



Evaluating the Feasibility of Esophagotomy Suture Line Reinforcement Using Platelet Rich Fibrin Membrane and Its Effect on Wound Healing

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Abstract

Objective- This study aimed to evaluate the feasibility of using platelet rich fibrin membrane as a novel on-lay patching biomaterial in canine esophagotomy and its effects on esophageal wound healing.

Design- Experimental study.

Animals- Eight adult mixed breed dogs of both sexes equally allocated to control and treatment groups.

Procedures- Longitudinal incisions measuring 3 cm were made in the cervical esophagus of all dogs (2 incisions in each dog). All incisions were sutured and on-lay patching was performed in four dogs using platelet rich fibrin. After 28 days, wound healing was assessed by macroscopic, histological and biochemical methods

Results- Patching resulted in less adhesion formation (18.24 vs. 61.67 mm, $p < 0.05$) and increase in tissue hydroxyproline content (91.31 vs. 74.31 mg, $p > 0.05$). Histologically, platelet rich fibrin membrane mostly influenced wound healing in the outer layers of the esophagus particularly the muscular layer although a slightly better wound healing was observed overall.

Conclusion and Clinical Relevance- Platelet rich fibrin membrane could be used as an alternative patching biomaterial in esophageal surgery although further investigations needs to be carried out particularly in clinical cases.

Key words- Platelet rich fibrin membrane, Onlay patching, Esophagus, Dog, Wound healing.

Introduction

Esophagotomy is commonly performed to remove foreign bodies and treat esophageal perforations or diverticula.^{1,2} Dehiscence and leakage are the two most important complications of esophagotomy with catastrophic results.^{3,4} Patching of the suture line with various tissues has been used to prevent leakage and improve wound healing.^{1,2,5,6} Despite their success, these patching techniques are technically demanding to perform and invasive leading to prolonged operative times and increased postoperative complications. Platelet rich fibrin (PRF) is a second generation platelet concentrate developed by Choukroun et al.⁷ Venous blood from the patient is collected into glass tubes without anticoagulants and immediately centrifuged.

The resultant product is a true biomaterial containing platelets, leukocytes and growth factors trapped inside a dense network of fibrin clot. It can be used either as a clot or membrane and the latter form can easily be sutured in place during surgical procedures. PRF has been used in oral, maxillofacial, ENT (ear, nose and throat), plastic and orthopaedic surgery.⁷⁻¹³

The purpose of the present study was to evaluate the feasibility of using PRF membrane as an autologous biomaterial to reinforce esophageal incisions in an experimental animal model. It was hypothesized that PRF membrane could prevent dehiscence and leakage and improve wound healing through the release of growth factors.

Materials and Methods

Animals & Study design

This study was approved by the experimentation ethics committee and research council of the Faculty of Veterinary Medicine, Islamic Azad University, Tabriz branch. It was carried out on 8 adult mixed breed dogs of both sexes with the body weight of 20.34 ± 5.23 kg

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Received 30 Aug 2015 ; accepted 17 Dec 2015

(mean \pm SD). They were housed individually with adherence to institutional guidelines for the care and use of laboratory animals in research. Their health status at the time of experimentation was determined based on findings from physical examination and laboratory tests (complete blood cell count, blood biochemistry profiles, and urinalysis).

The dogs were randomly allocated to two identical groups consisting of 4 animals per group. Cervical esophagotomy was performed on all animals and the esophageal suture line was reinforced with platelet rich fibrin membrane in the treatment group. The animals were kept for 28 days and then euthanized to evaluate the esophageal wound healing using macroscopic, histological and biochemical parameters.

