University of Mosul
College of Veterinary Medicine

The Role of Bone Marrow, Hyaluronic Acid and Magnesium Oxide Nanoparticles on Esophageal Anastomotic Healing in dogs

Radhwan Ryad Kadeem AL-Ajeli

Ph.D. Dissertation
Veterinary Medicine / Veterinary Surgery

Supervised By
Professor Dr.

Abdul-Haleem Mawlood Saleh Al-Hasan

Co.dvisor
Professor Dr.

Saevan Saad Al-Mahmood

2022 A.D. **1444 A.H.**

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A dissertation submitted
By

Radhwan Ryad Kadeem AL-Ajeli

To
The Council of the College of Veterinary Medicine
University of Mosul

In
Partial Fulfillment of the Requirements
For the Degree of Doctor of Philosophy

In
Veterinary Medicine / Veterinary Surgery

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Professor Dr.

Saevan Saad Al-Mahmood

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يُؤْتِيهِ مَنْ
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الْفَضْلِ
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سورة الجمعة
الاية (٤)

Acknowledgment

I thank God Almighty and praise Him for His protection in my life and the success in my studies.

I am very indebted and grateful to my supervisor, **Professor Dr. Abdul-Haleem Mawlood Saleh Al-Hassan** and my Co. Advisor **Professor. Dr saevan Saad AL-Mahmmoud** for them great help, support, endless advices, patience, and suggestions for presenting this work in the most comprehensive, clear, and accurate way.

I express my thanks and gratitude to the Dean of the College of the Veterinary Medicine/University of Mosul who provided me with all the facilities required for this study. I also, thank the Vice Dean for Graduate Studies and Scientific Affairs.

I also extend my thanks to the Head of the Department of Surgery and Theriogenelogy for the facilities he provided me to complete this study.

I would like to express my appreciation to all the academic and technical staff in the Department of Surgery and Theriogenology at the College of Veterinary Medicine / the University of mosul, for their tremendous support and patience that contributed to the success of this work.

Radhwan

Supervisor Certification

I certify that this dissertation entitled **The Role of Bone Marrow, Hyaluronic Acid and Magnesium Oxide Nanoparticles on Esophageal Anastomotic Healing in dogs "** was prepared under my supervision at the College of Veterinary Medicine / University of Mosul, as a partial fulfillment of the requirements for the degree of Ph.D. in Veterinary Medicine/Veterinary surgery.

Signature:
Name: Prof. Dr saevan Saad AL-Mahmmoud (Co. Advisor)
Date: / / 2022

Signature:
Name: Prof. Dr Abdul-Haleem Mawlood Saleh
Date: / / 2022

Linguistician Certification

I certify that this dissertation entitled **" The Role of Bone Marrow, Hyaluronic Acid and Magnesium Oxide Nanoparticles on Esophageal Anastomotic Healing in dogs"** has been linguistically reviewed and corrected for language and expression mistakes; therefore, it becomes ready for the defense as for as the integrity of the writing and expression.

Signature:
Name: Lecturer. Dr Radhwan Ameen Hussein
Date: / / 2022

Department of Surgery and Theriogenology Head Certification

Based on the supervisor and linguistician recommendations, I forward this dissertation for the defense.

Signature:
Name: Prof. Dr Mohammed Abdalelah Aziz
Date: / / 2022

Postgraduate Committee Director Certification

Based on the supervisor, linguistician and the Head of the Department of Surgery and Theriogenology recommendations, I forward this thesis for the defense.

Signature:
Name: Prof. Dr . Raad Abdulghany Al-Sengery
Date: / / 2022

Examination Committee Decision

We, the members of the Evaluation and Discussion Committee, have reviewed this dissertation and discussed the student **Radhwan Ryad Kadeem AL-Ajeli** in its contents on 20 / 11 / 2022, and certify that he deserves the degree of Ph.D in Veterinary Medicine / Veterinary Surgery

Signature:
Name: Prof. Dr. Layth Mahmoud Dawod
Member

Signature:
Name: Prof.Dr. Taha Hassan Taha
Member

Signature:
Name: Assist. Prof. Dr. Osama Hazim Ismail
Member

Signature:
Name: Assist Prof. Dr. Mohammed Ghassan Saeed
Member

Signature:
Name: Prof. Dr. Bahjat Taifor Abbas
Chairman

Signature:
Name: Prof. Dr Abdul-Haleem Mawlood Saleh
Member and Supervisor

Signature:
Name: Prof. Dr sevan Saad AL-Mahmmoud (Co.Adviser)
Member and Co. Advisor

College Council Decision

The College of Veterinary Medicine Council was met, the meeting, on / / 2022, and decided to award him a degree of Ph.D. in Veterinary surgery.

Council Registrar
Prof. Dr . Raad Abdulghany Al-Sengery

The Dean
Prof. Dr. Dhafer Mohammad Aziz

Abstract

The objective of this study was to evaluate the healing process of cervical esophageal anastomosis that induced experimentally in dogs through local application of some bone marrow ,hyaluronic acid and MgO NPs on the anastomotic site. Thirty-six healthy stray dogs from both sexes, weighted an average 25 kg ± 1.3 and aged an average 27 months ± 1.5 were used in present study. The experimental animals allocated into four main groups 9 of each. They were divided into four equal main groups with 9 dogs . Each main group was furtherly divided into 3 subgroups of 3 dogs each according to time after operation 7,15,30 days. The autologous aspirated bone marrow, hyaluronic acid and Magnesium oxide Nanoparticles(MgO NPs) were spread on the mucosa and the muscular layer at the esophageal anastomotic site in each group according to the following :-

Group 1: Control group the anastomotic site left without addition of any materials.
Group 2: Bone marrow group autologous aspirated bone marrow.
Group 3: Hyaluronic acid
Group 4: MgO NPs size 20 nm

Evaluation of the results were based on the five criteria clinical signs by monitoring of the animals for detection on dysphagia, in addition esophageoscopy for detecting any stich dehiscenc (as a post- operative investigations), gross pathological examination , contrast radiography, histological, Immunohistochemistry studies and their histological scoring. The results of clinical study showed decreasing in the severity of dysphagia with time after operation (7^{th}, 15^{th},30^{th} P.O.Ds) in the same group, as well as, the severity of dysphagia as a comparison among the groups was a recorded mild in MgO NPs, mild to moderate in bone marrow , moderate in hyaluronic acid, and obvious in control group . Esophageoscopy reveals absence of any stich dehiscence that cause esophageal leakage. The gross pathological examination showed continuously decreasing in clarity of

anastomotic line over time after operation (7^{th}, 15^{th},30^{th} P.O.Ds) in the same group, in addition, among the groups the best result of gross pathological examination based on clarity of anastomotic line was recorded in MgO NPs, then in bone marrow , and hyaluronic acid compared with control group. After operation, the radiographic examination over time (7^{th} 15^{th},30^{th} P.O.Ds) showed non –significant increasing in percentage degree of stenosis in group 1 at $P<0.05$, while , in group 2, 3 and 4, there were decreasing (non-significant in 2 and significant in 3 and 4 groups at $P<0.05$). Among the groups, and at any postoperative time, low percentage degrees of stenosis were recorded in MgO NPs ,then in bone marrow and hyaluronic acid group compared with control group.

Histological and immunohistochemistry studies examination showed that the healing process was best recorded in the MgO NPs group, then in the bone marrow, and then in hyaluronic acid group in comparison with control group.

In conclusion , based on investigated criteria , the biological (bone marrow and hyaluronic acid) and non -bioactive materials(MgO NPs) can be used successfully when applied locally on the esophageal tissue for enhancement healing esophageal process, as well as the MgO NPs group was best then bone marrow and hyaluronic acid compared to control group through lowering degree of dysphagia, decreasing clarity of anastomotic line and decreasing percentage degree of stenosis and accelerate healing process.

Contents

List of Figures

List of Tables

List of Abbreviations

Abbreviation	Total name
FGF	Fibroblast growth factor
MSCs	Mesenchymal stem cells
BMT	Bone marrow transplantation
ECM	Extra cellular matrix
HA	Hyaluronic acid
IL-6	Interleukin- 6
TNF	Tumor necrosis factor
IGF-1	Insulin growth factor -1
GERD	Gastro-esophageal reflux disease
LES	Lower esophageal sphincter
IL-6	Interleukin -6
IL-8	Interleukin -8
IGF-1	Insulin-like growth factor-1
bFGF	Basic fibroblast growth factor

CXCL8	Ckemokine (CXC motif) ligand 8
CXCL1	Ckemokine (CXC motif) ligand 1
CCL2	C-C Motif ckemokine ligend 2
PDGF	Platelets-derived growth factor
VEGF	Vascular endothelial growth factor
FGFs	Fibroblast growth factor
HGF	Hepatocyte growth factor
MMPs	Matrix metalloproteinases
PRF	Platelet-rich fibrin
PRP	platelet-rich plasma
AAMSCs	Autologous aspirated mesenchymal cells
FS	Fibrin scuffled
EAL	Esophageal anastomotic leakage
GAGs	glycosaminoglycans
HMW	high molecular weight
LMW	low molecular weight
CD44	Cluster of differentiation 44
BBB	Blood-brain barrier
BRB	Blood-retinal barrier
ETT	Endotracheal tube
P.O.Ds	Post operative days
EGF	Epidermal growth factor
P	Proximal
D	Distal

Chapter one
Introduction

Esophagus is a relatively narrow musculo-membranous tube (Robert and Michael,2013), Conveys food, water and saliva, to the stomach (Konig and Liebich, 2004). Esophagus can be affected by numerous diseases cause a range of clinical signs, like regurgitation is the most prevalent clinical symptom in dogs persistent vomiting, coughing ,dysphagia, nasal discharge, or ptaylism (Washabau, 2005; Elwood, 2006; Marks,2017).The most important affections of esophagus include Esophageal foreign bodies such as bones, fishhooks, needles, and sticks (Jorg ,2008; Marks, 2017), esophageal neoplasia (Marks, 2017) ,esophageal fistula (Broncho-esophageal fistula) and fistulation with the skin (Jorg ,2008; Kaminen, *et al*., 2014), cervical esophageal diverticulum (Jorg,2008; Robert and Michael,2013) and Esophageal stricture (Fossum, *et al*.,2007). The radiography (Gaschen, 2018) and esophagoscopy most commonly diagnostic methods of esophageal diseases (Gualtieri, 2001; Tolbert, 2017).

History of esophageal anastomosis began from the first resection and anastamosis of cervical part in humans in 1877, by Czerny, and Dobromysslow described the first intrathoracic esophageal resection in 1901. (Yuan, et al.,2015) . Esophagectomy is a major surgical procedure used to correct a variety of esophageal disorder (Lerut, *et al*.,2002 ; Chen, *et al*.,2014 ; Seungju, *et al* .,2020) with high rate incidences of various complication (Bardini, *et al*.,1994; Chen, *et al*.2014) such as leakages (Xiang, *et al*.,2019) , dehiscence (Shahnam, *et al*., 2016) and Stricture (Carroll and Arnold 2012) , that considers are the most common complication (Carroll and Arnold 2012) specially they can be developed

after cervical esophageal anastomosis (Heijl, *et al*.,2010) and most of the complications continue to be a burden of swallowing (Lerut, *et al*.,2002) The bone marrow transplantation (BMT) is a medical method used to treat a variety of disorders (Flowers and Kansu, 2000). Made up of hematopoietic precursors, their differentiated progeny, and stroma. The Mesenchymal stromal cells (MSCs) are a type of regenerative material that has the ability to differentiate into multiple lineages and could be used to restore damaged tissues (Chou, *et al*., 2014; Xiang, *et al*., 2019).

Hyaluronic acid (HA) is important in wound healing process by creating a suitable environment for growth, (Shengkun,*et al*.,2016) and stimulates vascular endothelial cell proliferation and migration during angiogenesis.(Erin and Pardue *et al*., ,2008; Fallacara, *et al* .,2018) in addition stimulates chemotaxis, leukocyte and releases of inflammatory cytokines such as IL-1, TNF, and IGF-1 (Tavianatou, *et al*.,2019)

Using of inorganic nanoparticle in the medicine is a new therapeutic option, unlike conventional tissue repair, recently using in soft tissue repair technique (Urie *et al*., 2018). Magnesium (Mg) is inorganic mineral has a critical role in the body by modulation of extracellular matrix (ECM) and its interactions , MgO NPs has been employed in soft tissue engineering to boost proliferation and differentiation of the fibroblast. (Hickey and Webster, 2015).

Due to presence of different diseases of esophagus that need esophageal anastomosis with high incidence of complications as a result to present many predisposing factors that delay esophageal healing such as lack of serosa and omentum, segmental blood supply, and continuous motion of the suture site, therefore the project is designed to :-

A. Enrich this subject with the best biological and non-biological materials that to enhance and accelerate the esophageal healing process when applied locally on anastomotic site, these materials are includes:-

i. Bone marrow

ii. Hyaluronic acid

iii. Magnesium Oxide as a nanoparticles (MgO NPs), where we did not find any previous study confirming the use of this substance in esophageal surgery .

B. Compare between all groups depending on the clinical signs and endoscope investigation (as a post-operative observation), gross pathological changes ,contrast radiography, histological changes , immunohistochemistry and scoring of histological sections for evaluating the anastomotic site and its healing process. .

Chapter two
Review of Literature

2-1: Anatomy of Esophagus

Esophagus is a relatively narrow musculo-membranous tube begins dorsal to the cricoid cartilage of the larynx and follows the trachea down the neck to end at the cardia of the stomach (Dyce *et al*., 2010 ; Robert and Michael,2013), Conveys food, water and saliva to the stomach (Konig and Liebich, 2004; Bexfield, *et at*, 2006 ; Robert and Michael, 2013), In carnivores the lumen of esophagus wide at the thoracic inlet and is prone to dilatation of esophagus which predisposed this species less susceptible to chock at thoracic inlet and diaphragmatic hiatus if a compared with ruminant and equine(Konig and Liebich, 2004; Dyce *et al*., 2010).

The esophagus is divided into cervical, thoracic and short abdominal part, the cervical part of esophagus passes within the visceral space of the neck(Jergens, 2010; Hussein, *et al*.,2017) ventrally to the sub vertebral muscles (longus coli muscles) (Konig and Liebich, 2004; Dyce *et al*., 2010) and follows the trachea dorsally, and becomes on the left side of it closely to the thoracic entrance (Dyce, *et al*., 2010;Gaschen, 2018), then the cervical part of esophagus returns to the median position above the trachea before or shortly after entering the thorax, at the long of the length of cervical part ,esophagus is surrounds by the left carotid artery , recurrent laryngeal nerves and vagosympathetic trunk (Konig and Liebich, 2004; Dyce *et al*., 2010).

Within the thorax the thoracic part , moves in the mediastinum and, continues just above the tracheal bifurcation, crosses the base of heart, continues ventrally to the ascending aorta with a slight dorsal inclination and enters the abdomen through the esophageal hiatus of the diaphragm, and then traverses over the dorsal border of the liver to joints with the

stomach at the cardiac opening (Konig and Liebich, 2004; Dyce *et al*., 2010; Robert and Michael,2013).The short abdominal portion of esophagus has the shape wedge ,that joins with the gastric at the cardiac opening (Dyce, *et al*., 2010; Gaschen, 2018) and lies slightly to the right of midline(Konig and Liebich, 2004; Dyce *et al*., 2010)

Structure of esophagus comparable to the remainder of the digestive system and has 4 layers from exterior to interior (Fossum *et al*.,2007 ; Dyce *et al*., 2010 ; Robert and Michael,2013; Jardim Gomes, 2019), The outer layer is adventitia (tunica adventitia)(Konig and Liebich, 2004; Glazer and Walters,2008) as a loose connective tissue in the neck connects the esophagus to neighboring structures and allows freedom to move during swallowing and when the animal bends its neck . In the thoracic part tunica adventitia replaced by serosa that originated from the pleura whereas in the abdominal part originate from peritoneum(Konig and Liebich, 2004).

The muscular layer (tunica muscularis) is weak and holds structure poorly(Konig and Liebich, 2004; Dyce, *et al*.,2010) consist of two striated muscles layer at a long of whole length of esophagus in dogs (Bexfield, et at, 2006), the outer layer longitudinal and an inner layers circular , at the pharyngeal end of the esophagus the fiber of the outer layer fused with the crico -pharyngeus muscle and forming the pharyngo-esophageal sphincter (Glazer and Walters,2008 ; Dyce *et al*., 2010; Jardim Gomes,2019) Closely to the stomach at the diaphragmatic hiatus, the outer layer gets more longitudinal, whereas , the inner becomes more circular to creates the cardiac sphincter. (Konig and Liebich, 2004; Dyce, *et al*.,2010; Pollard, 2012; Robert and Michael,2013) (Figure 1) .

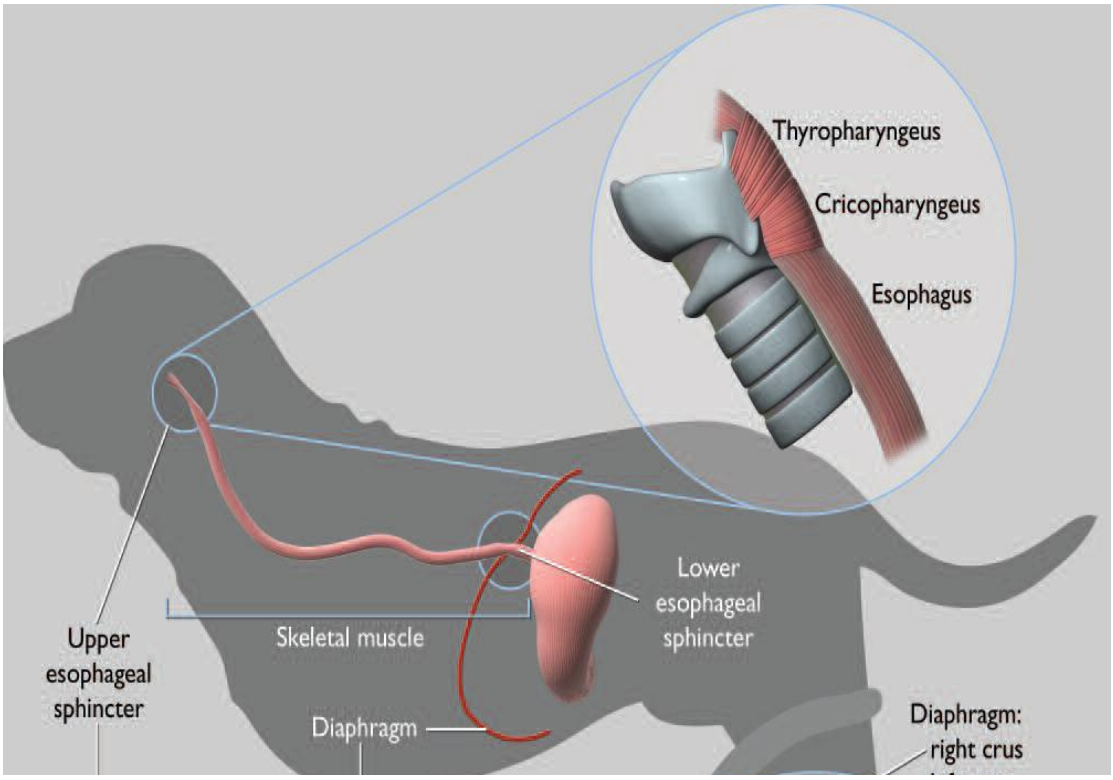

Figure 1: showing the upper and lower esophageal sphincter in dogs (Glazer and Walters,2008)

The inner part of the wall is divided between submucosa and mucosa (Figure 2). The submucosa in dogs contains mucous gland on the entire length of esophagus (Aughey, and Frye., 2001; Hussein, *et al*.,2017**)**, the submucosa also loosely connects the mucosa with musclaris thus enabling the mucosal layer to be thrown into longitudinal folds when the esophagus contracts. The mucosa is relatively stronger layers of esophagus and consists of three sublayers; epithelum mucosa (Dyce *et al*., 2010; Robert and Michael,2013) that covered with stratified squamous epithelium (William and Linda,2000; Konig and Liebich, 2004; Orlando, 2010), lamina properia mucosa and lamina muscularis mucosa that usually more prominent in the thoracic esophagus (Dyce *et al*., 2010; Robert and Michael,2013,).

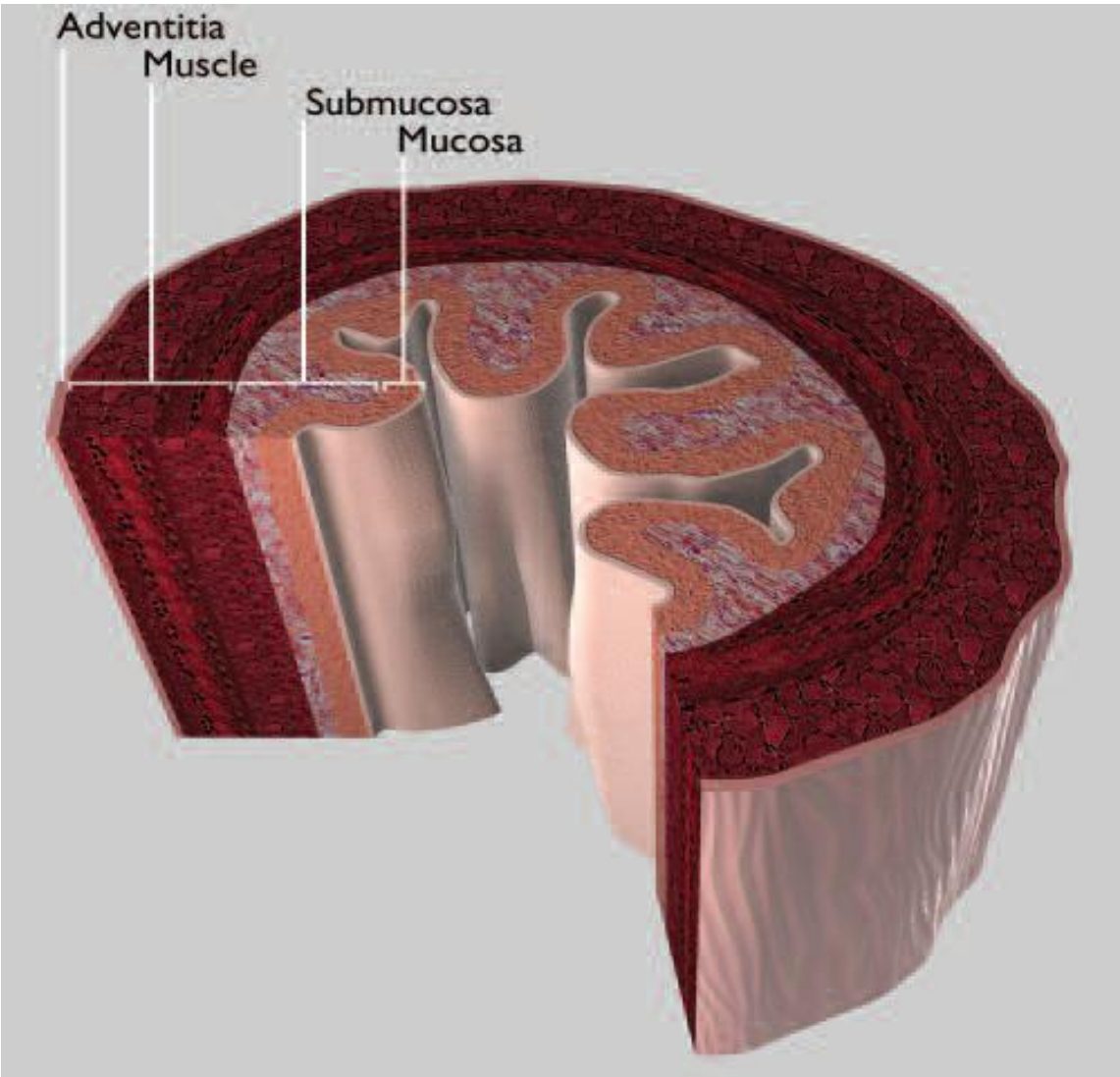

Figure 2: Showing the histological layer of esophagus
(Glazer and Walters,2008)

2- 2: The blood supply and lymphatic drainage

The esophagus has a segmental blood circulation , the cervical part receives blood from the cranial and caudal thyroid and esophageal branches of the common carotid arteries(Venker-van-Haagen, 2013), the cranial thyroid artery gives some branches that provides the pharyngo-esophageal lamina at the cranial esophageal sphincter , and the left caudal thyroid artery on the left side of thoracic inlet provides a small descending branch, and will connects with an ascending branch from broncho

esophageal artery, and finally will supply the cervical esophagus (Jardim Gomes,2019).

The two cranial thirds of the thoracic esophageal portion receive blood by broncho- esophageal artery (Venker-van-Haagen, 2013) while the aorta or intercostal arteries supply the caudal thoracic esophagus, however. The short abdominal part of esophagus receives blood from branch of the left gastric artery (Fossum *et al*.,2007; Gaschen, 2018).

The venous drain is achieved by external jugular vein and azygos veins, the blood from the cervical portion drains into external jugular vein whereas the blood leaves thoracic part drains into azygos vein (Venker-van-Haagen, 2013; Jardim Gomes,2019). The lymphatic drain of esophagus into the mediastinal lymph nodes and into deep cervical lymph nodes (Dyce *et al*., 2002; Konig and Liebich, 2004; Jardim Gomes,2019)

2-3: The innervation of Esophagus

The sympathetic and vagus nerves, as well as the recurrent laryngeal branches, responsible for innervation of esophagus (Dyce *et al*., 2002; Konig and Liebich, 2004). The cervical part is provided by the left and right recurrent laryngeal nerves and the proximal portion of thoracic part is feed by the left recurrent laryngeal nerve, while the distal aspect of thoracic portion and short abdominal part are enriched by the vagal trunk (Elwood, 2006; Dyce, *et al*., 2010; Venker-van-Haagen, 2013)

2-4: Physiology of Esophagus

Esophagus in the dog carries the food and liquids from mouth, pharynx to the stomach (Jergens, 2010; Gaschen, 2018). By functional mechanism, (swallowing) that include two phases the 1st phase is voluntary and the 2nd involuntary, The voluntary phase happens between the tongue that directed caudally to bring bolus to the oropharynx and contact with the pharyngeal mucosa (Dyce, *et al*., 2010), Followed by 2nd involuntary movement that shearing between the base of mouth and esophagus, when the bolus passes the cranial esophageal sphincter, (Pollard, 2012) the sphincter will be closed to prevents retrograde of ingesta (Pollard, 2012). After that the bolus pushed from the esophagus to the stomach by action of the two a peristalsis waves , The primary peristaltic is originated in the base of mouth and conveyed to the esophagus to pushes the meal caudally to the esophageal (Jergens, 2010; Pollard, 2012) any missing in this wave , the esophagus will distend and stimulate a secondary wave , after that the meal arrives the lower aspect of esophagus and the cardia relaxes for allowing the meal passes to the gastric (Elwood, 2006; Jergens, 2010; Pollard, 2012). Finally the cardiac sphincter closed to prohibits gastro-esophageal reflux (Jergens, 2010). The swallowing mechanism is regulated and controlled by cranial nerves including the trigeminal(V), facial (VII), glossopharyngeal (IX), vagus (X), hypoglossal (XII) (Pollard, 2012; Gaschen, 2018).

2- 5 : Affections of the Esophagus

The esophagus of dogs can be affected by numerous diseases cause a range of clinical signs (Elwood, 2006; Marks, 2017), esophageal foreign bodies, acute esophagitis, strictures, gastro-esophageal intussusception (Venker-van-Haagen, 2013), esophageal hiatal herniation and megaesophagus (Jardim Gomes,2019) are esophageal diseases manifested with Regurgitation (Venker-van-Haagen, 2013) that consider most prevalent clinical symptom in dogs with esophageal illness (Elwood, 2006; Marks, 2017). Depending on the illness development or subsequent consequences various clinical symptoms such as anorexia, persistent vomiting, coughing ,dysphagia, nasal discharge, or ptaylism, may be noted in esophageal disorder (Washabau, 2005; Elwood, 2006; Marks,2017).The most important affections of esophagus include:-

2-5-1: Esophageal hiatal herniation

Hernias of the esophagus, it is a congenital or acquired condition (Dvir, *et al*., 2003; Jergens , 2010; Reeve, *et al*., 2017). Rarely occurrence in dogs and cats due to translocation of any abdominal structure via the diaphragmatic esophageal hiatus, especiallywhen the phrenic esophageal ligament stretches and permitting herniation of the short abdomens part of esophagus portion, cardiac opening, gastric and any part of digestive system. (Dvir, *et al*., 2003; Pollard, 2012). This case surgically treated through correction of diaphragmatic hiatus or esophageopexey (Fossum *et al*, 2007). Hernias of the esophagus may be asymptomatic, or some time associated with recurrent signs such as regurgitation, retching and finished by vomiting.

The diaphragm weakness, elevation of intra-peritoneal pressure, and blocking of upper respiratory tract are a risks factors for occurrence of developed hiatal herniation in dogs and cats (Jergens, 2010; Reeve, *et al*., 2017). The lateral radiographs technique can reveals the sliding hiatal hernia as a smooth appearance or mixed smooth with gas closely located to the aorta and caudal vena cava (Gaschen, 2018) as well as the esophageoscoy also can assist in the diagnosis of sliding esophageal hiatal hernia, (Gualtieri, 2001).

2-5-2: Gastro- esophageal intussusception

Gastro-esophageal intussusception is uncommon illness of dogs usually occurrence in younger males dogs , with a potential life-threatening manifested by the intussusception of any abdominal organs such as the stomach , omentum, ,spleen, duodenum and pancreas, into the distal portion of thoracic esophagus (Pietra, *et al*., 2003; Venker-van-Haagen, 2013; Murphy, *et al*., 2015; Brady, *et al*., 2017; Gaschen, 2018) with high chance of impermanent of blood circulation of the intussuscepted organs , therefore its considered as a surgical urgent case (Brady, *et al*., 2017).

Gastro-esophageal intussusception may be occurs due to esophageal disease that causing weakness of esophagus such as myasthenia gravis, congenital abnormality of esophageal hiatus (Brady, *et al*., 2017) and dilated esophagus (Venker-van-Haagen, 2013). The clinical signs of this condition include abdominal pain, vomiting or haematemesis, and dyspnea (Pietra, *et al*., 2003).

Gastro-esophageal intussusception can be diagnosed by using endoscopy (esophageoscopy) through displaying a distended esophagus that, fully filled with stomach mucosal folds (Gualtieri,2001) or by contrast radiograph that, usually showing of the retende barium meal at the upper part of the esophagus, without reaching to the stomach or caudal thoracic

esophagus (Venker-van-Haagen, 2013) or can be diagnosed by CT scans that useful as s diagnostic imaging tool (Shum, *et al*., 2007).

2-5-3: Megaesophagus

Megaesophagus is a disorder of esophagus, may be congenital or acquired, (Wagner, 2008) and most commonly documented motility problem affecting the canine esophagus (Bexfield, *et at*, 2006) characterized by a hypomotile and focally or diffusely dilated esophagus (washabau,2003; Saravanan, *et al*,2010; Marks,2017). The Congenital Megaesophagus affects young dogs and can be hereditary or caused by developmental anomalies in the esophageal innervations. The clinical symptoms do not appear until the puppy is given solid food (Bexfield *et al*., 2006; Saravanan, *et al*,2010), while Acquired Megaesophagus develops in adult dogs as a result to the nerve damage, in cases Myasthenia gravis (distraction nerve and muscles) (Batmaz *et al.*, 1998), Lead poisoning, Addison's disease (hypoadrenocorticism), Thallium toxicosis Hypothyroidism and Polymyositis (Bexfield, et at, 2006, Saravanan, *et al*,2010).

In congenital megaesophagus regurgitation will be first noticed, through the nose especially after weaning, and also Weight loss, increased salivation, and gagging are asymptoms can be noted in this case.

In acquired megaesophagus disease, the affected animal show general weakness, coughing , dyspnea and difficult swallowing , and regurgitation of food and water that happens between a few minutes and many hours after a meal , lead to aspiration pneumonia that considers as a common complication of megaesophagus (Saravanan, *et al*,2010).The Most cases of megaesophagus are diagnosed by radiograph.(Hopper, *et al*.,2001 ; washabau, 2003), that clearly reveals the dilated esophagus filled with air or ingesta (Wray and Sparkes,2006) while , esophageoscopy is not always utilized to diagnose megaoesophageal illness, (Gualtieri, 2001).

2-5-4: Esophageal foreign bodies

Bones, fishhooks, needles, and sticks are the most prevalent foreign bodies are commonly occur in young canine (Jorg, 2008; Marks, 2017; Gaschen, 2018) causing either partial or total esophageal blockage (Elwood, 2006; Wagner, 2008). They usually become stuck at the thoracic entrance, or diaphragmatic hiatus, (Wagner, 2008; Marks, 2017; Gaschen, 2018). The objects that aren't obstructive, like fish bones and pins and other sharp items, prefer to reside in the pharynx (Gaschen, 2018). But when pass the pharynx can puncture the esophageal wall, causing a pneumothorax, pneumomediastinum, pleuritis, mediastinitis, and a tracheo-oesophageal fistula (Elwood, 2006; Wagner, 2008), many of complications such as stricture, esophagitis or aspiration penumonia can be noted if the foreign body stay in place

(Elwood, 2006; Jorg, 2008), the dislodge foreign body should be removed by endoscopy or surgically by esophgatomy or partial esophagectomy (Fossum *et al*, 2007)

Radiographs can be used to determine the location of esophageal foreign body and should start from the pharynx and extend distally to the stomach , (Elwood, 2006; Wagner, 2008; Gaschen, 2018) and esophageoscopy also can be used to detects and eliminates the lodge objects , as well as to assessment esophageal mucosa (Gualtieri, 2001).

