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Harendra Singh Rathore
 Division of Surgery, ICAR-
 Indian Veterinary Research
 Institute Izatnagar, Uttar
 Pradesh, India

PDS Raghuvansi
 Division of Surgery, ICAR-
 Indian Veterinary Research
 Institute Izatnagar, Uttar
 Pradesh, India

Deepesh Gautam
 Division of Surgery, ICAR-
 Indian Veterinary Research
 Institute Izatnagar, Uttar
 Pradesh, India

Divya Mohan
 Division of Surgery, ICAR-
 Indian Veterinary Research
 Institute Izatnagar, Uttar
 Pradesh, India

Ajit Kumar Singh
 Division of Surgery, ICAR-
 Indian Veterinary Research
 Institute Izatnagar, Uttar
 Pradesh, India

Anuj Pratap Singh
 Division of Surgery, ICAR-
 Indian Veterinary Research
 Institute Izatnagar, Uttar
 Pradesh, India

SK Maiti
 Division of Surgery, ICAR-
 Indian Veterinary Research
 Institute Izatnagar, Uttar
 Pradesh, India

Naveen Kumar
 Division of Surgery, ICAR-
 Indian Veterinary Research
 Institute Izatnagar, Uttar
 Pradesh, India

Correspondence
Naveen Kumar
 Division of Surgery, ICAR-
 Indian Veterinary Research
 Institute Izatnagar, Uttar
 Pradesh, India

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Use of the bubaline acellular diaphragm matrix (ADM) for repair of abdominal wall defects in four different species of animals

Harendra Singh Rathore, PDS Raghuvansi, Deepesh Gautam, Divya Mohan, Ajit Kumar Singh, Anuj Pratap Singh, SK Maiti and Naveen Kumar

Abstract

Decellularization of xenogenic biomaterials has been highly desired for implantation without considerable adverse inflammatory and immune responses. The present study was undertaken to decellularize the diaphragm of buffalo origin and to evaluate the efficacy of this acellular diaphragm matrix (ADM) for the repair of abdominal wall defects. Buffalo diaphragm was decellularized with 1% sodium deoxycholate (SDS) for 48 h. Microscopic examination of the detergent treated matrix confirmed complete extraction of stromal cells and orderly arranged collagen fibres. Prepared ADM scaffolds were used for repair of abdominal wall defects in four different species of animals. The abdominal wall defects repaired with ADM remained sound over a period of 3 months. All the defects repaired with ADM healed completely without graft rejections. The present study suggested that ADM may be used safely for repair of abdominal wall defects in different species of animals.

Keywords: abdominal wall defect, acellular diaphragm matrix, buffalo, cattle, goat, pig

Introduction

Surgical repair of large abdominal wall defects, such as ventral hernia, is a clinical challenge for surgeons. In most of the cases, these defects have resulted from trauma or weakness of abdominal musculature. Tight suturing to approximate and close the defect can lead to wound dehiscence, recurrent hernias and non-healing of the wound (Ober *et al.*, 2008) [18]. The use of prosthetic material for hernioplasty is required when the hernial ring size exceeds 3 cm in diameter (Vilar *et al.*, 2011) [22]. Although several repair techniques have been described, the use of nonabsorbable synthetic mesh material to achieve a tension-free closure of these abdominal wall defects is the most widely used reconstructive technique (Bellows *et al.*, 2008) [1]. The use of nonabsorbable synthetic mesh material has been reported to cause complications such as mesh extrusion, bowel adherence, fistula formation, wound infection, skin erosion and seroma development (Falagas and Kasiakou, 2005) [6]. To overcome the disadvantages of synthetic meshes, biological biomaterials may be preferable for the surgical repair of hernias (Wietfeldt *et al.*, 2009) [24]. Biological biomaterials have distinct advantages over synthetic mesh materials for the repair of abdominal wall defects, owing to their ability to resist infection, to minimize adhesion formation, to provide better scaffold for fibroblast proliferation, and neovascularization (Clarke *et al.*, 1996) [2]. Recently, acellularized biomaterials, composed of an extracellular matrix (ECM), harvested from a variety of allogenic and xenogenic tissues, have been utilized in an effort to address some of the limitations associated with synthetic materials (Franklin *et al.*, 2008; Kumar *et al.*, 2012; KUMAR *et al.*, 2013, a, b, c, d) [7, 11-15]. The potential benefits of biological materials include improved infection resistance, host tissue ingrowth, and less adhesion formation (GAERTNER *et al.*, 2007) [8]. However, in their native form biomaterials tend to be immunogenic and hence are decellularised to minimise their immunogenicity (Gilbert *et al.*, 2006) [9]. Gulati and COLE (1994) [10] observed less immunogenicity and better tolerance of acellular grafts in rats and rabbits.