PRF preparation method

Autologous PRF was prepared according to the method described by Dohan et al.¹⁴ Prior to induction of anesthesia, 20 ml of whole blood was collected from the jugular vein of each treatment animal into two sterile glass test tubes without any anticoagulants. The blood samples were immediately centrifuged at 3000 rpm (400 g) for 10 minutes using a laboratory centrifuge (Hermle Z 206 A, Germany). The PRF clot located in the middle section of the sample was removed from the test tube during surgery and the red blood cells at the bottom and acellular plasma at the top of the sample were discarded. The clots were pressed gently between sterile gauze sponges in order to obtain the PRF membrane which was sutured over the esophagotomy incision (Fig.1).



Figure 1. PRF produced in the middle layer of blood sample (arrow) immediately following centrifugation (a) and its clot (b) and membrane (c) forms

Surgical Procedure

The animals were fasted for 12 hours before surgery. A combination of ketamine (Ketamine 10%, alfasan, woerden, Holland) 5 mg/kg and acepromazine (Neurotranque 1%, alfasan, woerden, Holland) 0.05 mg/kg plus atropine (Atropine sulphate 0.5, Daroupakhsh Co., Iran) 0.03 mg/kg was injected intramuscularly as pre-medication. Anesthesia was

induced by injecting 2.5% solution of thiopental 10 mg/kg (Thiopental sodium 1 gr, Sandoz GmbH, Kundl, Austria) through an IV catheter placed in the cephalic vein and maintained by 1-1.5% halothane (Fluothane 250 ml, Nicholas Piramal India Ltd.) in oxygen after endotracheal intubation. Cefazolin (Cefazolin 1 gr, Loghman Pharmaceuticals, Iran) 20 mg/kg was given as preoperative antibiotic immediately after induction and lactated ringer's solution (Lactated Ringer 500 ml, Shahid Ghazi Pharmaceutical Co., Tabriz, Iran) 10 ml/kg/hr was infused during the surgery.

The dogs were placed in dorsal recumbency on the operating table and the ventral neck region was aseptically prepared. A ventral midline cervical incision was made on the skin beginning from the larynx and extending to the manubrium. The platysma muscle and subcutaneous tissues were incised and retracted. After separation of the sternohyoid and sternocephalic muscles and retraction of the underlying trachea to the right, access was gained to the cervical esophagus. Moistened gauze sponges were used to pack off the esophagus from the remainder of the surgical field. Stay sutures were placed and two longitudinal full thickness incisions measuring 3 cm were made in the cranial and caudal cervical esophagus of each dog therefore a total of 8 incisions /group were created. The esophageal lumen was flushed with warm saline solution and the incisions were sutured with single layer simple interrupted pattern using 3/0 nylon (Supalon, Supra Medical Devices, Tehran, Iran). The sutures were placed 2 mm from the wound edge and 2 mm apart. The integrity of suture line was checked by occluding the lumen, injecting saline and observing for any leakage between sutures after applying gentle pressure. In the treatment group, PRF membrane was sutured over the incision using simple interrupted pattern to reinforce the esophagotomy suture line (Fig.2). The incised muscles, subcutaneous tissues and skin were sutured routinely to complete the procedure.

Postoperatively, antibiotic therapy with cefazolin 20 mg/kg IV was continued for 5 days and the animals were given ketoprofen (Vetofen, Aburaihan Pharmaceuticals Co., Tehran, Iran) 2 mg/kg IM for 3 days as analgesic. Oral food was withheld for 24 hours and blenderized diet was offered for the next 3 days until the animals were gradually returned to their normal diet.



Figure 2. Completed patching of esophagotomy suture line using PRF clots sutured over the incisions

Macroscopic and histological examination

The dogs were first anesthetized with thiopental 10 mg/kg IV and subsequently euthanized with an overdose of the same drug on the 28th postoperative day. Access was gained to the cervical esophagus via the same approach used during the surgery. The esophagotomy incisions were identified and digital photographs were taken to measure the adhesion length. The adhesions were bluntly and carefully dissected and the cervical esophagus was totally removed. Each incision was then sectioned into two halves and the sections were used for histological and biochemical evaluations.

