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RESEARCH ARTICLE

Acellular Matrix of Swim Bladder for the Reconstruction of Abdominal Wall Defects in Rabbit

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ABSTRACT

The abdominal wall reconstruction with fresh swim bladder, a cellular swim bladder and autografts after creating an abdominal wall defect of 3x4cm was performed in rabbits and the biocompatibility of the grafts were studied. The study revealed no adhesions, herniation and eventration in the acellular graft group where as minimal adhesions in the fresh graft and autograft groups. Neovascularization on the peritoneal side of the graft at the reconstructive site was seen by day 14 in all the groups. Histologically all the sections showed inflammatory cells, like neutrophils, monocytes and macrophages, at the host graft interface, marked fibroblastic activity towards subcutaneous layer with the formation of thin fibrous tissue and neocapillaries on the peritoneal side on 14th postoperative day. Acellular graft and autograft groups showed early mesothelialization on the peritoneal side of the graft on 7th day which prevented adhesion formation with visceral organs where as in fresh graft group mesothelialization was noticed on 14th postoperative day. Absence of foreign body giant cells and tissue reaction suggested the biocompatibility of the both fresh and acellular swim bladders with the host tissue.

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INTRODUCTION

Most abdominal wall defects and hernia in animals can be repaired by primary closure. When the defect is large or there is tension on the closure, the use of a prosthetic patch material is indicated. Unlike scaffolds comprised of synthetic materials, xenogenic scaffolds are inherently suited to facilitate cell adhesion and native remodeling processes in abdominal wall reconstruction. The maintenance of collagen matrix integrity is important to provide a suitable environment for cell migration and to enable the tissue reorganization (Bader *et al.,* 1998 and Grauss *et al.,* 2005). Collagen is regarded as one of the most useful biomaterial due to its excellent bio-compatibility, biodegradability and weak antigenicity. The need of a readily available non-immunogenic and non-prosthetic biomaterial that could guide the regeneration of normal tissue is a fascinating possibility. Decellularized matrices have been suggested to be ideal natural bio-materials for tissue repair and could be used as supporting structures in tissue engineering applications (Schmidt and Baier, 2000).Fish swim bladders are thought to be collagen rich and cheaply available. Hence the present study was undertaken with an attempt to arrive at a new biological material that can overcome the disadvantages of the other grafting materials in abdominal wall repair.

MATERIALS AND METHODS

A total of 24 rabbits of either sex divided in to three groups were used for abdominal wall reconstruction with fresh swim bladder (Group I), acellular swim bladder (Group II) and autografts (GroupIII), after creating an abdominal wall defect of 3x4cm. Fresh swim bladders collected from fish were decellularized using 1M sodium chloride solution and 0.5% Triton X-100 under gentle agitation to prepare acellular swim bladder graft. Diazepam as @ 4mg/kg body weight intramuscularly as premedicant and 5% Thiopental sodium solution @ 30mg/Kg Body weight as intravenous agent through the marginal ear vein were used to induce and maintain anesthesia. Parenteral administration of enrofloxacin and daily dressing of the wound were carried as postoperative care.

The surgical wounds were examined for their gross appearance and the status of healing up to 14 post-operative days in all the groups. The degree of swelling at the site of reconstruction was graded on 1-4 scales as per the scores adopted by Kumar *et al.* (2002) and described as 1 = no swelling; 2 = mild swelling; 3 = moderate swelling; 4 = severe swelling while, the warmth at the site of reconstruction was compared with normal area touching with palm and described as 1 = normally warm;

2 = mildly warm; 3 = moderately warm; 4 = hot. The degree of exudation at the site of reconstruction was graded on 1-4 scale as follows: 1 = none (apparently dry wound); 2 = slight exudates (wound is moist, no oozing on pressing the wound); 3 = moderate exudates (wound is moist, slight oozing on pressing the wound); 4 = extreme exudates (exudates is visible and pressure lead to extensive exudation) as per the method adopted by Singh *et al.* (2008) where as the degree of pain was assessed by gently pressing the operation site and was graded on 1-4 scale as follows: 1 = no pain on extensive manipulation; 2 = mild (pain on extensive manipulation); 3 = moderate (pain on moderate manipulation); 4 = severe pain (pain on slight manipulation).