Although the results of the preclinical animal study have been promising, studies on the use of acellular diaphragm matrix grafts for the repair of hernias in clinical situations are not available.

Therefore, the present study was undertaken to acellularize diaphragms of buffalo origin and describes the successful use of acellular diaphragm matrix (ADM) for the reconstruction of large abdominal wall defects in four different species of animals, a new method for treating large hernias in animals.

Materials and Methods

All the chemicals used in this study were purchased from Sigma (St Louis, MO, USA), unless otherwise indicated.

Harvest and preparation of the acellular diaphragm matrix (ADM).

Fresh diaphragms of water buffalo (*Bubalus bubalis*) origin were collected from a local abattoir and immediately preserved in chilled (4 °C) sterile 1x phosphate buffered saline (PBS, pH 7.4) containing 0.1% amikacin (Mikacin, Aristo Pharmaceuticals Private Limited, Mumbai, India), and 0.2% ethylenediaminetetraacetic acid (EDTA). The tendinous portion of each diaphragm was excised and washed thoroughly with sterile PBS to remove all the adherent blood. After the initial washing, the diaphragm was cut into the desired size and then placed in 1% sodium deoxycholate (SDS) solution at a constant temperature (37 °C) on an orbital

shaker under physical agitation (180 rotations/minute) for 48 h. Tissue sample was collected, fixed in 10% neutral buffered formalin, serially dehydrated with ethanol, cleared in xylene and embedded in paraffin wax. Sections (5µm) were cut and stained with hematoxylin-eosin for histological analysis, to check the acellularity (Fig. 1, a, b). The resulting ADM was rinsed with PBS to remove the residual chemicals. Further, the prepared matrices were stored in a sterile PBS solution, containing 0.1% amikacin and 0.1% sodium azide at -20°C until use. The present study was in full compliance with the Institutional Animal Ethics Committee, ICAR-Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, India.

Animals

The present study was conducted on three client owned animals that were presented to the Surgery Unit of the Referral Veterinary Polyclinics, ICAR-Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, India. One animal (pig) was belonged to Institute Farm. Physical examination in each case revealed a painless, reducible soft swelling. At the time of presentation of the animals, heart and respiratory rates and temperatures were within normal reference ranges. The details are presented in table 1.

Table 1: Showing details of the clinical cases repaired with ADM

Case no.	Species	Age	Sex	Size of defect	Reconstruction of defect	Material used	Outcome
1	Buffalo calf	One year	Male	10 cm	Ventral abdominal hernia	Bovine diaphragm	cured
2	Cow calf	Six months	Male	8 cm	Lateral abdominal hernia	Bovine diaphragm	cured
3	Goat	Six months	Female	5 cm	Lateral abdominal hernia	Bovine diaphragm	cured
4	Pig	Six months	Male	8 cm	Umbilical hernia	Bovine diaphragm	cured