For histological analysis, the samples were fixed in 10% buffered neutral formalin and embedded in paraffin for routine sectioning. The 5 µm thick sections were stained with hematoxylin-eosin and Masson's trichrome and examined blindly under the light microscope to evaluate the healing of different layers of the esophagus. Digital photographs of the healed incision area were taken and the following histomorphometric measurements were made: thickness of the newly formed mucosal epithelium, thickness of the healing reaction defined as the thickness of the healed esophageal wall at the incision site without the mucosal layer and length of the healed area devoid of submucosal glands and muscular layer. The former two measurements were made at the center of the healed area and at the edges of the incision with normal esophageal tissue and the mean of the three measurements were used for each sample. All macroscopic and histological measurements were made using the ImageJ software (ImageJ 1.45s, National Institutes of Health, USA).

Hydroxyproline measurement

Tissue specimens harvested for biochemical analysis were preserved at -70 °C. The amount of tissue hydroxyproline was measured by modified spectrophotometric method described by Podenphant et al.¹⁵

Statistical analysis

One sample or unpaired t-test was used to compare the mean values of the quantitative data between the two experimental groups. The significance level was defined as $P < 0.05$. GraphPad Prism 5 software package (GraphPad Software Inc., La Jolla, CA) was used for data analysis.

Results

All animals survived the surgical procedure and no mortalities were recorded during the experimental period. Postoperative complications such as regurgitation, vomiting, dysphagia or infection were not

observed. Incisional swelling was observed to some extent in all dogs after the surgery which resolved in a few days.

At macroscopic examination, adherence of the incision site to the surrounding soft tissues was observed in all dogs. Although permanent adhesions were not seen and they were broken down by gentle blunt dissection, but stronger adhesions were observed in the control group (Fig.3). Also the extent of the adhesions in the treatment group was significantly lower in comparison with the control group (Fig.4). Wound dehiscence, leakage, stricture and fistula formation was not observed in any of the dogs. PRF membrane used in the treatment group was indistinguishable from the surrounding tissues.

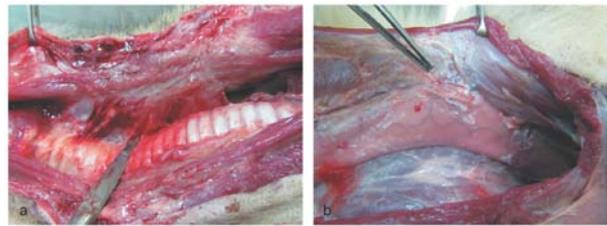


Figure 3. Adhesion of the esophagus to the surrounding tissues in control (a) and treatment (b) groups indicating stronger and lengthier adhesions in the control group.

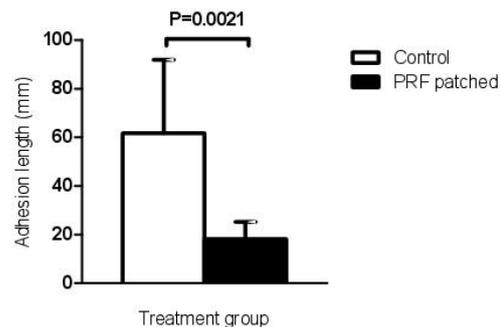


Figure 4. Mean adhesion length in the two treatment groups, error bars indicate standard deviation (SD) and the P value represents the statistical difference between the two groups

The incision site was readily identifiable in histological sections from the surrounding normal tissue due to alterations observed in the structure of the esophagus. Complete epithelial regeneration was observed in all 8 samples of the treatment group and 7 samples from the control group. The newly formed mucosal epithelium was thinner than the surrounding normal epithelium in both groups. The submucosal and muscular layers of the esophagus had lost their normal structure and were mainly replaced by a newly formed fibrous or collagenous reparative tissue in both experimental groups. The submucosal glands and muscles were confined to the edges of the incision (Fig.5). Results of histomorphometric measurements indicated better wound healing in the treatment group. Mean thickness

of the newly formed epithelial layer along with the healing reaction in the treatment group was more than the control group although the difference was not statistically significant. Mean length of the esophagus devoid of submucosal glands in the treatment group was insignificantly less than the control group whereas the mean length of the esophagus lacking any muscular layer was significantly less in the treatment group in comparison to controls (Fig.6).

