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Clinical application of animal based extracellular matrix in hernioplasty

Abstract

The structural and functional molecules of ECM are capable to establish proper attachment or communication with an adjacent environment of healing area of injury. Bovine pericardium has already been used for soft tissue, spinal, cranial, blood vessels (arms, legs and neck) and pericardial soft tissue repair. On the basis of the voluntary consent of owner nine animals were selected for this study. All the reconstructive surgeries were done with the routine surgical procedure with the use of bioengineered scaffolds. Post-operative of all animals was represented successful augmentation of reconstructive procedures with this newer approach.

Keywords: Collagen, caprine, bubaline and hernia, Pericardium, tissue reconstruction, cranial, blood vessels, ruminal, decellularization, Hernial ring, scaffolds, coccygeus muscles, aetiology, decellularization, endothelial proliferation

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Introduction

Biological scaffolds are composed of natural ECM (extracellular matrix) due to their origin from animal-based proteinous molecules; they have efficient in the potential of therapeutic applications. The ECM has full potential which increases constructive remodeling. The structural and functional molecules of ECM are capable to establish proper attachment or communication with an adjacent environment of healing area of injury. Their biocompatibility is responsible for positive signaling to recipient own matrix formation.[1] The ECM degradation with the promotion of native matrix is another property which makes it an ideal biomaterial.[2]

Various forms of intact ECM have been used in tissue reconstruction *i.e.* small intestine submucosa, skin, liver, pancreas and urinary bladder.[3] Many of these ECM have been commercialized for various therapeutic applications. Here, we have prepared ECM from forestomach components, pericardium, diaphragm and aorta of Indian buffalo (Bubalus bubalus) and forestomach of goat (*Capra hircus*). The ECM based biomaterial have all component *i.e.* collagen, fibronectin, vitronectin, laminin and hyaluronic acid, which facilitate cell adhesions and proliferation during the process of new tissue formation or healing cascade.[4] Bovine pericardium has already been used for soft tissue, spinal, cranial, blood vessels (arms, legs and neck) and pericardial soft tissue repair. Pericardium available in the market as Veritas, Dura- Guard, Vascu Guard and Peri Guard as dry and hydrated form respectively.

The bio-inductive properties of natural ECM facilitate reconstruction following in vivo transplantation due to the presence of various cell adhesive (fibronectin and vitronectin), visco-elastic and laminin for endothelial proliferation.[5] Angiogenesis host cell infiltration, mitogenesis and organization of new host ECM are the processes, responsible for the reconstructive outcome.[6]

Materials and methods

Bubaline and caprine based native tissuesa of forestomach, pericardium, diaphragm and aorta collected from the institutional and local abattoir and immediately preserved in chilled 1xphosphate buffer saline (PBS), (pH 7.4) solution containing 0.1% amikacin and 0.02% EDTA. Ruminal and other tissues were thoroughly washed with PBS to remove adhered debris. Then the tissues were cut into $5^{x}5$ cm pieces. Serosa and muscular layer of rumen were peeled off remaining reticular submucosa used as ECM. ECM of forestomach, pericardium, diaphragm and aorta were decellularized using 1% solution of sodium deoxycholate at room temperature in the wrist action shaker. A detergent solution of sodium deoxycholate was changed every 12 hours in the flasks. The degree of decellularization was evaluated by microscopic examination. DNA quantification and protein content of samples were done for native versus 24, 48, 72 and 96 hours.

Prepared ECMs have thoroughly washed in phosphate buffered saline (PBS) solution after completion of the protocol. The prepared acellular matrices derived from different animal tissues were stored at -20.0 C in PBS solution containing 0.1% amikacine till further use. The samples of native and processed ECM were fixed in 10% formalin for histological examination and mechanical tissue mincer was used to triturate tissues for DNA and protein analysis. All the ECM prepared was subjected to ethanol sterilization and PBS preservation a day before use. (Table 1) Volunteer animal owners documented their consent in Referral Veterinary Polyclinic, Indian Veterinary Research Institute, Izatnagar, Bareilly, was selected for above procedures.

All animal were subjected to 12 hours fasting, standard surgical site preparation and techniques, sites were prepared aseptically after clipping of hairs and topical application of povidone-iodine paint at the operative site. The anaesthetic regimen was opted for the different animal as per the following. (Table 2)

The surgical procedure, all animal were restrained on the operation table in standard operative manner and operated with suitable surgical techniques. Hernial ring exposed by incising skin with BP blade and subcutaneous tissues bluntly separated by mayo scissors and/or fingers avoiding any damage to underlying structures (hernia content). All adhesions were removed delicately and haemostasis was achieved by placing artery forceps. After reducing hernial content, the hernial

ring was felt and fresh with use of scissors or blade. ECM based scaffolds (ethanol dried and PBS hydrated) were used in appropriate size and shape as required. Scaffold reinforcement of the hernial ring was done in a different way like onlay / inlay to provide mechanical neutralization at the hernioplasty site. Suture material type and size was also selected as per mechanical stress (polyamide and/or PGA). Subcutaneous tissues apposition were achieved using PGA suture of desired sized as per standards. Skin apposition was done by polyamide suture ranging from No. 1 to 0 and removed undulating skin folds.

