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USE OF AUTOLOGOUS *LATISSIMUS DORSI* MUSCULAR FLAP FOR THE REPAIR OF IATROGENIC DIAPHRAGMATIC DEFECT IN DOGS

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ABSTRACT

The study was conducted to evaluate the viability and healing process of a pedicled latissimus dorsi muscular flap for use as an implant for herniorrhaphy in canine diaphragmatic muscle. After a three month acclimatization period, twelve clinically healthy mongrel dogs, of both sexes weighing 10 to 20 kg were induced with an iatrogenic diaphragmatic defect and divided into three groups A, B & C. A diaphragmatic defect, 8x8 cm in size, was created in the right muscular portion of the diaphragm through thoracotomy at the 9th intercostal space. The latissimus dorsi muscular flap with a dorso-lumbar pedicle was moved through the created window. Examination at day 15, 30, and 45 post-surgery, body temperature, pulse rate, respiratory rate and chest resonance varied significantly ($P<0.001$). Post-surgical accumulation of serous exudates also varied significantly ($P<0.001$) ranging from zero to 20.5ml at day-2 post-surgery, followed by a consistent decline till day-8 post-surgery. The abnormal limb function observed returned to normal by day-7 post-surgery. Post-mortem examination revealed no major pathological alterations and histopathological examination of the flap showed smooth healing without deterioration or any foreign body reaction. Use of an autologous latissimus dorsi muscular flap with a dorso-lumbar pedicle is a better and more economical choice for the repair of large diaphragmatic defects like hernia.

Key words: Autologous, Latissimus dorsi, Diaphragm, Hernia, Dogs

INTRODUCTION

The diaphragm is the major muscle of inspiration assisting ventilation, and plays a significant role in movement of lymphatics (Evans and Christensen, 1993). It is the second most important muscle after the heart, and the body relies on it fully for respiratory function. Any abnormality or malfunction of the diaphragm can lead to serious complications. Diaphragmatic hernia is a major pathological condition that occurs when the integrity of the diaphragm is compromised. Such a condition allows displacement of abdominal organs into the thoracic cavity (Tillson, 2000). Diaphragmatic hernia in small animals can be congenital if its development is incomplete and defective (Toulokian, 1978). Congenital diaphragmatic hernia may be pleuroperitoneal or more frequently, peritoneopericardial (Hosgood, 1996). Mostly it is acquired as a result of direct or indirect trauma to the diaphragm (Wilson and Hayes, 1986). A traumatic diaphragmatic hernia develops commonly as a result of animal attacks, vehicular or other blunt trauma. These are either circumferential, radial, or both. Circumferential hernias are most common; often through a tear ventral to the esophagus at the right costomuscular region (Garson *et al*., 1980). A sudden increase in abdominal pressure during trauma could also result in a diaphragmatic tear and the abdominal organs can be displaced into the thoracic cavity.

The majority of studies on the repair of diaphragmatic defects with prosthetic patches have resulted in reherniation and required reconstruction (Moss *et al.,* 2001). Moreover, recurrent hernia was a growing problem with the use of polytetrafluro ethylene (PTEF) (Lally *et al*., 1996). Other material like collagen coated vicryl mesh (CCVM) has also been reported as a short-term solution for the repair of diaphragmatic defects (Ramadwar and Carachi, 1997; Santtillan *et al*., 1996). Synthetic repair of large congenital or acquired diaphragmatic defects has invariably lead to recurrence, progressive wall deformity and restrictive pulmonary diseases (Moss *et al*., 2001). Bovine pericardium and dura matter are commercially available biological materials, but are expensive and risky. Thus, reconstruction of diaphragmatic defects with growing tissue would be helpful in avoiding such complications (Santtillan *et al*., 1996). Extensive use of a flap of latissimus dorsi muscle has been reported as an effective tool for reconstruction in a variety of body locations (Lally *et al*., 1996; Sydorak *et al*., 2003). However, the knowledge of the vascular anatomy of a muscle flap to be transformed locally or to a distant site would be of prime importance in determining its potential as a flap (Purinton *et al.*, 1992; Mathes and Nahai, 1981). The present study was thus designed to investigate the repair of a large diaphragmatic hernia using autologous tissue and aimed at developing a suitable methodology in the surgical repair of diaphragmatic hernia by practitioners/surgeons engaged in small animal surgery.

