Comparative evaluation of glycerolized bovine pericardium implant with prolene mesh for closure of large abdominal wall defects in dogs

Abouelnasr, K. S.[1]; Zaghloul, A. E.[1] and Karrouf, G. I.[1, 2*]

[1]Department of Surgery, Anesthesiology & Radiology, Faculty of Veterinary Medicine, Mansoura University, 35516 Mansoura, Egypt; [2]King Fahd Medical Research Center, King Abdulaziz University, Jeddah 21589, Kingdom of Saudi Arabia

Correspondence: G. I. Karrouf, King Fahd Medical Research Center, King Abdulaziz University, Jeddah 21589, Kingdom of Saudi Arabia and Department of Surgery, Anesthesiology & Radiology, Faculty of Veterinary Medicine, Mansoura University, 35516 Mansoura, Egypt. E-mail: gkarrouf@kau.edu.sa

Summary

The aim of this investigation was to evaluate the use of polypropylene mesh and glycerol preserved bovine pericardium (GBP) to repair abdominal wall defects in 24 clinically healthy mongrel dogs using inlay and underlay techniques. Twenty four healthy mongrel dogs were used in this study. The animals were divided into two main groups according to the type of the prosthetic material used. Each group was further divided into two subgroups according to the implantation technique. The prosthetic materials used were polypropylene mesh (Prolene, Ethicon, Johnson and Johnson, Belgium) and GBP. The two implantation techniques were the inlay and underlay techniques. Based on postmortem findings, biomechanical analyses and histopathological examinations, GBP patches were found to be successful alternatives for the reinforcement and repair of large abdominal wall defects as compared to prolene mesh.

Key words: Bovine pericardium, Abdominal wall defect, Polypropylene mesh, Dog

Introduction

The surgical repair of major abdominal wall defects remains a significant problem. Many abdominal wall defects can be repaired by primary closure; however, if the defect is large tensions on wound closure exist, the use of prosthetic materials is suggested (Kingsnorth and LeBlanc, 2003). Post-repair clinical complications such as wound infection, bowel fistula, erosion into abdominal viscera, increased recurrence rate of 25%, repair failure and mesh extrusion together with the high cost associated with synthetic material have initiated the search for safe and cheap biodegradable material that can be replaced by the recipient's tissue (Luijendijk *et al.*, 2000).

Several types of connective tissues and muscles have been used experimentally and clinically for re-constructing congenital or acquired soft tissue defects. These include fascialata (Saaverda *et al.*, 2001), pericardium (Hafeez *et al.*, 2004), duramater (Parizek *et al.*, 1997) and tunica vaginalis (Tung *et al.*, 2002). Glycerol preserved bovine pericardium (GBP) implant was gradually resorbed and replaced with recipient tissues at different rates seemingly delaying implant biodegradation and replacement by the host tissue compared with other types of preservation such as lyophilized, irradiated freeze drying methods (Hafeez *et al.*, 2005).

The aim of this study was to evaluate the effectiveness and economic aspect of using glycerolized bovine pericardium in comparison to polypropylene mesh to repair experimentally induced abdominal wall defects in dogs by using of inlay and underlay techniques.

Materials and Methods

Experimental animals, housing and feeding

Twenty four healthy mongrel dogs with an average age of 1.5-2 years and 17.4±1.6 kg body weight were used in this study. All dogs were kept in closed boxes at Mansoura Veterinary Teaching Hospital of the Faculty of Veterinary Medicine, and were fed dog food according to maintenance and *ad libitum* access to water. This study was approved by the ethical committee for animal care of the Laboratory Animal Center, Mansoura University, Egypt.

The animals were divided into two main groups according to the type of the prosthetic material used. Each group was further divided into two subgroups according to the implantation technique. The different prosthetic materials used were polypropylene mesh (Prolene, Ethicon, Johnson and Johnson, Belgium) and GBP. Implantation was carried out using inlay and underlay techniques.

Preparation of prosthetic material

The bovine pericardia were freshly harvested from an abattoir immediately after slaughtering the animals and processed according to Hafeez *et al.* (2005).