2-5-5: Esophagitis

Esophagitis is a frequent consequence of an underlying disease or secondary to esophageal disease, leading to decreasing of esophageal peristaltic motility and, in extreme conditions , esophageal stenosis (Jergens, 2010; Marks, 2017; Reeve, *et al*., 2017). It characterized by an acute or chronic persistent inflammation mucosal layer of the esophagus , which may spreads to the submucosa and muscularis (jorg,2008 ; Marks, 2017 ; Jardim Gomes, 2019).

Esophagitis also can be occurs due to ingestion of caustic agent, swallowing foreign body, thermal burns (jorg ,2008 ; Robert, and Michael. , 2013) chronic vomiting (Elwood, 2006), radiation injury, un explained causes megaesophagus, inflammation accompanied with malignant esophageal tumor (Venker-van-Haagen, 2013) and general anesthesia due to reflex gastric secretion toward the esophagus (Torrente, *et al*.,2017). The clinical symptoms of esophagitis are determined by the depth and severity of inflammation, (Jorg,2008 ; Robert and Michael. , 2013) either early symptoms caused by inflammation or sometime later signs caused by esophageal stenosis , dogs with mild esophagitis may not exhibit any clinical symptoms (Gualtieri and Olivero.,2006; Kook, *et al*.,2014) , but those suffering from more severe esophageal inflammation may have loss appetite, dysphagia,(Mazzei, *et al*., 2009) ptyalism, increased empty swallowing motions, extension of head and neck during swallowing, retching, vomiting, regurgitation, sudden unexplained discomfort, belching and drooling(Han, *et al*.,2003; Muenster, *et al*.,2017; Peter,2021)

The most sensitive technique of diagnosing esophagitis is an endoscope, (Peter,2021) . Erythema and edema, generally above the lower esophageal sphincter, are early symptoms of esophagitis can be noted through esophageoscopic examination, and some time we note increased vascularity that appear due to enlargement of capillaries near the mucosal surface as response to acid, with long term acidic injury leads to proliferation of submucosal esophageal glands (Van and Willems, 1998; Peter,2021) and their excretory ducts may be observed as round spot. Another typical symptoms is increased granularity and the mucosal surface seems to be rough and puckered,(Mazzei, *et al*.,2009 ; Peter,2021).

2-5-6: Gastro-esophageal reflux disease (GERD)

Manifested as a dysfunction of the cardiac sphincter that causes regurgitation of gastric or esophageal content (food or liquid) in humans and dogs. (Venker-van-Haagen, 2013; Muenster, *et al*., 2017; Torrente, *et al*., 2017). The affected animal can be suffering from repeated esophageal inflammation, regurgitation and pain as well as to the development of esophageal ulceration, esophageal strictures and epithelial metaplasia (Muenster, *et al*., 2017).

The fluoroscopy can be used for identification of GERD by revealing of the barium sulphate being reflects from the gastric back into the esophagus (Gaschen, 2018). Endoscopy can be used to diagnosis of GERD by provide a complete picture for the lower esophageal sphincter(LES) that always opened (Gualtieri, 2001).

2-5-7: Esophageal neoplasia

Esophageal neoplasia is quite uncommon in dogs (Venker-van-Haagen, 2013; Marks, 2017), might be due to esophageal, peri- esophageal e.g.(cervical lymph nodes) or metastatic origin (Marks, 2017). Neoplasm most often seen in the distal portion of thoracic esophagus, however leiomyomas are known to develop at the cardiac sphincter (Venker-van-Haagen, 2013). The most frequent tumors affecting the dog's esophagus are fibrosarcoma and osteosarcoma, both of which are caused by Spirocerca lupi's malignant growth . But Leiomyosarcoma, Leiomyoma, , chondrosarcoma, adenocarcinomas, lymphoma undifferentiated carcinoma, , and metastatic carcinoma are some of the less frequent tumors (Venker-van-Haagen, 2013; Wagner, 2008; Marks, 2017),

Esophageal tumor either localized or metastasized to the surrounding structure like cervical lymph nodes thyroid gland (Venker-van-Haagen, 2013) and treated by esophagectomy (Fossum,*et al*,2007) .

Radiographs utilize to recognition the mass lesion along the region of the esophagus (Gaschen, 2018), whereas barium sulphate radiography , or esophageoscopy may be necessary to distinguish esophageal masses from non-esophageal masses and esophageal foreign bodies (Gualtieri, 2001; Venkervan-Haagen, 2013; Gaschen, 2018).

2-5-8: Esophageal fistula

Esophageal fistula is a tract between the esophagus and the trachea (Jorg ,2008; Kaminen, *et al* ., 2014) , bronchus or in certain cases with the skin(Kaminen , *et al*.,2014) which has been described seldom in dogs (Della,2010) can be treated surgically (slatter,2003) Esophageal fistula might be congenital or acquired , the acquired air way esophageal fistula arises as a result to foreign body penetration of esophagus (jorg ,2008; Kaminen, *et al*.,2014) and a bronchoesophageal fistula is thought to be a potential cause of recurrent respiratory infections (Kaminen , *et al*.,2014), that manifested by, cough and dyspnea, regurgitation and aspiration pneumonia especially after eating or drinking (Jorg ,2008; Kaminen, *et al*.,2014) . The most reliable diagnostic method is fluoroscopy, Bronchoscopy may allow the fistula to be seen , but in case of tiny fistular opening gives a false negative , ligation and dividing of the fistula remained the successful method for treatment of esophageal fistula (Kaminen , *et al*.,2014)

2-5-9: Esophageal diverticulum

The diverticula in the esophagus are (sac) pouch-like dilatation of the esophageal wall that can be congenital or acquired (Jorg,2008; Robert and Michael,2013) is more occurrence in distal part of cervical esophagus or in distal thoracic region , the congenital diverticulum develops as a result of congenital weakness of the musclaris layer in esophageal wall or abnormal separation of tracheal and esophageal buds (Fossum *et al*.,2007) ,whereas, acquired divided into pulsion and traction diverticulum (Borku,

et al.2009; Durocher,2009) Pulsion diverticulum represent an out pouching of esophagus mucosa through muscularis and adventitia (Robert and Michael,2013) , It generally acquired occurs secondary to increased interluminal pressure by the foreign body (Gualtieri , 2001; Oliveira, *et al*.2018) or due to deep esophageal inflammation(jorg ,2008;), esophageal stricture, esophagitis, and hiatal hernia (Borku, *et al*.2009; Durocher,2009)

Traction diverticulum is usually caused by inflammation and results in the development of fibrous tissue, which contracts and pulls the esophagus wall outward. The contrast thoracic radiograph is the most often used technique which show esophageal dilatation(Jorg ,2008; Robert and Michael,2013, Oliveira, *et al*.2018) Small diverticula may not show clinical symptoms, while large diverticula allow food to be trapped, resulting in postprandial dyspnea, regurgitation, and odynophagia (Jorg,2008; Robert and Michael,2013).The diverticulum surgically treated by transect the pouch and suturing by end –to- end simple appositional layer(Fossum *et al*.,2007) .

2-5-10: Esophageal stricture

Esophageal stricture defined as intraluminal band causing partial or complete esophageal obstruction,(Fossum, *et al*.2007). It consider as a typical reasons of esophageal illness in canine and feline (Rance and Michael, 2003). Esophagitis following gastroesophageal reflux during anesthesia (Leib, *et al*.,2001;Wilson and Walshaw,2004; Bissett ,*et al*, 2009; Desmond, *et al*.,2018),fibrous tissue development after circumferential mucosal ulceration and erosion ,Mechanical traumas (Leib and Sartor,2008; Gianella,*et.al*.,2009), iatrogenic trauma during esophageal operation or esophageoscopy , congenital stenosis, and chemical wounds have all been classified as a causes of esophageal strictures (Desmond, *et al*.,2018),The most typical presenting symptom is

regurgitation, and stricture should be considered in animals who regurgitate frequently with presence previous esophageal trauma or esophageal surgery . (Fossum. *et al*. ,2007) esophageal stricture can be diagnosed by contrast esophageography or endoscopy(Fossum. ,2007). Treatment include medical management to controle esophageal reflux and subsequent mucosal damage, mechanical dilatation, stenting, or surgical resection and anastomosis(Bissett, *et al*.,2009; Lam, *et al*.,2013)(Figure3)

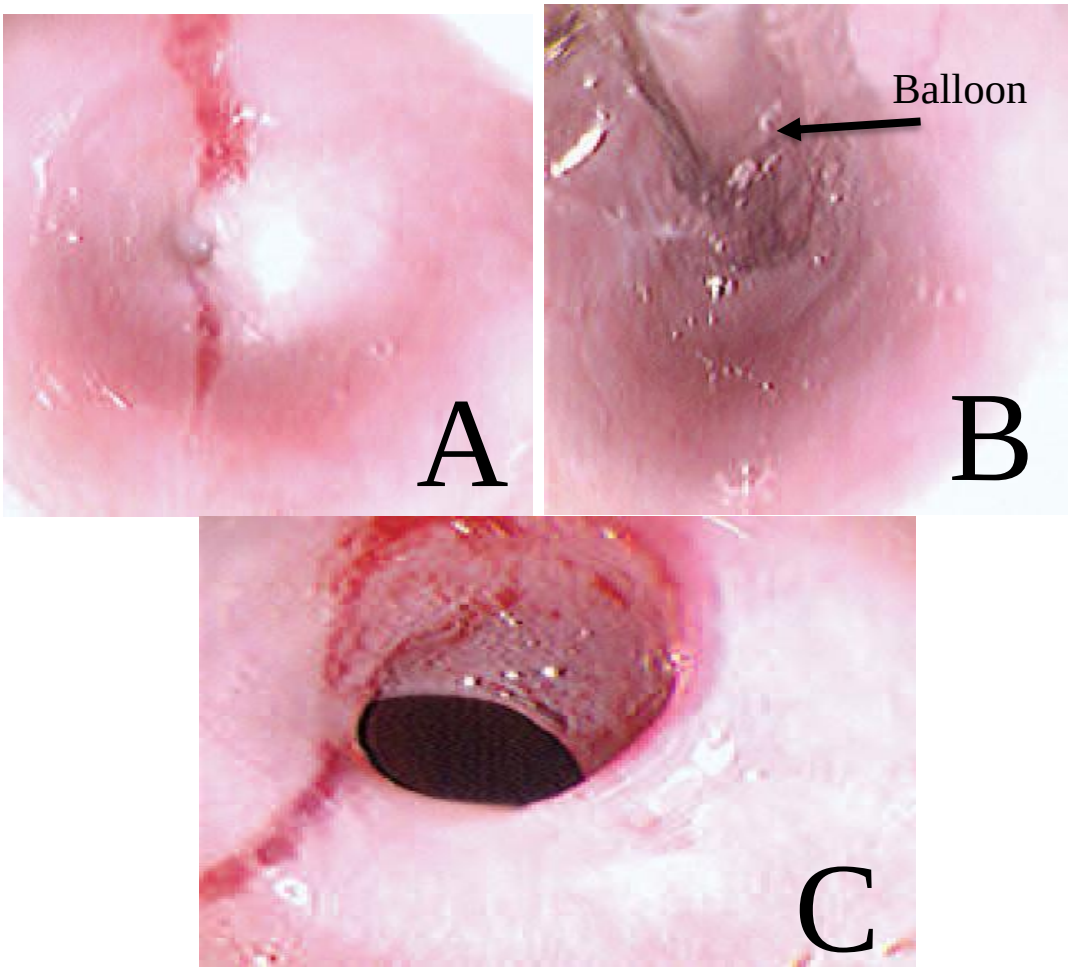

Figure 3: Showing the esophageal stricture and its treatment by balloon dilation

A: An esophageal stricture

B: Insertion of the balloon through the stricture site.The arrow indicates the balloon dilator

C: The stricture site after the dilation

(Glazer and Walters,2008)

2- 6: Diagnosis of Esophageal Disease

Plain or contrast radiography, computed tomography (CT) fluoroscopy, ultrasonography, and Magnetic Resonance Imaging(MRI) are some of the medical imaging techniques useful to examine the dogs esophagus for detection of abnormalities or disorder . However, radiography and ultrasonography are presently the most often used modalities in small animal clinic (Wisner, *et al*., 1991; Ridgway and Graves, 2010; Pollard, 2012; Baloi *et al*., 2013; Gory *et al*., 2014; Kirberger *et al*., 2014; Bristow, 2015; Gaschen, 2018).

2-6-1: Radiography

The radiographs of the esophagus from pharynx to the cardiac opening of the stomach are recommended to diagnosis of esophageal diseases (Gaschen, 2018) When the esophagus is empty, it cannot be seen on plain radiographs, the Plain radiographs, on the other hand, offer information on esophageal content and can be used to detect of many esophageal disorders such as esophageal dilation and foreign bodies (Elwood, 2006; Jardim Gomes,2019). Furthermore, utilize to detect secondary problems including aspiration pneumonia and pleural effusion (Jardim Gomes,2019). Using of barium sulphate suspension or paste as a contrast media in esophageal radiograph can provide more structural information about the size, contents, and outlines of radiolucent objects (Gaschen, 2018; Elwood, 2006), as well as esophageal masses, esophageal strictures, bronchoesophageal fistulae, esophageal perforation, and hiatal hernia (Bradley, 2005; Elwood, 2006; Jardim Gomes,2019). Barium aspiration is a possible complication of esophageal positive contrast radiography, small quantity of barium reaches the airways causing aspiration pneumonia(Gaschen, 2018)

The contrast radiography can be used for detection and estimation the degree of stenosis in digestive system , Al-Maseeh and Eesa, 2009 were

used contrast radiography(Figure 4) to determine the degree of stenosis after esophageal anastomosis in dogs by taking 10-12 centimeters of esophagus at the site of anastomosis after euthanasia of animals and application of the following formula 100 {1-2A/ (B+C)} as well as Al-Qadhi and Al-Hasan were used contrast radiography to determine the degree of stenosis after intestinal anastomosis and apply the same formula to calculate the degree of stenosis.

Figure 4: Contrast radiography Showing the site of esophageal anastomosis sutured with two layers simple interrupted suture pattern in dogs after 30 days of operation.
(Al-Maseeh and Eesa, 2009)

2-6-2: Fluoroscopy

Fluoroscopy defined as a dynamic radiographic technique with a contrast media (Barium meal) that allows for in-vivo monitoring of swallowing, esophageal activity , function of the cardiac opening (Elwood, 2006; Gaschen, 2018) and also used to detection on esophageal dilation (Reeve, *et al*., 2017; Gaschen, 2018). Contrast fluoroscopic techniques tends to be more accurate than contrast radiography in detecting esophageal disorders and mild or temporary disorders that might undetected. (Elwood, 2006; Gaschen, 2018). The barium meal(liquid) can be used in this technique (Bonadio, *et al*., 2009) .The fluoroscope technique can be applied by restraining the dogs on the sternum recumbence or in erect position or in lateral recumbency (Bonadio, *et al*., 2009; Gaschen, 2018).The sternum recumbence is preferable for estimation of the swallowing and esophageal passage times (Venker-van-Haagen, 2013).

2-6-3: Computed tomography

CT is an efficient method for identifying esophageal masses, it allows for examination of neighboring structures and its blood circulation (Kirberger, *et al*., 2014; Gaschen, 2018). Computed tomography of esophagus used for diagnosis of metastatic tumor , characterization of esophageal spirocerca nodules , (Kirberger, *et al*., 2014) and esophageal varices (Ledda, *et al*., 2015; Gaschen, 2018).

2-6-4: Magnetic resonance imaging (MRI)

MRI is a modern technology showing great soft tissue image not commonly utilized for esophageal examination in vet but it is frequently used to assess esophageal tumors in humans (Rossum, *et al*., 2015; Gaschen, 2018). Due to location of esophagus within the mediastinum, respiration , cardiac motion and lung blood supply , and peristaltic motion , the esophagus is difficult to visualize on MRI and make it restricted diagnostic technique (Riddell, *et al*., 2006; van Rossum, *et al*., 2013; Rossum, *et al*., 2015).

2-6-5: Ultrasonography

The transcutaneous sonography could be utilized to exam of the cervical and short abdominal parts of the dog esophagus (Wisner, *et al*., 1991; Gory, *et al*., 2014; Gaschen, 2018). The location of the esophagus, gas inside the lungs, the patient's body state, and the surrounding skeletal system factors are restrict and interference with the ultrasound of thoracic area in human and leading to partial visualization of esophagus , as well as, abdominal ultrasound in vet and human can be used for revealing of the abdominal esophageal part and esophageo- gastric sphincter (Shang-Yong, *et al*., 2004; Gory, *et al*., 2014), and detection on the many diseases of such as GERD ,esophageal carcinoma ,hiatal hernia , varices and leiomyoma(Shang-Yong *et al*., 2004).

The endoscopic ultrasonography can be used for detection on esophageal fistula, diverticula, and peri- esophageal masses (Gaschen, 2018) and can be applied in pet clinic by using a trans-esophageal transducer (Capitani, *et al*., 2014) but still uncommon used in vet than in human because of requires to heavy sedation or general anesthesia to prevents damaging of equipment by the animal teeth (Gaschen, 2018).

Jardim Gomes,2019 was using transcutaneous sonography of the canine cervical esophagus as left sided approach and found its possible visualization the whole length of the cervical esophagus. The esophagus on transverse images was appeared as oval shape and on longitudinal images was appeared a rectangular shape (Figure 5)

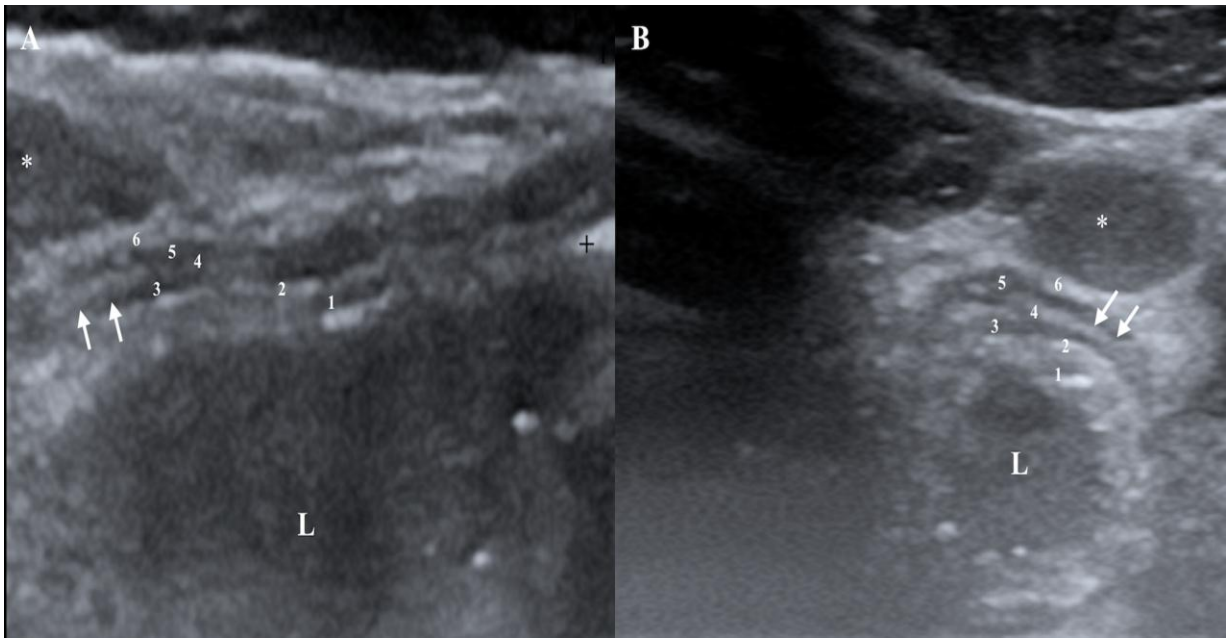

Figure 5: Showing structures of normal canine cervical esophagus by using the transcutaneous ultrasound

A: Longitudinal ultrasound image of the middle region

B: Transverse ultrasound image of the cranial region

1 mucosa,2 Submucosa; 3 Inner circular muscle; 4 Fibrous connective tissue; 5 Outer longitudinal muscle; 6 Adventitia , * Thyroid gland ,L esophageal lumen and white arrows hyperechoic fibrous layer between the muscular layer

2-6-6: Esophagoscopy

Esophagoscopy is a minimal invasive method for treatment and diagnosis esophageal disorder, permits the lumen and mucosal lining of the pharynx and esophagus to be examined (Gualtieri, 2001; Tolbert, 2017). It can be used to find esophagitis, strictures, dislodge objects, sample biopsies and early esophageal dysfunction (Gualtieri, 2001; Elwood, 2006; Tolbert,2017). esophageal fistulae, ulcers and neoplasia, (Gualtieri, 2001). endoscopy can be used to diagnose and treat the possible leakage in the same time at the site of anastamosis (Fabbi, *et al*., 2021) without any risks of anastomosis disruption (Page, *et al* .,2013).

2-7: Esophageal resection and anastomosis

History of esophageal anastomosis begins from The first cervical esophageal resection with re-anastomosis in humans in 1877, and Dobromysslow described the first intrathoracic esophageal resection in 1901. (Yuan, et al.,2015) . Esophagectomy is a major surgical procedure used to corrects a variety of esophageal disorder (Lerut, *et al*.,2002 ; Chen, *et al*.2014) , although it is most often used to treat esophageal cancer (Lerut, *et al*.,2002 ; Chen, *et al*.2014; Seungju, *et al* .,2020) with high rate incidences of various complication (Bardini, *et al*.,1994; Chen, *et al*.2014) continue to be a burden of swallowing (Lerut, *et al*.,2002). Esophageal anastomosis can be established by different techniques such as hand sewn , total mechanical anastomosis

with circular stapler, or semi- mechanical anastomosis with linear stapler, with or without simple modification and each one has it is advantage and disadvantage , the hand sewn anastomosis had been the predominant option for esophageal reconstruction for a long time until achievement of stapling device, (Yuan, *et al*.,2015). Hand sewn end to end anastomosis as a simple or continuous pattern can be done via different layer of

anastomosis with different size and types of suture materials(Al-Maseeh and Eesa, 2009; Yuan, *et al*.,2015).

Murakami, *et al*.,2000, found the esophageal anastomosis by using interrupted suture technique in double layers was better results as a comparison with other techniques while , Senyk and Rand ,1978 were found that single layer suture technique was better than double layers suture technique for esophageal anastomosis in pigs, dogs and cats (Al-Maseeh and Eesa, 2009), additional study was showing the two layer anastomosis is still more popular among surgeons and also reveal the anastomotic leakage rate with a double layer was lower than single-layer anastomosis , while the degree of stenosis was higher incidence double layer anastomosis than one layer(Zhu, *et al* .,2008) moreover, another study conducts that one-layer anastomosis can be finished rapidly than the two -layer technique and with same rate of complications (Burch, *et al*.,2000; Aslam, *et al*.,2008) on other hand, Zieren *et al* 1993, was noted the two layer anastomosis has not lower rate of leakage than one -layer during performing of cervical anastamosis by one and double layer techniques. Despite numerous research comparing various anastomotic procedures, there is still a point of contention, which one is better than the rest (Yuan, *et al*.,2015).

The ideal suture material for anastomosis should be sufficiently to maintain anastomosis firmly in first stage of healing process , additionally trigger little immunological reaction and scarring to avoid postoperative stricture and stenosis (Munday and McGinn, 1976, Yuan, *et al*.,2015) . polypropylene, catgut, and polyglycolic acid elicit milder inflammation reaction (Koruda and Rolandelli, 1990) As a comparing between the absorbable with non-absorbable sutures materials were generally considered that non-absorbable materials (silk and polypropylene) cause

less immunity response and less stricture formation and the preferable suture material was varied among several studies (Yuan, *et al*.,2015).

Usually the suitable size of suture materials 3-0 were a strong enough to maintain anastomosis with 4 mm apart among each bite (Al-Maseeh and Eesa, 2009; Yuan, *et al*.,2015).

2-8: Complications of esophageal resection and anastomosis

Anastomotic complications rates varied significantly between studies and It's important to remember that esophagectomy and reconstruction outcomes are influenced by a variety of factors, including technical factors such as adequate blood supply preservation, free of tension, gentle and precise handling of tissues, and a tight closure of anastomotic tissues especially in the mucosal layer (Urschel,1995; Alanezi and Urschel, 2004; Yuan, *et al*.,2015). these factors can be classified under experienced of surgeons that have a significant impact in the surgery's outcome and decrease chance of occurrences (Chen, *et al*.2014) as well as, the anatomical factors made the esophagus more susceptible to the post anastomotic complications, these factor include lack of serosa and omentum, longitudinal tension weakening of the esophageal tissues , segmental circulation , and continuous motion of suture site, (Al-Maseeh and Eesa, 2009; Seungju, *et al* .,2020). The postoperative complications includes Stricture (Carroll and Arnold 2012) , leakages (Xiang, *et al*.,2019) , dehiscence (Shahnam, *et al*., 2016). The delayed emptying or dumping syndrome, reflux, and chylothorax also considered as a postoperative complications but usually accompanied with replacement of esophagus stomach in case of esophageal cancer (Chen , *et al*.2014).

2-8-1: Esophageal Stricture or Stenosis

Stricture is a typical consequence can be developed after an esophageal cervical anastomosis (Heijl, *et al*.,2010; Carroll and Arnold 2012) especially when the anastomotic technique is poor (Carroll and Arnold 2012). The anastomotic stricture is two type can be classified according to the causes the first type occurs due to scar contraction and the second type stricture due to anastomotic leakage , the scar contracture stricture can be easily healed by dilation (Chen, *et al*.2014) and less life threatening than strictures caused by anastomotic leaks (Yuan, *et al*.,2015) , whereas dilatation for the second type is sometime harmful (Chen, *et al*.2014), and may require temporary stenting (Yuan, *et al*.,2015). According to Several studies, the stricture after esophageal anastomosis progressively reduced after a period of years without the requirement for dilation (Blackmon, *et al*.,2007). Esophageal strictures generally require dilation to improve the animal's condition , the dilation of stricture can be done by either bougie-nage or balloon dilation. The bougie-nage procedure involves gently pushing a long, narrow, rigid device (a bougie) through the stricture to gradually break down and stretch the scar tissue this procedure done by endoscopy (Wo and Waring , 1997) Moreover, The esophageal balloon dilatation method entails inserting an expandable bulb through the stricture under esophageoscopy or fluoroscopy supervision . The stricture is progressively stretched by expanding the balloon filled with normal saline or a diluteed contrast media to a predefined diameter (Cox, *et al*.,1994) (Figure 6).

Symptoms of stricture are dysphagia (Carroll and Arnold 2012; Chen, *et al*.2014; Jessie, *et al*., 2016) that characterized by regurgitation, appears a few days or weeks following esophageal surgery (Cheryl and Theresa,2007) .The anastomotic stricture can be diagnosed depending on

the symptoms of dysphagia (Kim, and Takabe, 2010; Chen, *et al*.2014) as well as endoscopy or a contrast radiograph (Carroll and Arnold 2012)

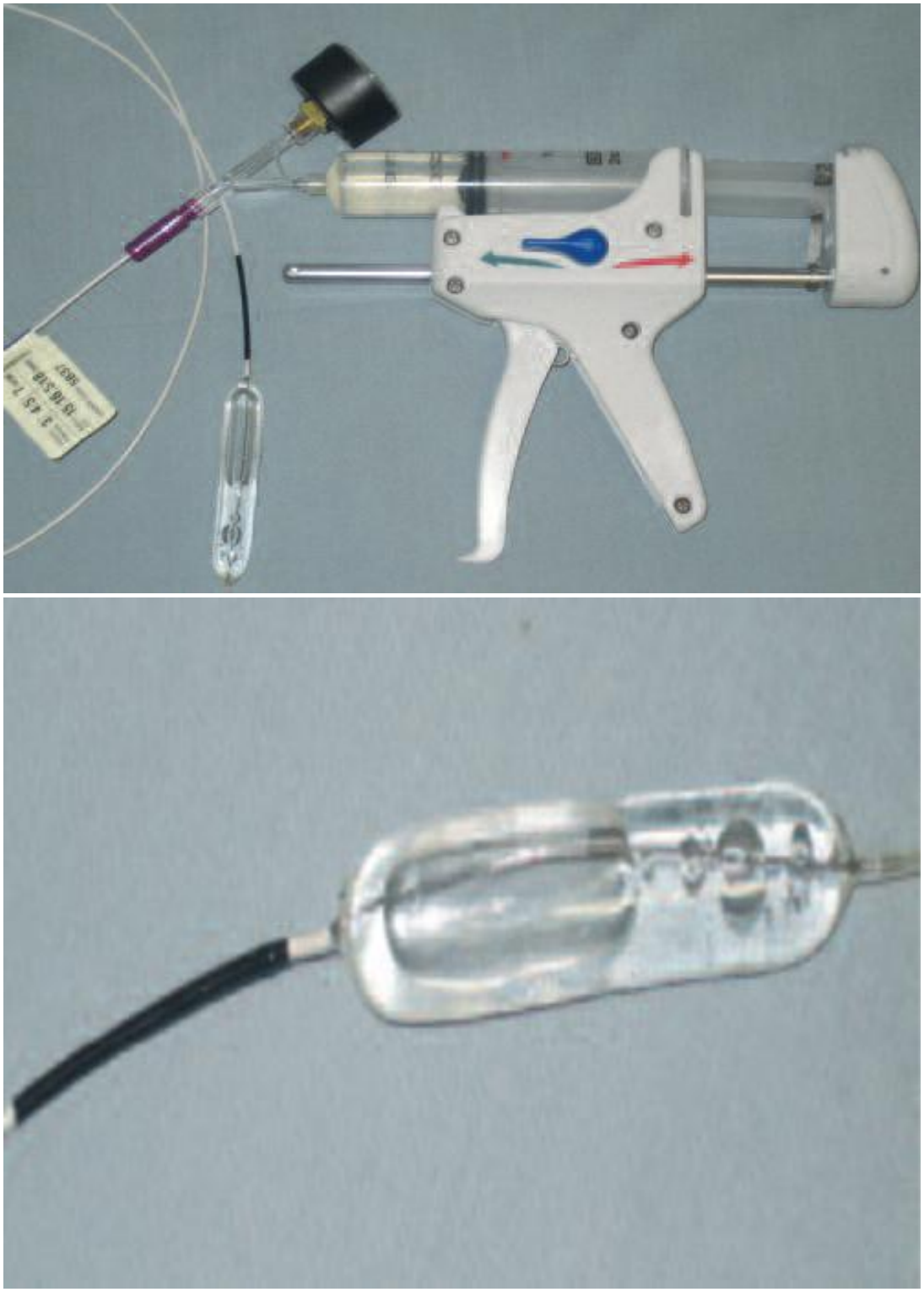

Figure 6: Balloon dilation equipment**.**

(Glazer and Walters,2008)

2-8-2: leakage

Esophageal anastomotic leakage remains one of the most severe consequences following esophagectomy (Bardini, *et al.*,1994; Bruce, *et al.*, 2001; Blewett, *et al.*,2001; Blencowe, *et al.*,2012; Yuan, *et al*.,2015; Xiang, *et al*.,2019), was mainfested by clinical symptoms like as a hematoma or seroma at the base of the neck peri-anastomotic collection, septicemia, peritonitis leak, local irritation, air or saliva evacuation Pneumothorax, mediastinitis, abscess, empyema (Bruce, *et al.*,2001 ; lerut, *et al*.,2002) and also resulted due to ischemia of the esophageal ends, or excessive tension at the anastomotic site (Carroll and Arnold 2012) or to segmental blood supply (Zheng, *et al.*,2015).

types of anastomosis play an important role to decrease chance of leakage, Nederlof, *et al*.,2014 study found the end-to-end cervical anastomosis tends to have a lower leakage rate than end -to- side anastomosis, furthermore, esophageal anastomosis dehiscence is common, and there are still debates over which type of anastomosis to use to reduce the risk of dehiscence (Shahnam, *et al*., 2016). Using interrupted suture in two layers is better than other methods to decrease chance of its occurrence (Murakami, *et al*.,2000).

Leakage is more common in the cervical region than in the thoracic but leakage from intrathoracic anastomosis are usually more life threatening. (Bardini, *et al.*,1994; Blewett, *et al*.,2001; Yuan, *et al*.,2015).

Management of anastomotic leakage is usually selective and based on patients' condition, position, and extension of leak. In case of neck leakage the conservative treatments should be applied for leak include peri-anastomotic drainage, nasogastric decompression, proper nutrition support, and antibiotic administration , while, intra- thoracic leaks may need more invasive procedures such as thoracotomy, thoracoscopic drainage, or even full gastric redirection (Yuan, *et al*.,2015) some times

fistulation of thoracic part develops and accumulation of fluid and pus in sac at the anastomotic site , a CT-guided thoracocentesis can be used to provide appropriate drainage until surgical intervention available.

To avoid a fatal anastomotic leakage, the surgeon should have the knoledgement about , physiology, anatomy, and esophageal disease, and skills in esophageal surgical methods (Chen, *et al*.2014.)

2-8-3: Delayed Gastric emptying

Is the most prevalent complication in persons with thoracic stomach movement impairment , with a 50% occurrence after esophagectomy (when the esophagus substituted by stomach in case of esophageal tumor), the reducing stomach size and weakened of expansion, leads to pyloric malfunction , which result in postpone gastric emptying , that characterized as vomiting(Andrews and Bingham, 1990; Chen, *et al*.2014.).

Treatment of delayed gastric emptying either medicine treatment or surgical treatment , the medicine treatment by using drugs as cisapride, metoclopramide, domperidone and bethanechol , through reducing the signs (Chen, *et al*.2014) , While , the surgical treatment by increasing the diameter of pylorus either myotomy, or pyloric balloon dilation , some of surgeon preferred the pyloric balloon dilatation on other procedures (Sutcliffe, *et al*., 2008 ;Ronald, *et al* ., 2014; Chen, *et al*.2014).