Macroscopic observations were recorded on days 7, 14, 21 and 28 postoperatively in all the groups after euthanizing two animals at each time interval. The adhesions if present at the site were graded according to the classification of Nair *et al.* (1974) as follows: 0 = no adherence; 1 = single adherence between two organs or between an organ and the abdominal wall; 2 = two adherences between organs or one organ and the abdominal wall; 3 = more than two adherences between the organs or a massive generalized adherence of the intestine with no adherence to the abdominal wall; 4 = generalized adherences between organs and the abdominal wall or massive adherence among all organs.The tissues collected from the operative site were preserved in 10% formal saline solution and then processed. The tissue sections were cut at 5 micron thickness and were stained with Haematoxylin and Eosin as per the method of Singh and Sulochana (1997). The sections were examined for microscopic changes.

RESULTS AND DISCUSSION

Clinical observations

All the animals were dull, depressed and partially anorectic with little faecal output for first two days after surgery. The degree of swelling at the site of reconstruction was mild to moderate at day 1 in all the groups that persisted up to day 5 in group II and day 7 in groups I and III. The average scores for degree of swelling were 3.0, 2.2 and 2.5 in groups I, II and III respectively indicating moderate swelling with fresh graft, mild swelling with acellular graft and mild to moderate swelling with autograft .The average scores for degree of exudation in group I, II and III were 2.63, 2.0 and 2.25 respectively indicating slight to moderate exudation in fresh graft and slight exudation with acellular and autografts. Mild to moderate warmthness was observed at day 1 in all the groups that persisted up to day 5 in group II and III and day 7 in group I. The swelling and exudation at the reconstructed site was attributed to hyperaemia and out pouring of protein rich fluid containing blood cells into the extra vascular tissue as reported by Singh *et al.* (2008).

The average scores for degree of warmthness were 3.0, 2.5 and 2.75 in group I, II and III respectively indicating moderate warmthness at the site of reconstruction in all the groups. The degree of pain was assessed by gentle pressure at the operation site and was graded as mild to moderate pain at day 1 in all the groups which persisted up to day 5 in group II and III and day 7 in group I. The average scores for pain for groups I, II and III were given as 3.25, 2.0 and 2.25 indicating moderate pain in fresh graft whereas mild pain in acellular and autografts. Mild to moderate warmthness was observed at the site of reconstruction at day one was attributed to the increased vascularity at the operative site. Increased vascularity at the operative site although of variable degree was the common feature in all groups. The vascular change at the site is a part of normal body response to injury and it was thought to be an attempt to increase resorption and removal of clot and debris from the wound site and finally helping in the laying down of fibrous tissue (Silver 1982).

All the animals following surgery were examined daily and complications were recorded In the present study the wound dehiscence was observed in 75.0, 12.5 and 0 percentage of the animals in group I, II and III while the abscessation of the wound was 50.0, 50.0 and 37.5 respectively. During healing process, 37.5, 0 and 37.5 percent of the animals in group I, II and III respectively showed fistula formation, where as the wound edges showed cicatrization in 50.0, 37.5 and 50.0 percent of the animals respectively with fresh swim bladder, processed swim bladder and autografts. In the present study majority of the animals with fresh graft showed wound dehiscence when compared to the other two groups while the abscessation of the wound was similar in fresh and processed graft followed by autograft animals. During healing process, fresh and autograft animals acquired fistula but none of the animals in processed graft showed fistula formation. The wound edges showed cicatrization in 50.0 percentage of fresh and autograft animals followed by processed graft. The cutaneous wound healed between 21-28th day in fresh graft group while the same was completed between 14-21st day in processed and autograft groups. The cutaneous wound healing is incomplete in two animals of auto graft group with scab formation.