MATERIALS AND METHODS

Animal/ Experimental model: Twelve clinically healthy mongrel dogs (10 to 20 kg in weight) irrespective of the sex were selected to study the repair of large iatrogenic diaphragmatic defects using autologous tissue, divided into three equal groups and housed at kennel of the Pet Centre, Department of Veterinary Medicine & Surgery, University of Veterinary and Animal Sciences Lahore, Pakistan for a period of three months (including 15 days adaptation and pre-operative period). All the dogs were thoroughly examined prior to experimentation to rule out the possibility of any systemic disease. Each dog was allotted a specific identification number and kept on similar pet food throughout the experiment. Each dog was vaccinated against rabies and dewormed prior to initiation of the experiment.

Animal preparation & surgical procedure: The experimental animals were fasted 12 hours prior to surgery in each case. They were administered 2% xylazine hydrochloride (Rompun 2%; Bayer, Canada) at the dose rate of 0.05 mg/kg intramuscularly as pre-anaesthetic. General anaesthesia was induced with 5% aqueous solution of thiopentone sodium (Pentothal sodium; Abbott laboratories, North Chicago, Illinois) at the dose rate of 7 mg/kg. The trachea was intubated with a cuffed endotracheal tube and anaesthesia was maintained with 2-bromo-2-chloro-1,1,1 trifluro-ethane (Halothane-Vet; Merial Animal Health, UK) at 2.5 V% vaporized in 100% oxygen and delivered at controlled artificial positive pressure ventilation during the surgery.

A 15 cm area from each side of the proposed incision line was surgically prepared to facilitate extension of the incision if needed (Fig. 1A). A flap of the latissimus dorsi was resected transversely equal to the size of the diaphragmatic defect and reflected caudally, maintaining the thoraco-dorsal pedicle up to 3cm of its insertion (Fig. 1B). A right lateral thoracotomy incision was extended through the 9th intercostal space caudal to 9th rib in order to open the thoracic cavity and expose the diaphragm (Fig. 1C). The flap was continuously irrigated with warm sterile saline solution during the surgical procedure.

A diaphragmatic defect, measuring 8×8cm was created in the right muscular portion of hemi diaphragm for the implantation of the muscular flap. Diaphragmatic herniorraphy was performed by suturing the flap was sutured with the borders of the diaphragmatic defect by interrupted mattress sutures using polypropylene 2/0 (Prolene; Ethicon, UK) (Fig. 1D).

The thoracic cavity was closed by applying simple continuous sutures with using No. 2 chromic catgut (Ethicon, UK). At thoracoraphy, care was taken not to compress the flap between the ribs. Following irrigation with copious amounts of sterile saline the cutaneous and subcutaneous tissues were closed with chromic catgut No.2 (Ethicon, UK). Penrose drain was incorporated in the subcutaneous and skin incision was close by applying interrupted horizontal mattress sutures using nylon monofilament suture ethilon (Ethicon, UK). Thoracostomy tube was inserted into the plural cavity ventro cranially at 7th intercostal space at the level of costochondral junction. The tube was fixed to the skin with adhesive tape using purse string suture connected with a valve for plural drainage.

In compliance with the rules of the National Society of Medical Research Principles of laboratory care of experimental animals and postoperative care was carried out for each group ending for a period of 45 days. Post-operative analgesia was accomplished with diclofenac sodium 2.0 mg/kg (Phlogin Inj; Brookes, Pakistan) and ampicillin trihydrate 10% (Ampi-kel; Hasco, Pak) 0.2mi/2kg body weight intramuscularly was administered as an antibiotic cover for at least 7 days. Ringer lactate (Ringo Lact; Otusuka Pharmaceutical, Hong Kong) and dextrose infusions 5% (Dextrose Infusion Ringo; Otusuka Pharmaceutical, Hong Kong) were administered for postoperative fluid therapy.

The dogs were examined radiographically in case of suspected respiratory insufficiency. Radiographs of each dog were taken immediately after surgery to confirm proper placement of the thoracostomy tube placed for aspiration of fluids from the chest cavity. Thoracostomy tube and skin sutures were removed at day 7 and 10 after surgery, respectively.

