# BIOMIMETIC DERMOID GRAFTS WITH ALLOGENIC BIOACTIVITY: AN INNOVATIVE APPROACH FOR HERNIA REPAIR IN BUFFALOES

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#### **ABSTRACT**

Hernia development is a common abdominal wall condition observed in calves, frequently manifesting in the umbilical region. This innovative biological dermoid scaffold is considered superior because of their inherent ability to combat infections through the release of antimicrobial peptides and non-complement fixing antibodies.

The Division of Surgery at ICAR-IVRI, Izatnagar, has pioneered the development of bioinspired-bioactive dermoid scaffolds, as an initiative aimed at addressing hernias in large animals. The *in vitro* evaluation of the scaffold included crucial parameters such as H and E staining, SEM evaluation, and scaffold-cell interaction. Histocompatibility, cyto-toxicity, cell attachment, and proliferation of the seeded scaffolds were assessed using DAPI staining, along with an examination of porosity and density.

All cases share a common history of a large swelling in the abdominal region. Physical examination, palpable hernial ring was identified followed by USG examination leading to the diagnosis of a large ventral hernia. Hematobiochemical analysis indicated a slight

metabolic alkalosis, along with mild anaemia and azotaemia. Subsequent exploratory laparotomy was performed, to address the large abdominal defect, we decided to repair it using an allogenic dermoid graft in an overlaid fashion. Postoperative care included a 7-day course of antibiotics, analgesics, and antihistamines. All buffaloes in the study exhibited uneventful recovery, with no recurrence observed during the 5-month follow-up period.

No reports on treatment of large abdominal wall defects with dermoid graft reported, which prevent reoccurrence of hernia. This study marks for the first time, where we outline the successful closure of a hernia in clinical cases.

**Keywords**: *Bubalus bubalis*, buffaloes, dermoid graft, allogenic, hernia, laparotomy, herniorrhaphy

#### INTRODUCTION

Protrusion of the body cavity contents through an opening in the wall of that cavity is defined as a hernia (Mohsina, 2014). Abdominal wall hernias or defects are characterized by loss of tissue function and structure in the load bearing

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muscle, fascia layer and tendon (Franz, 2008). The aetiologies of hernia may be congenital or acquired in origin. Umbilical hernia is the most prevalent congenital hernia in calves, which can affect any breed and is more common in females than males (Doijode, 2019). Inguinal and ventral hernias are more observed in adult animals (Abdin-Bey and Ramadan, 2001). Acquired hernias are introgenic or traumatic in origin (Pratschke, 2002). Abdominal defects caused by horn injuries from other animals is one of the main causes of acquired hernias in large animals (Al-Sobayil and Ahmed, 2007). Abdominal wall defects in large animals leads to morbidity, low production, poor performance and infertility. Recurrence of hernia and an increase in hernial ring diameter were found to be directly related to body weight in calves (Jaman, 2018). Owing to large size of the animals and massive volume of abdominal viscera, reconstruction of the abdominal wall using tension sutures has resulted in wound dehiscence and recurrent hernia. Therefore, repair of such hernias with large tissue defects, requires a tension free closure using a tissue substitute possessing high mechanical strength (Ober et al., 2008).

Conventional methods can be used to treat small hernial rings with reducible hernial contents while repair of large abdominal wall defects in animals is difficult due to increased hernial ring size and margin distortion. Frequent recurrence occurs in these cases because of compromised closure due to lack of tissue at the site (Mohsina *et al.*, 2014). Severe tension on the suture line and the existence of weaker tissue around the hernial ring, result in failure of hernia repair (Abdin-Bey and Ramadan, 2021). In such situations, special techniques, such as hernioplasty with biomaterial implantation, can be used (Mohsina *et al.*, 2014).

Surgical repair of hernias using a mesh

has become common procedure worldwide in the last few years, as this has been shown to lower the rate of recurrence (Falagas and Kasiakou, 2005). Placement of meshes may be intra or extra peritoneally, and the latter can be used either as outlay or inlay. Physical pressure between the layers of the abdominal wall, suturing with absorbable or non-absorbable material, or application of fibrin glue can all be used to secure prosthetic material. The inlay technique has the advantage of causing less dissection of soft tissue, resulting in less devascularised tissue, however it is also associated with a high recurrence rate. Whereas the disadvantage of the outlay technique is the substantial soft tissue dissection (Mohsina et al., 2014).