Macroscopic Studies

In the present study the macroscopic changes were recorded on day 7, 14, 21 and 28 postoperatively after euthanizing two animals at each time interval. Immediately after euthanasia the implant site was inspected grossly for any herniation, adherences of viscera with abdominal wall, integration of biomaterial with the host tissue, evidence of fibrosis, infection and inflammatory responses.

The average scores for degree of adhesions in groups I, II and III were 0.87, 0 and 0.125 indicating minimal adhesions with fresh and autograft whereas no adhesions with processed graft. Neovascularization on the peritoneal side of the graft at the reconstructive site was seen at day 14 in all the groups as evident by early vascularization with visible blood vessels. There were no signs of inflammation or fibrosis and the grafts were stably embedded in to the host subcutaneous tissue without any translocation.

In Group I, grossly, out of eight animals four (50.0%) showed adhesions of various degrees to the graft. Among them the graft showed adhesions with caecum and omentum on 7th and 14th day in one animal each. On 21st day the adhesions were noticed at host graft interface with mesenteric fat and small intestinal loop each with one

animal. The average score for degree of adhesion was 0.87 indicating minimal adhesions. There were no adhesions with any visceral organs at host graft interface at 28th day. Herniation of intestinal loops under the subcutaneous layer through a tear in the graft in one animal and eventration of intestinal loop through the fistulated wound in another animal were also observed. The graft was intact without any newly formed layer on 7th day while a thin fibrous layer of loose connective tissue was covered over the graft by 14th day.

In Group II, there were no adhesions between the graft and the surrounding tissues on any post operative day under study. At day 7, the graft was covered with glistening membrane indicating mesothelialization where as at day 14 the graft was covered with thin fibrous layer of loose connective tissue. As day progress the fibrous tissue proliferation was more which was evident as moderate layer on 21st day and thick layer of fibrous tissue with partial resorption of the graft .The average score for degree of adhesion was 0 indicating no adhesions. None of the animals showed herniation, eventration and translocation of the graft.

In Group III, out of 8 animals one (1.25%) animal showed omental adhesion on 7th post operative day. The graft was covered with glistening membrane at day 7 and thin fibrous layer of loose connective tissue by 14th day. The average score for degree of adhesion was 0.125 indicating minimal adhesions. Herniation, eventration and translocation of the graft were not seen in any of the animals.

In fresh graft group the peritoneal side of the graft was found intact without any structural changes like fibrous tissue covering and mesothelialization which would have eventually lead to the formation of adhesions to the abdominal viscera. Schneider *et al.* (2006) observed broad adhesions between caecum and abdominal wall after reconstruction with glue coated collagen sponge. Singh *et al.* (2008) observed moderate adhesions of caecum with porcine acellular bladder matrix. Ayele *et al.* (2010) noticed adhesions between caecum and non seeded bovine tunica vaginalis at 7th and 14th postoperative days. It appears that adhesions were found only between caecum and the abdominal wall by several researchers. Contrary to this in the present study adhesions were found with omentum, small intestinal loop, and mesenteric fat besides caecum. Clinically, the presence of omentum does not always prevent the formation of adhesions between bowel and the anterior abdominal wall (Becker *et al.* 1996). Judge *et al.* (2007) recorded adhesions to omentum, bowel when used sodium hyaluronate/carboxy-methylcellulose on to the abdominal wall defects.

Herniation of intestinal loops under the subcutaneous layer through a tear in the graft was observed in one animal on 28th postoperative day which might be due to the intrinsic weakness of the unprocessed graft along with its partial absorption. Singh *et al.* (2008) also made similar observations at day 90. Eventration of intestinal loop through the fistulated wound was observed in one animal on 14th post operative day. This could be due to a self-inflicted injury coupled with intrinsic weakness of the graft.