2-8-4: Dumping Gastric disorder

Dumping gastric phenomena is a typical symptom consequence to dysfunction of stomach after pulling in the thoracic cavity . A 5% percent of patients have a moderate symptoms While , 1 percent have severe symptoms (Chen , *et al*.2014).

Dumping 's causes include abnormal pyloric function , devascularization and reduced gastric capacity. The majority of dumping syndrome symptoms can be alleviated by changing person's food patterns and styles, such as consuming multiple small foods ignoring the need to drink more liquid immediately after a meal, limiting monosaccharide-containing foods (cookies, sugar, sweets), and substituting monosaccharide- containing food with polysaccharide-containing foods like pasta, potatoes, and other grains, withheld milk products , and raising ratio of fats and proteins (Berg and McCallum, 2016; Laura and Susan,2016) and Many patients may need to experiment with different kinds of diet to understand their food preference and tolerance(Ukleja, 2005; Laura and Susan,2016) .The Drugs like, verapamil, prednisolone, propranolol methysergide maleate, octreotide and acarbose can be used to treat severe instances (Engstad and Schipper, 2009; Chen, *et al*.2014).

2-8-5: Gastric Reflux

Reflux occurs as a result to the impermanent of the anti-reflux mechanisms at the cardiac sphincter (Chen, *et al*. 2014) , Reflux can also be aggravated by partial location of the gastric in the positive pressure of abdomen and slowed gastric emptying (Engstad and Schipper, 2009; Laura and Susan,2016). This case clinically manifested by coughing, pneumonia, vomiting , recurrent laryngitis due to reflux bile and stomach acid, and incapacity to lie on one's back (Chen, *et al*. ,2014 ;Laura and Susan,2016). Proton pump inhibitors or histamine 2 (H2) receptor blockers can be used to relieve the symptoms (Engstad & Schipper, 2009) Many strategies can be employed to assist reduce gastric reflux, including adequate stomach mobilization, excision of majority of the acid-secreting part , surgical operation that improve evacuation of stomach (Chen, *et al*.

2014) , modifying food pattern , avoiding lying down immediately after meals (Chen, *et al*.2014; Laura and Susan,2016) .

2-9: Esophageal healing

Normal esophageal anastomosis healing is multifactorial , healing fails when any phase of healing process is disrupted (Yuan, *et al*.,2015) and the esophagus, unlike other digestive organs, lack of serosa (which promotes a rapid seal due to exudation of fibrin) and omentum, longitudinal tension weakening of the esophagus tissue, segmental blood supply, and continuous motion of the suture site, consider a predisposing factors demonstrate poor healing (Al-Maseeh and Eesa, 2009; Seungju, *et al* .,2020) however, The wound healing mechanism of esophageal anastomosis parallels wound healing in other tissues (Shomaf,2003), the process of healing starts immediately after injury , and divided to four subsequence phases, hemostasis, inflammation, proliferation, and remodeling, each of which progress in different rates(Patrulea , *et al* ., 2019 ; Ribeiro, *et al* .,2019) . During these stages ,many factors are present in order to promote early wound closure and healing without scar formation , For example, platelet aggregation, release of proi-nflammatory cytokines such as interleukin IL-1 , IL-6, IL-8 and tumor necrosis factor (TNF-_) , transforming growth factor alpha, transforming growth factor beta, insulin-like growth factor-1 (IGF-1) and basic fibroblast growth factor (bFGF), which are very important during the inflammation phase (Ghatak, *et al*.,2015). The growth factors and cytokines are play essential role for activation of epithelial and fibroblast cells, to prepare the injured site for the proliferation phase (Ucuzian , *et al* .,2010). In other context the healing mechanisms divided to the 4 consequence phases or stages (Figure 7) :-

A. Hemostasis phase

This phase occurs as a result of vasoconstriction as a reaction to injury, and it diminishes quickly and the damaged (cut) blood vessels will then relax, enabling further bleeding if platelets are not involved. During vasoconstriction, platelets adhere to exposed collagen at the end of injured vessels , particularly that found in the basement membrane underlying damaged endothelial cells , and secrete vasoconstrictive substances to maintain transected vessels constriction, initiate the process thrombogenesis to close the leak in the vessel and prevent additonal damage and initiate blood vessel healing (angiogenesis) through, in part, the release of TGF-β and PDGF (Mcgavin and Zachary, 2017).

B. Inflammatory phase

This phase is second phase of wound healing, that fully established by 24 hours after vascular damage and can persist up to 96 hours (Mcgavin and Zachary, 2017). This phase starts by efflux of inflammatory cells and tissue growth factor to the injured tissue (Yuan, *et al*.,2015), whereas excessive infiltration of inflammatory cell reduces healing. Neutrophils and macrophages break down and eliminate cell debris arising from damaged tissue by phagocytosis and their enzymes. neutrophils produce, CXCL8, TGF, PDGF, and CXCL1, and the macrophages secrete a wide range of chemotactic and growth factors such as CS3CL1, CCL2, PDGF, VEGF, epidermal growth factor (EGF), TGF , TNF-, and IL-1-, which provide the microenvironment for proliferation (granulation). The Negatively charged ECM molecules like proteoglycans attract and bind to positively charged growth factors, chemokines, cytokines, MMPs, and other molecules.

Proliferative phase

During the proliferative phase (third phase) an d in early healing process, the basal cells begins migration from the overlying epithelium at the

transected edges the wound's margins , where they rapidly undergo hyperplasia in response to EGF, FGFs, IL -1, hepatocyte growth factor (HGF), VEGF, IL-1, and TGF-, there are released by epithelial cells, endothelial cells, and fibroblasts. The migrating basal cells multiply and expand to bridge the wound; however, once differentiated, the cells stop proliferating and migrating. The proliferation phase might continue three to four weeks depending on the extent of the incision , this phase characterized by formation of new endothelium ,epithelium and connective tissue fibroplasia to restore normal shape and function to the damaged tissue(Mcgavin and Zachary, 2017). and formed Collagen plays essential function in preserving tissue intensity, (Yuan, *et al*.,2015), Prescience of suture materials in this phase during anastomosis and may induce inflammation and excessive fibrosis, potentially leading to anastomotic stricture development, (Al-Maseeh and Eesa, 2009 ; Yuan, *et al*.,2015) To reduce the risk of postoperative stricture formation, the best suture material for anastomosis construction must be strong enough to hold the anastomosis in place during the early stages of healing while simultaneously causing little immunological response and scarring (Koruda and Rolandelli, 1990).

C. Remodeling or contraction phase

This phase starts about three to four weeks following injury , but only when the inflammatory and proliferative phases are completed . In this phase granulation tissue remodels by immature connective tissue and conversion of immature to the mature connective tissue via extracellular collagen formation in response to PDGF, TGF-β, MMPs , FGF-2(Mcgavin, and Zachary, 2017).

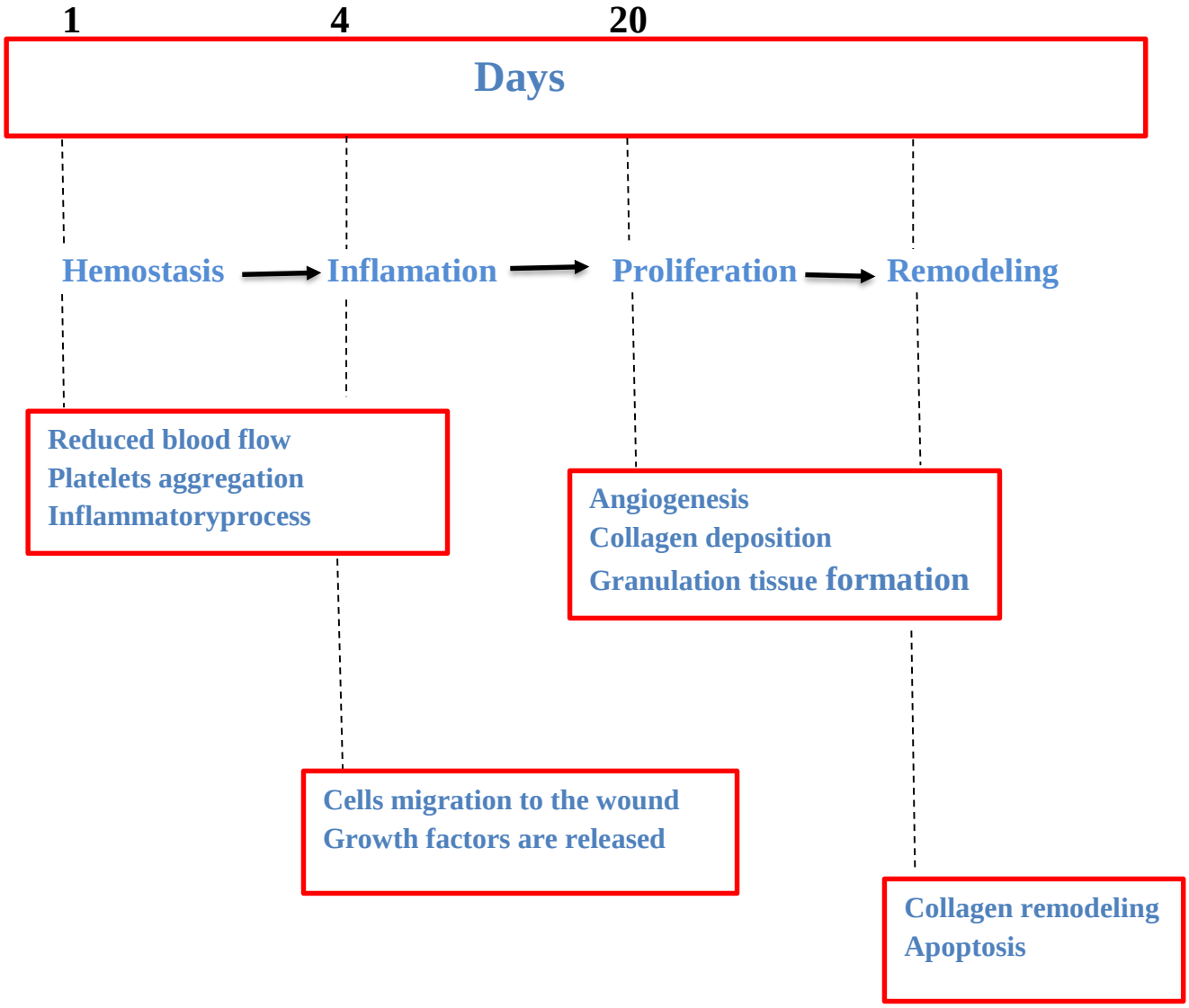

Figure 7: Showing wound healing process

(Francesco and Šeila, 2015)

2-10: Biological materials used to enhance healing

The biologic materials are less controllable and subject to natural variability, but they tend to create a friendlier host response and promote constructive tissue remodeling, as well as the capability to enhance different stages of the healing response by triggering a shift from inflammation and scar tissue formation to constructive remodeling and functional tissue restoration. Blood normally contains 94 percent red blood cells (RBCs), 5% platelets, and 1% white blood cells. Platelet-rich plasma (PRP), on the other hand, is made up of 95 percent platelets (Freymiller and Marx, 2004) and the platelets establish and regulate wound healing process through the activation of various biomolecules, including growth factors, angiogenesis, proliferation, and activation of linked cells, such as macrophages, stem cells, fibroblast and neutrophils (Sánchez-González, *et al.*,2012). The platelet-rich fibrin (PRF) and platelet-rich plasma (PRP) are well-known for its natural healing abilities (Al Deeb,2020) ,

PRP can be used as a source of growth factors to aid in the repair of both hard and soft tissues.(Saluja, *et al*.,2011; Sunitha and Munirathnam , 2008) and when are activated, they release growth factors (PDGF and TGF), which are important for wound healing (Sunitha and Munirathnam , 2008). and PRF is made up of cytokines, leukocytes, platelets, stem cells , that promotes micro- vascularization, and serves as a transport for cells that are essential for tissue regeneration (Temmerman, *et al.*, 2018).

In other context , Bone marrow represents the main hematopoietic organ, and a primary lymphoid tissue, able to produce of erythrocytes, granulocytes, lymphocytes, and platelets (Abboud and Lichtman, 2001) and contains mesenchymal stromal cells (MSCs), that considered as a type of regenerative biological material derived from a variety of tissues,

including bone marrow (Aghamir *et al*., 2016). The mesenchymal stem cells characterized by its ability to differentiate into many lines, can be used to repair damaged tissues (Xiang, *et al* ,2019) and have anti-inflammatory and immunity properties, as well as to the self-renew, expansion and differentiation (Mehrabani *et al*., 2013). Hyaluronic acid shows great promise in both animal and clinical tissue engineering study (Richard, *et al* 2005). And is a key component of the extracellular matrix (ECM) of vertebrates (Litwiniuk *et al*., 2016) aids in wound repair by creating a favorable environment for growth, resulting in accumulation of several matrix proteins (Shengkun, *et al*., 2016) and has been shown to modulate inflammation, cellular migration, and angiogenesis(Londono, and Badylak, 2015 ; Malgorzata, *et al* ., 2016) as well as signaling the body to build more blood vessels in the injured area by enhancing proliferation of endothelial cell (Malgorzata, *et al* ., 2016). All the events that induced by bio-materials are complex, involving both the immunity system and the mechanisms of stem cell recruiting , to growth and differences (Londono, and Badylak, 2015).

2-10-1: Bone marrow

Blood and bone marrow are two of the major organs in the body and are thought to be an important potential target organ (Gregory, 2006). Bone marrow can be found in the cavities of long bones, and defined as a complex tissue made up of two different compartments is now widely recognized (Krebsbachl, *et al*.,1999) hematopoietic tissue islands and adipose cells that are surrounded by vascular sinuses and interspersed inside a trabecular bone meshwork (Gregory,2006). In dogs, it accounts for about 2% of their body weight(Jain, 1986) .

Bone marrow represents the main hematopoietic organ, and a primary lymphoid tissue, responsible for the creation of erythrocytes, monocytes,

granulocytes, lymphocytes and platelets (Abboud and Lichtman, 2001).There are two forms of bone marrow, red (active) marrow and yellow (inactive) marrow. Red marrow can be found near the ends of long bones, the sternum, the ischium, and ribs (Rrywlin, 1985). The Bone marrow is made up of hematopoietic precursors, their differentiated progeny, and stroma, as a connective tissue network. The stroma is a heterogeneous collection of cells that includes reticulocytes , adipocytes, fibroblastic cells and endothelial cells (Jaiswal *et al*., 1997). The stroma comprises cells that differentiate into cartilage, bone, fat, and a connective tissue that promotes hematopoietic stem cell differentiation (Dexter and Testa, 1976; Beresford *et al*., 1992).

Bone marrow transplantation (BMT) is a medical method used to treat a variety of disorders (Majorana *et al*., 2000; Flowers and Kansu, 2000). Through removing stem cells from the donor, who could be the patient (autologous transplant) or another suitable donor (allogeneic transplant) (Flowers and Kansu, 2000; Martin,2008; Treister *et al*., 2010)

The field of regenerative medicine is modern therapy applied for esophageal repair, The main objective of regenerative medicine is the functional repair of lost or injured tissues, (Ricardo and Stephen, 2015) Mesenchymal stromal cells (MSCs) are a type of regenerative material that has ability to differentiate into multiple lineages and could be used to restore damaged tissues (Chou, *et al*., 2014; Xiang, *et al*.,2019), The Mesenchymal stem cells (MSCs) are derived from a variety of tissues, including bone marrow (Aghamir *et al*., 2016) also can be differentiated into fibroblasts and chondrocytes, and have ability to form connective tissues (Kadiyala *et al*., 1997, Aghamir *et al*., 2016) and have anti-inflammatory and immunity properties, as well as to the self-renew , expansion and differentiation (Mehrabani *et al*., 2013). Recently MSCs used as a regenerative therapy for treatment various diseases, such as

myocardial infarction (Chou, *et al*.,2014; Xiang, *et al*.,2019), spinal cord injury (Piltti, *et al*.,2013) liver cirrhosis (Puglisi, *et al*.,2011) ,gastric perforation(Caldas,2015) ,penetrating ulcers (Chiu, *et al*.,2015). Using transplanted precursor cells to the necrotic head of femur , non-unions or other bone healing problems have shown the first promising clinical results through enhancement healing of bone (Jäger, *et al*., 2009)

Study by Al-Hyani, 2019 have used bone marrow in the regeneration of the trachea by topical application of autologous aspirated bone marrow to rebuild the induced tracheal defect in dogs to enhance the degree of healing by formation and proliferation of more collagen fibers and regeneration of tracheal cartilage , moreover, another study by Al-Hyani, 2012 found the addition of autologous aspirated bone marrow on the nerve graft segment enhance and accelerate the degree of healing of injured sciatic nerve with improving the functional use of affected limb.

Now Tissue engineering appears as a promising alternative technique for esophageal replacement (Poghosyan, *et al*., 2016) MSCs accelerate the mature re-epitheliazation and early initiation of muscle cell colonization (Jonathan,*et al* .,2018). Study by Xiang *et al*,2019 was deals with Autologous aspirated mesenchymal cells (AAMSCs) in fibrin scaffold (FS) as therapeutic strategy for the treatment of esophageal anastomotic leakage (EAL) by suppressing inflammation response and alleviating fibrosis progression.

2-10-2: Hyaluronic acid

Hyaluronic acid (HA) belongs to the glycosaminoglycans (GAGs) class, which are the primary components of the extracellular matrix (ECM) (Litwiniuk, *et al*.,2016) of vertebrates which synthetize in cell plasma membarane (Shengkun,*et al*.,2016) . Characterized by simple structure and high molecular size (Litwiniuk, *et al*.,2016) and linear polysaccharide, made up of b-1,4-glucuronic acid repeating disaccharide units and b-1,3-

N-acetyl glucosamine (Laurent and Fraser.,1992; Lin and Gong.,2015) these units connect to gather more one of times to forms a structure molecular weight reaching 5 x 106 kDa (Litwiniuk, *et al*.,2016)(Figure 8)

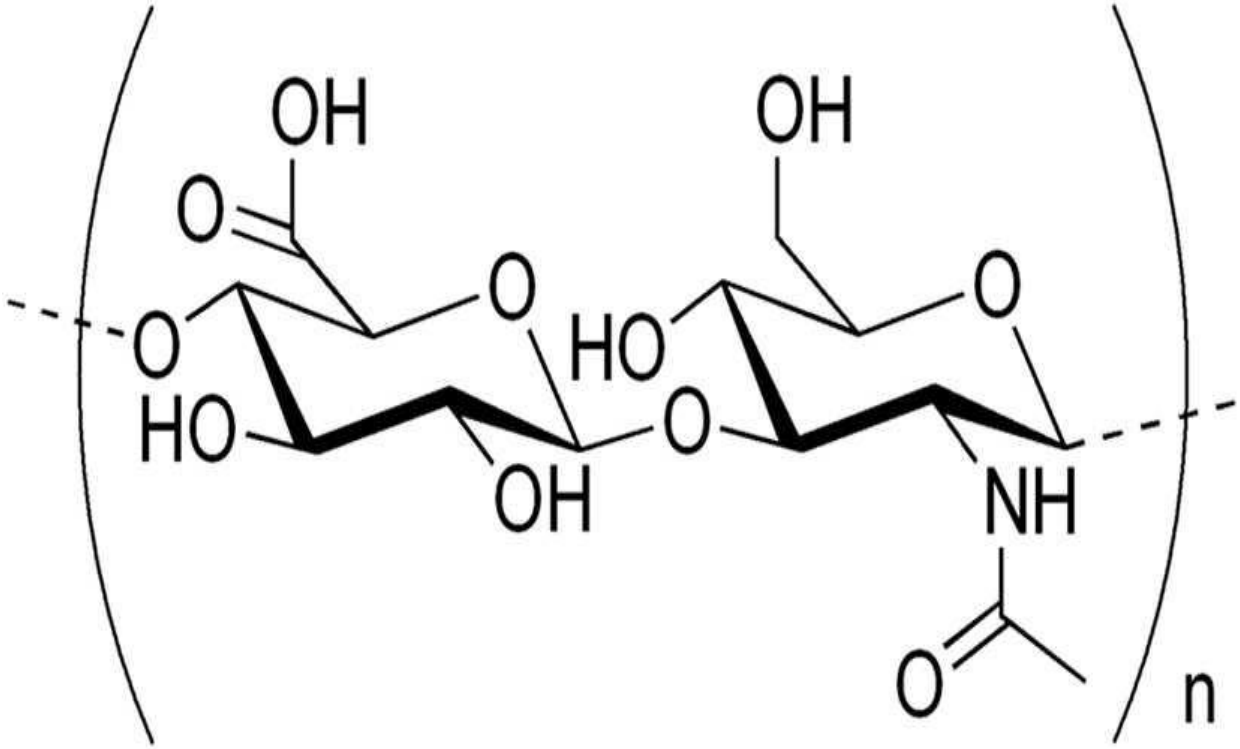

Figure 8: Chemical structure of Hyaluronic acid
(Gupta, *et al*., 2019)

Hyaluronic acid has a half-life of 3 to 5 mint in blood, less than a day in the skin and 1 to 3 weeks in the cartilage (Eleni, *et al.*,2012). The Hyaluronic acid is degraded by hyaluronidases to the different size fragments (HYAL), by hydrolyzing the hexosaminidic β (1–4) linkages between N-acetyl-D-glucosamine and D-glucuronic acid residues in HA. because of the negative charge of HA subunits, HA retains of water and supporting the ECM (Choi, *et al*.,2017), and creates a suitable

environment for growth, and accumulation of several matrix proteins (Kirker, *et al*.,2002), to serves as a scaffold for blood vessel formation (Slevin, *et al*., 2007) and fibroblast migration(Li, *et al* ., 2006),

Hyaluronic acid present in the body person with an average weight of 70 kg has approximately 15 g of HA (Gupta , *et al*.,2019) , distributed in different tissues such as skin(Tzellos, 2009), vitreous of the eye , umbilical cord, synovial fluid, (Eleni, *et al*.,2012) but it is also found in all tissues and fluids of the body, such as skeletal tissues,(Armstrong and Bell, 2002), heart valves (Toole, 2004) , lung,(Papakonstantinou and Karakiulakis, 2009) aorta (Papakonstantinou, *et al*.,1998) prostate(Goulas, *et al*.,2000) tunica albuginea, corpora cavernosa and corpus spongiosum of the penis(Eleni, *et al.*,2012) . During tissue injury and early stage of wound healing the synthesis of hayalorunic acid accelerate (Slevin , *et al* ., 2002) and HA modulates a various aspects of tissue repair, such as activation of inflammatory cells to improve immunity response (Teriete, *et al*.,2004) , fibroblasts (Bai,*et al*.,2005) and epithelial cells as a response to injuries (Jiang, *et al*.,2006) , Because of its high molecular size, hyaluronic acid acts as unique temporary structure during allows nutrients and waste materials to diffuse away from the injured site (Jeffrey and Vickie, 2012).

The functions of HA in the body are water hemostasis , lubrication , regulation of plasma protein distribution (Fraser , *et al* 1997) and immune regulation (Eleni, *et al*.,2012) , angiogenesis (Slevin, *et al*.,2007) , chondrocytes stimulation (Knudson and Knudson, 2004) and these functions depend on the concentration of HA in the tissues (Nakamura, *et al* .,1992; Lin and Gong.,2015).

The influence of hyaluronic acid on the tissues can be determined by the molecular weight (Moustafa , *et al*.,2016) , the high molecular weight form depression immunity response of the body while Low molecular weight

stimulate the immune cells response to tissue damage (Manuele, *et al*.2015) . During the inflammatory stage, via the action of hyalordenaze enzymes, the high molecular weight (HMW) HA is rapidly degraded into low molecular weight (LMW) (Tavianatou, *et al*.,2019) and helps in wound healing process through binding to certain cell-surface receptors, such as cluster of differentiation 44 (CD44) and receptor for hyaluronan-mediated motility (RHAMM) , and effects on cell signaling and activation of inflammatory cells (Turley , 1992 ; Shengkun, *et al*.,2016, Nyman, *et al*.,2019) and stimulates chemotaxis, leukocyte(Tavianatou, *et al*.,2019) and release of inflammatory cytokines and growth factors such as IL-1_, TNF-_, and IGF-1 (Tavianatou , *et al*.,2019; Matthew, *et al*.,2020) leading to accelerate of vascular endothelial cell proliferation and migration during angiogenesis (Erin and Pardue ,2008; Fallacara, *et al* .,2018; Matthew, et al.,2020) as a response to immunity (Teriete, *et al*.,2004) and damage of fibroblasts (Bai, *et al*.,2005) and epithelial cells (Jiang, *et al*.,2006), On other hand , high molecular weight hyaluronic acid has antiangiogenic effects by inhibiting endothelial cell migration and proliferation(Slevin, *et al*.,2007) .

As well as HA plays an important role to provides the framework for blood vessel formation (angiogenesis) (Slevin,*et al*.,2007; Eleni, *et al*.,2012). and supporting the migrated cells by formation of ECM (Collins and Birkinshaw, 2013; Patrulea, *et al* .,2019). Finally , the wound healing mechanism could be classified into three main steeps, each event include HA , The first step, a matrix enriched with HA is set down in a cell-poor space, second step the mesenchymal cell migration is promoted and the HA matrix is infiltrated by cells migrating from the nearby tissues, and the third step cells within the HA matrix produce both hyaluronidase, which degrades the HA, and sulfated GAGs and collagen(Gupta, *et al*., 2019) .

Clinically HA 0.3% eye drop was used for treatment corneal abrasion (Lin and Gong.,2015), relieve dry eye symptoms in case of ocular burning and irritation (Johnson, *et al* .,2006) and today used as tear substitutes for dry eyes , enhance and accelerate ocular surface wound healing (Yu.*et al* .,2013). As well as HA has been used as injection (intradermal at the site of incision) after experimentally induced skin incision, biopsies was taken after 24 of treatment for histological study ,showed complete re-epithelization of the tissue that interprets the role of HA on wound healing (Nyman, *et al*.,2019) and It was used to enhance Achilles tendon through topical application for reconstruction of tendon (Shengkun, *et al*.,2016) and showed a good result for repairing of connective tissue and tendon in rabbit (Oryan, *et al*.,2012) and enhance bone repair in rabbit and rats (Chen, *et al*. *et al*., 2014). In other context the topical use of HA 0.4% (10 mg/ml) has a positive effects on healing of colonic anastomosis in rats (Lima, *et al* ., 2020)

An additional study showed that the topical HA products are used to treat recurring aphthous ulcers, resulting in quick symptom relief (Volpi, *et al* .,2009; Massimo, *et al*.,2012) , Nawres , *et al*., 2020 was noted decrease healing period for patient suffuring from Oroantral Fistula (abnormal communication between maxillary sinus and oral cavity) during topical application 0.2% HA as gel after surgical correction , and Manuele, *et al*.,2015 were used HA as a supportive treatment following nasal and sinus surgery to help patients breathe more comfortably through their noses.

Hyaluronic acid is considered as a potential building element for a variety of medical applications. The polymer's chemical structure is easily changeable, allowing for the generation of a variety of hyaluronic acid physical types. The polymer's versatility is enhanced by the fact that each shape has its own specialist and biomedical use. Viscoelastic liquids,

hydrogels, electrospun fibers, scaffolds, flexible sheets, and nanoparticulate solutions are among the physical forms of HA .

i. Viscoelastic solutions form

The viscoelastic properties of HA solutions are ideal for simulating the synovial fluid present in joints. For most of the natural viscoelastic properties of synovial fluid could be attribute to the concentrations of hyaluronic acid found within it on the other hand, HA do not have long-term mechanical integrity (Kazuko, 1996; Eleni, *et al*.,2012) .

ii. Scaffolds form

Hyaluronic acid-based scaffolds are also commonly employed in the medical field for a variety of therapeutic objectives. HA scaffolds have been used in bone tissue regeneration, space filling, nerve and brain tissue regeneration, cell transport, and muscle regeneration (Watanabe and Yamaguchi , 1996) The scaffolds must meet certain specific criteria, including, the scaffold's surface should allow for cell attachment and development, as well as being biocompatible and not causing inflammation or an immunological reaction. The scaffold's surface disintegration should not release hazardous chemicals, and the scaffold should have the same physical and mechanical properties as the biological tissue it is attempting to imitate. The scaffold's porous should be sufficient to allow for cell development and nutrient diffusion (Fernanda, *et al*., 2017) .

iii. Hydrogel form

Hyaluronic acid hydrogels are considered bulk gels where the HA chains interconnected to gether randomly (Eleni, *et al*.,2012) as a networks of hydrophilic polymer chains that form 3-D structures(Daniel, *et al*., 2019).

These structures bulge in response to water and other factors as a temperature and PH , although they maintain their structure. which may also be considered as scaffolds, and widely used in the biomedical industry (Daniel, *et al*., 2019).

HA hydrogels form , different variations and molecular weights can produce HA- hydrogels with variable , density, stiffness, degradation rates and pore size(Eleni, *et al*.,2012)

iv. Hyaluronic acid sheets

Hyaluronic acid sheets are available in the form of skincare products. These sheets make use of hyaluronic acid's hydration and moisture-retention characteristics once more (Martina, *et al*., 2008) the idea of HA sheets is considered as a scaffolds which may still resemble a gel scaffold(Jason, *et al*.,2011). these sheets were used based on the moisture and hydration retention abilities of hyaluronic acid (Martina, *et al*., 2008; Eleni, *et al*.,2012). Furthermore, these sheets can include other substances such as vitamins and antioxidants additionally to HA for healthier skin (Juanfeng, *et al*., 2019).

v. HA nanoparticles form (HA-NPs)

Have a lot of value as a drug delivery system in cancer treatment, Many research have focused on the pharmacological potential of HA-NPs for anti-cancer treatments since HA can specifically attach to certain cancer cells that overexpress the CD44 receptor. The majority of HA's uses in cancer therapy are as a targeting moiety (Ki Young, *et al*., 2011) . Furthermore, as compared to water-soluble HA derivatives, HA-NPs have shown to accumulate better at the tumor location Surface chemistry, size, surface charge, and molecular weight all play a role in the overall efficacy of HA-NPs (Eleni, *et al*.,2012).

2-11: Nanotechnology and nanoparticles

2-11-1: Medical Nano- Technology

Nanotechnology is a potential method for restoring the function and regeneration of organs and tissues that have been damaged (Chaudhury *et al*., 2014; Jo *et al*., 2015), deals with materials with a grain size less than 100 nanometers (nm) or it is size greater than 1 nm and less than 100 nm (Sullivan *et al*., 2014; Bramhill *et al*., 2017). With the discovery of nanotechnology, regenerative medicine acquired a great development in recent years (Chaudhury *et al*., 2014; Jo *et al*., 2015), and Nano-medicine arisen as a specific application of nanotechnology in the health system and bring a new answers for medicine's unsolvable problems (Kargozar and Mozafari, 2018).

Nanotechnology is now being developed for a variety of medical uses, including, biosensors, diagnostic imaging and medication delivery (Sonaje, *et al* .,2010). In pharmacology (medicine) the nanotechnology use of nanomaterials (nanoparticles) allows the drugs to be delivered to precise diseased areas, and minimizing off toxicity in many drugs and treatments of diseases that are difficult to treat, such as cancer (Anderson, *et al* .,2016). The minimizing toxicity of various types of drugs specifically related to the usage of chemotherapeutics, as off-target reactions can produce major side effects in cancer patients. Furthermore, using of nanotechnology in the field of medical imaging can be improved by allowing precise diagnosis for targeting of damaged tissues at resolutions that are currently unavailable (Sonaje, *et al* .,2010; Anderson, *et al* .,2016) , and the capacity of nanomaterials as a diagnostic agents is related to their ability to improve magnetic resonance imaging and, as a result, For instance, help in identification of liver cancer (Rappeport, *et al*.,2007).

Other nanoparticles materials are being researched as antibacterial alternatives to traditional antibiotics, and can provide a higher local dosages of medicines to the target diseased area without excessive systemic levels therapeutic (Sonaje, *et al*.,2010; Anderson, *et al*.,2016).

2-11-2: Medical Nano-materials

Nanomaterials also can be used in regenerative medicine or tissue engineering (Kargozar and Mozafari, 2018) additionally to the diagnostic and therapeutic properties of various disease , uses of nanoparticles in medical science as a therapeutic or diagnostic method were attributed to the advantages of nanoparticles such as , Rapid onset of action due to rapid diffusion and dissolution of the drug, Increased adherence of nano - particulate drugs to mucosal surfaces that can lead to increased drugs residence duration at the site of action and the ability of Nanoparticles to cross biological barriers such as blood-brain barrier (BBB) and blood-retinal barrier (BRB) therefore, they can penetrate leaky vessels more easily (Buxton, 2006; Jo, *et al*., 2015).

As well as to the mentioned above , the flexibility, simplicity of surface modification , small sizes and wide surface to volume ratio, (Bindhu, *et al*., 2016) , can be a count as a properties of nanoparticles, the small size of nanoparticles improves efficiency for precise intracellular drug absorption and bio-distribution in the intended cellular sites(Semete, *et al*.,2010 ; Bao, *et al*., 2016) and the big surface area allows the particle to be easily manipulated into transporting high quantities of drug or other substances(Sabzichi, *et al*., 2016) .

In this context, nanoparticles possess a wide range of biological properties, including anti-inflammatory, anti-diabetic, anti-cancer, accelerate wound healing, anti-bacterial action and anti-oxidant (Sankar *et al*., 2015).Using of inorganic nanoparticle in the medicine is a new therapeutic option, recently applied in soft tissue repair technique , unlike conventional tissue

repair, NP aims to stimulate the damaged tissue to regenerate. Because of easy application, variety ,consistency, efficacy, rapidity, low invasiveness and functionality make inorganic nanoparticles employed in soft tissue repair (Urie *et al*., 2018). Depending on the size of nanoparticles we can determine their half-life and distribution in the circulation, particles less than 10 nanometers are excreted by the kidney, while those larger than 200 nanometers are phagocytosed by the spleen, Most therapeutic nanoparticles, have a size range from 10 to 100 nm allowing them to distribute throughout the circulation and pass through capillary blood vessels (Walmsley *et al*., 2015).

