

## The Role of a Combination of Autogenous Fresh Bone Marrow and Omental Free Graft in the Cervical Esophageal Wound Healing in Dogs

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**Abstract: Problem statement:** Esophageal surgery is associated with greater wound healing problems than surgery on other portion of the alimentary tract. Omentum and fresh bone marrow are rich source of angiogenic factors that promote the growth of blood vessels into wound tissue. The aim of this study was evaluation of the effects of a combination of autogenous fresh bone marrow and omental free graft in esophageal wound healing. **Approach:** This study was carried out on 8 male adult dogs, assigned into two equal treatment and control groups. All dogs underwent cervical esophagotomy and laparotomy operations, also bone marrow aspiratin, simultaneously. A 3 cm linear full thickness incision was made on the cervical esophagus and then sutured. In treatment group, A small piece of omentum was obtained from abdominal cavity and secured on esophageal suture line and then 1 mL of fresh bone marrow was injected into the omental graft and wound edge. In the control group, the suture line was left without any treatment and the harvested omentum and aspirated bone marrow were disposed. During this study, the dogs were examined clinically and they were euthanized 14 days after operation. The surgical site of esophagus was assessed macroscopically and then histopathologic samples were taken from the repaired tissue of esophageal wall. The samples were stained by hematoxylin-eosin and masson-trichrome. **Results:** The clinical evaluation of present study revealed that all dogs were healthy with no major postoperative complications. There was no significant difference based on macroscopic examination in surgical site of esophagus between the two groups. The microscopic study revealed that inflammatory cells, angiogenesis and granulation tissue formation in wound bed of the treatment group were significantly more than the control group. **Conclusion:** According to the results of this study, application of a combination of autogenously fresh bone marrow and free omental graft can improve wound healing process in esophageal incision wounds.

**Key words:** Bone marrow, autogenous, omental graft, esophagus

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### INTRODUCTION

Alimentary tract adhesion due to the surgical interference and foreign bodies is a major complication, especially in animal practice (Ismail *et al.*, 2007). The esophagus is regarded among surgeons as an infamous organ of the human body and even today it is a great challenge to perform certain types of esophageal operations (Pap-Szekeres *et al.*, 2005). Surgical repair of the esophagus requires meticulous surgical technique because it is more prone to postoperative dehiscence and leakage than other portion of the alimentary tract. Several characteristics of the esophagus make it a less than ideal surgical tissue: it lacks a serosal cover, which

may limit leakage by exuding fibrin; it is in constant motion as the result of swallowing and the movement of the diaphragm and adjacent organs; it tolerates longitudinal stretching and tension poorly and it lacks an omentum to help localize and seal a leak (Schunk, 1990). One of the most important features of the anatomy of the esophagus from a surgical aspect is the segmental blood supply of the organ (Pap-Szekeres *et al.*, 2005). During the operative preparation, small supply vessels are injured and the operation is therefore carried out on an organ with a poor blood supply. The postoperative complication rate in esophagus is high (Pap-Szekeres *et al.*, 2005) and cervical esophagus is more prone to the postoperative complication in comparison with the abdominal and

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thoracic esophagus, because the cervical part has no serosa or plural mesothelium (Kyles, 2003).

Augmentation of esophagotomy site with omentum, pericardium, local muscle flap, stomach, or intestine can aid healing by supporting sealing and revascularizing the surgical site (Kyles, 2003; Slatter, 2002). In ideal wound treatment but healing alone is not enough. Restoration of function of the part healed is the ultimate objective (Sharifi *et al.*, 2006a).

It has long been recognized that the greater omentum has great ability in revascularization of tissue and promote angiogenic activity in adjacent structures to which it is applied. Indeed the angiogenic materials obtained from the omentum is abundant in supply (Saifzade *et al.*, 2007). Because of this unique ability of omentum, it has been used as a pedicle flap in many frequent surgeries such as tracheal autograft (Nakanishi *et al.*, 1994) and ischemic limb (Bhargava *et al.*, 1997). Furthermore omental pedicle flap has been recommended for supporting of esophageal wounds. Omentum is exceptional for esophageal wound patching and closing small defects because it is vascular and available; esophageal stricture formation may also be minimized with omental patching (Slatter, 2002).

In the previous studies, transposition of free omental graft has been used in revascularization procedure involving the chronically injured spinal cord (Zhang *et al.*, 1989), head and neck deformities (Losken *et al.*, 2002) and bone fractures (Oloumi *et al.*, 2006). Bone marrow seems a logical candidate for treatment of chronic wounds as it contains inflammatory cell progenitors, mesenchymal stem cells and multipotent stem cells. Inflammatory cells have long been known to participate in wound healing and hematopoietic hormones such as granulocyte-monocyte colony-stimulating factors have been reported to accelerate wound healing (Badiavas and Falanga, 2003). The plasma of bone marrow includes various growth factors and cytokines that are benefit for wound healing process (Moon *et al.*, 2008). Some experimental and clinical studies have confirmed the therapeutic effects of bone marrow on wound healing in various organs (Oloumi *et al.*, 2008; Ichioka *et al.*, 2005; Badiavas and Falanga, 2003; Karaoglu *et al.*, 2009). According to the author's knowledge there is no documented data about the effect of combination of autogenous fresh bone marrow and free omental graft on esophageal wound healing process.