Post-operative clinical examination was conducted on a daily basis until the termination of the trial. Plain chest radiographs were taken on the first day (post-surgically) followed by every 7th, 15th, 30th and 45th day to evaluate the integrity of the diaphragm. The dogs of group A, group B and group C were euthanized at day 15, 30 and 60 post-surgery respectively, to investigate the macroscopic healing process of the flap and any other injuries to the thoracic and abdominal cavities. Histopathological examinations were also carried out to study the integrity of the anastamotic site and type of granulation tissue, allowing for the reconstruction of the diaphragm. The post surgical investigations were undertaken at three different stages, i.e. day-15, day-30 and day-45.

Post-surgical examination included the evaluation of body temperature, pulse rate, respiratory rate and chest resonance as well as looking out for signs of dyspnoea, orthopnea, hyperpnoea, shock, and cyanosis

Statistical analysis: The data were analyzed by computer programme SPSS using. General Linear Model (GLM) procedure and Duncan Multiple Range test to evaluate the difference between groups at different time period in terms of clinical examination, histological healing process and assessment of diaphragmatic integrity.

RESULTS AND DISCUSSION

All dogs were (P<0.001) severely dyspnoeic, orthopnoeic, and hyperpnoeic on the first day post-operation. However, none of them was severely dyspnpoeic from day-3 onward. A significant (P<0.01) improvement in the orthopnoeic condition was observed from day-6 post-surgery onward, and all of them eventually fully recovered. A higher proportion of the dogs (58.33% and 100%) recovered to a condition of moderate hyperpnoea on day 3 and 4 post-surgery respectively and no clinical signs of hyperpnoea were observed from day-8 post-surgery onwards.

No signs of cyanosis and shock were observed in any dog throughout the post-surgical period. A significant variation (P<0.01) in mean body temperature and pulse rate was found over the entire post-surgical period. Mean body temperature and pulse rate recorded in the evening was lower than that of the morning. Significant variations (P< 0.001) in mean respiratory rate were found over the entire post-surgical period. Higher respiratory rates were observed during the morning and evening on the first day post-surgery. A consistent decline in respiratory rates was observed and the values returned to normal on the fourth day post-surgery.

Mean volume of seroma collected from the chest cavity during the postoperative period in all dogs varied significantly (P<0.001). No seroma accumulation in the chest cavity was observed on the first day post-surgery. A significantly higher volume of seroma (20.5 ml) in the chest cavity was found on the second day post-surgery, followed by a consistent decline (17.17 ml, 12.62ml, 5.53ml, 5.24 ml, 2.53 ml and 0.89 ml, respectively) day 3, 4, 5, 6, 7 and 8 post-surgery (Fig. 2). A significant (P<0.01) variation in chest resonance was found on the first day post-surgery as compared the other days of the experimental period. No evidence of gastric tympany was found in any dog at any stage of the post-surgical period.

Significantly higher (P<0.01) variation in limb function and gait of the dogs during the post-surgical period was found. Limbs of all dogs were found to be non-functional on the first day post-surgery; however, all the dogs showed improvement as the days progressed to reach a stage of limited malfunction on day 6 post-surgery and a stage of full recovery to normal day 10 post-surgery. Diaphragmatic integrity was evaluated radiographically and at day 1, 7, 15, 30, and 45 of the post-surgical period, no displacement of any abdominal organ was seen. However, the image obtained on the first day post-surgery showed accumulation of gas in the thorax of 58.33% of the dogs. The radiograph also revealed an accumulation of fluid in the thorax on day 7 post-surgery but on the succeeding examination (day 15) no fluid was observed.

Blood supply was found consistent in the entire microscopic examination of the tissues and no blockage in blood supply was observed at the tissue level. No haemorrhages or foreign body reactions were observed. Connective tissue growth was consistent in all dogs throughout the experimental period. Inflammatory cells (premature granulating tissue) in all tissue samples were found at day 15 and 30 post-surgery (Fig. 4: A, B&C). However, no evidence of inflammatory cells was observed on day-45 post-surgery, the final examination day Fig. 4C. Variations in adipose tissue deposits and adhesion of the flap with surrounding tissue were observed upon post mortem examination. Rich deposits of adipose tissue at the grafted site were found at day-15 post-surgery in 75% of the dogs (Fig. 4D) and moderate deposits were seen in the rest of the dogs. Deposition of adipose tissue was found to be lower in all dogs on the final examination day, day 45 post-surgery. In 75% of the dogs, the adhesion of flap with the surrounding tissue was not observed. The muscular flap of the latissimus dorsi was easily resected in all dogs, according to the protocol (Grerory *et al*., 1988). The flap was resected atraumatically, in an attempt to preserve its vascular supply. Intercostal arteries between the 9th and 10th ribs were cut and ligated, without any impairment of flap viability, this was in accordance with the method used elsewhere (Grerory *et al*., 1988; Bianchi *et al*., 1983; Wallace and Roden, 1995). This result differs from those who reported deterioration of the flap due to these ligatures (Pavletic *et al*., 1987; Philibert and Fowler, 1996).