Adhesions were totally absent in the animals where reconstruction was done with acellular graft as the peritoneal surfaces of the implanted grafts in this group were completely covered by newly developed white fibrous connective tissue as well as neo-peritoneum with smooth and shinny appearance, which later on accompanied with the numerous fine new blood vessels (neovascularisation). Similar findings were also made by Ayele *et al.* (2010) after using myoblast-seeded-bovine tunica vaginalis and by Eberli *et al.* (2010) after using acellular human dermis in the repair of abdominal wall defect. It is well documented that mesothelial cells prevent adhesions because of their fibrinolytic properties that are associated with the secretion of tissue plasminogen activator (Baptista *et al.* 2000). Mesothelial cell membranes are rich in phospholipids that can play a lubricating role between the parietal peritoneum and the underlying viscera, avoiding the appearance of fibrin deposits (Holmdahl and Ivarsson, 1999). The formation of an organized neoperitoneum reduces the adhesions generated on this interface to a minimum (Brown *et al.* 1985). In autograft group one animal showed omental adhesion at the host graft interface on 7th post operative day as the peritoneal covering was not intact at the junction.

Adhesion formation after abdominal surgery can lead to infertility, bowel obstruction, chronic pelvic pain, increased medical expenses, result in postoperative morbidity, fistulization, and difficulties with reoperation (Ray *et al.* 1993). Contradictory to this, in the present study none of the animals exhibited such signs. Adhesions form as a result of defective healing processes related to blood coagulation. Inadequate fibrinolysis processes lead to persistent fibrin structures that subsequently mature into fibrous tissue, followed by organization into rigid, persistent fibrous adhesions, possibly containing blood vessels and nerve fibers (Thompson *et al.* 1998).

Fibrin is a filamentous protein which forms a network that entraps red blood cells, white blood cells, and platelets. This coagulum provides the framework for the growth of reparative cellular tissue. In normal circumstances, mesothelial cells migrate to the wound site. After serosal injury, the damaged mesothelium activates blood clot formation. A layer of fibrin will be deposited over the traumatized tissue. Subsequently they replicate and repair the mesothelial surface. Meanwhile the fibrin network is degraded by biochemical lysis. Persistent fibrin structures will result if fibrinolysis is inadequate. Surgery diminishes fibrinolytic activity by increasing the level of plasminogen activator inhibitors and by reducing the tissue oxygenation (Di Zerega *et al.* 1997). Subsequently the fibrous tissue forms persistent fibrous adhesions. Surgical trauma and intraabdominal infection causes the production of proinflammatory cytokines, which alters the fibrinolytic capacity of the peritoneum and leads to severe adhesions (Tuzuner *et al.* 2004).

Microscopic Observations

In group I, at 7th post operative day the graft on the peritoneal side was not covered with any tissue however the formation of granulation tissue at the host graft interface towards the subcutaneous layer was evident with the infiltration of inflammatory cells like neutrophils, eosinophils and monocytes (Fig.1) at the junction. The

host graft junction showed some fibroblastic activity with necrotic changes in the muscle bundles and edema .At 14th post operative day the graft showed marked fibroblastic activity towards subcutaneous layer with the formation of thin fibrous tissue and neocapillaries on the peritoneal side with the infiltration of inflammatory cells. At 21st postoperative day there was formation of neocapillaries on the subcutaneous layer side of the graft with fibroblast proliferation with infiltration of inflammatory cells. At 28th post operative day the graft showed neovascularized peritoneum, fibroblast proliferation and presence of inflammatory cells (Fig.2).