Nanotechnology is now being developed for a variety of science specially in medical uses, which , has been expanded rapidly due to development of a new nanomaterials , that have different biological effect as well as to its effect of the wound healing , for instance, as nanomaterials that used in medicine , silver that can be used to a accelerate of wound healing by increasing of collagen deposition furthermore, to its action as a wide range of antimicrobial agent(Sushovan, *et al*., 2014), gold nanoparticles also can be used as wound accelerator through enhancing of epithelization and increase collagen deposition(PikSuan, *et al*.,2017).

2-11-3:Pharmacokinetics and toxicology of nanoparticles

The most essential aspect in determining nanoparticle toxicity and effectiveness in the biological system is their behavior in vivo. Nanoparticles that are introduced locally usually stay in their surroundings for a long time and so have a therapeutic effect without producing toxicity. The most important component of studying nanoparticle toxicity and effectiveness in the biological system is their behavior in vivo. Nanoparticles that are introduced locally usually stay in their surroundings for a long time and so have a therapeutic effect without producing toxicity. Systemically introduced nanoparticles offer a variety of pharmacokinetic

features, as opposed to locally implanted nanomaterials, which have limited biodistribution. Nanoparticle pharmacokinetics and biodistribution are influenced by a number of interconnected biological and physicochemical aspects. Absorption, metabolism, excretion, and redistribution of nanoparticles are all measured to determine their pharmacological action (Chaudhury *et al*., 2014). Increased capillary permeability in tissues such the spleen, bone marrow, lungs, liver, and cancers leads to significant nanoparticle absorption. The effect of "enhanced permeability and retention" refers to the higher rate of nanoparticle absorption by tumours. Opsonization is a well-known purifying technique for microorganisms. Nanoparticles have also been seen to undergo opsonization, and as a result, they are quickly expelled from the body. Neutrally charged particles have been shown to have a substantially lower rate of opsonization than charged particles. The pharmacokinetic and biodistribution profiles of nanoparticles have been observed to be altered by chemical coating (Chaudhury *et al*., 2014).

2-11-4: Magnesium oxide nanoparticles MgO NPs

Magnesium (Mg) is mineral plays an important role in the body mediating cell extracellular matrix (ECM) interactions , it's insufficiency has been linked to blood pressure regulation , it has also been employed in soft tissue engineering via boost fibroblastic proliferation (Hickey and Webster, 2015). MgO NPs have been shown to improve bone cell proliferation and differentiation, as well as decrease osteoporosis and accelerate bone regeneration (Fooladi *et al*., 2013). MgO NPs have analgesic properties and can play a positive effect in cells and animals and may protect against oxidative stress, lipid peroxidation, cytotoxicity(Moeini, *et al*., 2017) , and also have, antifungal effect against Candida albicans (Karimiyan, *et al*., 2015 and Elham, *et al*., 2019)

and anti diabetes diseases by acting as an anti-inflammatory through peripheral and central mechanisms (Moeini, *et al*., 2017). As well as have a strong antibacterial action. (He, *et al*., 2016) more effective against the gram-positive than the gram-negative pathogen (Bindhu *et al*., 2016; Hayat *et al*., 2018). The MgO nanoparticles showed no cytotoxicity when the concentration was less than 200 μg / ml , whereas the relative growth rate (RGR) was low when the concentration was greater than 500 μg/ml , and MgO NPs demonstrated cytotoxicity. Endothelial cells were exposed to low concentrations of MgO NPs directly, which enhanced cell growth and It was suggested that the low concentration of MgO NPs suspension created a physical environment that did not interfere with cell growth (Ge *et al*., 2011). Bader 2020 use MgO NPs 20 nm in size , 100 μg MgO NP powder was dissolved in 1 ml distal water and the suspension was prepared as soon as at the surgical procedure then the suspension was sprayed on the stump with the dose was proportional to the surface area of the resected lobe (Bader,2020) calculated as 1 ml / cm3 (Chenthamara *et al*., 2019).

Magnesium oxide NPs can be absorbed by the skin, digestive system and lungs and accumulate in the tissues(Suzana ,2020) in the context, nanoparticles can be administrated through several routes including the skin, respiratory tract inhalation , parenteral administration, (Chenthamara *et al*., 2019). Currently MgO NPs are utilized to treat a variety of diseases, including sour stomach and heartburn, as well as as a cleansing agent and bone regeneration. (Sharma *et al*., 2017).

2-12: Cytokines

Cytokines are a class of immune and non-immune cell synthesis and fractionation a small molecule of peptides that regulate a variety of cellular physiological functions and it plays an important role in stress, such as trauma, pain and infection (Li *et al*., 2017). Cytokines directly or indirectly affect every cell in the body. Cytokines exert their function on any cell that bears receptors specific to them and may act in autocrine, paracrine or endocrine fashion. Cytokine production is genetically, pigenetically and post-transcriptionally controlled, and the net effect of an entire cytokine signaling cascade ultimately determines each cytokine's effects in the balance between health and disease (Richter *et al*., 2018). Cytokines are classified into lymphokines (cytokines that are secreted by T cells and regulate immune responses), proinflammatory cytokines (cytokines that amplify and perpetuate the inflammatory process, such as TNF-α, IL-1β, and IL-6), growth factors (cytokines that promote cell survival and result in structural changes), chemokines (cytokines that are chemotactic for inflammatory cells) and anti-inflammatory cytokines (cytokines that negatively modulate the inflammatory response), although many of these functions may overlap (over 50 cytokines have now been identified) (Li *et al*., 2017; Richter *et al*., 2018).

Cytokines play multiple, integral roles in the immune defense against infection and neoplasia, but they are also mediators of both inflammatory and autoimmune diseases (Richter *et al*., 2018). Cytokines play a key role in orchestrating the chronic inflammation by recruiting, activating and promoting the survival of multiple inflammatory cells (Barnes, 2008).

Currently known Interleukin (IL), interferon (IFN), colony stimulating factor

(CSF), tumor necrosis factor (TNF), nerve growth factor (NGF) and transforming growth factor (TGF) Etc., they play a very important

regulatory role in the immune system, in abnormal situations it can also lead to pathological reactions (Li *et al*., 2017).

Cytokines are produced by many different cell types and often show overlapping activities regulating proliferation or differentiation, depending on the type and developmental state of the target cells involved. The cytokines IL-6, IL-1β and TNF-α are elevated in most, if not all, inflammatory states and have been recognized as targets of therapeutic intervention (Scheller *et al*., 2011). TNF-α and IL-1β also activate transcription factors to produce IL-6 (Tanaka *et al*., 2014a).

It is recognized that the cytokine network plays a pivotal role in inducing the acute-phase inflammatory and immunologic response to surgical trauma. Several laparoscopic procedures have been shown to be associated with decreased release of cytokines compared with their open counterparts. There have been, however, few investigations comparing cytokine productions after video-assisted thoracic surgery (VATS) versus the conventional approach (Yim *et al*., 2000).

Tissue trauma generates a wide range of immunologic responses. Cytokines, chemokines, growth factors, lipids and other mediators are released by activated leucocytes, macrophages, fibroblasts and endothelial cells and are involved in thelocal injury response and in the systemic reaction. Although there are parallel and interacting processes involved, from a surgical perspective, a major initiating process involves the activation and recruitment of macrophages and monocytes by tissue damage leading to release of IL-1β and TNF-α, which cause the release of IL-6. These cytokines are those most studied in the surgical literature. IL-1β and TNF-α have been implicated in the immune response to surgery (Walker and Leaver, 2007; Li *et al*., 2017).

Elevated concentrations of IL-6, IL-1β and TNF-α in serum are good early markers of severity of surgical injury and may reflect development

of postoperative complications and subsequently lead to more prompt administration of proper treatment which might decrease morbidity and mortality (Lesina *et al*., 2014; Guisasola *et al*., 2015).

Interleukin 6 (IL-6) is one of major proinflammatory cytokines responsible for immune response activation. It is a soluble mediator cytokine with redundant pleiotropic activity. It is also able to influence hormonal balance and some endocrinological abnormalities (Schaper and Rose-John, 2015).

IL-6 synthesis is tightly regulated by transcriptional andposttranscriptional mechanisms. When infections and tissue injuries occur, IL-6 synthesis is promptly induced and provides an emergent signal that contributes to host defense through the stimulation of acute-phase responses, immune reactions and hematopoiesis. After the environmental stress is removed from the host, the production of IL-6 is terminated.

However, dysregulated continual synthesis of IL-6 is involved in the development of chronic inflammatory autoimmune diseases (Schaper and Rose- John, 2015).

IL-6 is produced by various types of lymphoid and non-lymphoid cells, such as T cells, B cells, monocytes, macrophages, fibroblasts, keratinocytes, endothelial cells, mesangium cells and several tumor cells. The production of IL-6 by T cells requires the presence of monocytes. IL-6 has important roles in many processes, such as the development of humoral and cellular responses, triggering of the inflammatory response, regulation of hematopoiesis, supervision of cellular differentiation and reproduction and wound healing. Any inflammatory stimulus can increase the IL-6 concentrations and thus can serve as a trigger for other cytokines, such as IL-1β and TNF-α (Hunter and Jones, 2017). Furthermore, IL-1β is a strong stimulator of IL-6 synthesis in fibroblasts. Since IL-1β is also induced upon stimulation of Toll-like receptors (TLRs), IL-1β is part of a

feed-forward loop which increases the production of IL-6 (Schaper and Rose-John, 2015).

2-13: Vascular Endothelial Growth Factor (VEGF)

Vascular endothelial growth factor (VEGF), also known as vascular permeability factor (VPF), was originally described as an endothelial cell-specific mitogen VEGF is produced by many cell types including tumor cells, macrophages platelets keratinocytes, and renal mesangial cells. The activities of VEGF are not limited to the vascular system; VEGF plays a role in normal physiological functions such as bone formation, hematopoiesis, wound healing, and development. (Ferrara, *et al*.,1992; Chintalgattu, *et al*., 2003) also consider as a key regulator of physiological angiogenesis during embryogenesis, skeletal growth and reproductive functions. VEGF has also been implicated in pathological angiogenesis associated with tumors, intraocular neovascular disorders and other conditions (Napoleone, *et al* ., 2003)

Chapter three
Materials and Methods

3-1: Animals

In the current study , thirty six local breed mature dogs of both sexes were used. The average weight 25 kg ± 1.3 and aged an average 27 months ± 1.5was. The animals were clinically healthy and housed in the dog house at the College of Veterinary Medicine, University of Mosul and were given free food and water.

3-2: Experimental design

Thirty six dogs were divided randomly into four equal main groups with 9 dogs . Each main group was furtherly divided into 3 subgroups of 3 dogs in each according to time after operation 7,15,30 days to study the effect of additive agents on the healing through study of clinical signs, gross and histopathological study ,imunohistochemistry , contrast radiograph and endoscopy. All animals in main and subgroups underwent esophageal anastomosis in the cervical part of esophagus . The anastomotic site was closed in the same manner , the mucosa and muscularis were treated with follow:-

Group 1 (9 Animals): without addition any materials (Control) .

Group 2 (9 Animals): Local application of autologus aspirated bone marrow.

Group 3 (9 Animals): Local application of pure hyaluronic acid 0.7%

Group 4 (9 Animals): Local application of magnesium oxide nanoparticles with size 20 nm. 100 µg powder of MgO NP were dissolved in the distal water (1 ml) was prepared immediately.

The results were evaluated depended on Clinical signs , Esophageoscopy , Gross changes , Contrast radiography , Histological changes and Immunohistochemistry , invistegated where biopsy collection at anastomotic site at 7^{th} 15^{th} ,30^{th} days post surgery. Scoring of histological and immunohistochemistry

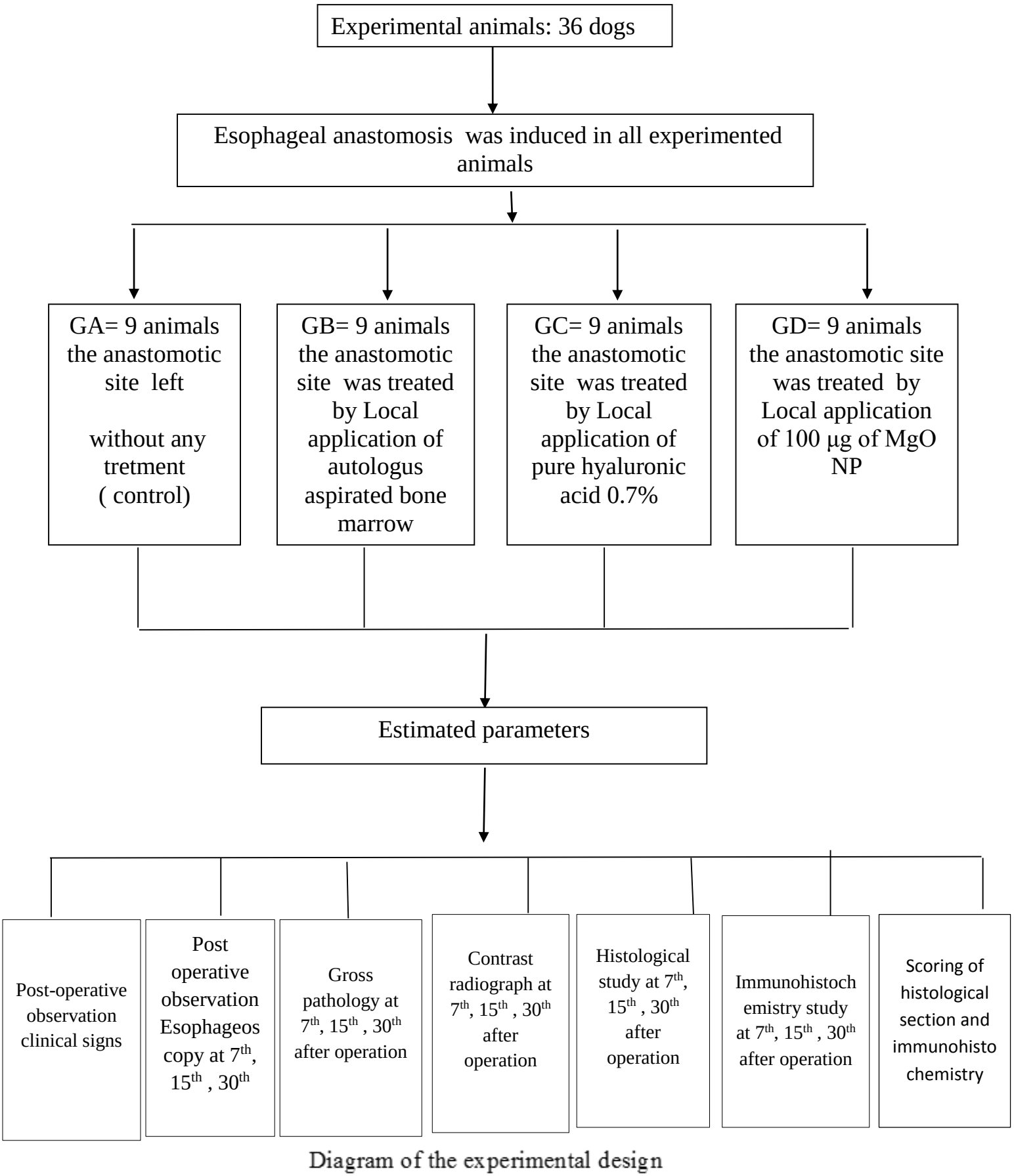

Diagram of the experimental design

3-3: Preoperative preparation

All animals underwent operation were kept 24 hours without food and 6 hours without water before the surgical operation. Area of cervical region was prepared for aseptic surgery by clipping and shaving of the hair from the mandible to the sternum region or thoracic inlet. After that the area was washed with soap and water, then antiseptic by the application of 5% tincture of iodine . Then the dog was controlled on the surgical table in a dorsal recumbency and all four leg were fixed to the surgical table.

3-4: Anesthesia

Before administration of general anesthesia all dogs were given atropine sulphate (Vapco, Jorden) at dose 0.04mg/kg BW/SC as a premedication. Then general anesthesia was given to all dogs , i.m injection of ketamine 10% HCl(Rotexmedica , Germany) at 15 mg/kg, with xylazine 2% (Interchemie, Holand) at 5mg/kg B.w. Both drugs are given as a single bolus injection, at femoral muscle. Where necessary ketamine alone was given repeatedly (Green, and Thurmon, 1988).

3-5: Surgical technique

A 10-centimeter skin incision was made on the ventral midline of the neck at the junction of the middle and lower third. The neck muscles brachiocephalic and sternohyoidus were bluntly dissected by scissors to expose esophagus. The esophagus was isolated carefully from the neighboring tissue with minimum damage to its blood supply and adventitia. High care was paid to avoid damage of surrounding vital structures such as trachea ,common carotid artery, recurrent laryngeal nerve, vagus nerve. The esophagus fixed by stay sutures placed at equal distances from two sides(Figure 9). and the esophagus was incised completely (Figure 10). In all groups end to end esophageal anastomosis was applied and the mucosa was closed with 2/0 silk in a simple interrupted pattern and the knot tied within the lumen (Figure 11). , while

, the muscular layer was closed with 2/0 polygalactin (Vicryl) in an interrupted horizontal mattress (Figure 12). finally the neck muscles were closed with polygalactin size 1 using simple continuous and the skin was sutured with silk size 1 using simple interrupted pattern .

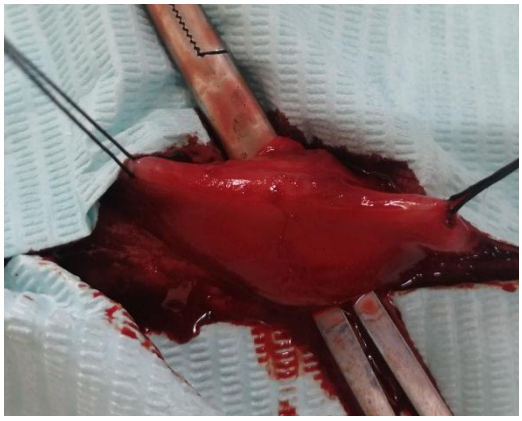

Figure 9: Exposed esophagus

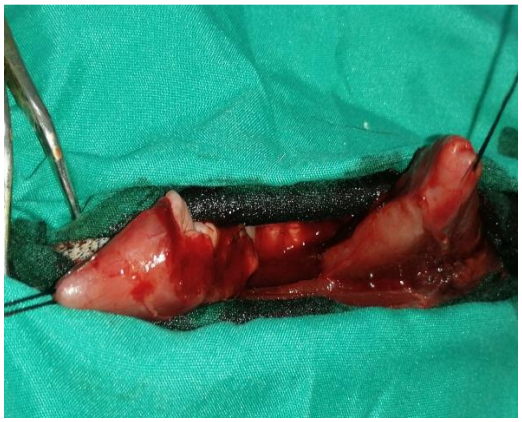

Figure 10: Complete resection Esophagus

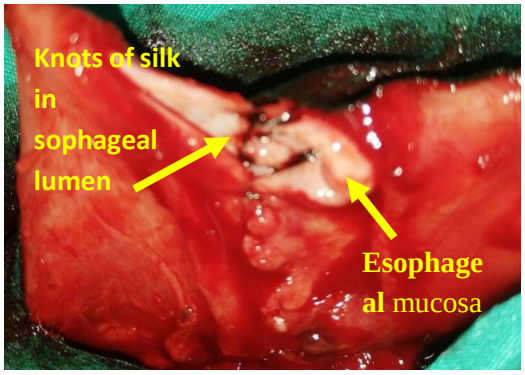

Figure11: Esophageal mucosa sutured with 2/0 silk in a simple interrupted pattern

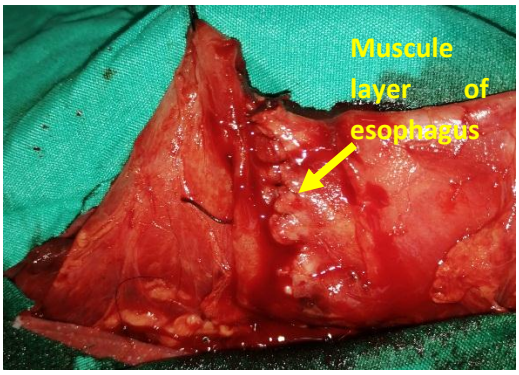

Figure 12: Esophageal muscularis sutured with 2/0 in an interrupted horizontal mattress

The autologous aspirated bone marrow, hyaluronic acid and MgO NPs were spread on the mucosa and the muscular layer at the esophageal anastomotic site in each group as follows:-

Group 1: Control group

In this group the mucosa and muscularis at the anastomotic site were left without treatment by any biological or non biological materials .The esophageal layers, mucosa and muscular layer of esophagus were sutured as discussed above , neck muscles and skin incision has been closed routinely.

Group 2 : Bone Marrow group

The 2 ml autologous aspirated bone marrow was collected under aseptic conditions from the head of femur bone using 18 gage needle(Figure 13). The mucosa and the muscular layer of esophagus were sutured as mentioned above ,after suturing of mucosa one ml of fresh marrow was spread at anastomotic mucosal site (Figure 14). and left for several minutes to clot, while , the reminder of aspirated marrow has been spread on the muscular layer of esophagus(Figure 15). Finally the neck muscles and skin inscion were closed routinely

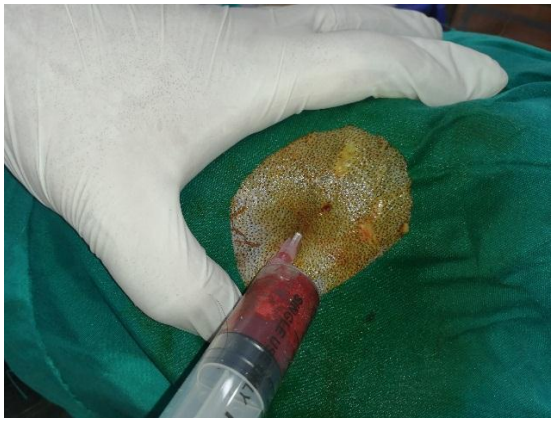

Figure 13: Site of bone marrow Aspiration

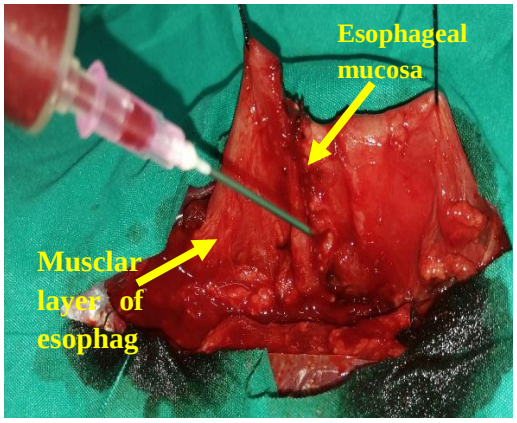

Figure 14: Fresh marrow spread on closed esophageal mucosa

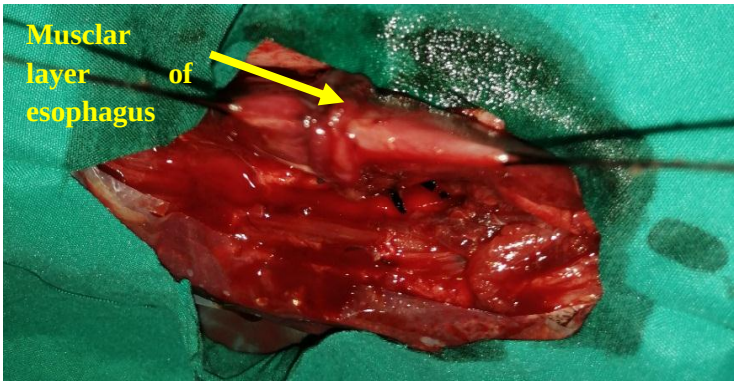

Figure 15: Fresh marrow spread on closed muscularis layer of esophagus

Group 3: Hyaluronic acid

One ml of pure hyaluronic acid (3.5% /5 ml) manufactured by Mccosmatic/ spain (Figure 16) was used in this group. The esophageal anastomosis, surgical operation and suture pattern were used in this groups similarly to previous groups . The mucosa at anastomotic site was coated with 0.5 ml of HA(Figure 17) . And the muscular layer of esophagus was coated with another half (Figure 18).

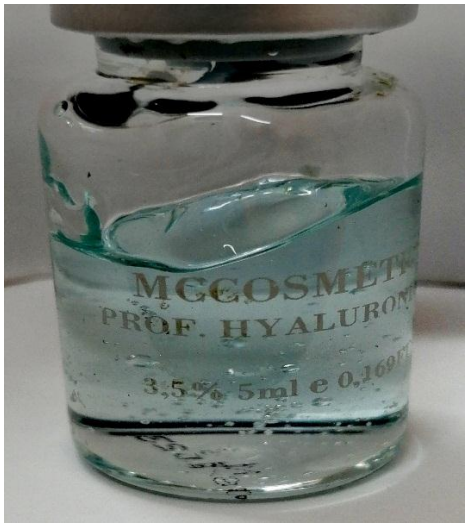

Figure 16: Hyaluronic acid Mccosmatic /spain

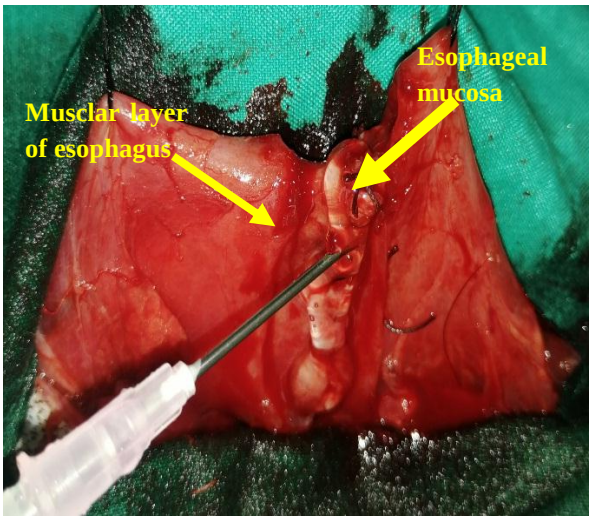

Figure 17: HA spread on closed esophageal Mucosa

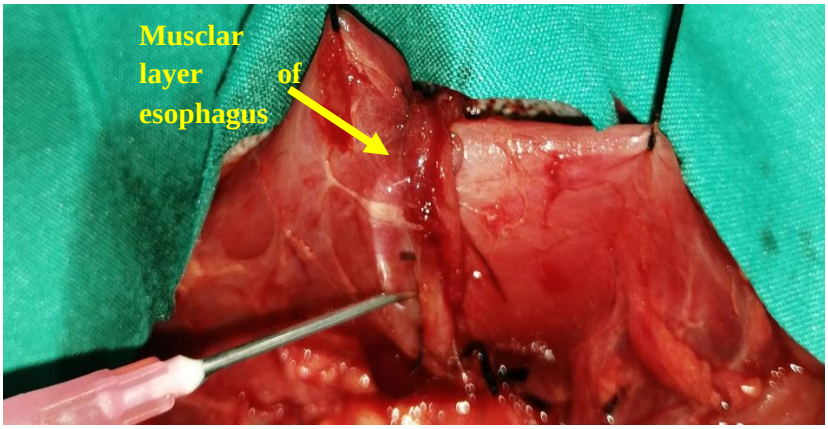

Figure 18 : HA spread on closed muscularis layer of esophageal

Group 4: Magnesium Oxide Nanoparticles

Local application of magnesium oxide nanoparticles with size 20 nm manufactured in Iran (Venmaterials company) (Figure 19) . 100 µg powder of MgO NP were dissolved in the distal water (1 ml) (Bader,2020) then spread 0.5 ml of suspension on the mucosa (Figure 20). while, the reminder was spread on the muscularis (Figure 21). This group had the same surgical procedure and suture pattern as group 1, 2 and 3.

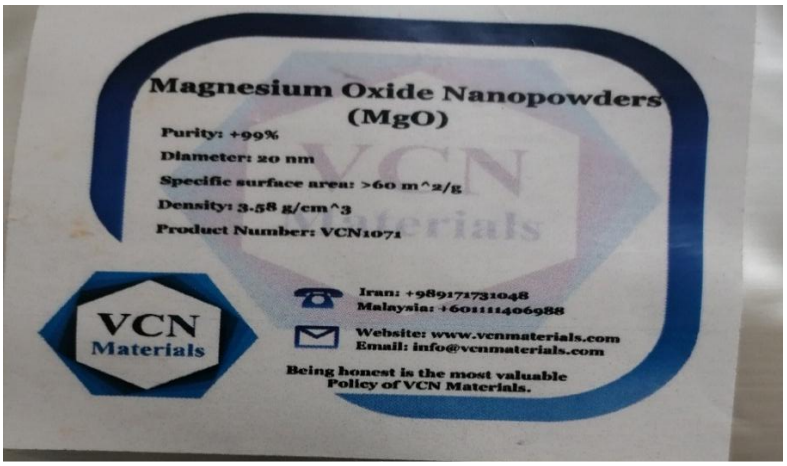

Figure 19 :MgO NPs (Venmaterials company / Iran)

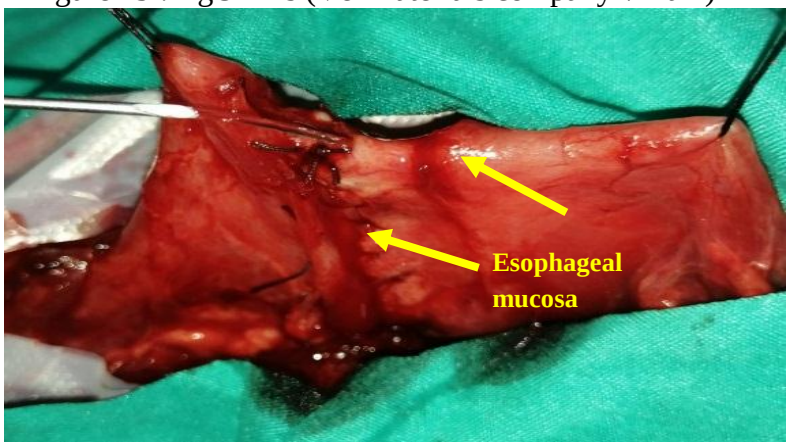

Figure 20: MgO NPs spread on
Closed esophageal mucosa

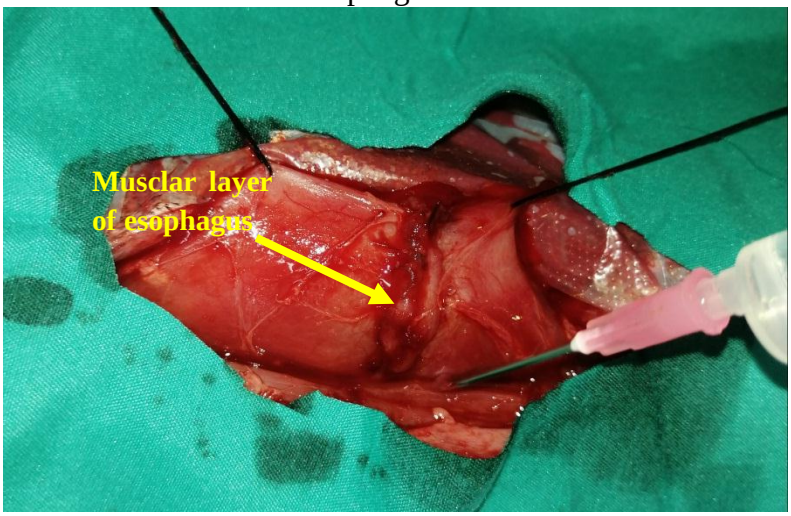

Figure 21: MgO NPs spread on closed muscularis layer of esophagus

3-6: Post-operative care

The experimental animals were received penicilline – streptomycin(kanavet, Canadian), in a dose of 10.000 I.U /Kg / B. W. Penicilline and 10mg/kg /B. W. streptomycin for 3 days by I.M route injection at femoral muscle . all animals were given intravenous fluid therapy for two days after surgical operation and oral feeding starts in third day after operation with milk as a liquid food for 5^{th} day , then gradually returned to the normal solid food. Skin sutures were removed 7 days after the operation (except animals those euthanized at the seventh day after operation)

3-7: The appraisal of the esophageal anastomosis was based on the following parameters:

1- **Clinical signs** by monitoring of the animals for detection any complications that could be occur after esophagectomy such as dysphagia and leakage .

2- **Esophageoscopy** was used to viewing the esophageal lumen and mucosal lining at the anastomotic site for detection on the presence of any stich dehiscence that leads to leakage.

In this study the borescope made in china was used instead of medical endoscopy, its defined as an optical tool used to help in the visual inspection of narrow, difficult-to-reach cavities. The borescope consists of a flexible wire 3.5 M with a HD camera 7mm in diameter on the one end and the computer's USB socket on the other end , as well as to photo control switch(Figure 22). The lens of camera built-in 6 white leds lights for illumination. The camera creates an internal image(video or picture) , which is magnified and displayed on computer through its own software version AN 98 .This models of boroscope can be used in medical treatment (according to manufacturer) and offered improved features such as lower cost, better resolution,

adjustable illumination or replacing the built-in display with a computer connection, such as a USB cable.