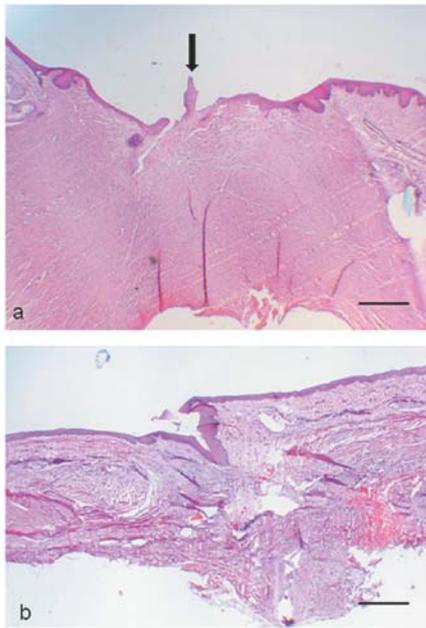


Figure 5. Representative histological sections of the healed esophageal tissue at the site of the incision. Incomplete mucosal re-epithelialisation (arrow) can be seen in the control group (a) while complete re-epithelialisation is observed in the treatment group (b) (Hematoxylin-eosin staining, bars= 2.04 μm).

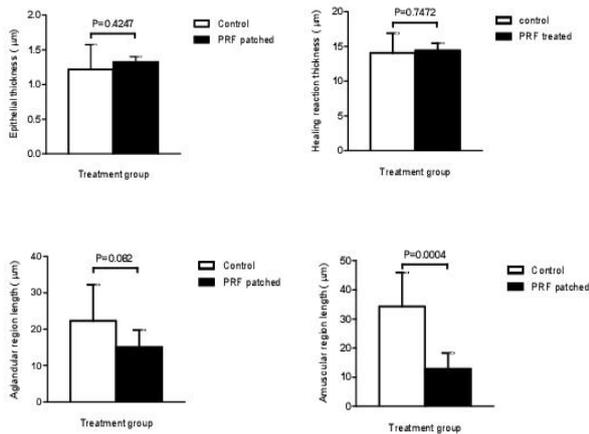


Figure 6. Mean values of the histomorphometric measurements in the two treatment groups, error bars indicate standard deviation (SD) and the P value represents the statistical difference between the two groups.

Mean tissue hydroxyproline content of the treatment group was more than the control group but there was no statistically significant difference between the two groups (Fig.7).

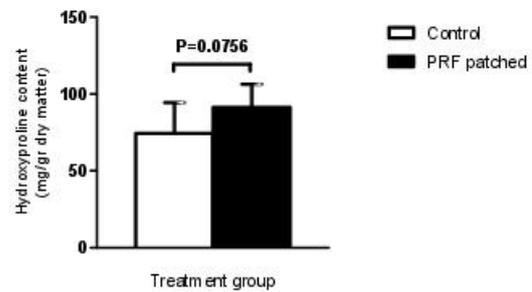


Figure 7. Mean tissue hydroxyproline content in the two treatment groups, error bars indicate standard deviation (SD) and the P value represents the statistical difference between the two groups