In group II, at 7th post operative day a mesothelial lining on the peritoneal side of the graft and mild infiltration of inflammatory cells at the host-graft junction was observed.At 14th post operative day the peritoneal side of the graft showed the evidence of neocapillary formation and migration of young muscle bundles with the infiltration of inflammatory cells and proliferation of fibroblasts (Fig.3). At 21st post operative day a thick fibrous tissue formation towards the peritoneal side of the graft with neocapillaries and granulation tissue formation on subcutaneous layer side were evident. The section on 28th day showed neocapillaries on peritoneal side (Fig. 4), migration of muscle bundles at the subcutaneous layer side of the graft and necrosed muscle bundles at the junction were observed along with infiltration of inflammatory cells.

In group III at 7th postoperative day the graft showed thin fibrous tissue formation on peritoneal side with haemorrhage and infiltration of inflammatory cells at the junction (Fig. 5). At 14th post operative day fibrous tissue formation with neovascularization on the peritoneal side of the graft was evident. There was infiltration of the eosinophils, neutrophils and monocytes at the host-graft junction. At 21st day the graft showed thick fibrous tissue formation on peritoneal side and neovascularization on subcutaneous layer side with fibrous tissue proliferation around it. At 28th post operative day neovascularization with migration of young muscle bundles on the subcutaneous layer side of the graft and thick fibrous tissue deposition on the peritoneal side of the graft (Fig.6) were observed. And there was infiltration of the inflammatory cells like neutrophils, eosinophils at the host graft interface.

Neovascularization with visible blood vessels on the peritoneal side of the biomaterial at the reconstructive site was seen at day 14 in all the groups. There were no signs of inflammation or fibrosis and the biomaterials were stably embedded in to the host subcutaneous tissue. The vascular change at reconstructive site is a part of normal body response to injury. It is an attempt to increase resorption and removal of clot and debris from the wound site and finally helping in the laying down of fibrous tissue (Silver 1982).

The infiltration of inflammatory cells at 7th post operative day in all the groups was in accordance with the findings of Ayele *et al.* (2010). The early inflammatory response indicates an immediate response initiated by surgical trauma (Gamba *et al.* 2002, Gangwar *et al.* 2006, and Singh *et al.* 2008). The intact graft on the peritoneal side in fresh graft group and thin fibrous connective tissue formation on the peritoneal side of the graft in processed and autograft groups were in agreement with the findings of Singh *et al.* (2008). They also observed a thin fibrous layer of loose connective tissue covering the peritoneal side of the graft when used porcine acellular biomaterials.

At 14th post operative day the grafts showed marked fibroblastic activity towards subcutaneous layer with the formation of thin fibrous connective tissue and neocapillaries on the peritoneal side with moderate infiltration of inflammatory cells. There was infiltration of eosinophils, neutrophils and monocytes at the host graft junction. Ayele *et al.* (2010) observed marked presence of fibroblasts, collagen fibers with neovascularization and newly formed young muscle in case of myoblast-seeded bovine tunicavaginalis at 14th day post-implantation in rabbits.

Thick fibrous tissue towards the peritoneal side of the graft and granulation tissue formation on subcutaneous layer side with neocapillaries on the peritoneal side, fibroblast proliferation, and infiltration of neutrophils, eosinophils and macrophages at the host-graft junction were evident in all the grafts. Milburn *et al.* (2008) demonstrated inflammatory cells at the tissue-graft junction, with cellular ingrowth and new capillary formation within the acellular dermal matrix grafts by day 21 of implantation. Under ischemic conditions, often associated with surgical injury, the normal fibrinolytic activity of tissue associated with mesothelial repair is compromised, allowing the fibrin matrix to persist and gradually mature into an organized fibrous adhesion (Becker *et al.* 1996).