An ideal biomaterial graft has been proven to be able to withstand bacterial infection, provide a scaffold for tissue regeneration, yield better tissue integration, and degrade completely over time (Bellows *et al.*, 2007). Xenogenic or allogenic tissues exhibit cross species reactions or antigenicity owing to the cellular contents. Therefore, tissues and organs that have undergone decellularization have been successfully used in a variety of tissue engineering applications (Hezbollahi *et al.*, 2013). Weak antigenicity, as well as outstanding biocompatibility and biodegradability make collagen most practical as a tissue substitute (Mohsina *et al.*, 2014).

A collagenous scaffold provides a structural template for penetration and adherence of cells and new tissue formation (Ghodbane and Dunn, 2016). Various biological materials have been used for the repair of hernial defects including small intestinal mucosa, dermal collagen of porcine origin, tensor fascia lata and full thickness autologous skin and dermis (Mohsina *et al.*, 2014)..

The biogenicity, biocompatibility and non-

toxicity of these grafts have already been evaluated in vitro and in vivo (Bhat et al., 2015). Keeping in view the superior mechanical strength of dermoid scaffolds, it was hypothesized that a graft composed of dermoid tissue incorporated with collagen gel and growth factors would be suitable for the repair of abdominal wall defects, having properties of biocompatibility, enable tissue integration, provide a scaffold for cell attachment and proliferation and provide a source of collagen during the tissue remodelling process. But there have not been any studies so far, where bubaline derived dermoid scaffolds incorporated with collagen gel sheet are used to treat hernial gap defects.

#### MATERIALS AND METHODS

This study was conducted in the Division of Surgery, Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh. The study consists of both *in vitro* and *in vivo* testing of the bubaline derived bioactive-dermoid scaffolds for hernia repair.

#### Decellularization of dermoid tissue

Decellularization of the bubaline dermoid was done as per the standard protocol in DBT laboratory, Division of Surgery, ICAR-IVRI, Izatnagar. The bubaline dermoid tissue was harvested in an aseptic manner from a slaughter house and stored at -40°C until processing. Blood and unwanted debris were first be removed from the samples by washing 3 to 4 times with 1x PBS in aseptic condition. The samples were then subjected to decellularization process involving chemical and physical treatments following standard protocols.

# Preparation of dermoid grafts

The decellularized dermoid grafts were cut into desired size and shape (Figure 1), they were subjected to sterility testing of grafts. Later on, collagen gel sheets were incorporated to scaffolds and graft was ready to use.

# In vitro evaluation of bioinspired-bioactive dermoid scaffolds

#### Histology

A 4% formaldehyde solution was used to fix the dermoid samples for 24 to 48 h at room temperature, after which they were embedded in paraffin, and cut longitudinally into 5  $\mu$  thick sections. Hematoxylin and Eosin staining was done as per the standard procedure.

# Scanning electron microscopy (SEM)

The samples were fixed in 2.5% glutaraldehyde in PBS overnight at 4°C. After removal of fixative, samples were dehydrated in a graded series of ethanol and sputter coated with gold. Samples were observed by SEM (Jeol, JSM-6610LV).

#### Cell-scaffold interaction

Scaffolds (2 to 4 mm in height, 15 mm in diameter) were sterilized by soaking in 70% ethanol aqueous solution prior to introduction of cells. The scaffolds were transferred to 24-well plates and seeded with 1×10<sup>4</sup> cells per sample (suspended in suitable media). Sterile scaffolds (3x2 mm) were transferred to 24-well plates and seeded with 1×10<sup>4</sup> cells per sample suspended in complete media containing 84% DMEM, 15% FBS and 1% Penicillin- Streptomycin (10,000 U/ml; 10,000 µg/ml). The cells that were seeded directly on tissue are observed for cell attachment, spreading and viability were evaluated using fluorescence

microscopy after DAPI staining on day 3, 7 and 14 (Figure 6a, b and c).

# In vivo evaluation of bioinspired-bioactive dermoid scaffolds in clinical case

The prepared dermoid scaffolds were evaluated for hernia repair in 10 different clinical cases of buffalo.

# Haematology

Complete blood count was evaluated before surgery and on day 3, 10 and 20 post operatively. An increase in WBC count helped predict the occurrence of post operative complications after incisional hernia repair. Hematobiochemical analysis revealed slight metabolic alkalosis with mild anaemia and azotaemia was observed.