Preoperative preparation and anesthesia

All dogs received a preoperative dose of flummox (EIPICO, ARE), were pre-medicated with intramuscular injections of atropine sulphate (1 mg/ml, Misr Co., Egypt) at a dose of 0.1 mg/kg B.W. followed by an IM injection of xylazin Hcl (Xylaject-ADWIA) at a dose of 1 mg/kg B.W. General anesthesia was induced and maintained using thiopental sodium 2.5%.

Surgical technique

A full thickness abdominal wall defect (10 × 6 cm), including muscles and peritoneum was created. After controlling the bleeding, prosthetic materials proportionate to the defect sizes were implanted using inlay or underlay techniques (Figs. 1A-B, respectively).
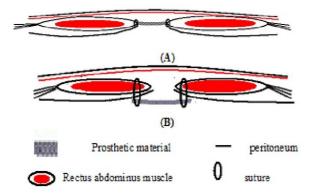

Fig. 1: Inlay (A) and underlay (B) techniques of implantation

Four dogs from each group were humanly euthanized at 60, 120 and 180 days. The abdominal wall defect areas were examined grossly before removal of the skin to assess the skin wound healing, presence of prosthetic imprint and detection of adhesion between the skin and the implant.

After removal of the skin, a 6 cm wide rectangular shape including the prosthetic implant was examined for the presence of connective tissue covering and implant shrinkage. Afterwards, three borders of the full thickness abdominal wall defect were removed and studied to detect the development of neoperitonium, presence or absence of inner revascularization and adhesion grade using the adhesion scoring system described by Jenkins *et al.* (1983), in which a qualitative assessment of adhesion was classified into four grades:

(0) No adhesion
(1) Minimal
(2) Moderate
(3) Dense

Biomechanical evaluation

Transverse strips 4 cm wide and 10 cm long including the implanted material and bilateral adjacent body walls were harvested at each euthanasia and placed into the grips of a ensile strength testing apparatus (LLOYD).

The samples were elongated until failure at a crosshead speed of 50 mm/min. Load at failure and extension percentages were recorded simultaneously on a digital screen connected to the load frame. Tensile strength was calculated by dividing sample load at failure by sample width. The anatomic location of the break was noted for each specimen.

Histological examination

Tissue specimens were collected from the patch grafts at 60, 120 and 180 days following hernioplasty. All collected specimens were fixed in a 10% formalin solution for 48 h, trimmed to suitable sizes, washed, dehydrated, cleared in xylol, embedded in paraffin wax, sectioned at 5-6 µ thickness, and mounted onto glass slides stained with haematoxylin and eosin (H & E) Masson's trichrome and von kossa for evaluation using a conventional light microscope according to Bancroft *et al.* (1996). The amounts of fibrosis were graded during histological examinations according to the fibrosis grading scale described by Hooker *et al.* (1999) as following:

0: Nil
1: Minimal
2: Moderate
3: Dense

Statistical analysis

Data analyses were performed using the statistical software program Graph Pad Prism, version 3.0, USA. Mean values and standard deviations for each assessed variable at each time point were calculated. Repeated measures ANOVAs (with repeated measures on treatment and time) were used to determine the main effect of implants and time. Where the repeated measures ANOVA indicated a statistically significant difference between groups, a one way ANOVA with a Duncan post hoc multiple comparison test was used to identify the group which was statistically different from the others. Differences between means were considered significant at $P<0.05$.

Results

Animals

Prosthetic materials including prolene mesh andglycerolized bovine pericardium were effective in reconstructing experimentally induced abdominal wall defects in dogs (Table 1). All experimental dogs tolerated the surgical procedure well and survived until the determined date of euthanasia.