At 28th post operative day in group I, the graft showed neovascularized peritoneum, fibroblast proliferation and infiltration of neutrophils, macrophages at the host graft interface. In group II, the graft showed neocapillaries on peritoneal side. Migration of muscle bundles at the subcutaneous layer side of the graft and necrosed muscle bundles at the junction were also observed. Werkmeister *et al.* (1998) also noticed a mesothelial cell lining on the peritoneal surface, and complete tissue incorporation on the dermal side of the wet collagen composite patches at 4 weeks after implantation in rabbits. Gamba *et al.* (2002), Schneider *et al.* (2006), Eberli *et al.* (2010) and Ayele *et al.* (2010) observed neovascularization on peritoneal side of the homologous diaphragm matrix, glue coated collagen sponge, acellular human dermis and bovine tunica vaginalis respectively at 30th postoperative day following abdominal wall reconstruction.

In all the groups there was infiltration of neutrophils, monocytes macrophages and fibroblasts during the first 30 days following grafting. Infiltration of cells was observed only at the host graft junction. The absence of cell at the center of tissues probably results from tightly compacted network of collagen bundles, blocking the migration of cells (Armour *et al.* 2004). The implants provoked cellular responses which led to invasion by various cells such as macrophages, and fibroblasts (Lai *et al.,* 2006). This cellular activity during the early stages of wound healing is particularly important in peritoneal repair. Macrophages play an important role in the healing process by secreting substances such as platelet-derived growth factor, glucans, and transforming growth factor beta that modulate tissue repair (Mustoe *et al.* 1987 and Browder *et al.,* 1988).

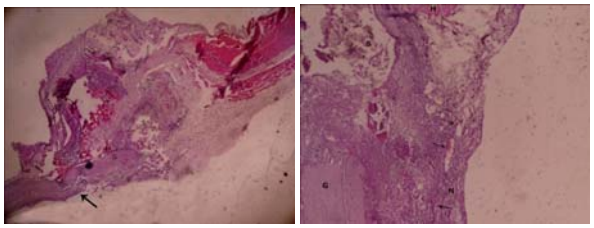

Fig. 1: Showing silk material (S) and infiltration of inflammatory cells at host (H) – graft (G) interface .Note the graft (G) without mesothelialization on peritoneal side (→) - H & E X 40 (7POD, Group I). **Fig. 2:** showing neovascularization (→) on peritoneal side of the host (H) - graft (G) junction, silk (S) H & E X 100 (28th POD Group I).

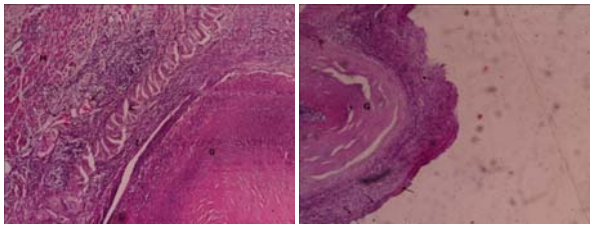

Fig. 3: Showing neovascularization (→), migration of young muscle bundles (M), collagen bundles (C) on the peritoneal side of the graft (G) – H & E X 100 (14th POD Group II)**. Fig. 4:** Showing neocapillary formation (→) on peritoneal side of the graft (G) with cellular infiltration – H & E X 100 (28th POD Group II).

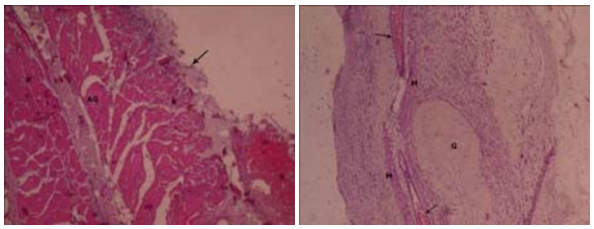

Fig . 5: Showing thin fibrous tissue formation on peritoneal side of the autograft (AG) – H & E X 100 (7th POD Group III). **Fig. 6:** Showing neovascularization (→), young muscle bundles migration (M) on peritoneal side of the graft (G) – H & E X 100 (28th POD Group III)

Proteolytic enzymes such as collagenase secreted by macrophages degrade the acellular matrix allowing fibroblasts from the host tissue (Liang *et al.,* 2004). Macrophages produce a variety of angiogenic factors and are of importance during neo-vascularization (Leek *et al.,* 1996).