In this study, we have evaluated the role of fresh autogenous bone marrow and free greater omental graft in the healing of experimental cervical esophageal incision, base on clinical, macroscopic and microscopic examinations.

## MATERIALS AND METHODS

In this study, eight male adult mixed breed dogs, 2-3 years old and weighting between 20 and 25 kg, were used. They were determined to be healthy on the basis of physical (body temperature, respiratory rate and heart rate) and hematological examination (CBC, WBC, PCV, Hb, platelet counts and total protein). The dogs were randomly designed to either an experimental or a control group; 4 dogs in each group. All ethical considerations using animals were considered and the experimental protocol was approved by the Ethics Committee of KUMS.

The animals were premedicated with 0/05 mg kg<sup>-1</sup> acepromazine (KELA Laboratory, Belgium) intramuscularly. Anesthesia was induced intravenously with 10 mg kg<sup>-1</sup> thiopental sodium (Thiopental, Caspian, Iran) and maintained with halothane 2% (Halothane BP, Nicholas, India). After induction of anesthesia, surgical preparation was carried out in the ventral part of abdomen and neck, also proximal part of left humerus in standard method.

In this study, all dogs underwent esophagotomy and laparotomy operation; also bone marrow aspiration. To operate cervical esophagotomy, an incision was made on the midline skin on the ventral surface of the neck, beginning at the larynx and extending caudally to the manubrium. After separation and retraction of local muscles, the trachea was retracted to the right and cervical esophagus was exposed. After this, a stab incision was made into the lumen of esophagus and a 3 cm linear incision was extended longitudinally. Then surgical site was rinsed by normal saline and esophagotomy incision was closed using 3-0 nylon (Tebkeihan, Iran) with simple interrupted pattern, in both groups.

To carry out laparotomy, the abdominal cavity was approached through a 12-15 cm ventral midline incision midway between the umbilicus and pelvic inlet. A 3×2 cm<sup>2</sup> piece of the omentum was isolated by two ligatures and cut free from the remaining parts and placed over the esophagotomy incision of treatment group and secured in place with the tacks of sutures. Then 1 mL of fresh bone marrow was aspirated from proximal part of left humerus by bone marrow aspiration needle and immediately injected into the part of the omental graft that covered esophagus incision, at 3 different points using a syringe with gauge 20 needle. In control group, esophagotomy suture line was left without graft and bone marrow and the isolated omentum and aspirated bone marrow were disposed. Finally, all surgical sites closed routinely.

Table 1: Criteria for histological scores

Score	Re-epithelialization	Cell infiltration	Granulation tissue	Angiogenesis	Collagen deposition
1-3	Minimal re-epithelialization	Wound covered with thin to moderate cell layer	Granulation tissue around wound edge only	500>capillary bud mm <sup>-2</sup>	Low density
4-7	Moderate re-epithelialization	Wound covered with thick cell layer	Granulation tissue around wound edge and in 30-50% of wound bed	500-1000 capillary bud mm <sup>-2</sup>	Moderate density
8-10	Complete re-epithelialization	Wound covered with very thick and densely populated cell layer	Granulation tissue around wound edge and in >50% of wound bed	1000<capillary bud mm <sup>-2</sup>	High density

Postoperative management included: withheld of oral intake was for 24 hours, intravenous fluids therapy for 2 days, antibiotic (Cefazoline, 20 mg kg<sup>-1</sup>, IV, tid for 5 days) and anti-inflammatory (Dexamethasone, 0.5 mg kg<sup>-1</sup>, IM, single dose) administration in the all dogs.

During this study, the dogs were inspected clinically and then, they were euthanized 14 days after surgery by intravenous injection of sodium thiopental solution. All the soft tissues around the esophagus were retracted and surgical site were studied grossly. A 2 cm full thickness samples were taken from repair site of esophagus and fixed in 10% phosphate-buffered formaline. The samples were embedded in paraffin wax afterwards. Five-micrometer serial section of the samples were cut and stained with hematoxylin-eosin and masson-trichrome and then examined in a blinded fashion under light microscope. Each slide was given a histological score ranging from 1-10 according to the following parameters: re-epithelialization, wound bed cellularity, angiogenesis, granulation tissue formation and collagen deposition. The amount of collagen deposition was evaluated by masson-trichrome staining and the other parameters were examined in hematoxylin-eosin stained sections. Angiogenesis was assessed morphometrically by examining three fields per section under  $\times 400$  magnifications and presented in number per mm<sup>2</sup>.