# Ultrasonographic examination

Ultrasound investigation provides a dependable method for detecting defects in the abdominal wall (Spangen, 1975). Ultrasonographic examination using sector probe at 7.5 to 12 MHz was done prior to surgery to assess the extent of the hernial defect (Figure 2a), and immediately post operative, and on 14 after surgery to assess the extent of healing (Figure 2b), presence of exudation and integrity of the implant.

# Surgical procedure

The animals were appropriately restrained, and the surgical site was shaved and prepared aseptically for surgery (Figure 3 and 4). Followed by local infiltration of 2% lignocaine hydrochloride along with epidural anaesthesia. An elliptical incision was made around the hernial swelling, making sure to avoid abdominal veins. After identification of the hernial ring, the hernial content was reduced into the abdominal cavity. The

abdominal muscles were brought into apposition as much as possible with non- absorbable suture material using a vest-over pant technique. The prepared dermoid scaffolds were secured to the abdominal defect in an outlay pattern using absorbable suture material. The subcutaneous tissue was closed in simple continuous fashion and skin with horizontal mattress.

## Post operative care and management

Ceftriaxone at dose rate of 25 mg/kg body weight was administered for 7 consecutive days along with administration of analgesic meloxicam at dose rate of 0.3 mg/kg body weight for 5 days. Daily antiseptic dressing with povidone iodine solution was done until the incisional wound was completely healed and the skin sutures were removed after 14 day post-operatively.

#### RESULTS

The skin of buffalo procured from slaughterhouse, decellularization protocol was being standardised and optimized by Division of Surgery, ICAR-IVRI, Izatnagar. Skin was subjected to decellularization process and assessed based on H and E staining and SEM evaluation to ensure complete decellularization. The duration of the process was approximately 14 to 21 days because of the incubation periods required to remove the epidermis and detergent buffers. The scaffolds were subjected to appropriate in vitro tests, Histocompatibility, cytotoxicity was assessed upon seeding of cells on scaffold and check for cells attachment, proliferation and migration. Then preevaluated scaffolds were used for in vivo studies on clinical cases, showing profound promising results.

## Histology

Histopathology of dermoid scaffold was done for examining matrix of the samples, to ensure complete decellularization, and to evaluate the quality of the matrix and orientation of collagenous structure of scaffolds (Figure 5). Relative absence of cells in the decellularized dermoid tissue proves the efficacy of the decellularization process. The number of pores in the field for decellularized dermoid graft (Mean ± SEM) obtained was 0.2±1.01 and found to be highly significant when compared to that of native dermoid tissue 38.9±1.05 The structural integrity, crimp, and alignment of the collagen fibers was observed to be retained in the decellularized tissue. The results of the study were similar to those obtained by Bhat *et al.* (2015).

## Scanning electron microscopy

SEM of decellularized dermoid samples was done to evaluate the surface morphology of the matrix and tissue, number of pores and pore diameter (Figure 6). Higher porosity in a scaffold allows sufficient nutrient and gas exchange, number of pores per field was found to be significantly higher (Mean  $\pm$  SEM) 76.2 $\pm$ 4.58 in dermoid scaffold when compared to native scaffold  $30.2\pm2.44$ . The pore diameter was found to be ranging from  $1.89\pm0.52$  to  $9.76\pm0.91$  which was highly significant than native dermoid scaffold, ranging from  $1.46\pm0.07$  to  $2.66\pm0.32$ .

#### **Cell-scaffold interaction**

4′,6-Diamidino-2- phenylindole (DAPI) staining is done to visualise the cellular nucleus. DAPI images reveal that canine adipose derived stem cells were able to attach and proliferate on the scaffold. The growth of cells on dermoid scaffold was evaluated on 3,7 and 14 days (Figure 7), (Mean  $\pm$  SEM) was 30.3 $\pm$ 1.4, 78.6 $\pm$ 1.9 and 126.5 $\pm$ 2.6

respectively.

#### **DISCUSSION**

Abdominal wall hernias or defects are characterized by a loss of tissue function or tissue structure at the load bearing muscle or fascia layer. The underlying biologic processes are muscle layer weakness leading to recurrence of hernia and wound dehiscence (Franz, 2008). Dermoid tissue in turn promotes collagen deposition at defect site which forms a main constituent of the ECM and prevents recurrence (Jansen *et al.*, 2004).