Neutrophils are the highly chemotactic and first to arrive at the site of injury. Mononuclear cells include monocytes, macrophages and lymphocytes. The main function of these cells is to complete the destruction of irritant through their powerful enzymes and remove the necrotic tissue from the area and help in antigen presentation because of this mononuclear cells are also known as second line of cell defense whereas the lymphocytes also appears in chronic inflammation and participate in the immune response (Vegad 1995). Absence of foreign body giant cells was noted in all the groups. This could suggest the biocompatibility of the scaffolds with the host tissue (Ayele *et al.,* 2010).

In conclusion the processed swim bladders were well accepted by the host tissue and are ideal for abdominal wall reconstruction in rabbits followed by fresh swim bladder graft. However the fresh swim bladder graft has the drawbacks of complications in wound healing, more adhesion formation, more tissue reaction

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REFERENCES

Armour A, J Fish, K Woodhouse and J Semple, 2004. A comparison of human and porcine acellularized dermis: interactions with human fibroblasts in vitro. Plastic and Reconstructive Surgery, 117: 845-856.

Ayele T, ABZ Zuki, BMA Noorjahan and MM Noordin 2010. Tissue engineering approach to repair abdominal wall defects using cell-seeded bovine tunica vaginalis in a rabbit model. Journal of Materials Science: Materials in Medicine, 21: 1721-1730.

Bader A, T Schilling, OE Teebken, G Brandes, T Herden and G Steinhoff, 1998. Tissue engineering of heart valves: human endothelial cell seeding of detergent acellularized porcine valves. Eur J Cardio-Thoracic Surg, 14: 279-84.

Baptista ML, ME Bonsack, I Felemovicius and JP Delaney, 2000. Abdominal adhesions to prosthetic mesh evaluated by laparoscopy and electron microscopy. J Amer Coll Surg, 190: 271-280.

Becker JM, MT Dayton and VW Fazio, 1996. Prevention of postoperative abdominal adhesions by a sodium hyaluronate based bioresorbable membrane: a prospective, randomized, double-blind multicenter study. J Amer Coll Surg, 183: 297-306.

Browder W, D William and P Lucore, 1988. Effect of enhanced macrophage function on early wound healing. Surg, 104: 224.

Brown GL, JD Richardson, MA Malangoni, GR Tobin, D Ackerman and HC Polk, 1985. Comparison of prosthetic materials for abdominal wall reconstruction in the presence of contamination and infection, Annals surg, 201: 705-711.

Di Zerega GS, 1997 Biochemical events in peritoneal tissue repair. Eur J Surg, 577: 10-16.

Eberli D, S Rodriguez, A Atala and JJ Yoo, 2010. In vivo evaluation of acellular human dermis for abdominal wall repair. J Biomed Mat Res, 93A: 1527-1538.

Gamba PG, MT Conconi, R Lo Piccolo, G Zara, R Spinazzi and PP Parnigotto, 2002. Experimental abdominal wall defect repaired with acellular matrix. Pediat Surg Inter, 18: 327-331.

Gangwar AK, AK Sharma, N Kumar, N Kumar, SK Maiti, OP Gupta, TK Goswami and R Singh, 2006. Acellular dermal graft for repair of abdominal wall defects in rabbits. J the South Afric Vet Assoc, 77: 79-85.

Grauss RW, MG Hazekamp, F Oppenhuizen, CJ van Munsteren, AC Gittenberger-de Groot and MC DeRuiter, 2005. Histological evaluation of decellularized porcine aortic valves: matrix changes due to different decellularization methods. Eur J Cardio-Thoracic Surg, 27: 566-571.

Holmdahl L and ML Ivarsson, 1999. The role of cytokines, coagulation, and fibrinolysis in peritoneal tissue repair. Eur J Surg, 165: 1012-1019.