Figure22: Borescope

Esophageoscopy after esophagectomy was studied in all experimental animals. This examination was performed under general anesthesia by the same anesthetics agents that, used in the surgical operations then all the dogs were euthanized after esophageoscopy . A suitable size endotracheal tube (ETT) induced in the trachea to facilitate breathing and prevent asphyxia, followed by gently introducing camera protected within another endotracheal tube(ETT) inside the esophagus for exploration of anastomotic site , the second endotracheal tube acts as a guide to facilitate manipulation of flexible camera (Figure 23)

Figure 23: borescope with the two endotracheal tube

3- **Gross pathological examination** and autopsy collection after esophagectomy and euthenasia was studied in all groups at 7th, 15th , 30th postoperative days, to evaluation healing of esophageal anastomotic site grossly, depending on, clarity and binding line of anastomotic site .

4- **Contrast radiography** for assessment the degree of stenosis at anastomotic site, contrast radiography was applied by taking 10 cm of esophagus at the point of anastomosis after euthanasia of animals by over dose of ketamine . then was filled with (100% w/v) barium sulphate and the degree of stenosis was calculated by using the equation below.

i. Stenosis index % = 100 {1-2 a / (b + c)}
ii. a: the diameter of esophagus at the anastomotic site in centimeters
iii. b: the diameter of esophagus in centimeters at 2 cm pre-anastomotic site

iv. c: diameter of esophagus in centimeters at 2 cm post- anastomotic site (Mc Adams, *et al*.,1969; Al-Maseeh and Eesa, 2009)

5- Histological and Immunohistochemistry studies, that performed where samples collection at anastomotic site , 7^{th} 15^{th} ,30^{th} days after operation.

A. Histological study

After tissue collection, the samples were fixed in 10% neutral buffered formalin (prepared by adding 10 ml of 37-40% formaldehyde to 90 ml of distilled water containing 4 grams of sodium phosphate monobasic salt and 6.2 grams of sodium phosphate dibasic salt) to start the tissue fixation, and this stage lasts at least 72 hours, later these samples were washed in running tap water for one hour, then processed to be embedded in paraffin wax (Luna, 1968; Suvarna, *et al*.,2013). The samples were later carried in tissue cassettes and labeled with each group and animal code, and then these samples were dehydrated to remove the water from tissues using ethyl alcohol; this process starts with 70% ethyl alcohol overnight, then 80% ethyl alcohol for one hour, 90% ethyl alcohol for one hour at two changes, then the samples transferred to absolute alcohol for one hour at two changes (Luna, 1968). At this stage, the water was excluded from tissue and ready to be cleared with xylene, which was used as pure as its supplied for thirty minutes at two changes, with visual examination until the tissue had a clear yellow to brown gelatinous appearance to complete the clearing process and at this stage, the tissue was ready to be infiltrated with paraffin wax (Luna, 1968). After the clearing was complete, the samples were transferred to hot paraffin wax at 55-58ºC for one hour at three changes (the first changes should have 50% xylene and 50% paraffin

wax, the second changes should have 25% xylene and 75% paraffin wax, the third changes should be 100% of hot paraffin wax) at the end of this stage the samples should have a solid, firm and natural tissue color which mean the tissue was completely infiltrated with paraffin wax (Suvarna, *et al*.,2013). The tissue samples are then embedded in paraffin wax by using paraffin mould where the mould is filled with hot paraffin wax, and the tissue sample is localized in the center of the mould and filled mould to the cassettes, then left to be cooled at the room temperature (Luna, 1968). The paraffin block sectioning using rotary microtome at 4-6 µm then transferred to the floatation water bath, and lifted using labeled clean glass slide, and dried at room temperature for 24 hours, then slide put on a hot plate to complete drying for one hour at 55-60ºC, the slide was ready to stain (Luna, 1968 ; Suvarna, *et al*.,2013).

i. Harris hematoxylin and alcoholic Eosin

The glass slides were stained with routine Harris hematoxylin and alcoholic eosin stain using the standard protocol described by Luna 1968:

1- The paraffin wax was removed using xylene for three changes, ten minutes each.

2- The slides rehydrated by ethyl alcohol, 100%, 90%, 80% 70%, two changes, five minutes each.

3- The slides floated with tap water for 10 minutes to complete the rehydration.

4- The slides were stained with Harris hematoxylin for ten minutes.

5- Slides were washed with tap water and blue using saturated lithium carbonate solution.

6- Slides washed in running tap water to develop the sky blue color.

7- Slides rinsed with 90% ethyl alcohol for one minute.

8- Slides stained with alcoholic yellow eosin for two minutes.

9- Slides were dehydrated using ethyl alcohol 70%, 80%, 90%, 100% tow changes, five minutes each.

10- Slides cleared in xylene three changes, fifty minutes each.

11- Slides cover with glass slide using DPX tissue mount media.

12- the result of this stain was, the blue color and their shadows stain the basophilic cell element such as the nucleus and nuclear material, while the red and pink color stain the eosinophilic components such as cytoplasm (Luna, 1968 ; Suvarna, *et al*.,2013) .

ii. Trichrome stain - Masson's trichrome

The glass slides were stained with routine Harris hematoxylin and alcoholic eosin stain using the standard protocol described by Luna 1968:

1- The paraffin wax was removed using xylene for three changes, ten minutes each.

2- The slides rehydrated by ethyl alcohol, 100%, 90%, 80% 70%, two changes, five minutes each.

3- The slides floated with tap water for 10 minutes to complete the rehydration.

4- The slides were stained with Weigher's hematoxylin solution for ten minutes, then washed with tap water for ten minutes, and rinsed in distilled water quickly.

5- The slides were stained with Biebrich scarlet and acid fuchsine solution for two minutes, then rinsed in distilled water quickly.

6- The slides floated with 5% phosphotungstic acid for 15 minutes.

7- The slides were stained with 1% aqueous light green or anilin blue solution for one minute, then rinsed in distilled water quickly.

8- The slides were differentiated in 1% aqueous glacial acetic acid for five minutes.

9- Slides were dehydrated using ethyl alcohol 70%, 80%, 90%, 100% tow changes, five minutes each.

10- Slides cleared in xylene three changes, fifty minutes each.

11- Slides cover with glass slide using DPX tissue mount media.

12- the result of this stain was the nucleus black in color, the other tissue elements red in color, the collagen fiber has a green colour (Luna, 1968 ; Suvarna, *et al.*,2013) .

B-Immunohistochemistry study

Immunohistochemistry was achieved by using the avidin-biotin immunoperoxidase technique. The adhesive slides were dewaxed and rehydrated. Endogenous peroxidase was blocked in 3% hydrogen peroxide-methanol solution for 30 min. Then, the slides were washed in phosphate buffered saline at pH 7.2, the nonspecific proteins were blocked by blocking solution for 1 hour at room temperature. The slides were incubated with primary antibodies for IL-6 (post *et al*., 2016), IL-12 (Lemchak and Akilov, 2016) and EVGF-A (Maae *et al*., 2011) they was used as Rabbit Polyclonal antibody at dilution equal to 1:200 (MyBioSource, USA) for overnight at 4ºC. Once washing with PBS, the slides were incubated with poly-HRP Goat Anti-Rabbit IgG at dilution 1:200 (Wuhan Fine Biotech, China) for 1 hour at 37ºC. After another PBS washing, the reaction was amplified with an avidin-biotin complex. The slides were counterstained with haematoxylin, rinsed in distal water, dehydrated and cover slipped. Using Image J program, IL-6, IL-12 and EVGF-A staining was evaluated by find out the density of positive nuclear of cytoplasmic.

C-The histological sections were scored according to the following criteria.

The histological scoring is used to convert non parametric healing process into numerical data that can be easily processed statistically to clarify the differences between different groups, in which the main features of normal histological healing process where taken under investigation, these parameters were explained in Table 1 (Yurtçu et al., 2011), in which the high scoring rank were express optimized healing process:

1- The inflammatory reaction and cellular infiltrations.

2- Formation of granulation tissue and maturation.

3- Formation of newly blood vessels.

4- The integrity of surface mucosal epithelial cells.

5- Collagen fiber deposition.

6- Expression of IHC markers included IL-6, IL-12 and VEGF-A.

Table 1: Microscopic scoring criteria that included in current study (Tabola *et al.*, 2016; Mcgavin and Zachary, 2017)

Criteria	0+	1+	2+	3+	4+
Inflammation	Severely infiltration with fibrosis	Moderate infiltration with hemorrhages	Few infiltration with edema	Few infiltration	Absent
Granulation tissue	Absent	Discrete	Moderate	Intense	Complete
Angiogenesis	Absent	Discrete	Moderate	Intense	Complete
Re-Epithelization	Absent	Discrete	Moderate	Intense	Complete
Fibrosis	Absent	Few	Spread	Extensive	Stretch
IL-6	Negative	Weak	+Positive	++Positive	+++Positive
IL-12	Negative	Weak	+Positive	++Positive	+++Positive
VEGF-A	Negative	Weak	+Positive	++Positive	+++Positive

3-8: Statistical analysis

Using SPSS version 22.0 to measures the significant differences at $P<0.05$ to compare between different mean for different treatments, in which the One Way ANOVA with Duncan's test were used to measures the significances between different groups at $P<0.05$. In contrast, Paired T test was used to measure the differences in the mean stenosis percentages in different treated groups with different post- operative days at $P<0.05$ (Barton and Peat, 2014).

Chapter four
Results

4-1: Post-operative Observations

The results of the post-operative examination were studied by clinical observation and endoscopic examination as adjunctive diagnostic method , clinical study showed that there was swelling at the site of surgical operation which subsided in five days after operation . In the group 2 (bone marrow group), two animals and in group 1 (control group) one animal developed hoarseness . Regurgitation and dysphagia were observed in all animals when solid food were offered at the 6^{th} P.O.Ds with various degrees of severity at 7^{th}, 15^{th},30^{th} P.O.Ds in each group furthermore among groups. The dysphagia was clear at 7^{th} and less at 15^{th},30^{th} P.O.Ds respectively in each subgroup while , among the groups the animals in the group 1 (control group) suffered from obvious dysphagia while group 3 (Hyaluronic acid) had a moderate one . Moreover , moderate to mild dysphagia was seen in the group 2 (bone marrow) while it was mild in the group 4 (MgO NPs).

Clinically none of the animals noted suffering from signs of esophageal leakage, and this result is confirmed by employing endoscopic as adjuvant diagnostic method for detection on any stich dehiscence ,using esophageoscopy was safe without any damage to the anastomotic site, the figures 24 ,25,26 and 27 are showing endoscopic examination and confirm complete absence of any dehiscence at anastomotic site

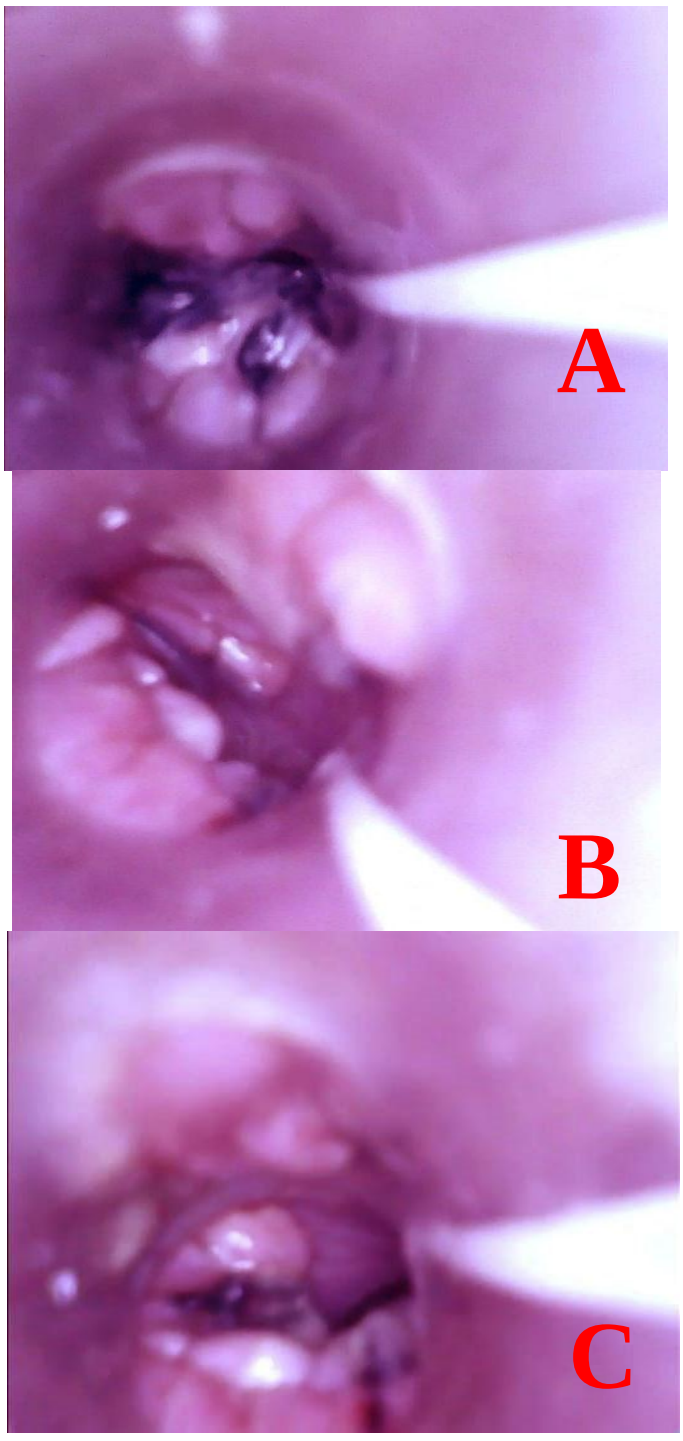

Figure 24 : Endoscopic examination showing esophageal mucosa of group1 (Control group)
A: At 7th P.O.Ds
B : At 15th P.O.Ds
C : At 30th P.O.Ds

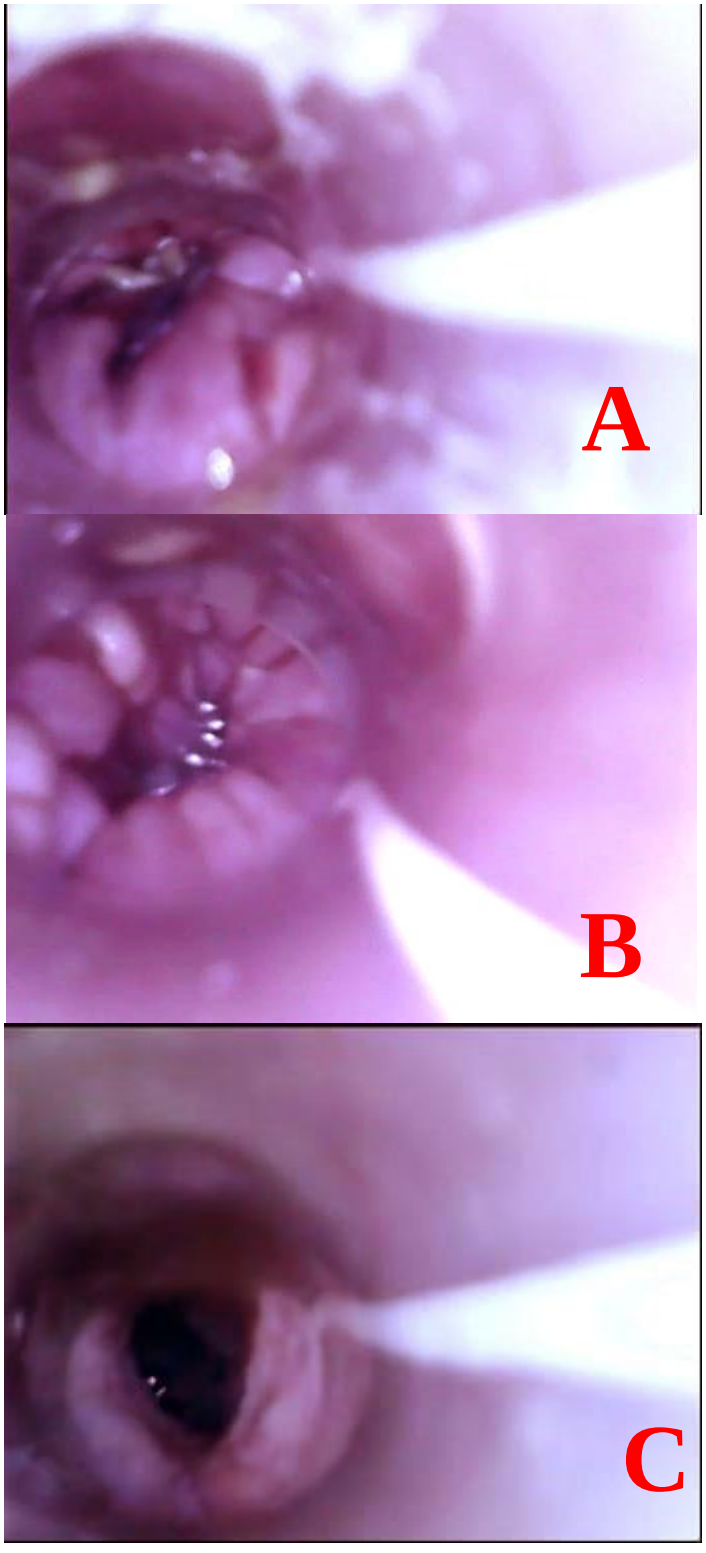

Figure 25: Endoscopic examination showing esophageal mucosa of group 2 (Bone marrow group)
A: At 7th P.O.Ds
B : At 15th P.O.Ds
C : At 30th P.O.Ds

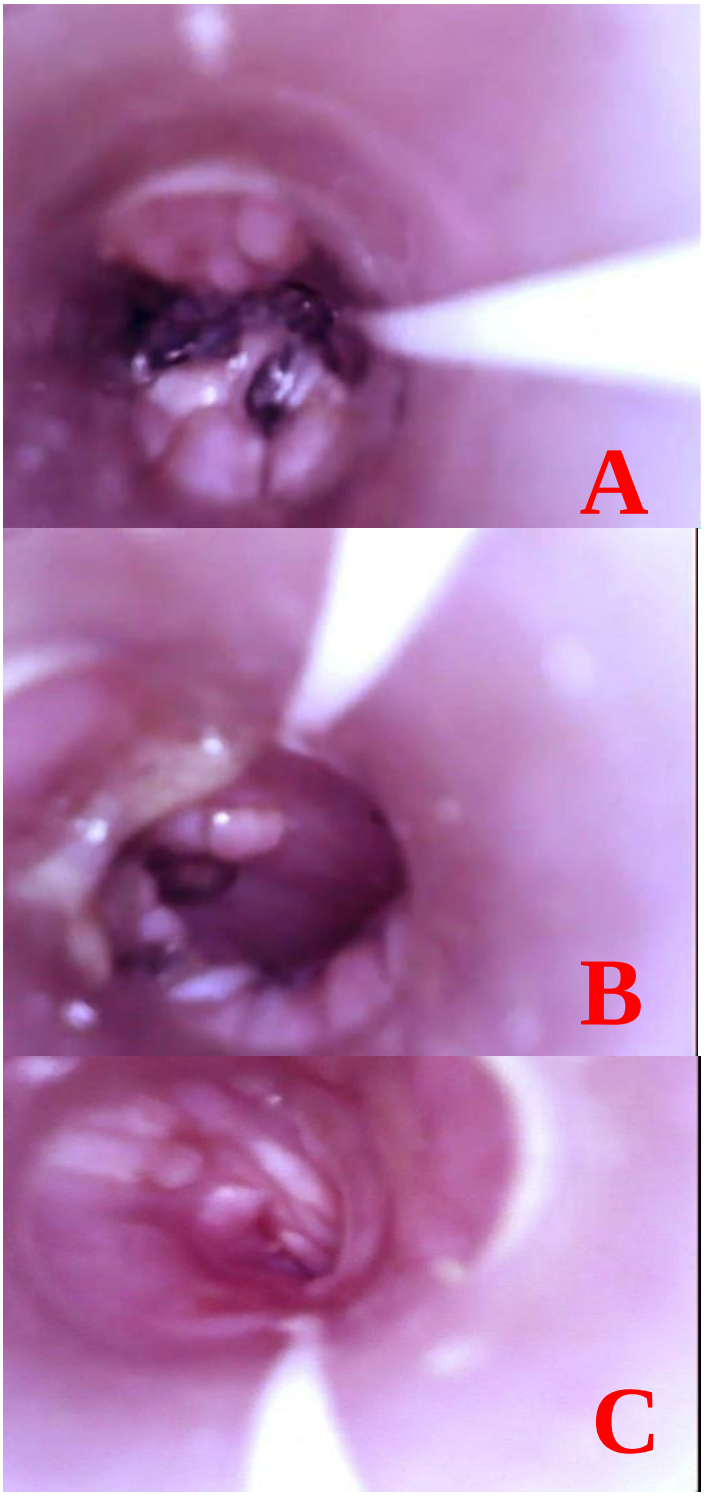

Figure 26: Endoscopic examination showing esophageal mucosa of group 3 (Hyaluronic acid group)
A: At 7th P.O.Ds
B : At 15th P.O.Ds
C : At 30th P.O.Ds

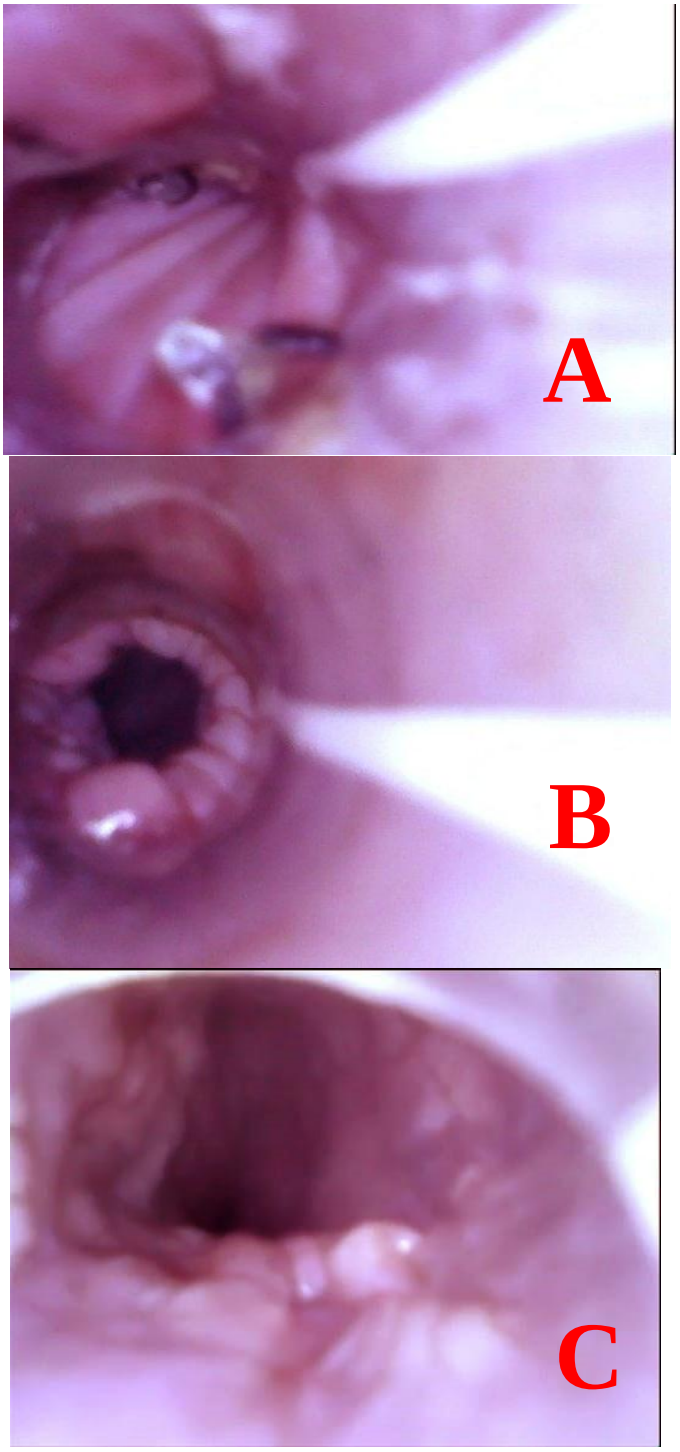

Figure 27: Endoscopic examination showing esophageal mucosa of group 4 (MgO NPs group)
A: At 7th P.O.Ds
B : At 15th P.O.Ds
C : At 30th P.O.Ds

4-2: Gross pathology

The gross pathological examination showed different degrees of clarity of anastomotic line. In each subgroup the degree of clarity of anastomotic line was noticed clearly at 7^{th} and became less clear at 15^{th}, and even less at 30^{th} P.O.Ds.

Among the groups, the degree of clarity of anastomotic line was obviously clear in the group 1 (control group) while, it was less in the group 3 (hyaluronic acid) , 2 (bone marrow) and 4 (MgO NPs) groups, respectively (Figures 28 , 29 , 30 and 31)

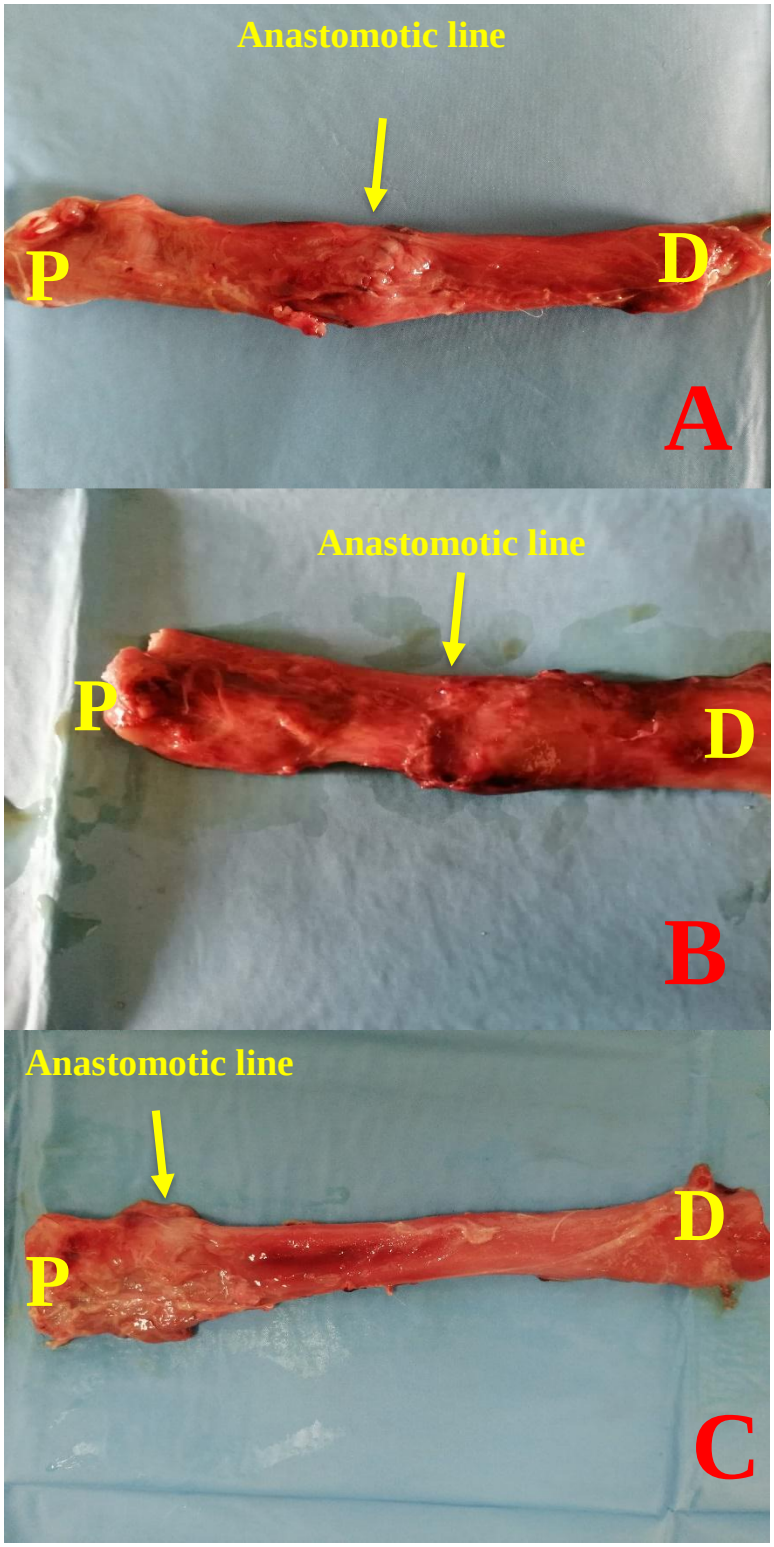

Figure 28 : Grose examination showing degrees of clarity of anastomotic line in group 1
(Control group)
A: At 7th P.O.Ds
B : At 15th P.O.Ds
C : At 30th P.O.Ds

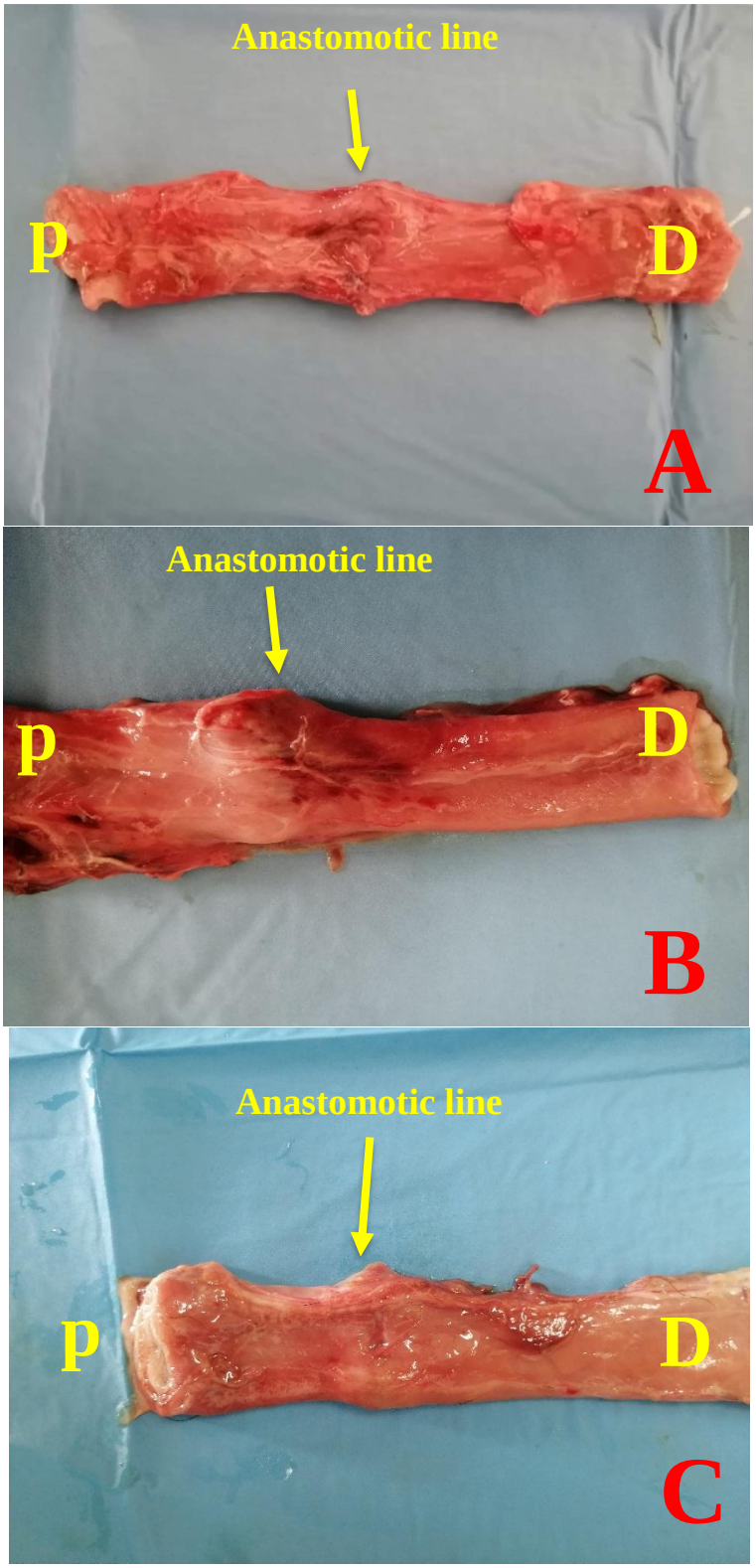

Figure 29 : Grose examination showing degrees of clarity of anastomotic line in group 3
(Hyaluronic acid group)
A: At 7th P.O.Ds
B : At 15th P.O.Ds
C : At 30th P.O.Ds