Treatment of hernias depend upon the size and severity of the abdominal wall defect (See et al., 2020). Surgical repair of abdominal wall defects has developed over the years. Smaller abdominal wall defects were previously repaired by simply apposing muscles with suturing. These sutures are to be placed with enough strength to ensure a secure closure, without affecting the normal function of the abdominal wall (Pratschke, 2002) An increase in hernial ring diameter was found to be directly related to body weight in large animals (Jaman et al., 2019). Reapproximating muscle fascia is difficult in cases of large hernias and when done, tension closure leads to an increase in intra-abdominal pressure, predisposing to hernia recurrence due to wound dehiscence (Eskandaros and Darwish, 2017; Ober et al., 2008). A tension free closure of the hernial ring is therefore essential (Ober et al., 2008). Tension may be overcome by using muscle or fascial flaps. However, if these techniques prove to be insufficient or unfeasible, hence prosthetic implants are to be used (Pratschke, 2002). It is associated with lesser rate of recurrence. but increased complications such as infection and discomfort (Mohsina et al., 2014).

An ideal graft for hernia should theoretically a) be strong enough to withstand physiologic stresses, b) conform to abdominal wall, c) promote strong ingrowth of host tissue, d) resist adhesion formation, e) resist infection, f) should be non-antigenic and g) non carcinogenic (Robinson et al., 2005). Biological graft materials are superior than synthetic materials due to the ability to fight infection by the liberation of antimicrobial peptides, and non-complement fixing antibodies. They also promote host cell migration and stimulate angiogenesis. Various disadvantages however, include their high cost, rapid biodegradation, and graft loss in infected sites (Bellows et al., 2007). When compared to synthetic materials, biologic material trigger a less inflammatory response and are more biocompatible, but have a good mechanical strength (Zhou et al., 2021).

The ultimate aim of tissue scaffolding strategies is to mimic the actual microenvironment that is the ECM. Placement of biological grafts may be intra or extra peritoneal, and the latter can be used either as outlay or inlay (Mohsina et al., 2014). Outlay technique involves placement of the scaffolding material between the muscle and the subcutaneous tissue, while inlay technique is when the graft is secured in between the edges of the abdominal defect, creating a bridge. Sublay implantation involves placement of the graft below the muscle layer (See et al., 2020). Physical pressure between the layers of the abdominal wall, suturing with absorbable or nonabsorbable material, or application of fibrin glue can all be used to secure the prosthetic material to the abdominal wall. Inlay implantation is associated with less dissection of soft tissue, resulting in less devascularized tissue, but it also associated with a high recurrence rate (Mohsina et al., 2014).

The presence of micropores (<10 µm) in a

biomaterial is essential for, cell attachment and mobility which create a greater surface area on the scaffold (Silvipriya et al., 2015). It stimulates ion exchange and protein adsorption and faster tissue ingrowth (Aldridge and Simson, 2001). It favors deposition of invading tissue elements, whereas synthetic meshes have absence of micropores (Fang and Lake, 2017). The use of surgical meshes has been proved to be efficient in repair of large hernial defects. The purpose of these scaffolding materials was to strengthen and replace tissue for stabilization of the abdominal wall and allow a tension- free repair that facilitates the integration of fibro-collagenous tissue, thereby strengthening the weakened area (Baylón et al., 2017). Porosities of 50 to 80% are suitable for cell attachment and neovascularization whereas, biologic scaffolds with porosity of 34.4% enabled invasion of fibroblasts (Lee et al., 2001). collagen scaffolds with 79% porosity- for hernia repair.

Dermoid scaffold when used as a porous matrix it exhibited a high cytocompatibility to support the growth and performance of various types of cells and chemotactic in nature, promote cellular motility, and migration of fibroblasts in the fibrillar scaffold, similar to the studies of Mohsina et al. (2014) and also found that, growth factors trapped within the natural ECM supply sufficient stimuli to hasten cell differentiation. After implantation of a biological scaffolds, proteins such as albumin, immunoglobulin G and fibrinogen surround the mesh and are involved in the inflammatory process along with PMN cells, macrophages, monocytes and platelets (Elango et al., 2014). The triple helical structure of collagen induces cell signalling pathways by interacting with cell receptors, integrins and other signalling agents (Elango et al., 2014). Scaffolds after decellularization have increased number of exposed ligand binding sites



Figure 1. Decellularized dermoid graft.



Figure 2a. Presence of intestines in hernial sac.



Figure 2b. Closure of defect wall 14th day post-operation.



Figure 3. Surgical procedure for case I (ventral hernia).