All animals were subjected to an antibiotic (cefotaxime) for 12 to 15 days post-operatively and analgesics (meloxicam) for 3 days post-operatively. The antiseptic dressing of surgical wounds was done up to the removal of skin sutures. Out of nine, six animals were recovered uneventfully on day 15. Swelling around the operative sites was recorded in two animals (calves) between 3- 6 days postoperatively, which were subsided on day 9-10 completely. One animal piglet was reported with dehiscence on day 3, were subjected to re-operation and the wound was healed on day 17.

Table 1 For clinical application, out of different ECM based scaffolds rumen, omasum, pericardium, diaphragm and aorta were used for hernioplasty as given below.

s.no.	**Patients description**			**Hernia ring diameter (fingers)**	**Type of hernia**	**ECM used as scaffold**
	Animal	**Age**	**Sex**			
1	piglet	3 month	M	3	Umbilical	Diaphragm
2	piglet	3.6 month	M	4	Umbilical	Rumen caprine
3	piglet	4.5 month	M	4	Umbilical	Rumen buffalo
4	Bovine calf	8 month	F	5	Ventro-lateral	Rumen buffalo
5	buffalo	1 year	F	3	Umbilical	Rumen caprine
6	calf kid	4 month	F	6-May	Ventral	Rumen caprine
7	canine	1 year	M	4-Mar	Ventral	aorta
8	canine	7 year	M	3	Perineal	Diaphragm
9	canine	8 year	M	3	Perineal	Pericardium

Table 2 animal as per the following table.

S no.	**Animal species**	**Premedication**	**Induction local/ analgesia**	**Maintenece**
1	Swine	xylanine@2 mg/kg B.Wt. IM	Ketamine @ 10-15 mg/kg B.Wt., IM plus local infiltration of 2% lignocaine hcl at site of operation	Ketamine@3 mg IV SOS
2	Bovine/bubalione and caprine	Midazolam@0.5 mg/kg B.Wt., IV	Local infiltration of 2% lignocaine hcl at site of operation	
3	Canine	Atropine@0.04 mg/kg B.Wt., IM followed by butorphenol@0.2 mg/kg B.Wt., IV and Diazepam @ 0.5 mg/kg	Thiopentone sodium @ 10 mg/kg B.Wt., IV	2% isoflurane

Results and discussion

Piglets having umbilical hernia were due to congenital malformation and improper apposition of umbilical ring and presence of remnants of umbilical cord may cause interference in cicatrization of the ring after furrowing. All the animals repaired for an umbilical hernia by using biomaterials were showing after 18 days post-operatively. In case of the bovine calf, one case may be due to blunt injury but another of an umbilical hernia have similar aetiology as the piglet. In kid, the hernia due to accidental injury all case showed complete apposition except one calf have swelling at repairing site of an umbilical hernia may be due to infection or self-mutilation

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against the floor, which took longer time (24 day) in comparison of 12-16 days of other cases. In canines, a perineal hernia may be due to the weakening of perineal muscles and due to constipation and difficult micturition get weakened coccygeus muscles. After surgical correction, all wounds were repaired uneventfully.

Collagen scaffolds retained their critical components both structural and functional to truly recapitulate native tissue. A number of collagen-based biomaterials developed during this era of advancement in biomedical engineering. The pioneer work of Badylak and co-workers on the development of intact and native ECM biomaterials, demonstrated the biochemical and in vivo benefits of native ECMs, over reconstituted collagen products as reported by Badylak et at[7], Simcock[8] was used ovine forestomach matrix as a substrate for single-stage split-thickness skin repair with successful reconstructive results. Successful augmentation of the tendon of the rotator cuff with OFM was reported by Street et al.[9]. The smooth surfaced ECM prosthesis should have acted as a barrier creating a peritoneal interface and should have prevented further adhesion formation and facilitating a rapid formation of complete mesothelial lining to the prosthetic mesh as per Pascual et al[10],Remya[11] also reported successful augmentation of full-thickness abdominal wall repair with less adhesion in their experimental studies. (Figure 1and 2)

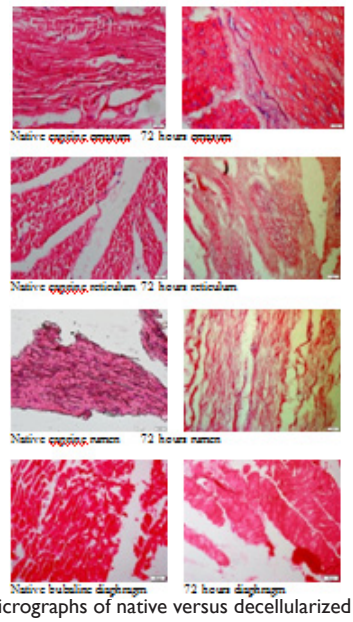

Figure 1 Photomicrographs of native versus decellularized scaffolds from different collagenouns animal tissue.

Figure 2 Photographs of pre, para and completion of surgical procedures.

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Conflict of interest

There is no conflict of interest

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None

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