Clinical findings

Mild to moderate inflammatory signs including hotness, pain, redness and abdominal discomfort were observed in animals implanted with bovine pericardium 24 h postoperation and disappeared within 2 weeks. For animals implanted with prolene mesh, inflammatory responses were more intense at first but decreased gradually until disappearing totally within 3 weeks. Seroma formation was recorded in 6 animals 2 days postoperatively, bur disappeared within 2 weeks without interference. No dogs showed evidence of herniation

Table 1: Results of prosthetic herniorrhaphy in dogs

Prosthetic materials	Inlay technique			Underlay technique		
	Excellent	Good	Poor	Excellent	Good	Poor
Prolene mesh	3	2	1	5	1	-
Pericardium	5	1	-	4	2	-
Total	8	3	1	9	3	-
			24			

Excellent: Rapid healing, no infection, no recurrence. Good: No recurrence, skin wound dehiscence, seroma formation and delayed healing. Poor: Recurrence, infection, wound dehiscence

Table 2: Grades of adhesion of the biomaterials with the underlying structures (n 24)

	Technique	Prolene mesh		GBP		Total
		Inlay	Underlay	Inlay	Underlay	
Grades of adhesion	0	-	-	-	3	3
	1	2	4	5	3	14
	2	3	2	1	-	6
	3	1	-	-	-	1
	Total	6	6	6	6	24

except for one belonging to the prolene mesh group using the inlay technique. This animal showed an orange size herniation swelling on its right side which developed at the third day after implantation.

No post-operative complications were observed for 2 months and the abdominal wall regained its normal integrity without abnormalities by 4 months. At 6 months, the implantation site became firmer in animals implanted with prolene mesh, while in animals implanted with bovine pericardium, the site felt more pliable and the imprint of the prosthetic materials was more difficult to outline.

Post-mortem findings

Gross inspection of the subcutaneous surface of the abdominal wall at the implantation site at 2 months revealed that the implanted materials were covered with a thin layer of white fibrous connective tissue and outer neovascularization which became more prominent at 4 and 6 months (Figs. 2A-C, respectively). The visceral surface of the implanted prolene mesh was encapsulated with fibrous tissue, remained without marked structural changes and showed irregularities and foldings of the mesh. Nevertheless, the implanted GBP was gradually coated with a connective tissue layer and did not show any irregularity or folding (Figs. 3A-B, respectively). Revascularization and new peritoneum was more prominent in animals implanted with GBP and was observable by the naked eye.
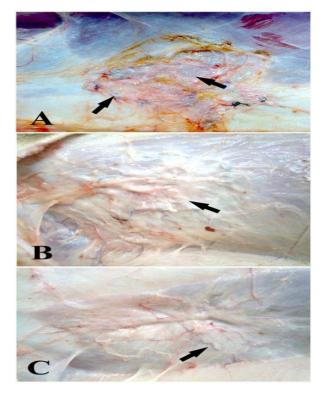
Formation of intra-abdominal adhesion

There was no evidence of adhesion between the biomaterial and the underlying visceral organs in any dog at any interval except for one dog implanted with prolene mesh and euthanized at 60 days, where the 3rd grade of adhesion was recorded. Frequent formation of adhesion was observed between the peritoneal side of the biomaterial and the greater omentum of grades 0 to 3 according to the implanted material and implantation technique (Table 2).
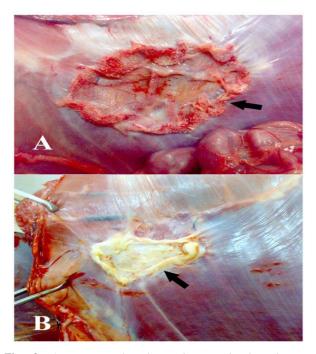
Tensiometric evaluation

Twenty four tested strips were broken either at the implant-muscle interface or at the muscle on either end of the implant.

The average mean differences of load at failure and healing tensile strength of the prolene mesh and GBP implants were not statistically significant at any time interval. However, the healing tensile strength of the abdominal wall implanted with different types of graft materials was generally significantly affected by time (P<0.01). Moreover, a one way ANOVA showed

Fig. 2: The prolene mesh was covered with a thin layer of connective tissue at 2 months (A) that increased in thickness at 4 months (B) and completely covered the implant with a clearly observable neovascularization at 6 months (C) (arrows)

Fig. 3: At two months, the prolene mesh showed some irregularities and folding after the inlay technique (black arrow, A) while the pericardium retained their original shape without folding (black arrow, B) after the underlay technique

significant differences (P<0.05) at different times (Tables 3 and 4). The different values of the extension percentage of the two types of implants were statistically significant (P<0.001) at all time points with different types of implants (Table 5).