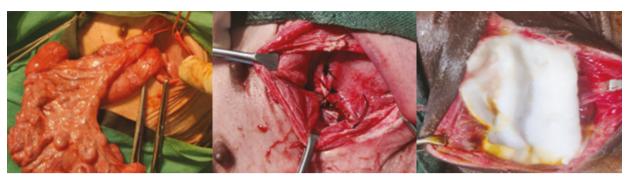


Figure 4. Surgical procedure for case II (Inguinal hernia).



Figure 5. Acellular scaffolds with tightly packed collagenous structure.

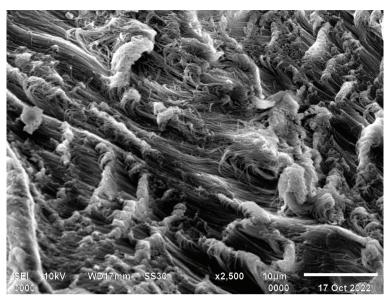


Figure 6. Closely aligned collagenous fibres with presence of pores with increased diameter.

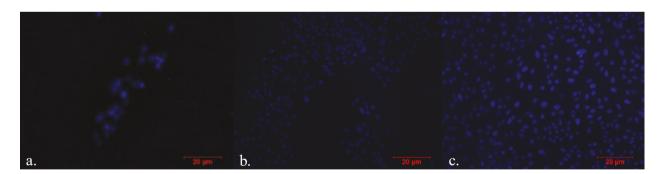


Figure 7. DAPI staining at a.) Day 3; b.) Day 7 and c.) Day 14.

Table 1. In vitro evaluation of scaffolding material.

P	Parameters	Native dermoid (Mean ± SEM)	Decellularized dermoid graft (Mean ± SEM)
Histology	Number of cells/Field	$38.9 \pm 1.05$	$0.2{\pm}1.01*$
SEM confined	Number of pores/fields	$30.2\pm2.44$	76.2±4.58*
SEM evaluation	Pore diameter	$1.46\pm0.07 - 2.66\pm0.32$	$1.89\pm0.52-9.76\pm0.91*$
Coeffeld 2011	Day 3	-	30.3±1.4
Scaliblu-cell	Day 7	-	78.6±1.9*
IIICIACIIOII	Day 14	ı	126.5±2.6**

\* and \*\* = indicates mean value differs significantly at P<0.05.

Table 2. In vivo application on clinical cases.

S. No.	Breed	Age	Sex	Hernia type	Hernial ring diameter in centimeters	Size of dermoid graft
1	Non-descript	1 year	Male	Ventral	8 cm	10.5x10
2	Bhadawari	6 months	Male	Umbilical	6 cm	13x7.5
3	Bhadawari	8 months	Female	Umbilical	6 cm	13x7.8
4	Non-descript 1.5 years	1.5 years	Male	Ventral	10 cm	11x11
5	Bhadawari	9 months	Male	Umbilical	8 cm	10x9.5
9	Non-descript 5 months	5 months	Male	Umbilical	7 cm	11x8
7	Bhadawari	6 months	Female	Ventral	6.8 cm	6.5x7
∞	Non-descript 1.8 years	1.8 years	Female	Ventral	15 cm	16x10
6	Non-descript	1 years	Male	Ventral	12.9 cm	13x10.5
10	Non-descript 1.2 years	1.2 years	Male	Inguinal	14 cm	13x13

and growth factors (Sackett *et al.*, 2018). Growth factors, namely, platelet derived growth factor, fibroblast growth factor, transforming growth factor  $\beta$ , insulin- like growth factor and epidemic growth factor are activated, which play a major role in hernia repair by acting as chemotactic agents, promoting fibroblast proliferation and extracellular matrix protein production (Liu *et al.*, 2021).

Tissue engineering encompasses the utilization of scaffolds, cells, growth factors, and various biochemical and physiochemical factors to design materials suitable for use as scaffolds. The ultimate aim of tissue scaffolding strategies is to mimic the actual 3D microenvironment that is the ECM results in replacing the biological tissue. The Bioinspired-Bioactive dermoid scaffold emerges as a promising alternative to synthetic mesh, exhibiting optimal characteristics such as biomimetic to extracellular matrix of host tissue and offering favourable biomechanical properties. This scaffold facilitates effective processes like cell seeding, attachment, proliferation, and migration, contributing to the remodelling of host tissue. Furthermore, it maintains adequate mechanical strength to endure the load imposed by abdominal organs.

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