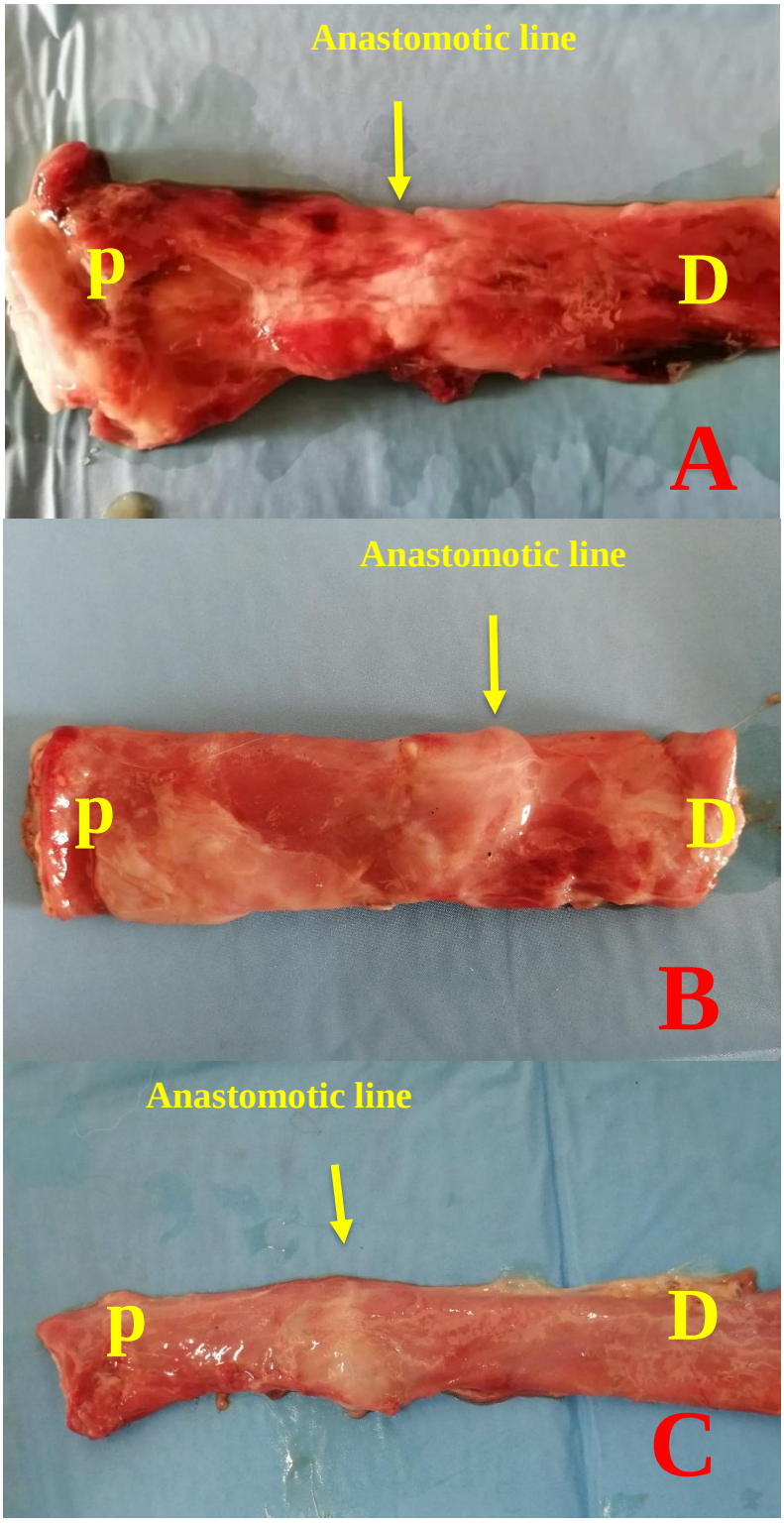

Figure 30 : Grose examination showing degrees of clarity of anastomotic line in group 2
(Bone marrow group)
A: At 7th P.O.Ds
B : At 15th P.O.Ds
C : At 30th P.O.Ds

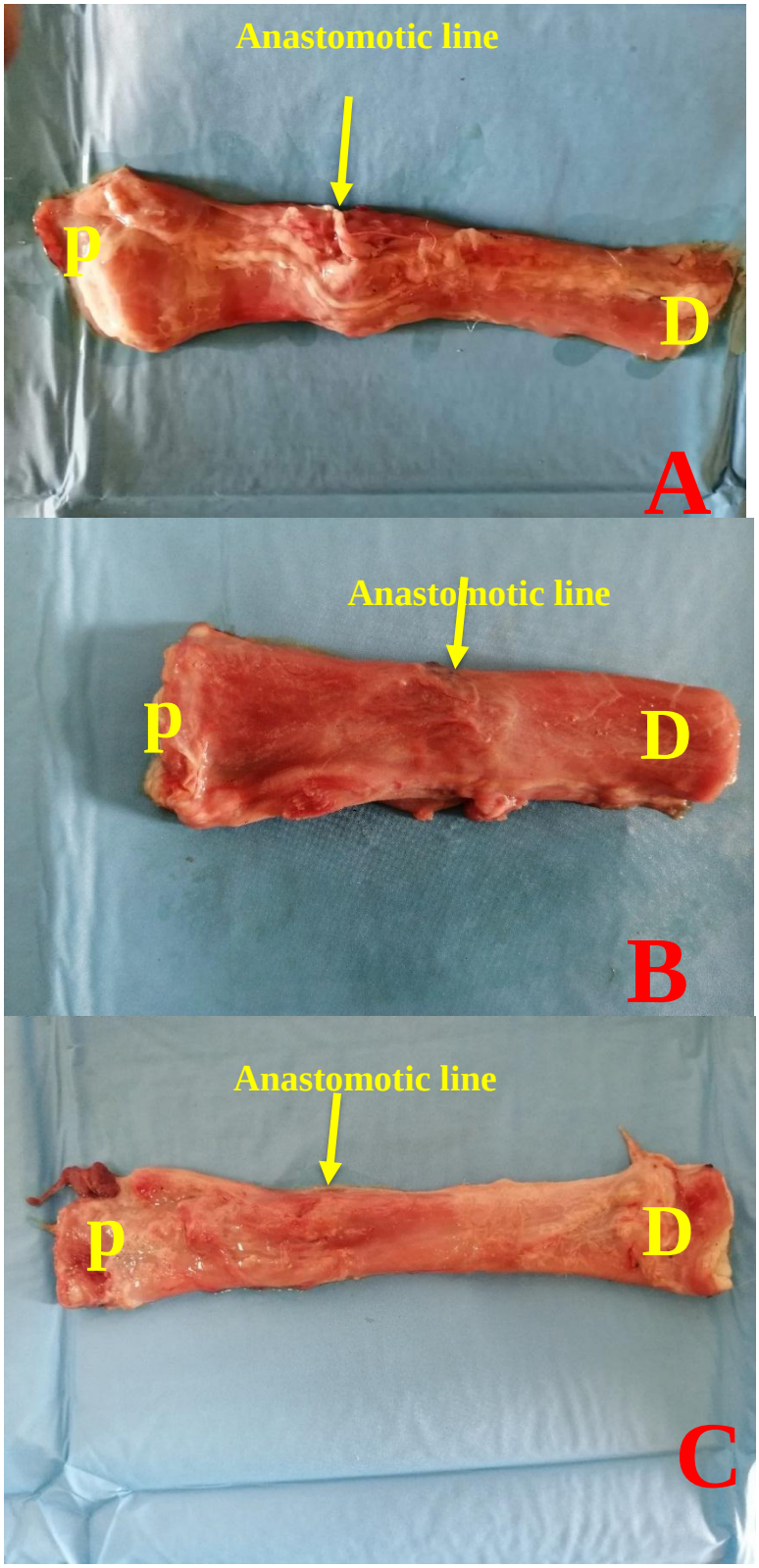

Figure 31 : Grose examination showing degrees of clarity of anastomotic line in group 4
(MgO NPs group)
A: At 7th P.O.Ds
B : At 15th P.O.Ds
C : At 30th P.O.Ds

4-3: The Radiographic Examination

The percentage degree of stenosis in control group (1) was non- significant increasing with time after operation (7^{th} , 15^{th} and 30^{th} P.O.Ds) (Figures 32) (Table 2). On the other hand, the percentage degrees of stenosis in bone marrow(2) , hyaluronic acid(3) and MgO NPs(4) groups were decreasing over time after operation (7^{th}, 15^{th} and 30^{th} P.O.Ds) (Figures 32,34,35) (Table 2) , these decreasing were non-significant at $P<0.05$ in group 2 (Bone marrow) ,while in group 3 (hyaluronic acid) and group 4 (MgO NPs) the decreasing were significantly at $P<0.05$ (Table 2) .

In other context, as a comparison among the groups, and at any postoperative time, the percentage degrees of stenosis in control group was high , at the same time it was diminished in hyaluronic acid group , moreover , in bone marrow group more diminishment was noticed and even more in MgO NPs group (Table 2) . At the 7^{th} P.O.Ds the decreasing percentage degrees of stenosis were significantly different in group 4 (MgO NPs) from 2 , 3 and 1 groups, whereas no significant differences between the groups 2 and 3 as well as the groups 2 and 3 were significantly different from the group 1.

At the 15 P.O.Ds the decreasing percentage degree of stenosis had no significant difference among the groups 2,3 and 4 while all (2,3 and 4) had a significant difference from group 1.

At 30 P.O.Ds the percentage degrees of stenosis were significantly different among the groups 2 , 3 ,4, and 1.

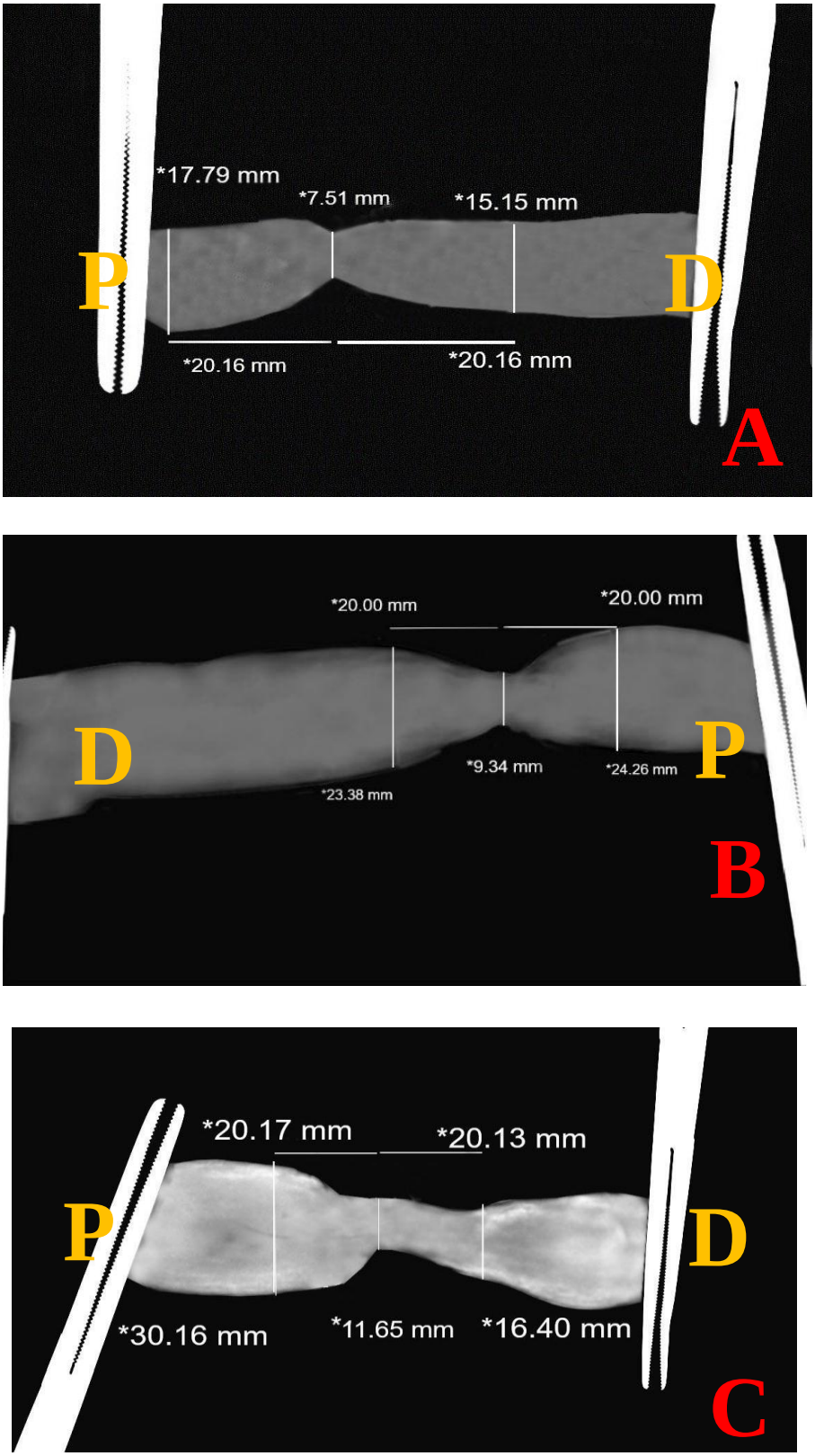

Figure 32 : Radiological examination showing degrees of stenosis at anastomotic line
(Control group)
A: At 7th P.O.Ds
B : At 15th P.O.Ds
C : At 30th P.O.Ds

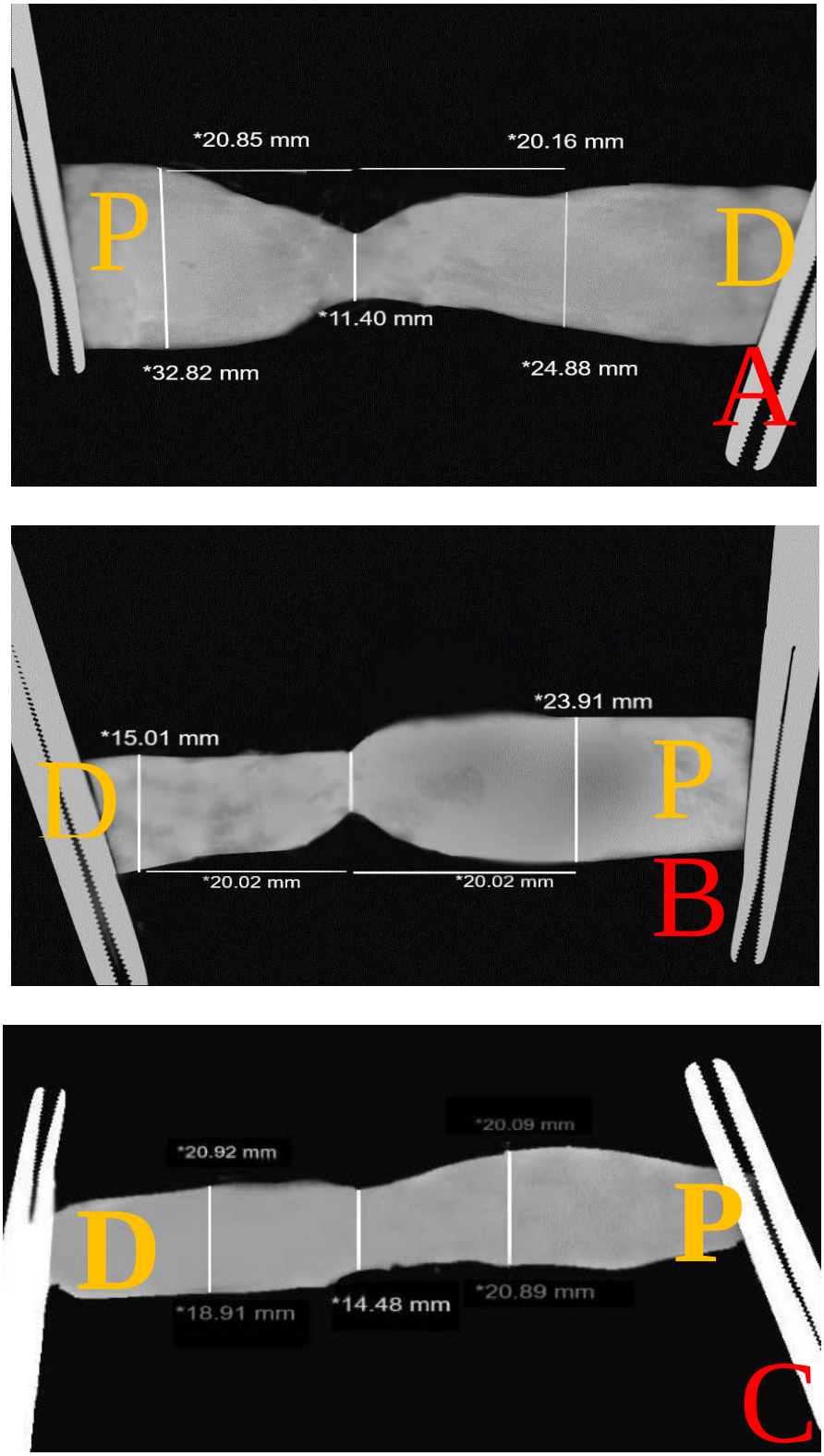

Figure 33 : Radiological examination showing degrees of stenosis at anastomotic line in
(Bone marrow group)
A: At 7th P.O.Ds
B : At 15th P.O.Ds
C : At 30th P.O.D

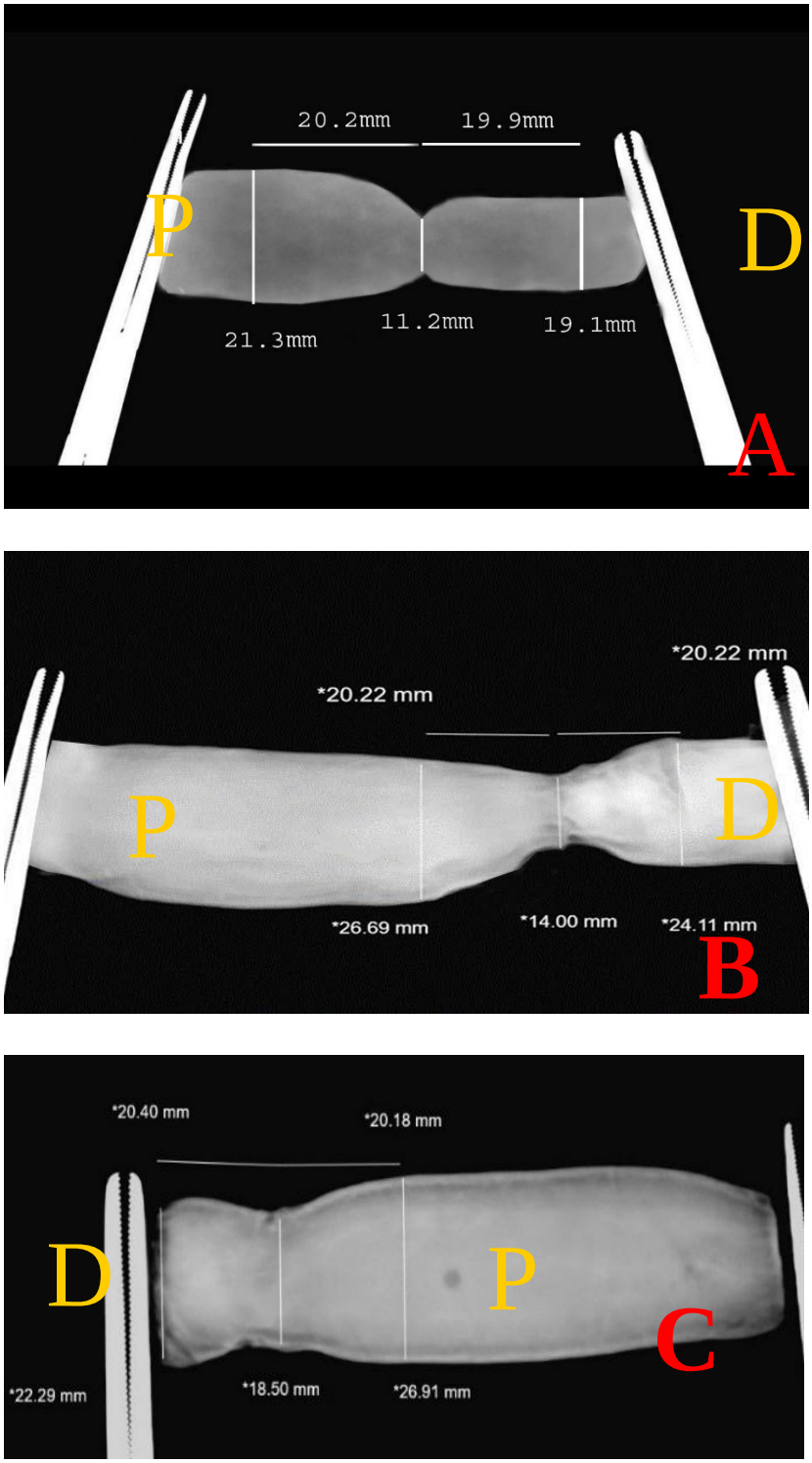

Figure 34 : Radiological examination showing degrees of stenosis at anastomotic line
(hyaluronic acid group)
A: At 7th P.O.Ds
B : At 15th P.O.Ds
C : At 30th P.O.Ds

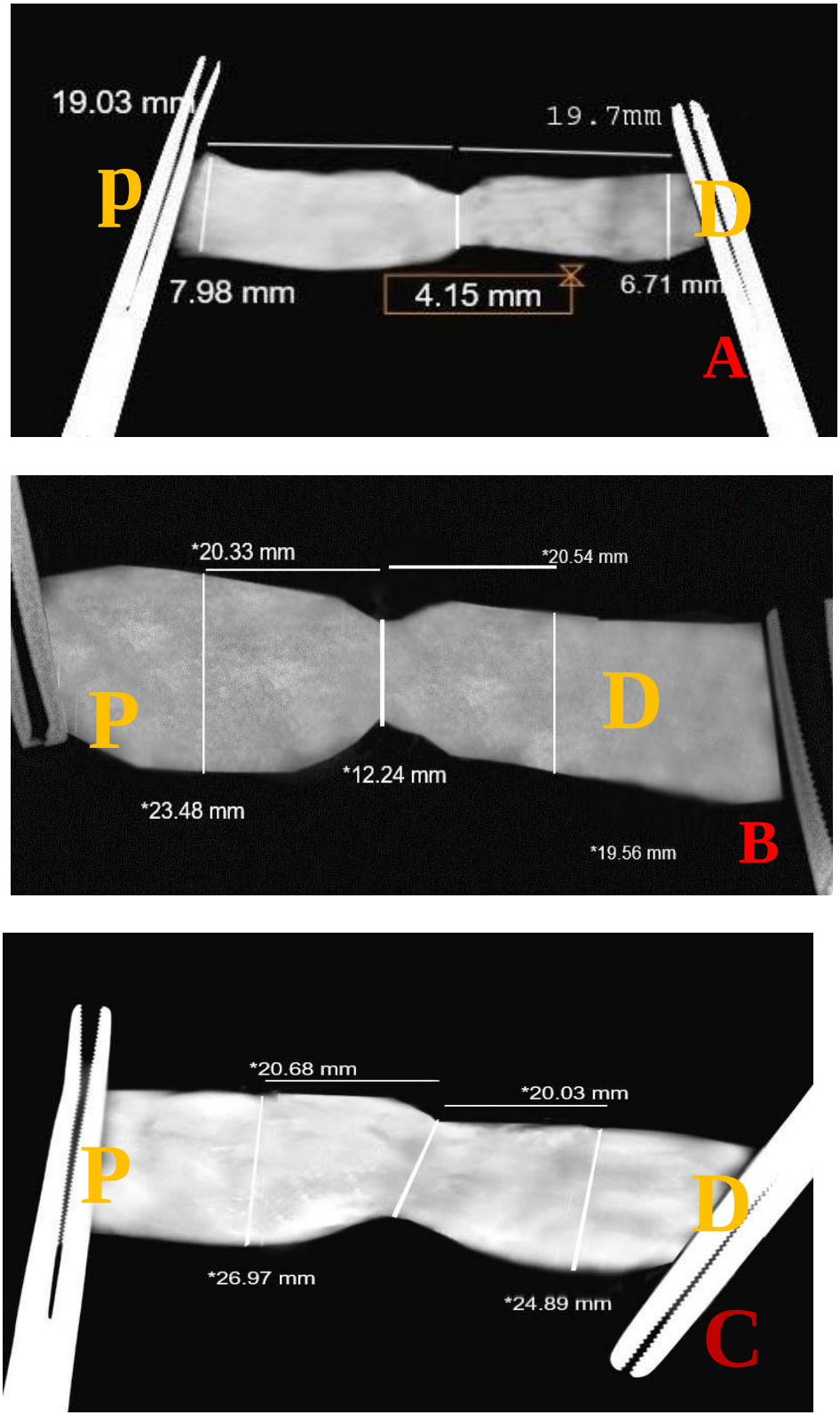

Figure 35 : Radiological examination showing degrees of stenosis at anastomotic line
(MgO NPs group)
A: At 7th P.O.Ds
B : At 15th P.O.Ds
C : At 30th P.O.Ds

Table 2: Percentages of moderate degree of stenosis at the site of anastomosis in the four experimental groups at 7,15 and 30 days after operations.

Time / groups	7 Days after operation	15 Days after operation	30 Days after operation
Control (1)	$^{A}36.33\%\pm6.55^{a}$	$^{A}39.56\%\pm5.80^{a}$	$^{A}42.20\%\pm2.42^{a}$
Bone marrow (2)	$^{AB}27.20\ \%\ \pm8.52^{a}$	$^{B}21\%\pm7.17^{a}$	$^{BC}14.66\ \%\pm5.81^{a}$
Hayluronic acid (3)	$^{AB}31.67\ \%\ \pm5.58^{a}$	$^{B}26.13\ \%\pm4.53^{ab}$	$^{B}20.83\%\pm4.02^{b}$
MgO NPs (4)	$^{B}23.23\%\pm4.66^{a}$	$^{B}17.16\%\pm4.63^{ab}$	$^{C}8.60\%\pm4.73^{b}$

Horizontal small different letters mean there is a significant difference at P<0.05.
Vertical capital different letters mean there is a significant difference at P<0.05.
The data were expressed as mean ± SE.

4-4: Histological and immunohistochemistry studies

4-4-1: Histological study :

In group 1 (control group) the histological study at 7th P.O.Ds , revealed necrosis with hemorrhage of epithelial layer at the site of anastomosis(Figure 36) , other view showing necrosis and sloughing to the epithelial layer and deposition of collagen fibers with infiltration of mononuclear inflammatory cells (Figure 37), the Masson's trichrome stain showing deposition of collagen fibers that take a bright green colour
(Figure 38). At 15th P.O.Ds showing re-epithelization of the esophagus epithelial layer and deposition of collagen fibers with necrotic tissue

(Figure 39) and other view showing few infiltration of mononuclear inflammatory cells with deposition of immature collagen fibers , with few fibrocytes showing hyperplasia (Figure 40) . the Masson's trichrome section study revealed deposition of immature collagen fibers that take a bright green color in layer of granulation tissue(Figure 41)

At 30th P.O.Ds the histological study showing a weak re-epithelization process and increase in collagen fibers deposition with necrotic tissues, and increase in the granulation tissue (Figure 42). There was in other view showing weak re-epithelization process and immature collagen fibers with hyperplasia of fibrocytes and infiltration of few inflammatory cells (Figure 43) ,the Masson's trichrome showing immature deposition collagen fibers that take a bright green color within granulation tissue (Figure 44) .

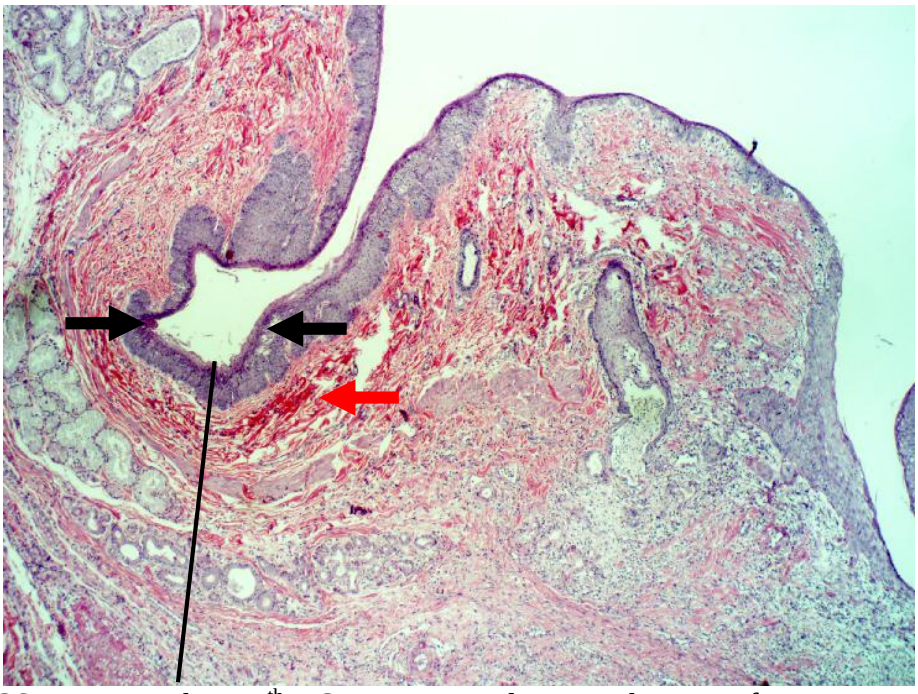

Figure 36: Micrograph at 7th P.O.Ds in control group the site of anastomosis (**line**), showing signs of epithelial layer necrosis (**arrow**) with hemorrhage (**arrow**). H&E, 40x.

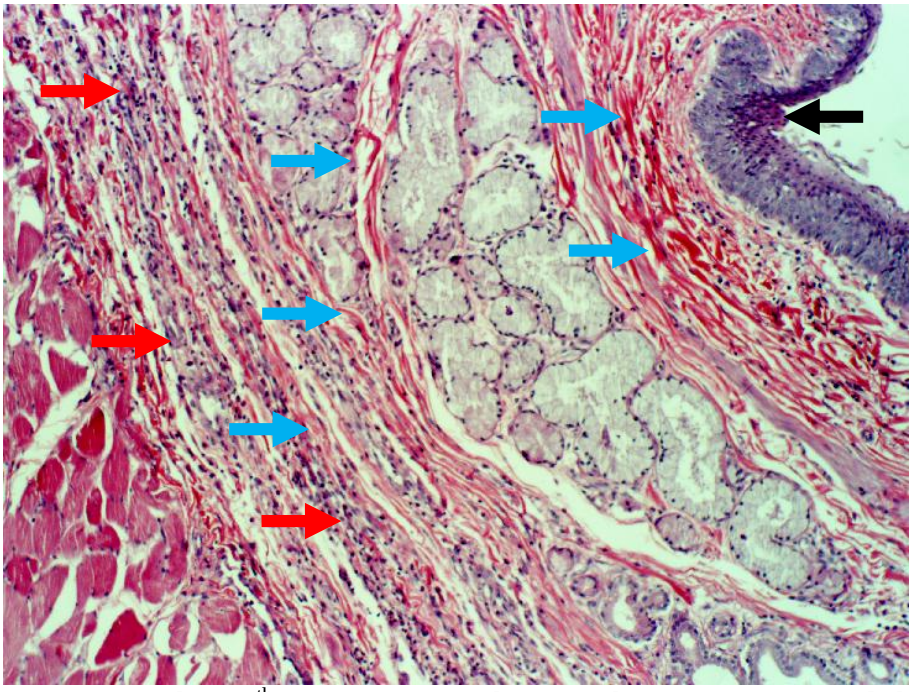

Figure 37: Micrograph at 7th P.O.Ds in control group showing necrosis and sloughing to the epithelial layer (**arrow**) deposition of collagen fibers (**arrow**), infiltration of mononuclear inflammatory cells (**arrow**). H&E, 100x.

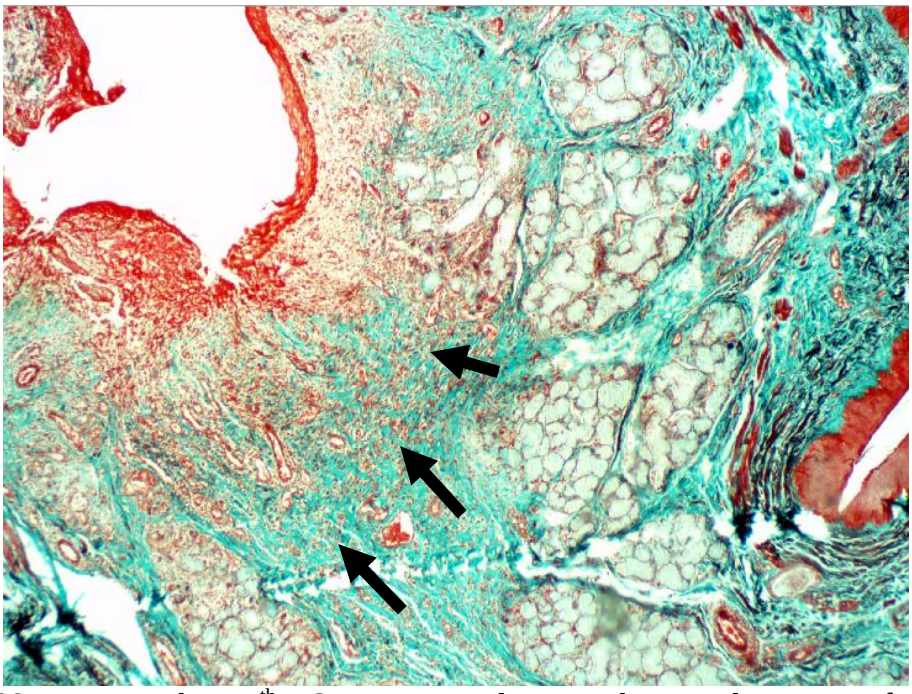

Figure 38: Micrograph at 7th P.O.Ds in control group showing deposition of collagen fibers that take a bright green color. Masson's trichrome, 100x.