Discussion

Esophagotomy or esophageal surgery in general has always been associated with higher postoperative complication rates in comparison with other parts of the gastrointestinal tract. Several factors have been held responsible including the presence of adventitia instead of serosal layer leading to ineffective fibrin production and sealing of the incision site, segmental blood supply, lack of omentum, constant motion and distension resulting from deglutition and respiration and the inability of the esophagus to tolerate tension.^{1, 2, 5} It has also been suggested that suture placement in the mucosa is made more difficult due to its considerable retraction from the cut margin of the esophagus which has been attributed to the unusual mobility of the mucosa resulting from the fat content of the submucosal layer.¹⁶ Low vascularity and excess tension at the suture line appear to be the major reasons for delayed wound healing and problems encountered in esophageal surgery.¹⁷ Apart from that, collagen metabolism is the most important influential factor in esophageal wound healing. The polymerized collagen present in normal esophageal tissue is replaced by immature and mechanically weaker newly formed collagen hence the probability of dehiscence and leakage increases at days 4 to 7 following surgery.¹⁸ To minimize the incidence of wound dehiscence and esophageal leakage, patching of the suture line with various tissues has been recommended. Apart from the periesophageal muscles, greater omentum has been used for this purpose by several investigators.^{17,19,20} Omental patching prevents leakage and reduces adhesion of the incision to the surrounding tissues. But it appears that the most important benefit of using omentum lies in its ability to increase revascularization and neovascularization of the healing esophageal tissue through the release of its lipid

angiogenic factors particularly vascular endothelial growth factor (VEGF).¹⁹ Omentum must be harvested from the abdominal cavity via celiotomy which increases tissue destruction leading to increased patient morbidity therefore its use outside the abdominal cavity is infrequent.

PRF is a true biomaterial which can be prepared easily and inexpensively from the patient's own blood before or during the surgical procedure. It is a rich source of several growth factors including platelet derived growth factor (PDGF), VEGF, transforming growth factor β (TGF β) and thrombospondin-1 which promote wound healing mainly by stimulation of collagen production and increase in wound strength.²¹ Due to the absence of any anticoagulants in the preparation of PRF, platelet activation is triggered resulting in the release of growth factors and cytokines slowly over an extended period of time in the site of PRF use.²²⁻²⁴ Apart from the growth factors, large amounts of fibrin present in PRF also promotes wound healing by allowing recruitment, migration, adhesion and proliferation of cells needed for wound repair.²⁵

PRF is not the same as plasma and there are differences between these two blood products. Plasma is the supernatant obtained following centrifugation of a whole blood sample which is taken with an anticoagulant and contains only acellular components of blood including hemostatic proteins. The addition of calcium chloride and thrombin to plasma results in the formation of diffuse fibrin clot and entrapment of all the cellular components of blood in the clot. As previously mentioned, PRF is produced by immediate centrifugation of whole blood without any anticoagulant. The absence of anticoagulant results in the activation of platelets and release of fibrinogen which is concentrated as a result of centrifugation in the middle part of the test tube. Therefore, PRF or fibrin clot is formed naturally during centrifugation in the middle part of the sample and platelets plus leukocytes are trapped inside the fibrin network. The red blood cells at the bottom and the supernatant acellular plasma or correctly termed serum can easily be separated from the PRF clot.^{14,22,23}

Dogs were chosen as the animal model to carry out this study based on the recommendations of Dohan Ehrenfest and colleagues. They argue that studies involving the use of PRF should be carried out on large animal species like dogs. True PRF clots which can be used in various surgical procedures can be obtained in these species as opposed to poor quality PRF like fibrin which is produced in laboratory animals.²⁶ The reason for making two esophageal incisions in the cervical region of each animal in this study was to limit the

number of animals used while creating acceptable number of samples for analysis. Regarding the choice of suture pattern for closure of esophageal incisions, we opted for single layer instead of double layer simple interrupted sutures to rapidly complete the operations and decrease anesthesia time. Although it has been accepted that traditional double layer single interrupted pattern results in greater immediate wound strength, better tissue apposition and improved esophageal wound healing, but it takes longer to perform and single layer closure can be a rapid, safe and effective alternative which has been used successfully in clinical cases.^{1,2,5} Non absorbable suture materials were used to readily identify the incision site during sampling.

