group



Increased fibroblasts, (arrows) regenerated muscle fibres with central nuclei, (**) multinucleated myotubes formed around the graft area. H&E staining (2A×10, 2B×40, 2D×40) and Masson trichrome staining (2C×40). E and F: PPM-grafted area at day 7. Newly formed muscle fibres (arrows) in the muscle tissue and collagen fibres (blue) between them. Masson trichrome staining (2E×10, 2F×40).

Fig 2: A, B, C and D: bSIS-grafted area at 7 days (*).

had a higher number of muscle fibres with centralised nuclei (Fig 2C) and myotubes with nuclei compared to the control group (Fig 2D).

The G2M1 group had regenerated muscle fibres extending to the graft site and increased collagen fibres and capillaries between these muscle fibres (Fig 2E and 2F).

In the G1M2 group, shrunk defect site and decreased inflammatory cells and angiogenesis were noted. Furthermore, fibrosis caused by increased collagen fibres was noted and there was an increase in the number of newly developing muscle fibres (Fig 3A and 3B).

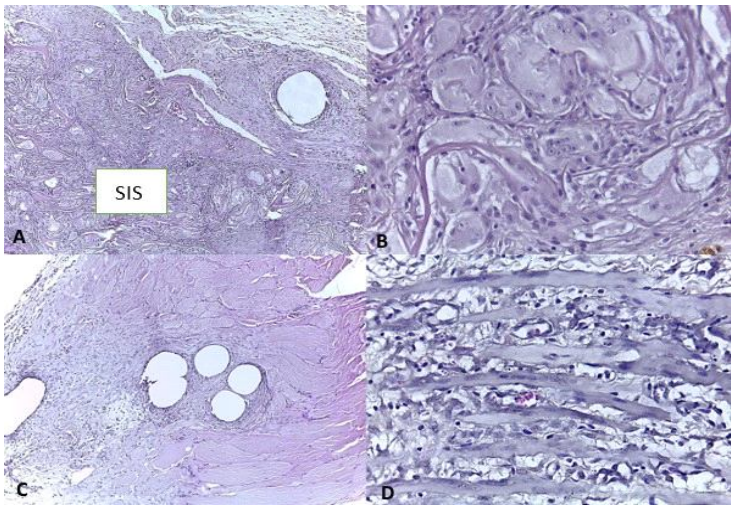
The G2M2 showed an increase in the regenerated muscle fibres and myotubes in the vicinity of the graft. In addition, fibrosis was evident (Fig 3C) and the macrophage cells were decreased compared to the control groups (Fig 3D).

Organisms' reaction to a foreign substance may be related to fibroblast formation in the early postoperative period. The thickness of fibroblasts formed around the material is an indicator of the severity of the reaction (Wang *et al*., 2018). Although intense inflammatory cells and increased fibroblastic activity were observed in the graft site in G1M1, this rate was >25%, however inflammatory cells (especially macrophages) and fibroblastic activity in G2M1 areas were >25% and >50%, respectively (Fig 2). These values showed that biograft had higher tissue compatibility and produced fewer reactions. In addition, formation of myotubes and muscle fibres that started during the early period in the biograft areas was significantly higher than in the PPM groups at 28 days ($p<0.05$). The graft area was covered with a large number of muscle cells, newly formed muscle fibrils and myotubes were abundant and clearly detectable (Fig 3).

An increase in the thickness of the implants appeared to be necessary to maintain the integrity of the abdominal wall during remodelling of the defect area and biodegradation of the grafts and this increase reportedly occurs because of the inflammatory response (Liu *et al*., 2011). The outcomes of the present study showed abdominal wall thickening as a result of increased collagen in both groups and this was more prominent for the biograft.

Statistically no significant difference was observed between G1C1- G2C1 and G1C1- G2C2 in terms of inflammation, fibrosis and muscle regeneration ($p>0.05$); G1M1 and G2M1 showed no significant intergroup differences in any of these results ($p>0.05$). But the ratio of areas containing inflammatory cells was significantly lower in G2M2 than G2M1 ($p>0.05$). While areas with new muscle fibres (muscle regeneration) were significantly less in G1C1 than in G1M1 ($p>0.05$), no significant differences in terms of inflammation and fibrosis ($p>0.05$). Comparison of G2M1 and G2C2 revealed no significant difference in any of the results ($p>0.05$). But the proportion of areas with fibrosis was significantly larger in the G2M2 than G2M1 and higher in G2C2 than in G2C1 ($p>0.05$). The proportion of areas with new muscle fibres was significantly higher in G1M2 than in G2M2 ($p<0.05$). The proportion of fibrosis areas was significantly lower in the G1M2 than in G1C2 ($p>0.05$). G1M2 and G1C2 showed a significant difference in terms of muscle regeneration ($p>0.05$), with a larger proportion of areas with new muscle fibres in the experimental group than in the control groups. G2M2 and G2C2 had no significant difference in any of the parameters ($p>0.05$); (Fig 4).

Wang *et al*. (2003) said that biografts promote angiogenesis by increasing the biocompatibility of the host



H&E staining. C and D: The muscle tissue in the PPM-grafted area at 28 days. The graft area is quite closed and has fibrotic tissue and muscle fibres (3C×10) and newly regenerated myotubes. The macrophages are absent in the connective tissue between the muscle fibres (3D×40). H&E staining.