Table 3: Means±SD of load at failure (N) for prolene and pericardium and at examined times

Time	Prolene	Pericardium
Two months	71.40 ± 1.65^a	70.85 ± 1.61^a
Four months	87.27 ± 1.03^b	85.57 ± 2.36^b
Six months	74.47 ± 1.61^c	73.60 ± 1.25^a

Means with different superscript letters at the same column are significantly different at P<0.05

Table 4: Means±SD of tensile strength (N/CM) for prolene and pericardium at examined times

Time	Prolene	Pericardium
Two months	14.28 ± 0.33^a	14.17 ± 0.32^a
Four months	17.45 ± 0.20^b	17.11 ± 0.47^b
Six months	14.87 ± 0.33^c	14.67 ± 0.26^a

Means with different superscript letters at the same column are significantly different at P<0.05

Table 5: Means±SD of extension percent for prolene and pericardium at examined times

Time	Prolene	Pericardium
Two months	36.50 ± 3.41^a	29.50 ± 1.29^a
Four months	35.75 ± 1.50^a	40.50 ± 2.38^b
Six months	44.00 ± 1.82^b	40.25 ± 1.70^b

Means with different superscript letters at the same column are significantly different at P<0.05

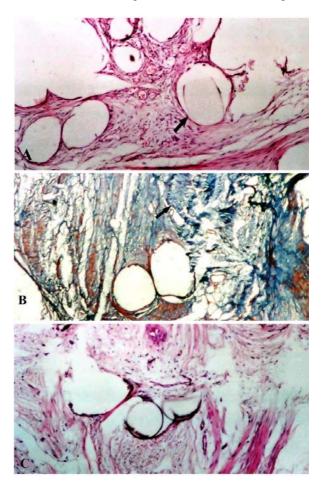## Histopathological examination
Prolene mesh

At 2 months, the inflammatory response was represented by a small number of mononuclear cells, plasma cells and lymphocytes, which were widely scattered especially around the mesh fibers (Fig. 4A). As confirmed by Masson's trichrome, the first grade of fibrosis including an immature fibrous tissue and a newly developed connective tissue layer consisting of delicate collagenous fibers was observed (Fig. 4B).

At 4 months, the number of inflammatory cells decreased and only a small number of macrophages and neutrophils remained between the mesh fibers (Fig. 4C). The newly developed connective tissue was proliferating and remodeling into a moderate dense fibrous capsule around the mesh implant which represented the 2nd grade of fibrosis (Fig. 5A).

At 6 months, plasma cells were evident in some areas but few eosinophils at the adjacent area and neo-angiogenesis were seen (Fig. 5B). Fibroblasts were seen among mesh fibers that appeared unaltered and the fibrous capsule appeared denser, representing grade 3 of fibrosis (Fig. 5C). Moreover, as confirmed by the von kossa stain, dense collagen fibers were observed together

Fig. 4: (A) Few mononuclear cells, plasma cells and lymphocytes widely scattered especially around the mesh fibers (H&E, ×10). (B) Immature fibrous tissue and a newly developed connective tissue layer (Masson's trichrome, ×10). (C) Few macrophages and neutrophils still present between prolene mesh fibers (H&E, ×10)
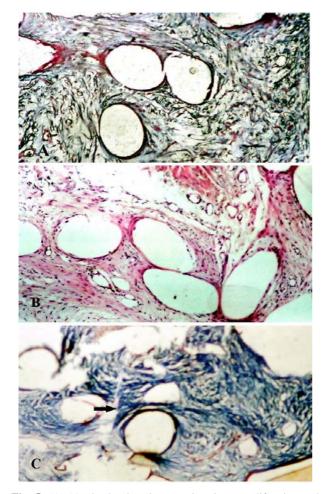
Fig. 5: (A) Newly developed connective tissue proliferating and remodeling into a moderate dense fibrous capsule around the mesh (Masson's trichrome, ×10). (B) Plasma cells in areas with few eosinophils at the adjacent area around the mesh fibers (H&E, ×10). (C) Fibroblasts among mesh fibers that appeared unaltered and the fibrous capsule appeared more dense (Masson's trichrome, ×10)
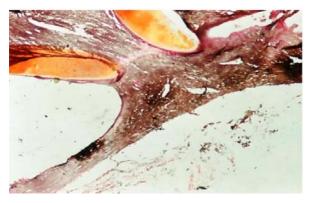