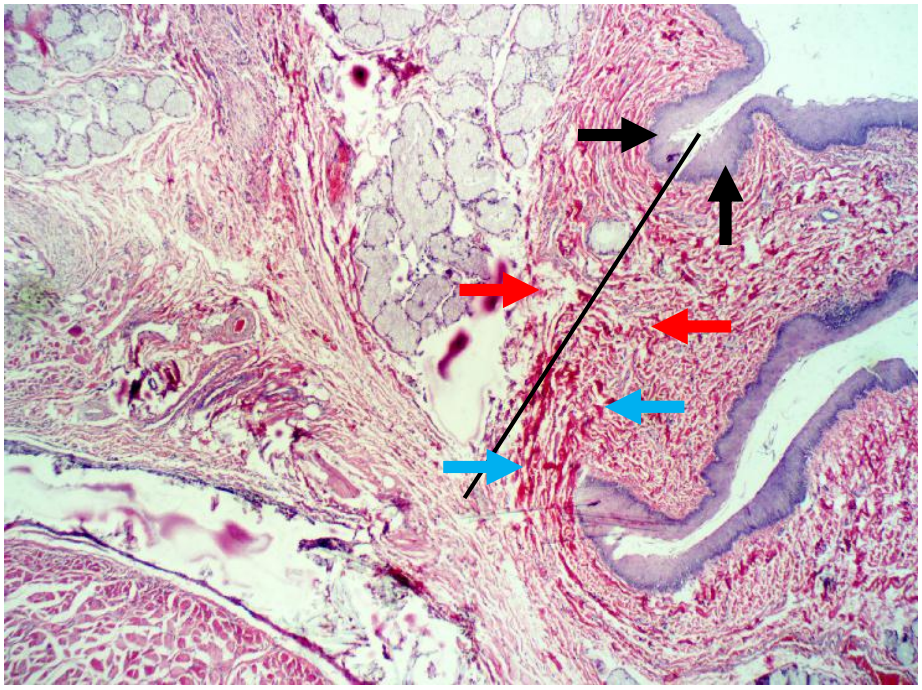

Figure 39: Micrograph at 15th P.O.Ds in control group the site of anastomosis (**line**) showing re-epithelization of the esophagus epithelial layer (**arrow**) deposition of collagen fibers (**arrow**) with necrotic tissue (**arrow**). H&E, 40x.

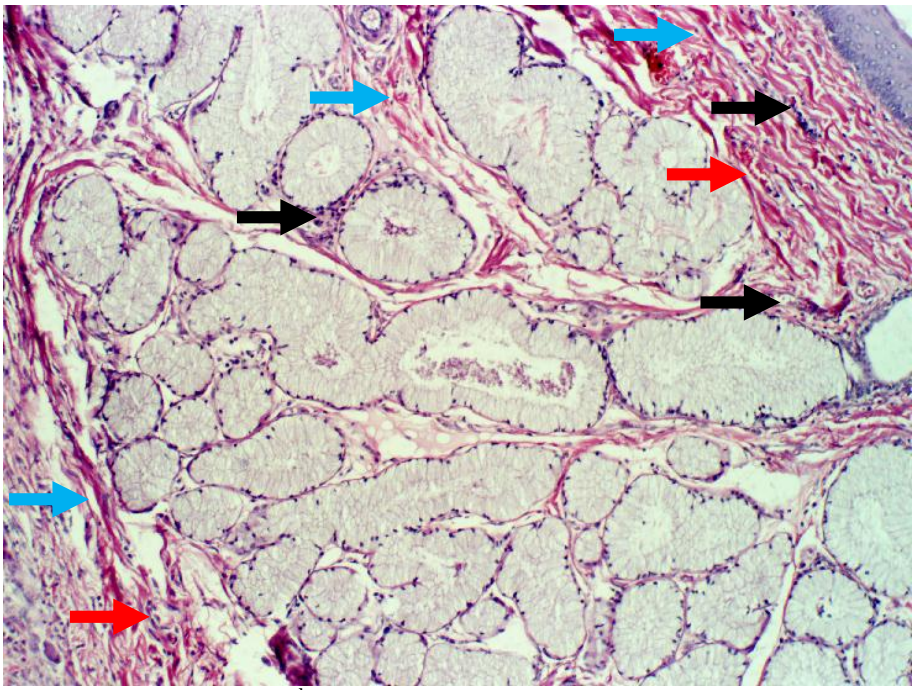

Figure 40: Micrograph at 15th P.O.Ds in control group showing few infiltration of mononuclear inflammatory cells (**arrow**) with deposition of immature collagen fibers (**arrow**), with few fibroblast proliferation showing hyperplasia (**arrow**). H&E, 100x

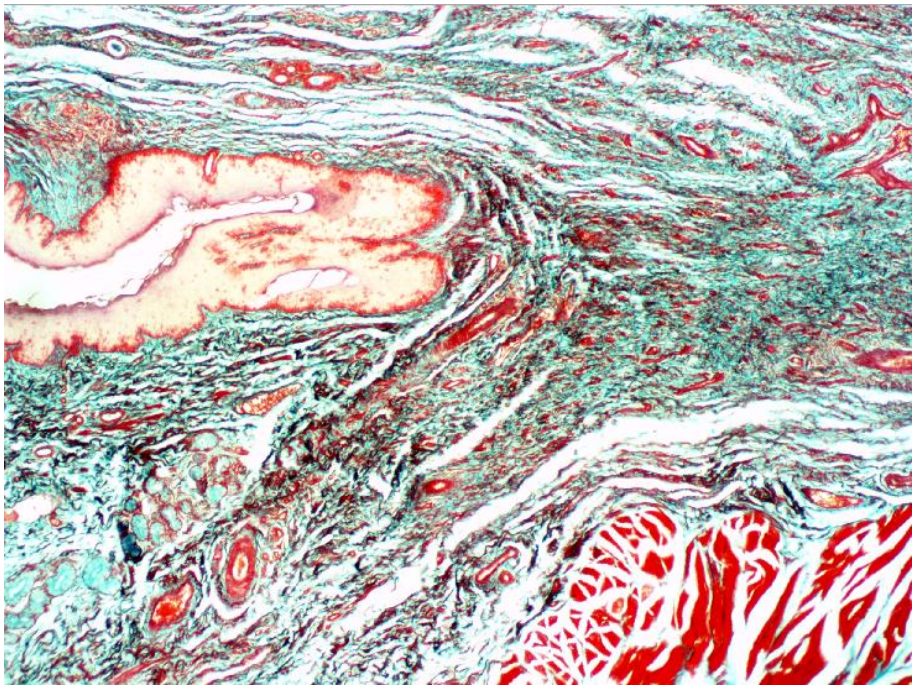

Figure 41: Micrograph at 15th P.O.Ds in control group showing deposition of immature collagen fibers that take a bright green color in site of granulation tissue. Masson's trichrome, 100x.

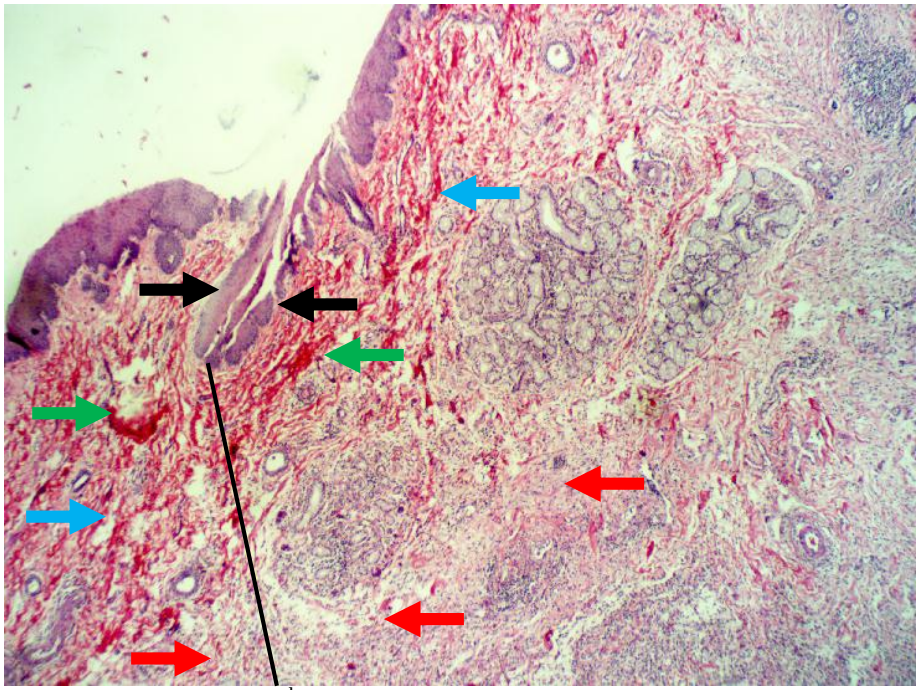

Figure 42: Micrograph at 30th P.O.Ds in control group the site of anastomosis (**line**) showing weak re-epithelization process (**arrow**) increase in collagen fibers deposition (**arrow**) with necrotic tissues (**arrow**), and increase in the granulation tissue (**arrow**). H&E, 40x.

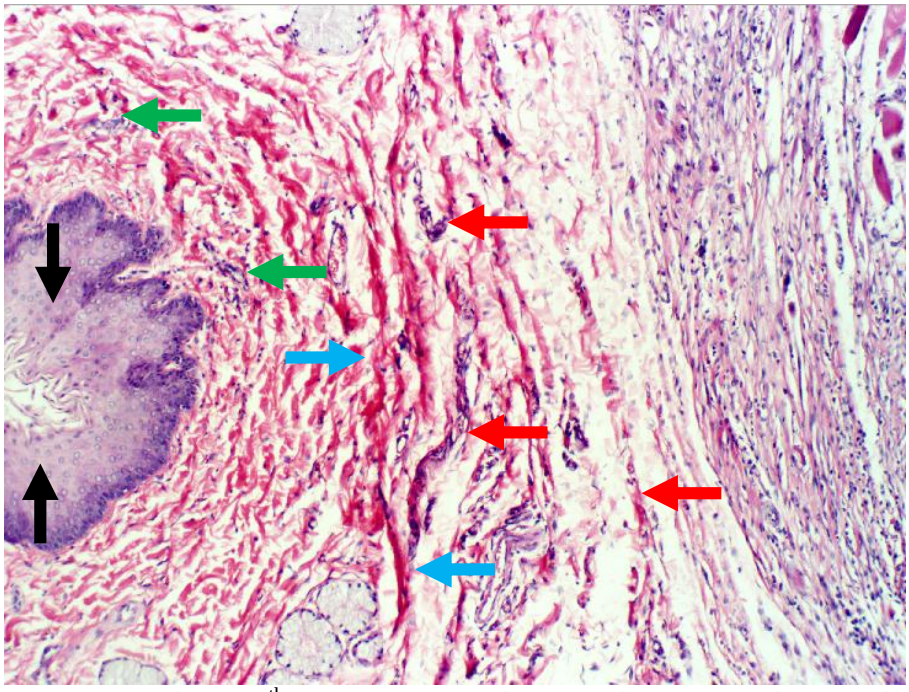

Figure 43: Micrograph at 30th P.O.Ds in control group showing weak re-epithelization process (**arrow**) immature collagen fibers (**arrow**) hyperplasia of fibroblast (**arrow**) with few infiltration of inflammatory cells (**arrow**). H&E, 100x.

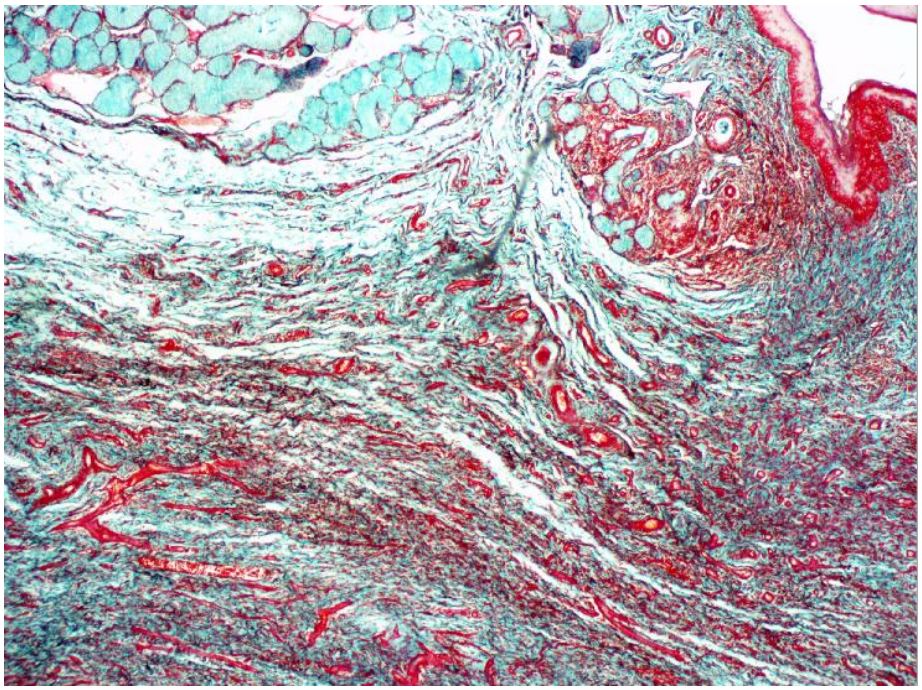

Figure 44: Micrograph at 30th P.O.Ds in control group showing immature deposition collagen fibers that take a bright green color within granulation tissue. Masson's trichrome, 100x.

In the group 2 (Bone marrow) the histological examination at 7th P.O.Ds at the site of anastomosis was showing signs of epithelial losing in addition to inflammatory infiltration of mononuclear inflammatory cells with hemorrhage(Figure 45) other section was revealed deposition of collagen fibers (Figure 46), staining with Masson's trichrome showing few deposition of collagen fibers that take a bright green color in the granulation tissue in the thickened mucosal layer(Figure 47). After 15th P.O.Ds. at the site of anastomosis was showing re-epithelization of the esophagus epithelial layer with presence of active granulation tissue and hemorrhage (Figure 48) as well as to the inflammatory infiltration of mononuclear inflammatory cells with increase in deposition of collagen fibers, and hyperplasia of fibrocytes (Figure 49), the staining with Masson's trichrome showing increase in the amount of collagen fibers that take a bright green colour in the granulation tissue in the thickened mucosa layer (Figure 50) . At 30th P.O.Ds the microscopic examination was revealed at the site of anastomosis complete re-epithelization process and stretching the collagen fibers with restoration of normal esophagus histological elements (Figure 51) and associated with a newly blood vessels (Figure 52), the Masson's trichrome was revealed a complete contraction of collagen fibers that take a bright green colour (Figure 53)

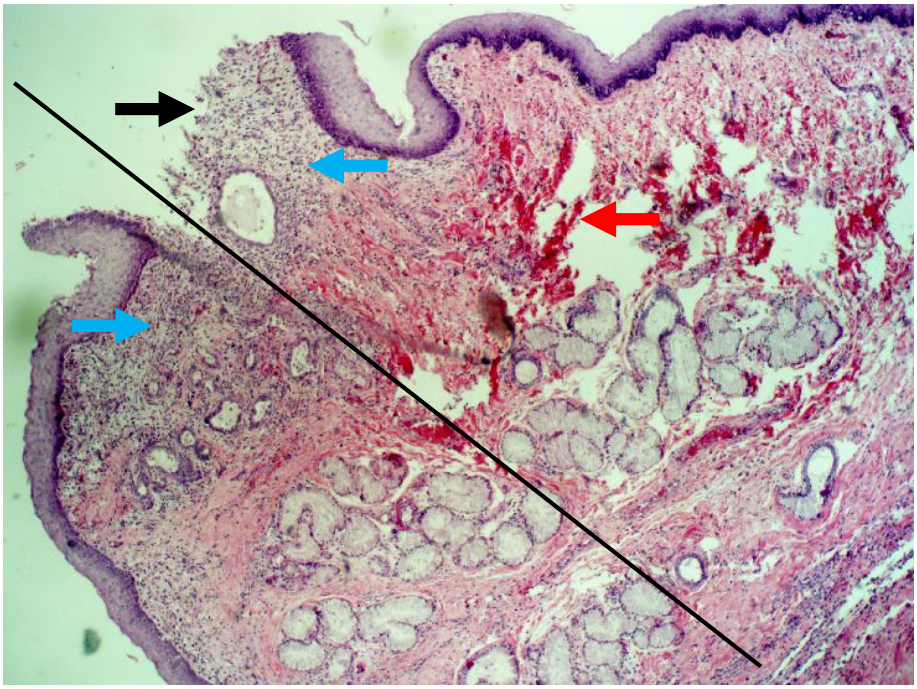

Figure 45: Micrograph site of anastomosis (**line**) in bone marrow group at 7th P.O.Ds, this location showing signs of epithelial losing (**arrow**) in addition to inflammatory infiltration of mononuclear inflammatory cells (**arrow**) with hemorrhage (**arrow**). H&E, 40x.

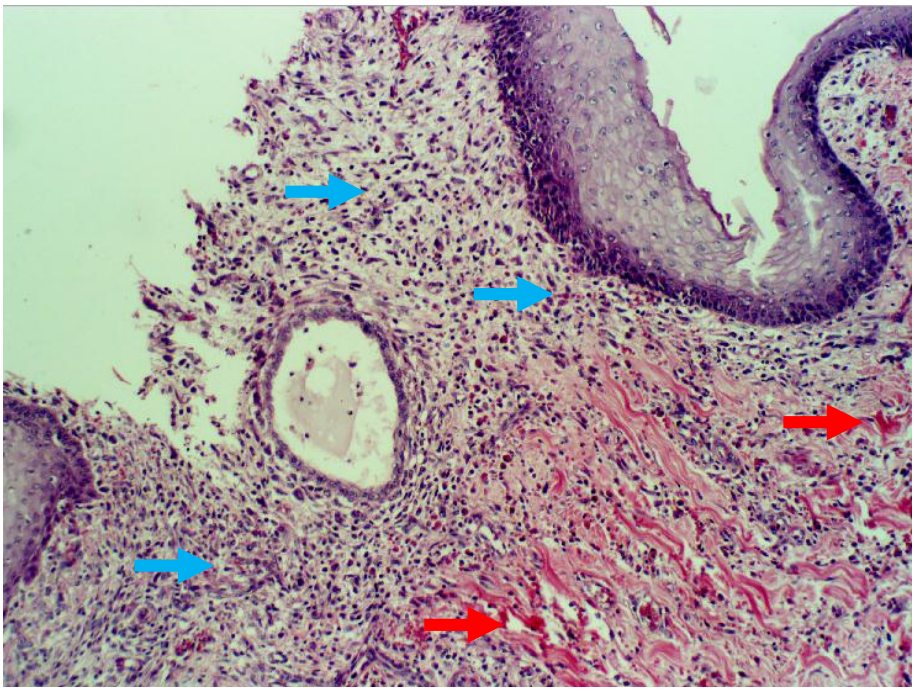

Figure 46: Micrograph at 7th P.O.Ds in bone marrow group showing inflammatory infiltration of mononuclear inflammatory cells (**arrow**) with deposition of collagen fibers (**arrow**). H&E, 100x.

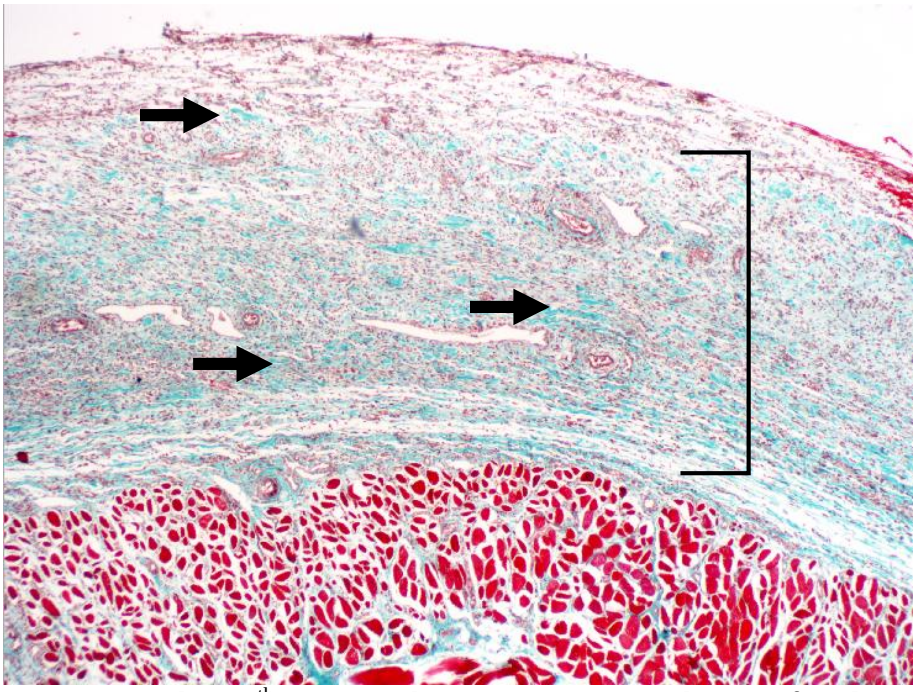

Figure 47: Micrograph at 7th P.O.Ds in bone marrow group showing few deposition of collagen fibers that take a bright green color (**arrow**) in the granulation tissue in the thickened adventitial layer (**curve**). Masson's trichrome, 100x.

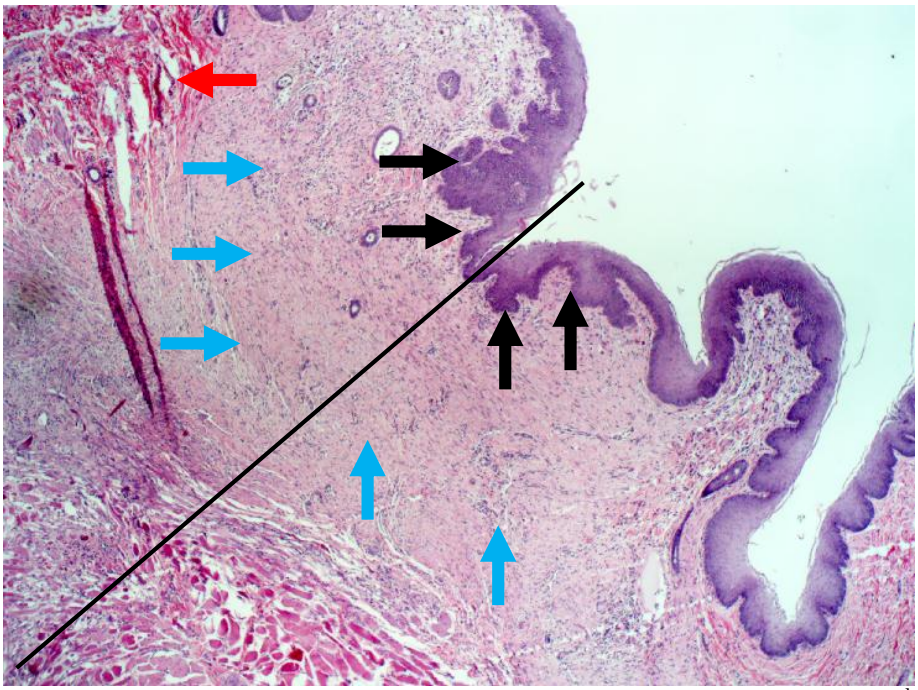

Figure 48: Micrograph Site of anastomosis (**line**) in bone marrow group at 15th P.O.Ds this location showing re-epithelization of the esophagus epithelial layer (**arrow**) presence of active granulation tissue (**arrow**) with hemorrhage (**arrow**). H&E, 40x.

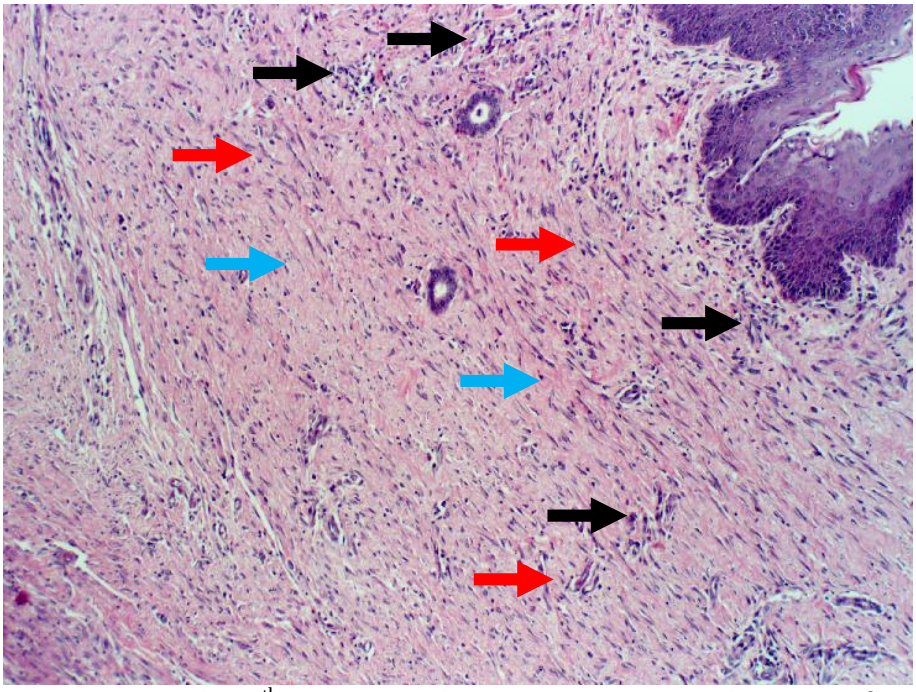

Figure 49: Micrograph 15th P.O.Ds in bone marrow group showing inflammatory infiltration of mononuclear inflammatory cells (**arrow**) with increase in deposition of collagen fibers (**arrow**), and hyperplasia of fibrocytes (**arrow**). H&E, 100x.

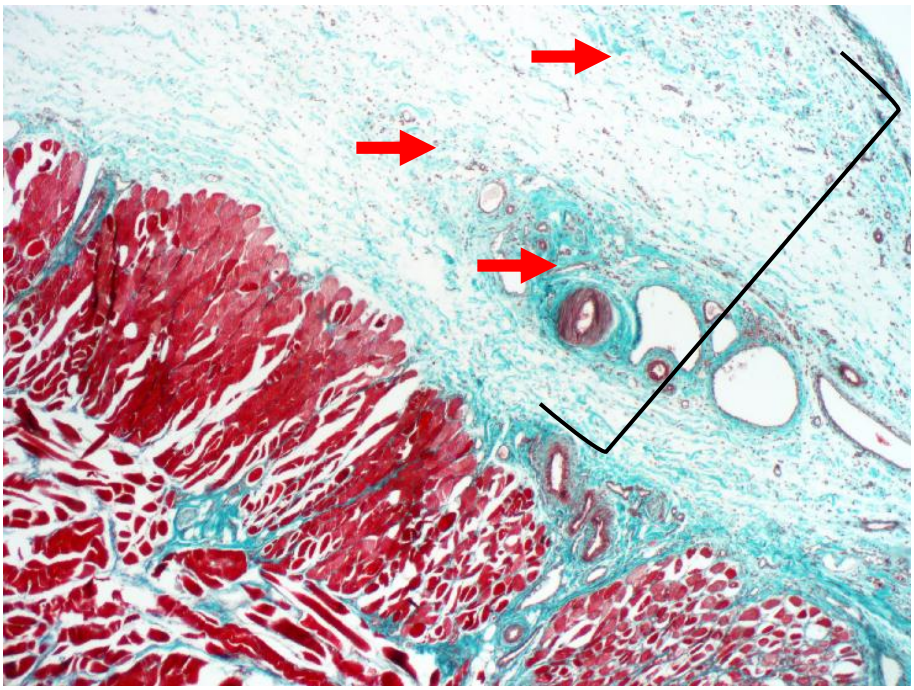

Figure 50: Micrograph 15th P.O.Ds in bone marrow group showing increase in the amount of collagen fibers that take a bright green color (**arrow**) in the granulation tissue in the thickened mucosa layer (**curve**). Masson's trichrome, 100x.

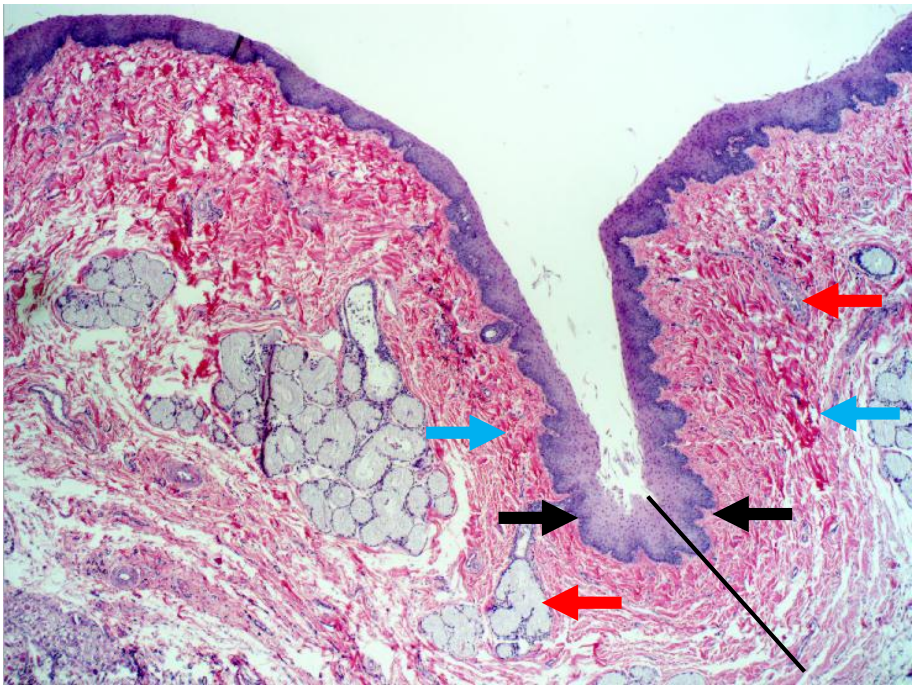

Figure 51: Micrograph Site of anastomosis (**line**) in bone marrow group at 30th P.O.Ds this location showing complete re-epithelization process (**arrow**) stretching the collagen fibers (**arrow**) with restoration of normal esophagus histological elements (**arrow**). H&E, 40x.

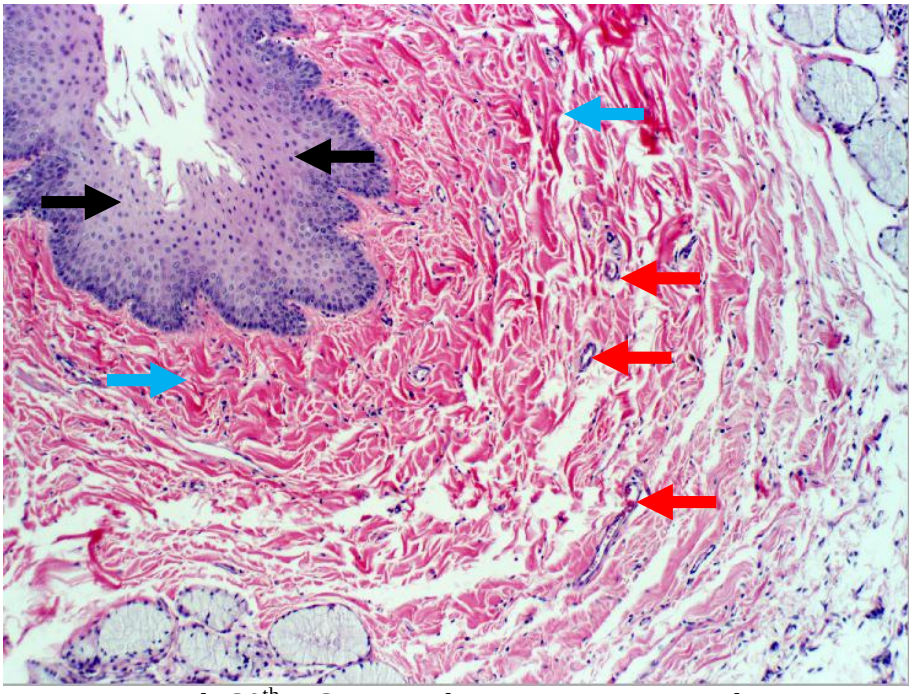

Figure 52: Micrograph 30th P.O.Ds in bone marrow group showing complete re-epithelization process (**arrow**) stretching the collagen fibers (**arrow**) newly blood vessels (**arrow**). H&E, 100x.

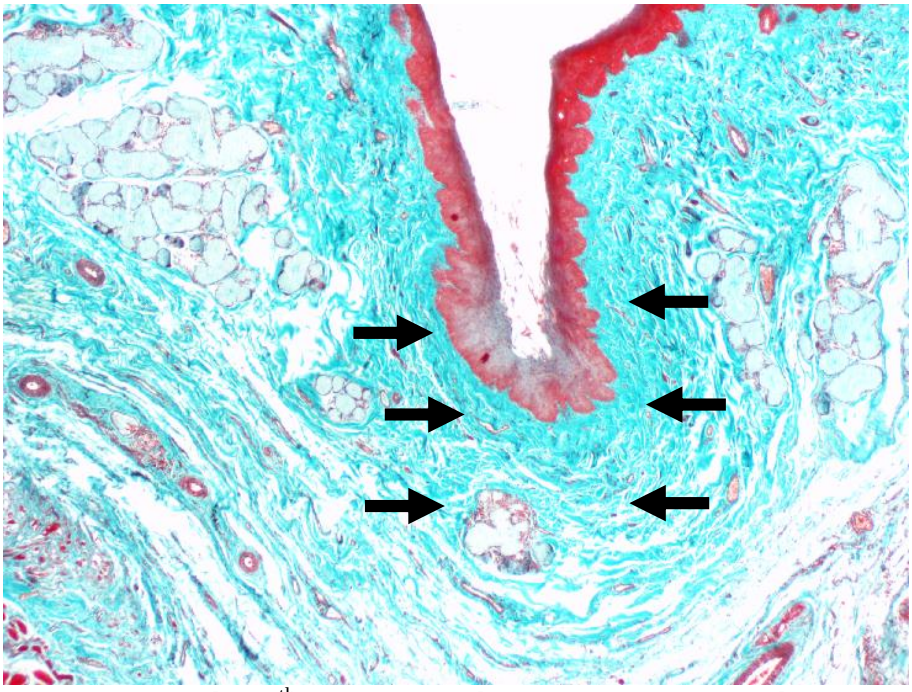

Figure 53: Micrograph 30th P.O.Ds in bone marrow group showing complete contraction of collagen fibers that take a bright green color (**arrow**). Masson's trichrome, 100x.