Surgical Procedure

The animals were fasted for 24h and deprived of water for 12h prior to anaesthesia. Each animal (ruminant) was premedicated with meloxicam (0.5 mg/kg IM) and ceftriaxone sodium (10 mg/kg IM) 1h prior to surgery, and sedated using 2% xylazine hydrochloride (0.1 mg/kg IM). Circular infiltration analgesia surrounding the hernia was performed using 2% lignocaine hydrochloride. In pig a pre-operative intramuscular injection of 25 µg/kg atropine sulfate (Atropine Sulphate, Morvel Laboratories Private Limited, Mehsana, India) and an intravenous injection of 4.0 mg/kg xylazine HCl (Xylaxin, Indian Immunologicals Limited, Hyderabad, India) was given. General anaesthesia was induced with an intravenous injection of 8 mg/kg ketamine HCl (Aneket, Neon Laboratories Limited, Thane, India) and maintained with repeated boluses of xylazine HCl and ketamine HCl, as per need. To expose the abdominal wall defect (hernial sac) a fusiform skin incision was made that spanned the length of the hernia and extended 2 cm beyond the cranial and caudal margins of the hernial ring. The hernial sac was dissected from overlying skin and the dissection was continued laterally to expose the hernial ring. The hernial sac was opened and an appropriately sized piece of AAM graft was placed and sutured as inlay graft with synthetic absorbable suture material. The abdominal wall muscles and sub-cutis was sutured in a continuous fashion with chromic catgut and skin was sutured with polyamide by horizontal mattress sutures. While the graft was being implanted, the surgical site was lavaged periodically with sterile PBS containing 0.1% amikacin. After recovering from anaesthesia, the ventral abdomen of each animal was moderately compressed with a sterile bandage to protect the surgical site from the external environment and to minimise postoperative oedema. Postoperative analgesia was provided by meloxicam (0.2

mg/kg IM, once daily) for 3 days. Ceftriaxone (10 mg/kg IM, twice daily) was administered for 5 days. The bandage was replaced every day (for 12 days) and the suture line was dressed daily with diluted povidone iodine solution. Skin sutures were removed on postoperative day 12. To assess the integrity of the repair, clinical evaluation of calves was performed at weekly intervals up to 3 months.

Results

A histological microphotograph of the tendinous portion of the diaphragm, with no treatment, showed cellularity (Fig. 1, a). Treatment with 1% sodium deoxycholate for 48h at 37°C under physical agitation resulted in acellular matrix where all the cells had been completely removed and the collagen fibres were orderly arranged (Fig. 1,b). Abdominal wall defects in four different species of animals were successfully repaired using acellular diaphragm matrix (ADM) (Fig. 2-5). The size of the hernial ring ranged between 5 and 10 cm (mean, 7.75 cm) in diameter. Mean surgical time (skin incision to closure) was 45 min (range, 40–60 min). No abnormal behaviour indicating pain or distress was observed after surgery. Temperature, pulse and respiration rates were increased during the first 48 h after surgery, but gradually subsided and disappeared within 3–4 days. Signs of parietal pain on palpation were observed in all the animals for 24–72h postoperatively, but were mild, and satisfactorily managed with the administration of non-steroidal anti-inflammatory drugs in all cases. Mild inflammatory oedema developed during the first week after surgery, but reduced daily until complete resolution occurred in all cases between day 10 and 15 post-implantation. The skin wounds in all the animals were healed by first intention. All the animals had an uneventful recovery without clinical signs of wound dehiscence, infection or recurrence of the hernia. In all the animals, the

integrity of the AAM graft was maintained for up to 3 months after hernioplasty.

Discussion

Prosthetic materials are required to enable tension-free repair of large abdominal wall defects and avoid hernia recurrence (Cobb *et al.*, 2005) [3]. Extracellular matrix scaffolds may be useful for hernia repair because of their potential capacity to resist infection and induce a milder inflammatory response, angiogenesis and host cell migration (BELLOWES *et al.* 2008; Gaertner *et al.*, 2007) [1, 8]. In our laboratory, a technique for decellularisation of harvested tendinous diaphragm has been developed through a unique chemical cleaning sequence that preserves the distinctive, natural, three-dimensional structures of collagen. The technique of repair of abdominal wall defect used in the present study appeared to be a satisfactory treatment regimen for repair of defect in all four animals. The results of this study demonstrated the uncomplicated healing of the repaired area, without recurrence or graft rejection in any one of the four different species of animals. Similar results were reported in another study in which six horses with ventral hernias underwent hernioplasty using xenogenic acellular dermal matrix (Kumar *et al.*, 2012) [11]. In another study successful use of acellular dermal matrix was reported in 6 goats with abdominal wall hernias underwent hernioplasty. Natural collagenous materials are used for surgical repair because of their inherent low antigenicity and their ability to integrate with surrounding tissue (Van Der Laan *et al.*, 1991) [20]. Furthermore, acellular tissue matrices are biocompatible, slowly degrade upon implantation and are replaced and remodelled by the extracellular matrix proteins, synthesized and secreted by ingrowing host cells (Pariente *et al.*, 2001) [19].