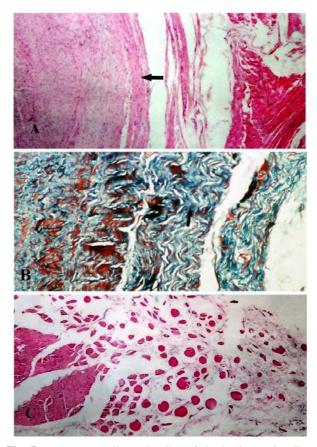**Fig. 6:** Presence of a mild degree of calcification around mesh fibers at 6 months, (von kossa stain, ×10)

with the a mild degree of calcification (Fig. 6).
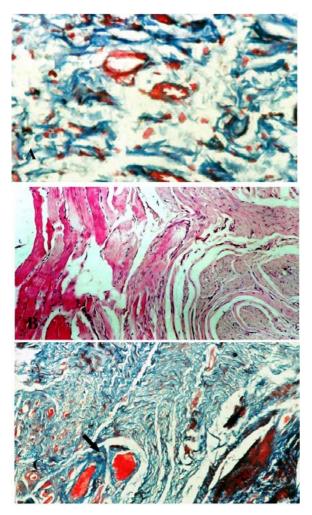
Bovine pericardium

At 2 months, dense collagen bundles of the bovine pericardium implants were present and surrounded by few mononuclear cell (plasma cells, lymphocytes and macrophage) infiltrations. Myoblasts were observed in

the vicinity of the native host muscles at junctional areas (Fig. 7A). The presence of newly formed fibrous tissues and collagen were confirmed by Masson's trichrome (Fig. 7B). At 4 months, inflammatory reactions completely reduced and young muscles were observed at junctional areas close to native host muscles and infiltrates through the implant. Neoperitoneum and mesothelial cells were observed under the surface of the biomaterials at 4 months (Fig. 7C). Gradual resorption and degradation of the dense collagen bundles were observed and confirmed with Masson's trichrome (Fig. 8A), while at 6 months, the formation of new blood vessels was observed within the fibrous tissue. Myoblasts became more clearly defined with peripherally located nuclei and expanded further into the biomaterial (Fig. 8B). The loose connective tissue was gradually changed to form a dense fibrous tissue, which represented the 2nd grade of fibrosis (Fig. 8C). No calcification or foreign body giant cells were observed at any time (Fig. 9).

Discussion

Post-operative wound infection and inflammatory changes encountered using pericardium implantation were less intense than those associated with the

Fig. 7: (A) Dense collagen bundles of the bovine pericardium implant present and surrounded by few mononuclear cells (H&E, ×10). (B) Newly formed fibrous tissues and collagen around bovine pericardium (Masson's trichrome, ×10). (C) Development of young muscles at the junctional area close to native host muscles infiltrating through the pericardium (H&E, ×10)

Fig. 8: (A) Gradual resorption and degradation of dense collagen bundles (Masson's trichrome, ×10). (B) Formation of new blood vessels within the fibrous tissue infiltration of myoblasts through the pericardial implant (H&E, ×10). (C) Loose connective tissue gradually changing to form a dense fibrous tissue (Masson's trichrome, ×10)

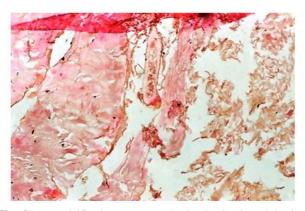**Fig. 9:** No calcification at 6 months in the implanted bovine pericardium (von kossa stain, ×10)

polypropylene mesh. Moreover, the higher elasticity of bovine pericardium made the implantation site more pliable compared to the abdominal wall rigidity caused by the inadequate elasticity of the prolene mesh. These findings agree with those of Hafeez *et al.* (2005) and Kader *et al.* (2005) who maintained that clinical

complications associated with synthetic materials and the difficulty in obtaining and preserving autografts and allografts make alternative xenografts preferable.