The criteria used for histological scores of wound are summarized in Table 1. The recorded scores of two groups for each parameter were analyzed by using the nonparametric mann-whitney test. A value of  $p \leq 0.05$  was considered as significant.

## RESULTS

All dogs were depressed and lost their appetite for 48 hours post surgery and regained their appetite after that. They were fed with liquid food and gruel in first 7 days after surgery and then gradually returned to their normal diet until the end of the study. All animals remained healthy throughout the study.

The clinical evaluation showed that there is no major postoperative complication such as dysphasia, regurgitation and vomiting in all examined dogs during this study.

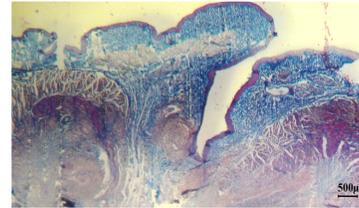


Fig. 1: Complete re-epithelialization with large amount of granulation tissue in treatment group (Masson-trichrome staining, Bar = 500  $\mu$ )

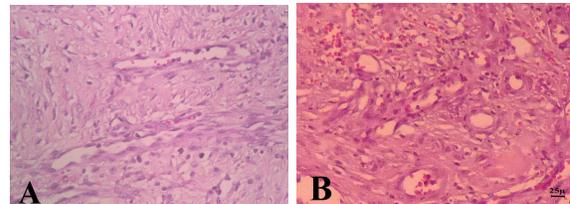


Fig. 2: Angiogenesis; (A): low vessel density in control group and (B): High vessel density in treatment group (hematoxylin-eosin staining, Bar = 25  $\mu$ )

The macroscopic assessment of surgical site revealed that in all dogs, esophagus was adhered to the surrounding soft tissue and there was no noticeable difference in the extent of adhesion between the two groups. No complications such as infection, dehiscence, stricture and fistula were observed in the surgical site of all dogs. In the treatment group, greater omentum was completely attached to the incision site.

Microscopic study of esophageal wounds showed that the new epithelial cells covered the surface of all esophageal wounds in both control and treatment groups and there was no significant difference in the re-epithelialization score between the groups ( $p > 0.05$ ) (Fig. 1). The number capillary buds in treatment group were significantly higher than the control group ( $p < 0.05$ ) (Fig. 2). The inflammatory cell in the wounds of the treatment group was significantly more than the control group. Presence of inflammatory cells especially macrophages around the wound of the treatment group were significantly more than the control group ( $p < 0.05$ ).

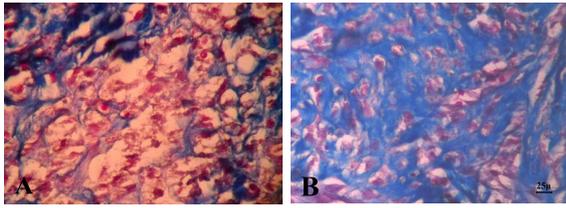


Fig. 3: Amount of collagen deposition; (A): Low collagen density in control group, (B): High collagen density in treatment group (Masson-trichrome staining, Bar = 25  $\mu$ )

The formation of granulation tissue in treatment group appeared to be significantly thicker and larger than control group ( $p < 0.05$ ). The amount of collagen deposition in the treatment group was significantly more than the control group ( $p < 0.05$ ) (Fig. 1 and 3). Due to the injection of fresh bone marrow in the wound site of treatment group, noticeable populations of hematopoietic cells were observed in some part of the wound.

### DISCUSSION

Esophageal surgery is historically associated with greater incisional dehiscence than surgery on other portion of the alimentary tract. Several factors may contribute to the high complication rate, including lack of serosa, the segmental nature of the blood supply, lack of omentum, the constant motion of swallowing and respiration and tension at the surgical site (Pavletic, 1981; Slatter, 2002). The risk of leakage from cervical anastomosis is higher than intrathoracic anastomosis (Muller *et al.*, 1990). There are numerous recommendations to avoid postoperative complications after esophageal surgery, the important ones being-gentle handling, meticulous technique of suturing, good vascular supply and omental wrapping (Thakur *et al.*, 2004). In recent studies, the omentum was used to cover soft tissue defects, to contribute to enhance healing, to control adhesion and to combat infection (Slatter, 2002). Recent studies have revealed that omentum, apart from being a great source of various growth factors, neurotransmitters, neurotrophic factors and inflammatory mediators, also contains omnipotent stem cells that can differentiate into a variety of cell type (Alagumuthu *et al.*, 2006). The omentum has the highest Vascular Endothelial Growth Factor (VEGF) secretion rate as well as the highest concentration VEGF protein in comparison with other organ in rat. It has been suggested that VEGF is the major angiogenic factor produced by omentum and possibly underlies the omentum-induced angiogenesis mechanism (Zhang *et al.*, 1997). Matoba *et al.* (1996) used omental implantation to repair perforated collagen ulcer in rat. Greater anti-