The incidence of post-operative complications associated with retroperitoneal placement of a synthetic mesh material, such as tearing of the internal abdominal oblique muscle and incisional edema and drainage, is considerably high (Elce *et al.*, 2006). However, in the current study, no post-operative complications were observed after retroperitoneal placement of an ADM graft in any animals for at least up to 3-months after the hernioplasty. Use of acellular aortic matrix for repair of umbilical hernia in buffalo calves (Kumar *et al.*, 2012) [11], acellular dermal matrix in a buffalo (Kumar *et al.*, 2014) [17], acellular aortic matrix in cow calves (Kumar *et al.*, 2013, a) [12], acellular dermal matrix in goats (Kumar *et al.*, 2013, b) [13], acellular dermal matrix for ventral hernias in horses (Kumar *et al.*, 2013,c) [14], crosslinked acellular aortic matrix for inguinal hernias in horses (Kumar *et al.*, 2013,d) [15] have been reported. The acellular matrix possesses the appropriate mechanical properties and induces appropriate interaction with the host cells that results in the regeneration of functional tissues (Voytik-Harbin *et al.*, 1998) [23]. Recurrence of the hernia is a relatively common complication of herniorrhaphy and has been reported after repair using sutures alone or after a synthetic mesh was implanted either inside or outside the hernial ring (Cook *et al.*, 1996; Van Der Laan and Klein, 1994) [4, 21]. Kumar *et al.* (2015) [16] evaluated efficacy of acellular diaphragm matrix (ADM) scaffolds for the repair of umbilical hernia was evaluated in 12 crossbred Landrace pigs. Treatment with 2% sodium deoxycholate lead to complete acellularization of the bubaline diaphragm at 48 h. Microscopic examination of the detergent treated matrix confirmed complete extraction of stromal cells and orderly arranged collagen fibres. All the hernias repaired with ADM remained sound over a period of 3 months.

In the present study, when the ADM graft was implanted inside the hernial ring (as an inlay graft), none of the hernias recurred within 6 months of their repair, as in the equine study (Kumar *et al.*, 2013, c) [14]. The greater success rate might be due to the acellular nature of bubaline diaphragms, which are composed of collagen. This may reflect the fact that ADM is able to withstand high pressures such as occur in the arterial vascular system. In the present study, no postoperative complications were observed after placement of the ADM as inlay graft in any of the animals up to 6 months after repair.

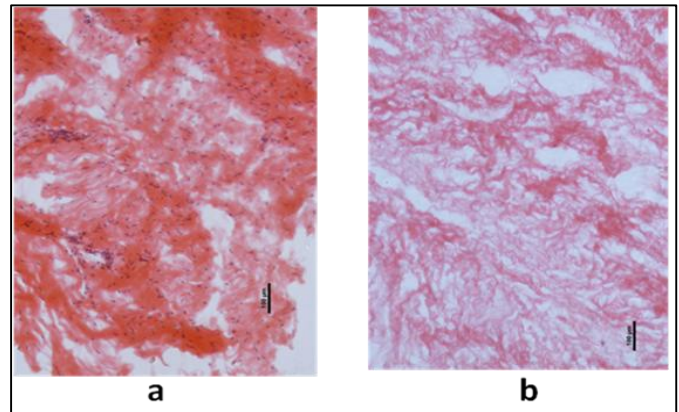


Fig 1: (a) histological examination showing native diaphragm matrix with abundance of cell nuclei (b) acellular diaphragm matrix (ADM) showing complete removal of all cells.