The length of the flap was ideal for reconstruction of the diaphragmatic defect. Thoracotomy at the 10th intercostal space allowed creation of a large pedicle flap of latissimus dorsi muscle. The size of this flap generally exceeds the size needed for most clinical situations (Nicoll *et al*., 1996). Excellent access to the costal portion of the diaphragm was provided through the same incision at the 9th intercostal space (Sydorak *et al*., 2003; Wallace and Roden, 1995), and all the procedures needed for the accomplishment of the surgery could be done through the same intercostal incision. The physical and clinical abnormalities observed in the first few days of the post-surgical period did not appear as recurrent clinical or physical signs. The postoperative mild pneumothorax observed in few of the dogs upon radiographic examination could be due to improper chest intubation. The problem was subsequently managed and the air was evacuated with a sterile syringe. The diaphragmatic contour was intact in all radiographs and a clear demarcation was seen between pleural and peritoneal cavities.

Signs of chest pain, abdominal pain and dyspnoea observed were same as observed for routine diaphragmatic surgeries in the first three to five days post-surgery (Payne and Yellin, 1982). Due to the excessive tear of the diaphragm and muscle resection at the axilla respiratory distress were observed. However, the condition subsided within a few days post-surgery and no clinical abnormalities were found from that stage onward. Some of the transient post operative complications observed in this study, like, pyrexia, orthopnoea and hyperpnoea were all successfully treated with fluid and antibiotic therapy. The weight-bearing forelimb lameness noted in all dogs during the first 24-48 hours following surgery could have been due to massive muscle resection. This complication was mild and transient and resolved in all dogs by day-9 post-surgery. These findings suggest a rapid compensation of the impaired muscle function. Decreased resonance of the chest cavity due to a steady accumulation of seroma in the pleural cavity of all dogs until day-8 post-surgery was subsequently corrected by aseptic aspiration of the seroma.

Adhesion of the flap progressed to a significant degree ($P<0.01$) with the advancement of the post-surgical period and in the end, complete adhesion of the flap with the surrounding tissue was observed in all dogs. The flap adhesion with the stomach tissue, liver and omentum and liver and stomach was found in 25, 50 and 25 percent of the dogs, respectively. Similar findings have also been reported following utilization of autologous muscle or any other autologous tissue for the repair of diaphragmatic defects (Matsumoto and Miyake, 1996; Mazzanti *et al.*, 2001; Sydorak *et al.*, 2003). The authors also mentioned that the liver, lungs and omentum, were the structures most prone to adherence. The absence of a serosal layer over the flap and the large extension of the flap was probably the cause of the adhesion found in this experiment. However, clinical alterations due to these adhesions were not seen. Moreover, no ischemia of the flap or deterioration of the flap after dissection was seen contrary to what has been reported (Bianchi *et al*., 1983; Pavletic *et al*., 1987; Philibert and Fowler, 1996). Findings of the present investigation therefore suggest successful use of large pedicle flaps of latissimuss dorsi for the repair of large diaphragmatic hernia in dogs.

In conclusion, all the dogs had post-surgical recovery with minor complications in the first week following surgery. Post-mortem examination revealed adhesions of the grafted muscle with neighboring viscera without major pathological alterations. Similarly, histopathological examination of the flap showed healing without foreign body reaction and deterioration. Findings of the study suggest that a latissimuss muscular flap with a dorso-lumbar pedicle can effectively be used as an alternative option for reconstruction of large diaphragmatic defects in dogs through intercostal thoracotomy. Moreover, incorporation of the autologous living tissue decreases the chances of infection.