inflammatory and angiogenic activity and accelerated collagen synthesis were seen in omental implantation group. Oloumi *et al.* (2006) investigated the role of free autogenous omentum in vasculogenesis during the process of bone healing. They stated that free omental graft significantly promoted angiogenesis in repaired tissue. Pap-Szekeres *et al.* (2003) performed transplantation and microsurgical anastomosis of free omental graft after experimental esophagectomy in dogs and declared that vital reactions, including granulation tissue and angiogenesis were present in the histological slides. There are several studies about the application of pedicle omental flap to help wound healing process in various tissues and organs, but there are some possible complications after omental transposition including; seroma formation, herniation through the omental exit hole, hemorrhage and flap necrosis (Slatter, 2002). Also in order to transpose omental pedicle, a major laparotomy must be done (Saifzade *et al.*, 2007). In our study the greater omentum was transplanted to the esophagus wound without vascular anastomosis. Oloumi *et al.* (2006) suggested that considering the angiogenic factors present in the greater omentum, it can be used at defects with no need to direct vascular connection with the recipient bed.

On the other hand, autogenous bone marrow aspirate is a useful adjuvant to enhance soft tissue healing because it contains hematopoietic cells that differentiate into red and white blood cells, platelets and macrophages and mesenchymal cells. Mesenchymal cells are multipotent and can evolve into several cell types involved in tissue repair depending on the environment they are transferred to and include osteoblasts, chondrocytes, fibroblasts and myogenic cells. Bone marrow is also the primary source of mesenchymal cells (Roukis *et al.*, 2009; Yamaguchi *et al.*, 2005; Chong *et al.*, 2007; Muschler *et al.*, 1997). Harvest of autogenous bone marrow aspirate can be performed rapidly, is simple to perform, easily reproduced and is associated with low financial cost (Roukis *et al.*, 2009; Schweinberger and Roukis, 2007). Although other reported materials such as growth factors and cultured cells must also significantly are more effective to improve of wound healing (Oloumi *et al.*, 2008; Badiavas and Falanga, 2003), from a practical standpoint, the use of bone marrow is more attractive because we can use the patient's own fresh cells with routine aspiration technique, allowing minimal systemic side effects (Ichioka *et al.*, 2005).

In a clinical study, fresh bone marrow after aspiration from iliac crest, was applied directly on the chronic wound surface and injected it into the wound edges, in human. It has been suggested that all patients showed clinical improvement in their wounds within

days following administration of bone marrow aspirate (Badiavas and Falanga, 2003); also the autogenous bone marrow aspirated from tibia plateau was used to treatment of experimental burn in rabbit skin, topically. The results demonstrated that fresh bone marrow enhanced burn wound healing (Oloumi *et al.*, 2008). Ichioka *et al.* (2005) showed that bone marrow-impregnated collagen matrix can promote the wound repair process through augmentation of angiogenesis. Karaoglu *et al.* (2009) evaluated effect of bone marrow or periosteum on tendon-to-bone tunnel healing in a rabbit model. The results of his study showed that both periosteal graft and fresh bone marrow had benefit effect on tendon healing. They suggested that a combination of wrapping periosteum and injecting bone marrow to the tendon graft would have a synergistic effect. In another study periosteal graft has a positive effect in healing of articular cartilage (Sharifi *et al.*, 2006b)

The most prevalent grafts are autografts, allograft, xenografts and synthetic substances. Because of many questions about the immunologic problems of the use of allograft and xenografts (Sadi *et al.*, 2010) and also because of easy availability of omentum and bone marrow autogenously, in the current study, autografts were applied.

In this study esophageal wound healing were evaluated histopathologically in three parts of a wound: Inflammatory exudate, Granulation tissue formation and proliferation of connective tissue (Azarabad *et al.*, 2006).

In regard to the beneficial effects of both fresh bone marrow and free omental flap to promote wound healing and presence of several risk factors responsible for delaying in wound healing of cervical esophagus incision, the current study was designed to evaluate the combination of bone marrow and omentum free graft to improve cervical esophageal wounds repair.

According to the results of present study, simultaneous application of autogenous fresh bone marrow and free omental graft improved esophageal wound healing by noticeable angiogenesis, early proliferation of inflammatory cells and fibroblasts and acceleration in granulation tissue formation.

### CONCLUSION

The current study presented the simple and practical method, which is immediately applicable without any processing procedure and major side effects to improve wound healing in esophagus. To approve this hypothesis long-term investigation should be performed.

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