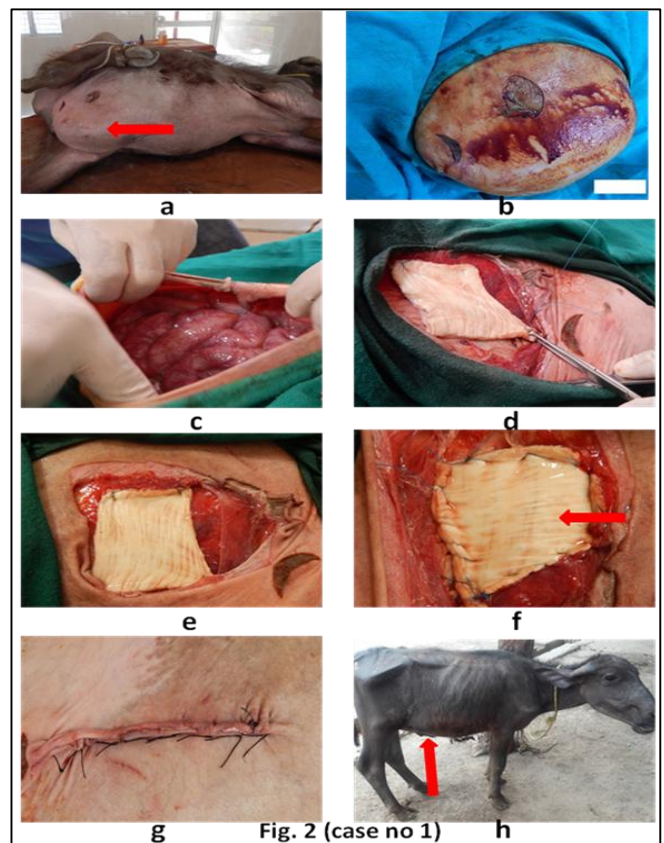


Fig 2: (a) swelling at the inguinal region in a year old buffalo calf (arrow) (b) close up view of large ventral hernia prepared for surgery (c) opening of hernial sac showing hernial contents omentum and intestines (d) application of ADM graft for reconstruction of defect (e) application of ADM graft (f) ADM graft fixed in inlay manner (arrow) (g) complete closure of skin incision (h) two months postoperatively showing complete recovery.

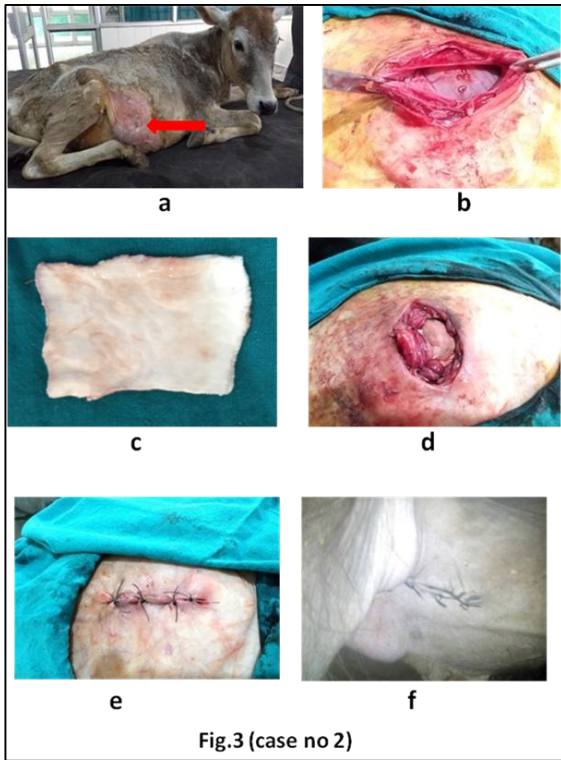


Fig.3 (case no 2)

Fig 3: (a) swelling at the lateral abdominal region in a 6 month old female cow calf (arrow) (b) opening of hernial sac (c) ADM ready to implant (d) application of ADM graft in inlay manner (arrow) (e) immediated closure of skin incision (f) skin incision on day 7 postoperatively.