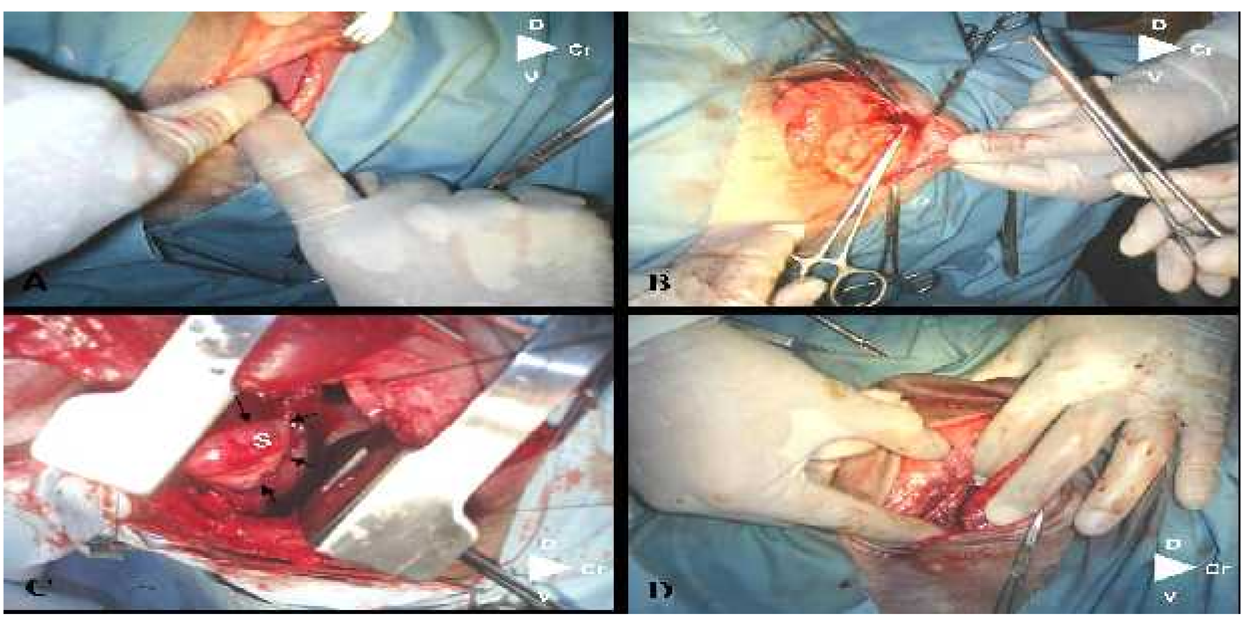

Fig.1. Intra-operative visualization of the diaphragmatic defect in the right lateral recumbency **(A)** Skin and subcutaneous tissue incision at 9th intercostal space originating at the level of the costochondral junction and extending in a gentle curve along the line of the rib **(B**) Harvested latissimus dorsi subcutanous muscular flap. **(C)** Retraction of the intercostal space with the help of finochietto rib spreader. Black arrows indicate the smooth edges of the diaphragmatic defect, whereas S indicates herniated stomach in the abdominal cavity. **(D)** Anastamosed flap of pedicled latissimuss dorsi with diaphragm. **D:** Dorsal **Cr:** Cranial **V:** Ventral

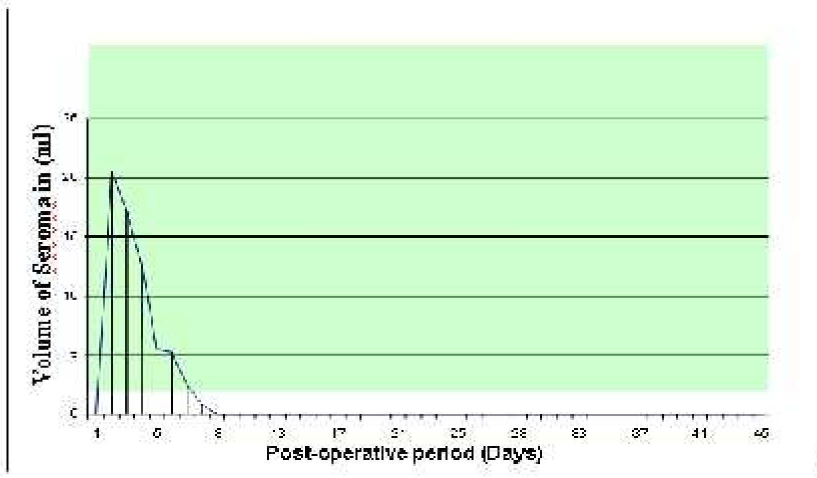

Fig. 2 Mean volume of seroma (ml) accumulation in chest cavity during post-operative period in all dogs of each group