Judge TW, DM Parker and RC Dinsmore, 2007. Abdominal wall hernia repair: A comparison of sepramesh and parietex composite mesh in a rabbit hernia model. J Amer Coll Surg, 204: 276-281.

Kumar N, AK Sharma, GR Singh and OP Gupta, 2002. Carbon fibre and plasma preserved tendon allografts for the gap repair of flexor tendon in bovines: Clinical, radiological and angiographical observations. J Vet Med Series, A49: 161-168.

Lai PH, Y Chang, SC Chen, CC Wang, HC Liang, WC Chang and HW Sung, 2006. Acellular biological tissues containing inherent glycosaminoglycans for loading basic fibroblast growth factor promote angiogenesis and tissue regeneration. Tissue Eng, 12: 2499-2508.

Leek RD, CE Lewis, R Whitehouse, M Greenall, J Clarke and AL Harris, 1996. Association of macrophage infiltration with angiogenesis and prognosis in invasive breast carcinoma. Cancer Res, 56: 4625-4629.

Liang H, Y Chang, C Hsu and M Lee, 2004. Effects of cross linking degree of an acellular biological tissue on its tissue regeneration pattern. Biomater, 25: 3541-3552.

Milburn ML, LH Holton, TL Chung, EN Li, G Bochicchio, NH Goldberg and RP Silverman, 2008. Acellular dermal matrix compared with synthetic implant material for repair of ventral hernia in the setting of peri-operative Staphylococcus aureus implant contamination: A rabbit model. Surg Infec, 9: 433-42.

Mustoe RA, GF Pierce, A Thomason, P Gramates, MB Sporn and TF Deuel, 1987. Accelerated healing of incisional wounds in rats induced by transforming growth factor-beta. Sci, 237: 1333.

Nair SK, IK Bhat and AL Aurora, 1974. Role of proteolytic enzyme in the prevention of postoperative intraperitoneal adhesions. Arch Surg, 108: 849-853.

Ray NF, JW Larsen, RJ Stillman and RJ Jacobs, 1993. Economic impact of hospitalizations for lower abdominal adhesiolysis in the United States in 1988. Surg Gynecol Obstet, 176: 271-276.

Schmidt CE and JM Baier, 2000. Acellular vascular tissues: Natural biomaterials for tissue repair and tissue engineering. Biomater, 21: 2215.

Schneider A, J Bennek, K Olsen, J Weiß, W Schmidt and U Rolle, 2006. Experimental study evaluating the effect of a barrier method on postoperative intraabdominal adhesions. Diges Dis Sci, 51: 566-570.

Silver IA, 1982. Basic physiology of wound healing in horse. Equine Vet J, 14: 7-15.

Singh J, N Kumar, AK Sharma, SK Maiti, TK Goswami and AK Sharma, 2008. Acellular biomaterials of porcine origin for the reconstruction of abdominal wall defects in rabbits. Trends in Biomater Artific Org, 22: 34-44.

Singh UB and S Sulochana, 1997. A practical manual of histopathological and histochemical techniques, Kothari Publications, Bombay.

Thompson J, 1998. Pathogenesis and prevention of adhesion formation. Diges Surg, 15: 153-157.

Tuzuner A, MA Kuzu, B Akin, S Karaca and S Hazinedaroglu, 2004. The effect of hyaluronan-based agenst on adhesion formation in an intraabdominal sepsis model. Diges Dis Sci, 49: 1054-1062.

Vegad JL, 1995. Textbook of Veterinary General Pathology. Vikas Publishing House Pvt. Ltd. Jangpura, New Delhi.

Werkmeister JA, GA Edwards, F Casagranda, JF White and JAM Ramshaw, 1998. Evaluation of a collagen-based biosynthetic material for the repair of abdominal wall defects. J Biomed Mat Res, 39: 429-436.