The results of the present study indicated the positive influence of PRF use on esophageal wound healing. The PRF patch had reinforced the suture line and increased its strength similar to omental graft but its preparation was much faster and easier than obtaining omentum from the abdominal cavity. Also the use of this autologous biomaterial significantly reduced the amount of adhesion formation. Collagen production assessed by measuring the amount of tissue hydroxyproline was also increased due to PRF use. Although angiogenesis and neovascularisation was not quantified in the present study by employing specific techniques like immunohistochemistry, but due to the release of VEGF from PRF patch, these features of wound healing could also be expected to be increased by using PRF patches to support esophagus.

Based on the results of the histological evaluations of the present study, it seems that the growth factors released from the PRF membrane mostly influenced the wound healing process in the external layers of the esophageal wall particularly the muscular layer. This seems logical due to the fact that the PRF membrane was sutured to the most external layer of the esophagus i.e. the adventitia.

To the authors' knowledge, this is the first study describing the use of PRF membrane as an onlay patch in esophageal surgery therefore the results could not be compared with similar studies. Guinot et al²⁷ have successfully used autologous PRF membrane for urethroplasty coverage in distal hypospadias surgery of human patients. They concluded that PRF patch is a safe and efficient coverage technique helping to reduce postoperative complications in circumstances where healthy tissue is unavailable for coverage.

Based on the results of the present study, it seems that PRF membrane can be used as an alternative to other methods of esophageal suture line reinforcement techniques safely and effectively although further studies should be carried out in clinical surgical cases.

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چکیده

ارزیابی امکان بکارگیری غشاء فیبرین غنی از پلاکت جهت استحکام محل بخیه در عمل برش مری و تأثیر آن بر التیام زخم

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هدف- هدف از این مطالعه ارزیابی امکان استفاده از غشاء فیبرین غنی از پلاکت به عنوان ماده زیستی خودی جهت وصله محل برش و افزایش استحکام محل بخیه به عنوان روشی جدید در عمل برش مری بود.

طرح - مطالعه تجربی

حیوانات- هشت قلابه سگ بالغ نر و ماده از نژاد مخلوط

روش کار- حیوانات بطور تصادفی به دو گروه مساوی شاهد و تیمار تقسیم شدند. در قسمت مری گردنی هر حیوان دو برش به طول ۳ سانتیمتر ایجاد شد. تمامی برشهای ایجاد شده بخیه زده شد و در گروه تیمار از غشاء فیبرین غنی از پلاکت به عنوان وصله بر روی خط برش استفاده شد. پس از سپری شدن مدت زمان ۲۸ روز التیام زخم در محل برش با استفاده از روش های ماکروسکوپی، میکروسکوپی و بیوشیمیایی مورد ارزیابی و مقایسه قرار گرفت.

نتایج- استفاده از غشاء فیبرین غنی از پلاکت منجر به کاهش معنیدار میزان چسبندگی (۱۸/۲۴ میلیمتر در مقایسه با ۶۱/۶۷ میلی- متر) و افزایش غیر معنیدار میزان هیدروکسی پرولین بافت التیامی (۹۱/۳۱ میلیگرم در هر گرم بافت در مقایسه با ۷۴/۳۱ میلیگرم در هر گرم بافت) شد. همچنین غشاء فیبرین غنی از پلاکت تأثیر بیشتری در التیام لایه های خارجی جداره مری بخصوص لایه عضلانی ایجاد کرده بود هرچند التیام کلی زخم در گروه تیمار بهتر از گروه شاهد بود.

نتیجهگیری و کاربرد بالینی- با توجه به نتایج حاصله به نظر میرسد که غشاء فیبرین غنی از پلاکت میتواند جایگزین مناسبی جهت بکارگیری به عنوان وصله روی خط برش در مقایسه با سایر روش ها باشد هر چند مطالعات بالینی بیشتری مورد نیاز میباشد.

کلمات کلیدی- غشاء فیبرین غنی از پلاکت، وصله خط برش، مری، سگ، التیام زخم.