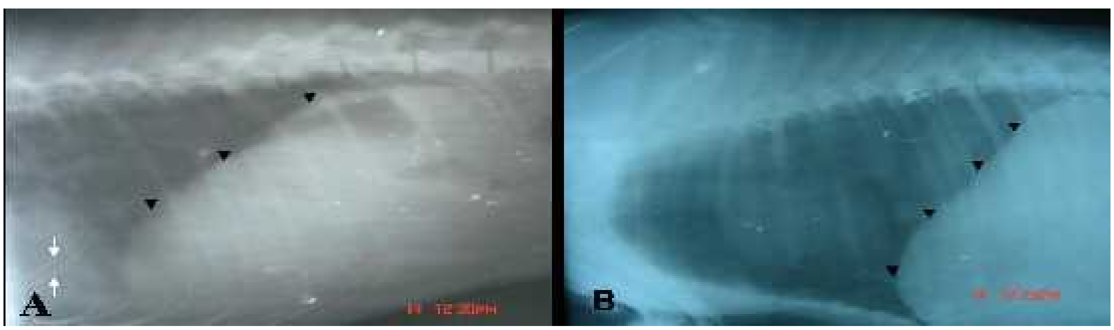

Fig. 3 Radiographic evaluation for diaphragmatic integrity. **(A)** Right lateral thoracic radiograph at Day-1 (representative figure for all group). Arrows indicate intact diaphragmatic outline and placement of thoracostomy tube at day 1st post-surgery. **(B)** Right lateral thoracic radiograph at day-15 post-surgery. Arrows indicate intact diaphragmatic outline and clear demarcation of abdominal and chest cavity.

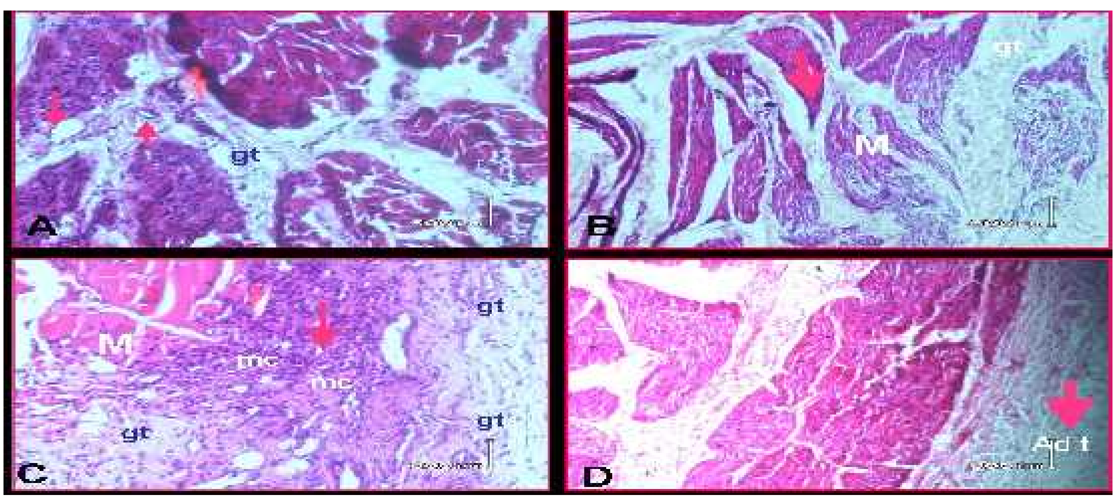

Fig. 4 Microscopic findings during post-surgical period in dogs reconstructed with latissimus dorsi **(A)** Histological findings at day 15 after reconstruction, the patch is covered by granulating tissue formation, arrows indicating new capillaries formation and intact blood supply **(B)** Findings at day-30 post-reconstruction, a specimen reconstructed with latissimus dorsi shows muscle fiber interdegitation, viable cells and blood vessels. **(C)** Mature granulating tissue, no inflammatory reaction and uniformity with surrounding tissue is observed. **(D)** Findings at day-15 after reconstruction, the patch is covered by mature granulating tissue and rich adipose tissue deposition at abdominal side.

Hematoxylin & Eosin-Stain, A, C &D (x 200), B (x 100): gt: granulation tissue, **m:** muscle, **mc**: macrophages, **Ad t:** Adipose tissue

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