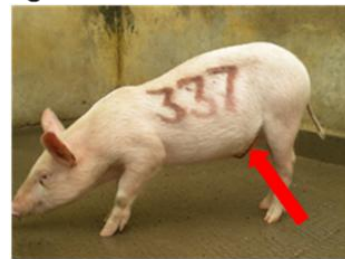
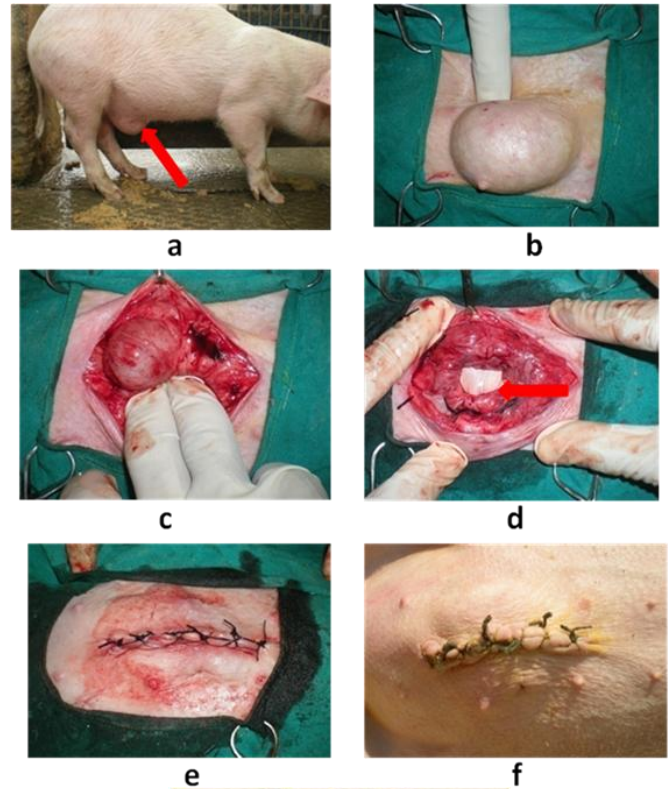


Fig.5 (case no 4)

Fig 5: (a) pig showing umbilical hernia (b) clinical examination revealed hernial ring (c) opening of hernial sac (d) application of ADM graft in inlay manner (arrow) (e) immediated closure of skin incision (f) skin incision on day 10 postoperatively (g) complete recovery after removing of sutre on day 10 postoperatively.

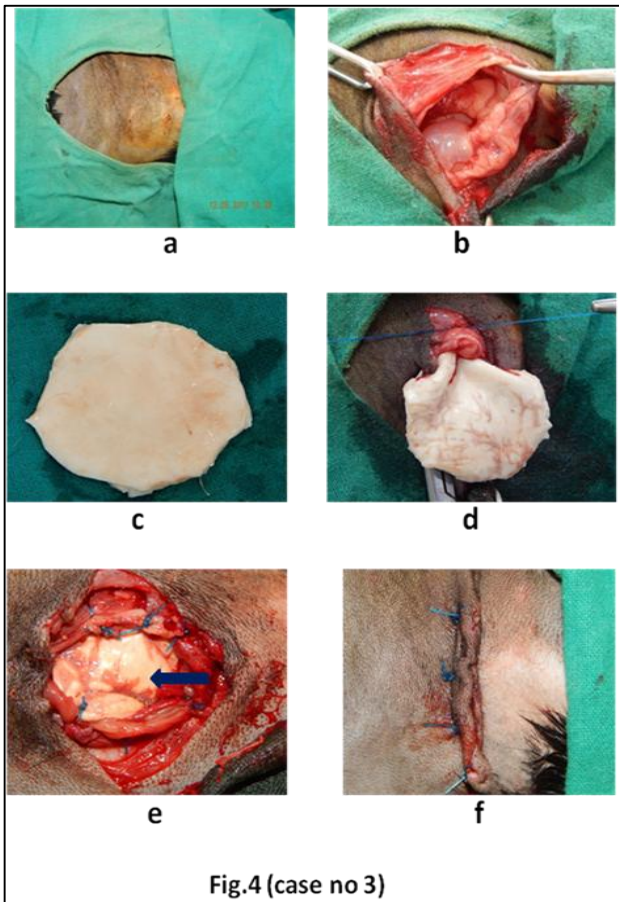


Fig.4 (case no 3)

Fig 4: (a) goat showing ventral abdominal hernia (b) opening of hernial sac (c) ADM ready to implant (d) application of ADM graft (e) application of ADM graft in inlay manner (f) closure of skin incision.

Conclusion

Buffalo diaphragm was decellularized with 1% sodium deoxycholate (SDS) for 48 h. Microscopic examination of the detergent treated matrix confirmed complete extraction of stromal cells and orderly arranged collagen fibres. Prepared ADM scaffolds were used for repair of abdominal wall defects in four different species of animals. The abdominal wall defects repaired with ADM remained sound over a period of 3 months. All the defects repaired with ADM healed completely without graft rejections. The present study suggested that ADM may be used safely for repair of abdominal wall defects in different species of animals.

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