The acceptability of bovine collagenous tissues for long term implantation has also been explained as being a result of either the homology of the collagen structure from different species (a low level of foreignness) or to certain structural features associated with collagen (Timple, 1982).

Implantation techniques of prosthetic materials are thought to play important roles in hernia recurrence (Schumpelick *et al.*, 2004). The underlay technique with omentalization gave more satisfactory results than the inlay technique. Although the inlay technique is the simplest form of repair, it lacks implant fixation by intra-abdominal pressure due to the minimal surface area of contact between the implant and the adjacent tissue, leading to high relapse rates. On the other hand, in the underlay technique, the position of the implant behind the rectus muscles (where the force of abdominal pressure holds the prosthesis against the deep surface of the abdominal muscle wall) minimizes hernia recurrence rates. The same explanation was offered by Rainier *et al.* (2006).

The continuous suture pattern used in the inlay technique, in which the breakdown of one stitch leads to the dehiscence of the whole suture line, explained the presence of the postsurgery swelling recorded in this study. These observations were completely in line with Ladurner *et al.* (2001) who found that interrupted sutures used for fixation of the implant in the underlay technique provided multiple points of non tension fixation which helped divide stress evenly over the mesh and reduced mesh crimping and bulging.

Higher degrees of adhesion formation were observed in animals implanted with the inlay technique without omentalization as compared with the underlay technique with omentalization. This could be attributed to the direct contact between the mesh and the abdominal viscera. Similar findings were reported by Halm and Jeekel (2005) who advised the interposition of the omentum to act as a physical barrier between the implant and the viscera.

Neoperitoneum formation recorded in animals implanted with GBP can act as a physical barrier that decreases adhesion formation. Processed bovine pericardium is proved to be placed in direct contact with underlying viscera without stimulating intra-abdominal adhesion. However, in the present study, adhesion was formed between the peritoneal side of the implant and the greater omentum, which may be attributed to the lesion caused by abrasion, ischemia and foreign bodies. Similar results were found when preserved bovine pericardium was used for dogs (Hafeez *et al.*, 2004).

Adhesion was more intense in animals implanted with prolene mesh, and was of grade 1, 2 and 3, requiring aggressive blunt dissections. This result agreed with Baptista *et al.* (2000) who believed that when using polypropylene mesh to repair abdominal wall defects, extensive visceral adhesions led to intestinal fistula due

to their higher ability to incorporate the surrounding tissues of the abdominal wall.

Postmortem examination revealed that bovine pericardium showed no signs of fragmentation, retained their original shape and position and decreased in diameter by time. This could be attributed to the contraction of the fibrous connective tissue after maturation of the healing sites around the patches (Zaghloul and Mosbah, 2006). On the other hand, in the prolene mesh, despite the rolling edges and moderate tension suturing, wrinkles and folds were observed due to fibrous tissue encapsulation which encourages mesh wrinkling. These findings were similar to those recorded by Amid (1997).

Microscopically, prolene mesh appeared without change in the mesh fibers and was surrounded by a delicate layer of fibrous tissue and collagen fibers that became denser at months 4 and 6 postoperation. This dense fibrous capsule was responsible for the firm incorporation between the mesh and the host tissue, although it might have also caused mesh wrinkling, as reported by Elliott and Juler (1979).

Mild calcification was recorded at 6 months in animals implanted with prolene mesh, while in animals repaired with GBP no calcification was observed. This indicates the histocompatability of these materials with the recipient tissue, and gives an advantage to glycerol preservation over other types of preservatives such as glutaraldehyde which have been reported by Jayakroshnan and Jameela (1996) to be associated with cytotoxic effects and calcification formation.

All tissue failures in tensiometric evaluations of this study occurred either at the implant-muscle interface or at the muscles on either end of the implant. This type of failure indicates lower tensile strengths of the muscle and fascia than the repair site. Similar results were recorded by Rainier *et al*. (2006).

The differences among overall load mean values at failure and healing tensile strengths of prolene mesh and GBP implants were not statistically significant at any time interval. These results suggest that the implanted materials were sufficiently strong to maintain abdominal wall integrity. Similar results were obtained by Hafeez *et al*. (2004, 2005).

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