Fig 3: A and B: The muscle tissue in the bSIS-grafted area at 28 days.

cell and help the tissue to regain its functions, while Liu *et al*. (2011) reported that inadequate angiogenesis in the graft site leads to contraction of the implants and subsequently to fibrosis and necrosis as a result of malnutrition. In the current study, newly formed vascular areas were significantly higher in the G2M1 group than in the G1M1 group (p>0.05); whereas at 28 days, angiogenesis was significantly higher in both groups, albeit their values were lower than those in the 7-day groups. The newly formed vascular areas decreased significantly in G2M2 compared with G2M1 (p=0.050).

However, although fibrosis decreased, it was more dominant in the PPM group. Lower shrinkage and higher fibrosis in the PPM groups is attributable to the porous structure of this material (Wang *et al*., 2018).

No statistically significant difference was observed between all control groups and G1C1- G1M1; G1M2- G2M2; G1C1-G2C2 and G2M1- G2C2 in terms of angiogenesis (p>0.05); (Fig 4).

Adhesion of the implant materials to the intra-abdominal organs is an important criterion. Adhesions are caused by intraoperative bleeding, the inflammatory response caused by the graft material and the accumulation of fibrin matrix into organised fibrous adhesions due to plasminogen activator suppressed during inflammation (Liu *et al*., 2011).

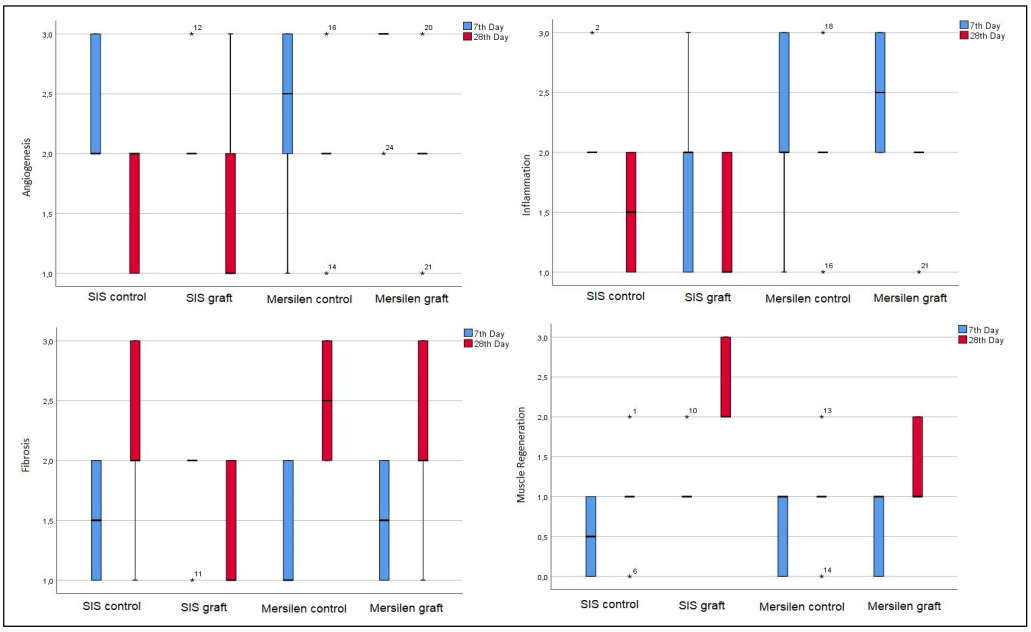


Fig 4: Statistical intragroup field comparison findings.

Table 1: Comparison of adhesions for bSIS and PPM materials at 7 and 28 days (Wang *et al*., 2018).

	The 7th day bSIS (G1M1)	The 7th day PPM (G2M1)	The 28th day bSIS (G1M2)	The 28th day PPM (G2M2)
Rat 1	0	0	4	2
Rat 2	0	0	4	2
Rat 3	0	0	0	2
Rat 4	0	0	3	1
Rat 5	0	0	2	1
Rat 6	0	0	2	1

Table 2: Histological scoring (Cianforlini *et al*., 2020).

Score rating	Neovascularisation	Inflammation	Fibrosis	Muscle regeneration
0	No	No	No	No
1	Newly formed vascular areas <25%	Areas filled with inflammatory cells<25%	Fibrosis areas <25%	Areas with new muscle fibres <25%
2	Newly formed vascular areas 25%-50%	Areas filled with inflammatory cells 25%-50%	Fibrosis areas <25%-50%	Areas with new muscle fibres 25%-50%
3	Newly formed vascular areas >50%	Areas filled with inflammatory cells>50%	Fibrosis areas >50%	Areas with new muscle fibres >50%

Liu *et al.* (2011) reported that adhesions were abundantly present in the early period, in the pSIS graft in particular, but these adhesions subsequently decreases. Wang *et al.* (2018) and Khansa *et al.* (2015) stated that the adhesions had formed as a result of placing the graft immediately subcutaneously in the muscular tissue; this is because the space formed between the graft and the muscle tissue provides space for organ movements and thus causes adhesions. The outcomes of the present study showed a more pronounced adhesion in the biograft group compared to the PPM group at 28 days, albeit statistically non-significant (p>0.05). The adhesion results were given in Table 1.

CONCLUSION

This study has several limitations. The commercial biografts derived from bSIS were not tested for tissue strength and were not analysed for inflammation markers. Additionally, although widely used commercial or non-commercial collagen-based biografts derived from porcine SIS have been documented in numerous studies to cause minimum immune reaction, the bSIS material has not yet been investigated to the best of our knowledge.

The commercial biograft (Matrisis™) used in this study had good tissue compatibility; tissue formation closer to the original tissue and caused less inflammatory reaction. Therefore, it appeared to be superior to PPM in the above-mentioned regards. However, this issue warrants further investigation to expand our knowledge on these types of grafts.

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Conflict of interest

All authors declare that they have no conflict of interest.

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