In the group 3 (Hyaluronic group) at 7th P.O.Ds the histological study was showing at the site of anastomosis signs of epithelial losing and wide distribution of necrotic tissue with hemorrhage (Figure 54), other section showing inflammatory infiltration of mononuclear inflammatory cells with deposition of collagen fibers and necrotic tissues and edema (Figure 55), the staining with Masson's trichrome showing a few deposition of collagen fibers that take a bright green color in form of waved sheets(Figure 56) . After 15th P.O.Ds the re-epithelization process of the esophagus epithelial layer was noted with increase in collagen fiber deposition with hemorrhage (Figure 57), as well as to infiltration of mononuclear inflammatory cells with increase in deposition of collagen fibers in form of waved sheets, and hyperplasia of fibrocytes and newly blood vessels (Figure 58), the Masson's trichrome was showing increase in the amount of collagen fibers that take appear as waved bright green sheets (Figure 59). At 30th P.O.Ds the re-epithelization process at a line of anastomosis was a completed and maturation of collagen fibers with edema were noted (Figure 60) and formation of new blood vessels (Figure 61) , Masson's trichrome staining showing a complete contraction and maturation of collagen fibers that take a bright green colour (Figure 62)

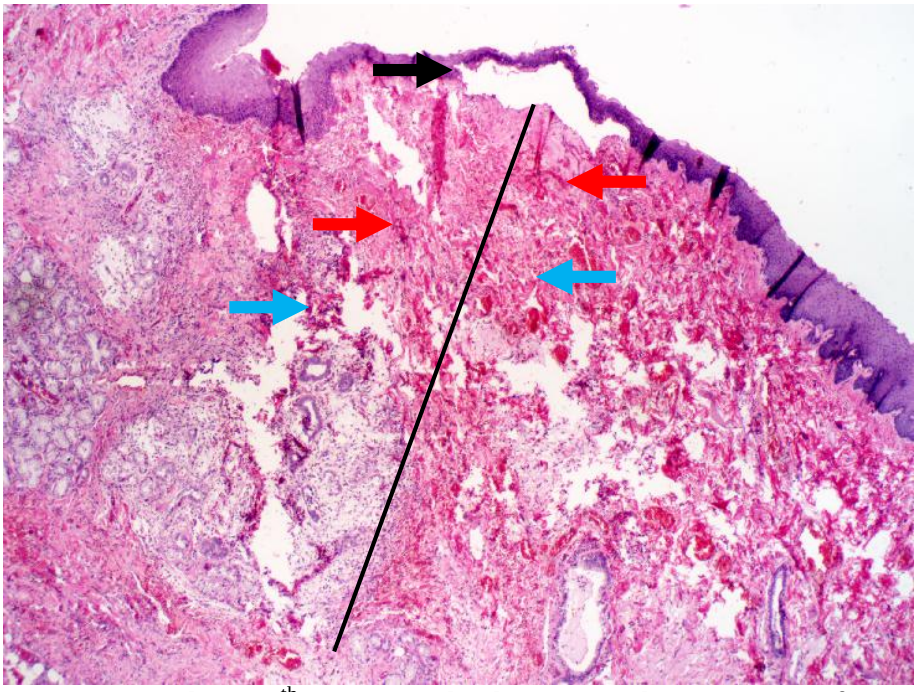

Figure 54: Micrograph at 7th P.O.Ds in hyaluronic acid group site of anastomosis (**line**), this location showing signs of epithelial losing (**arrow**) and wide distribution of necrotic tissue (**arrow**) with hemorrhage (**arrow**). H&E, 40x.

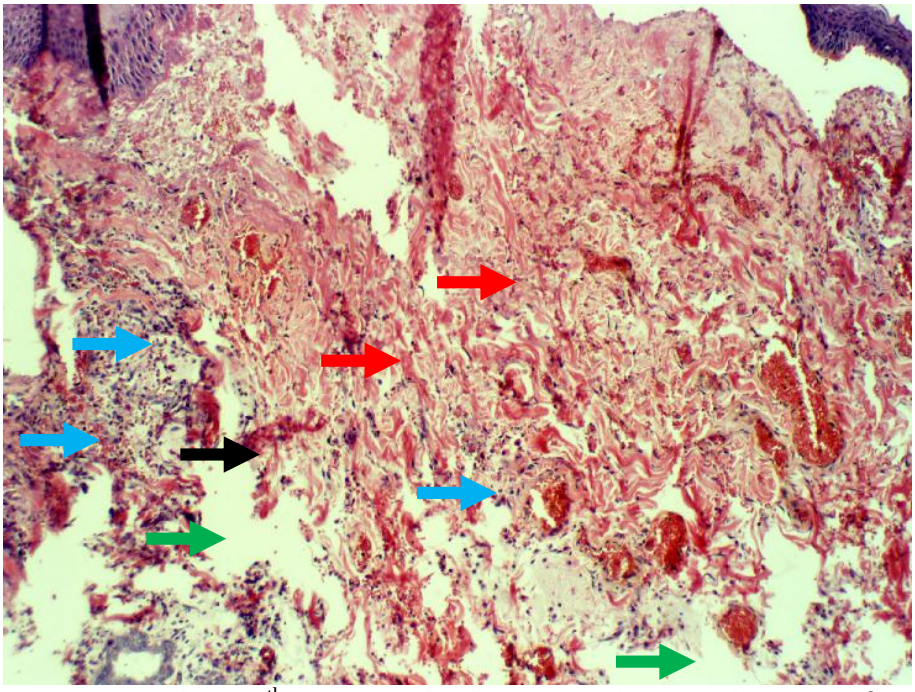

Figure 55: Micrograph at 7th P.O.Ds in hyaluronic acid group showing inflammatory infiltration of mononuclear inflammatory cells (**arrow**) with deposition of collagen fibers (**arrow**) and necrotic tissues (**arrow**) and edema (**arrow**). H&E, 100x.

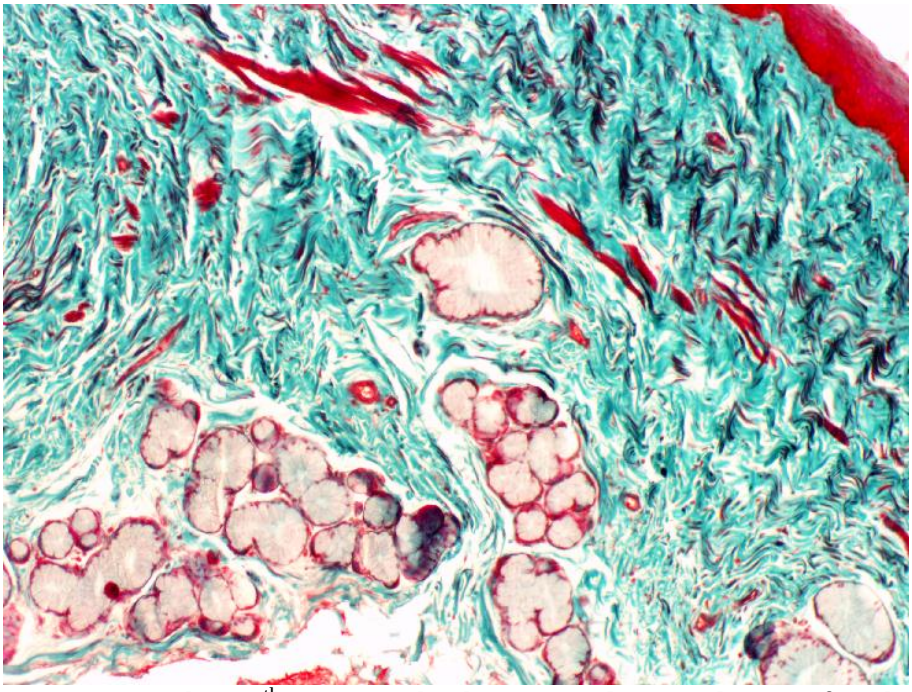

Figure 56: Micrograph at 7th P.O.Ds in hyaluronic acid group showing few deposition of collagen fibers that take a bright green color in form of waved sheets. Masson's trichrome, 400x.

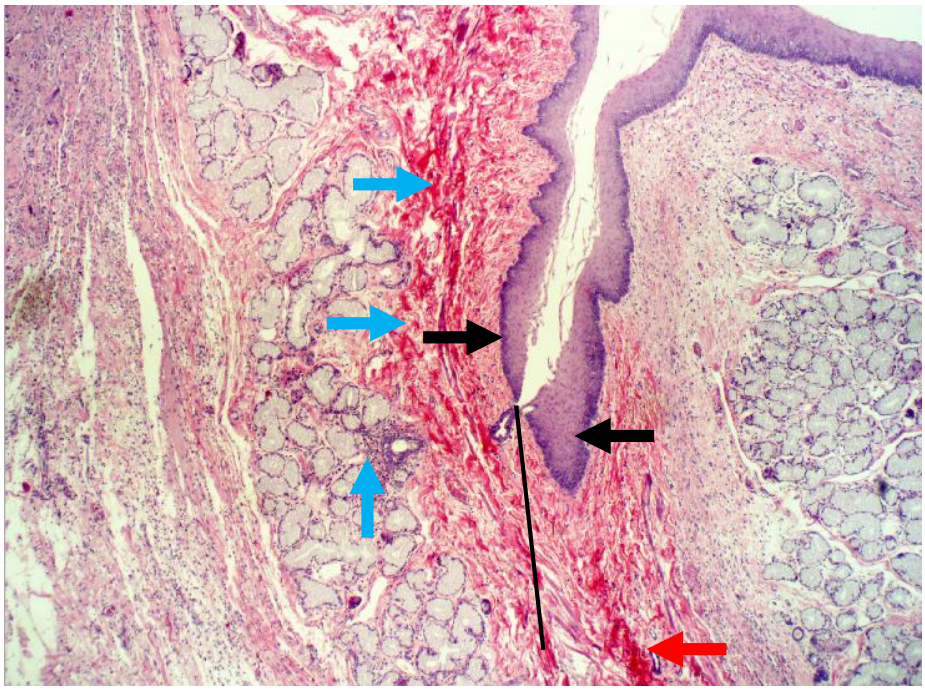

Figure 57: Micrograph at 15th P.O.Ds in hyaluronic acid group site of anastomosis (**line**) showing re-epithelization of the esophagus epithelial layer (**arrow**) increase in collagen fiber deposition (**arrow**) with hemorrhage (**arrow**). H&E, 40x.

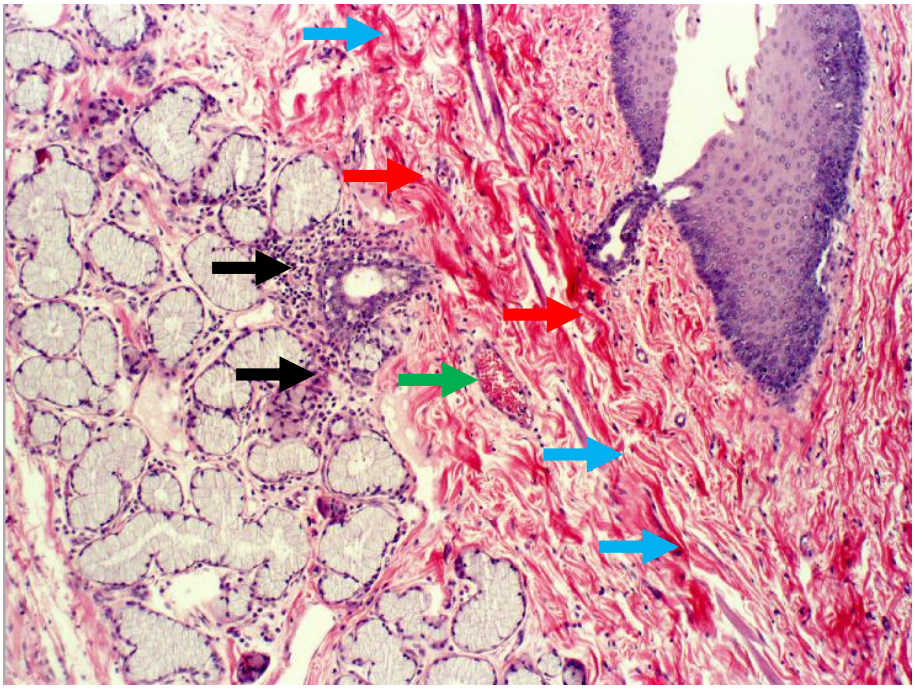

Figure 58: Micrograph at 15th P.O.Ds in hyaluronic acid group showing inflammatory infiltration of mononuclear inflammatory cells (**arrow**) with increase in deposition of collagen fibers in form of waved sheets (**arrow**), and hyperplasia of fibrocytes (**arrow**) and newly blood vessels (**arrow**). H&E, 100x.

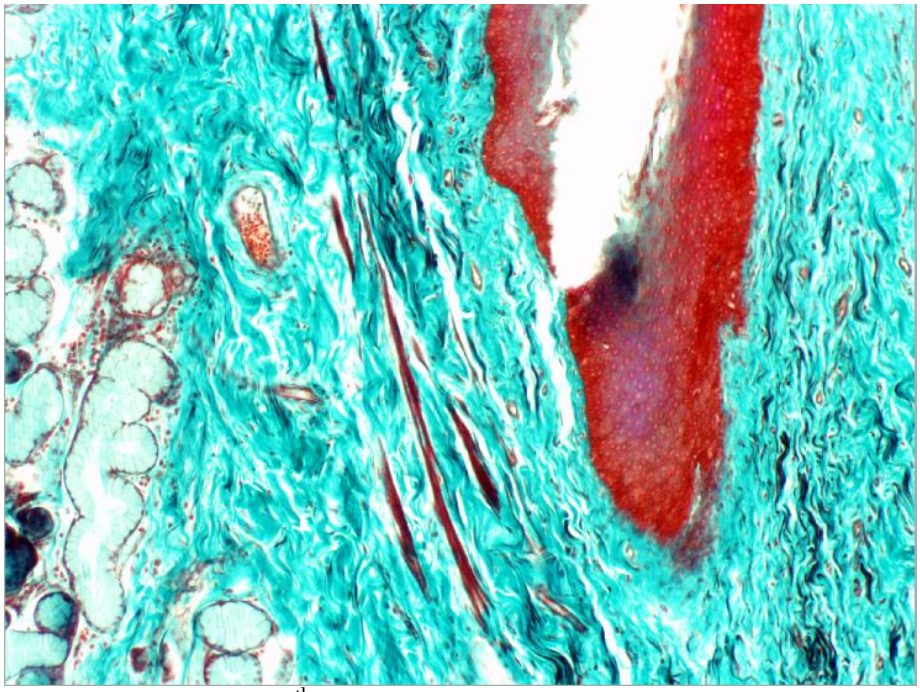

Figure 59: Micrograph at 15th P.O.Ds in hyaluronic acid group showing increase in the amount of collagen fibers that take appear as waved bright green sheets. Masson's trichrome, 400x.

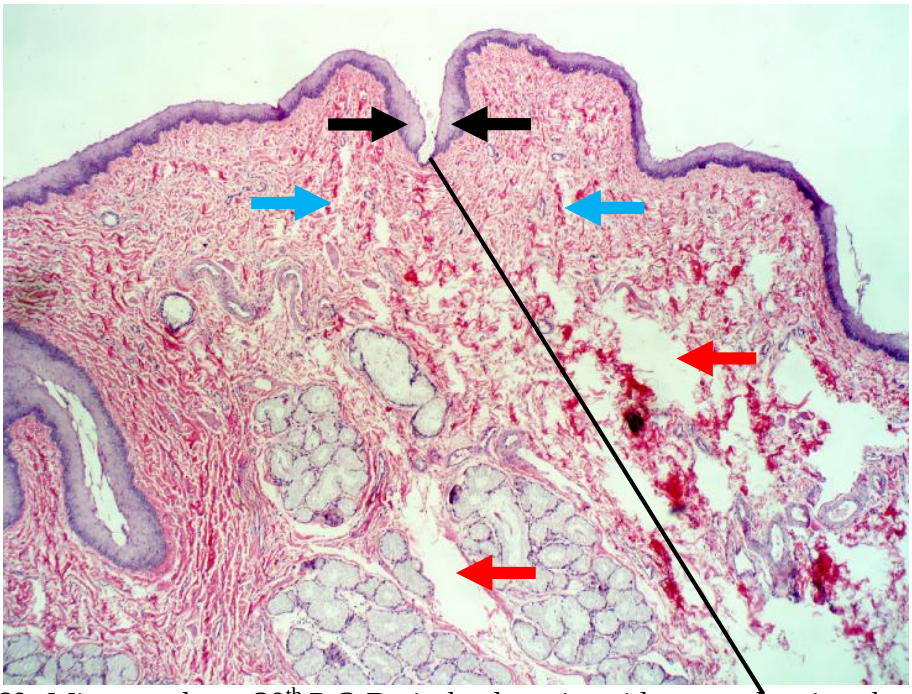

Figure 60: Micrograph at 30th P.O.Ds in hyaluronic acid group showing the Site of anastomosis (**line**), with a complete re-epithelization process (**arrow**) maturation of collagen fibers (**arrow**) with edema (**arrow**). H&E, 40x.

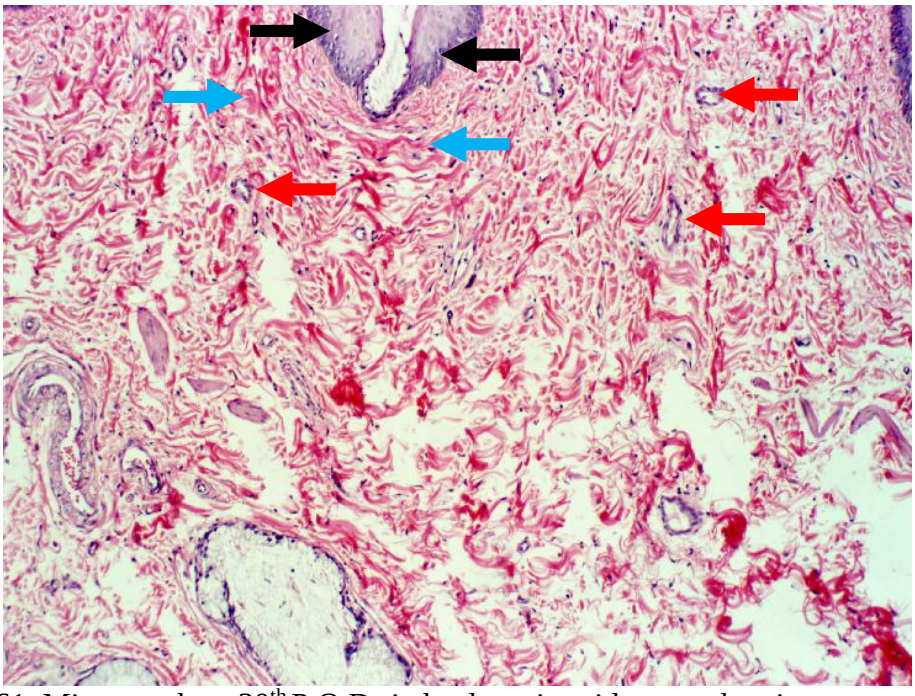

Figure 61: Micrograph at 30th P.O.Ds in hyaluronic acid group showing a complete re-epithelization process (**arrow**) maturation of collagen fibers (**arrow**) with newly blood vessels (**arrow**) at the site of anastomosis . H&E, 100x.

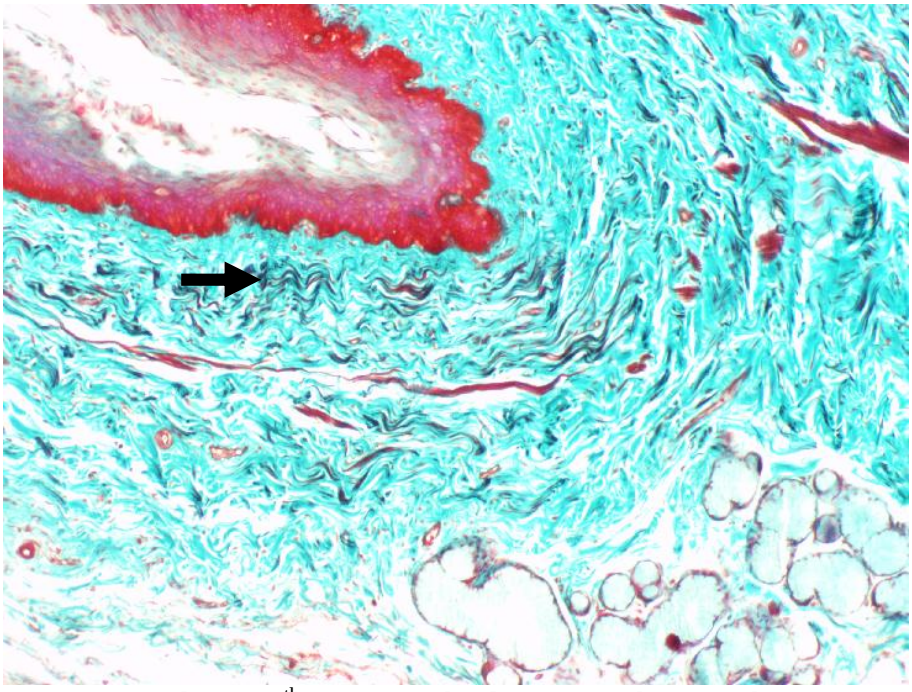

Figure 62: Micrograph at 30th P.O.Ds in hyaluronic acid group showing a complete contraction and maturation of collagen fibers that take a bright green color (**arrow**). Masson's trichrome, 400x.

In group 4 (MgO NPs), at 7th P.O.Ds., losing of epithelial and mucosal layer as well as to the presence of edema with deposition of collagen fibers were noted at the anastomotic site (Figure 63), there was inflammatory infiltration of mononuclear inflammatory cells with deposition of immature collagen fibers and hyperplasia of fibrocytes and edema (Figure 64), the Masson's trichrome study was showing few deposition of collagen fibers that take a bright green colour (Figure 65).

At 15th P.O.Ds Site of anastomosis showing re-epithelization of the esophagus epithelial layer with increase in collagen fiber deposition (Figure 66) there was inflammatory infiltration of mononuclear inflammatory cells with increase in deposition of collagen fibers and hyperplasia of fibrocytes (Figure 67) . Masson's trichrome staining showing increase in the amount of collagen fibers that appear as bright green colour. (Figure 68) .

At 30th P.O.Ds the anastomotic site showing re-epithelization process ,increase in collagen fibers deposition with hemorrhage and congestion (Figure 69) other view to site of anastomosis, showing re-epithelization process immature collagen fibers with congestion of blood vessels (Figure 70) ,the Masson's trichrome stain showing deposition of collagen fibers that take a bright green colour(Figure 71)

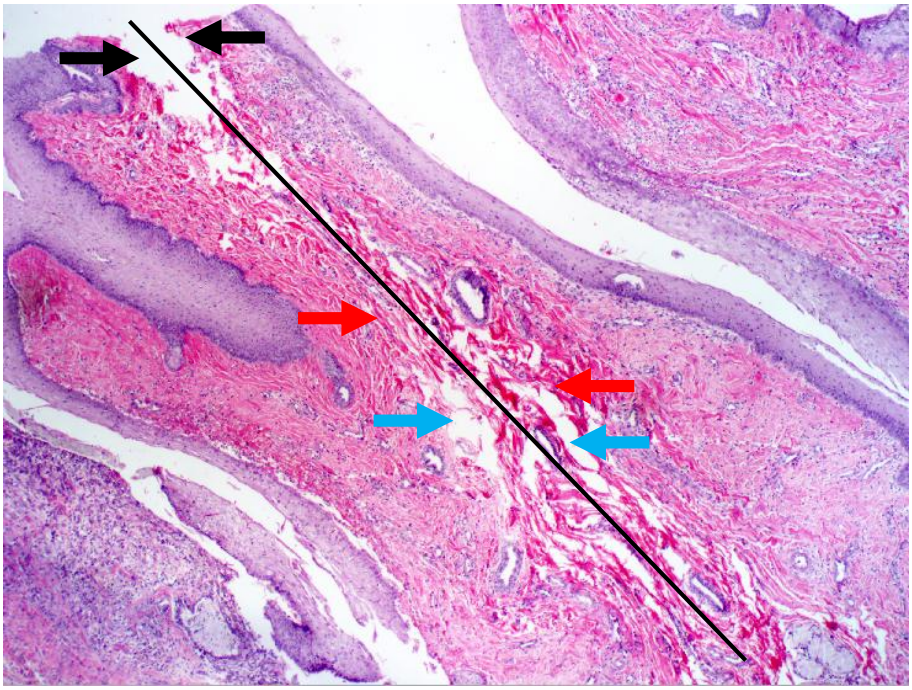

Figure 63: Micrograph at 7th P.O.Ds in MgO NPs group the Site of anastomosis (**line**), showing signs of epithelial and mucosa layer losing (**arrow**) presence of edema (**arrow**) with deposition of collagen fibers (**arrow**). H&E, 40x.

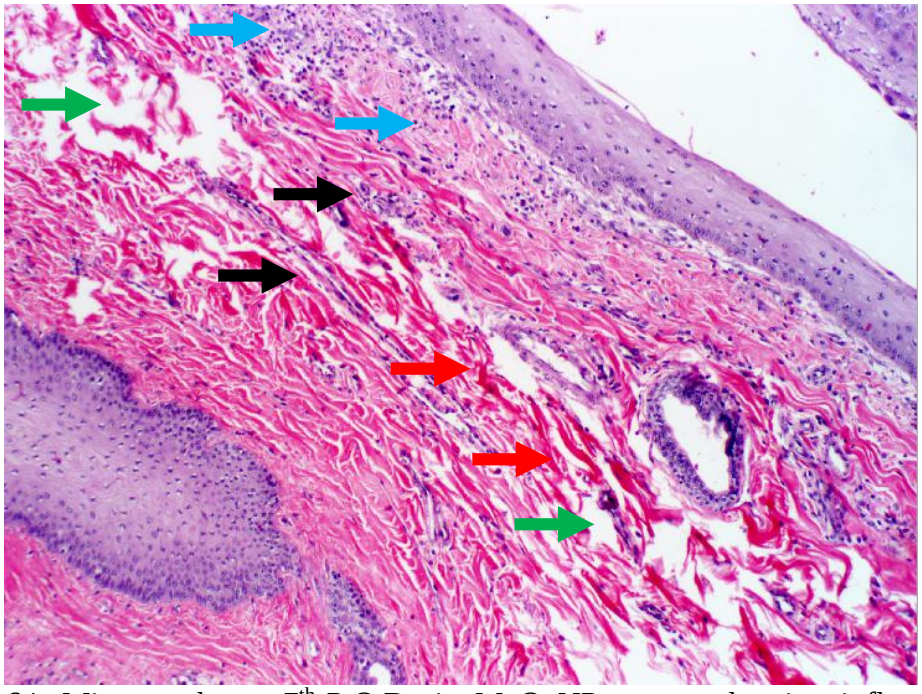

Figure 64: Micrograph at 7th P.O.Ds in MgO NPs group showing inflammatory infiltration of mononuclear inflammatory cells (**arrow**) with deposition of immature collagen fibers (**arrow**) and hyperplasia of fibrocytes (**arrow**) and edema (**arrow**). H&E, 100x.

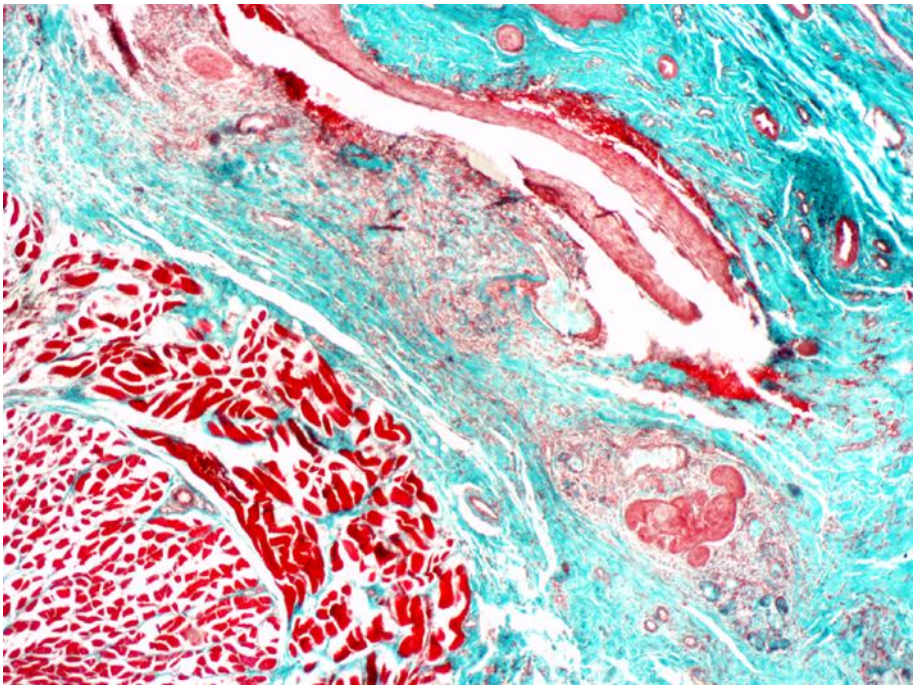

Figure 65: Micrograph at 7th P.O.Ds in MgO NPs group showing few deposition of collagen fibers that take a bright green color. Masson's trichrome, 400x.

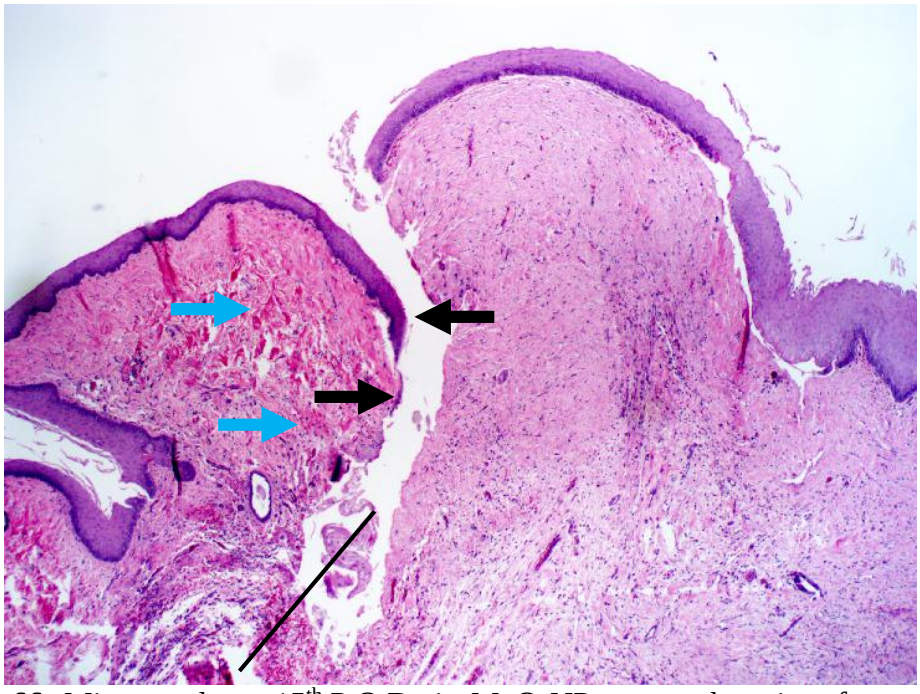

Figure 66: Micrograph at 15th P.O.Ds in MgO NPs group the site of anastomosis (**line**), showing re-epithelization of the esophagus epithelial layer (**arrow**) increase in collagen fiber deposition (**arrow**). H&E, 40x.

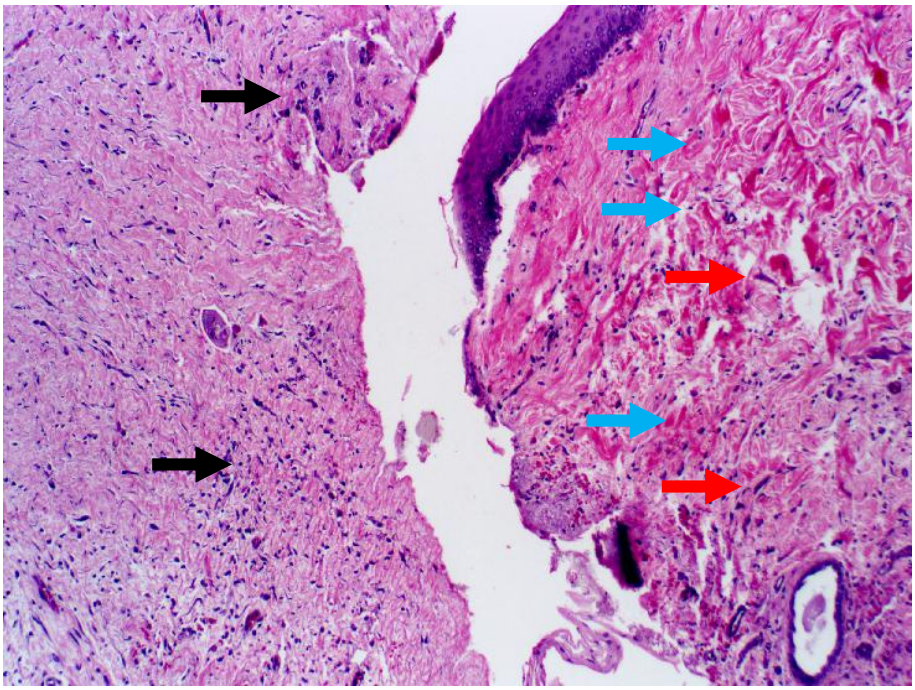

Figure 67: Micrograph at 15th P.O.Ds in MgO NPs group showing inflammatory infiltration of mononuclear inflammatory cells (**arrow**) increase in deposition of collagen fibers (**arrow**), and hyperplasia of fibrocytes (**arrow**). H&E, 100x.

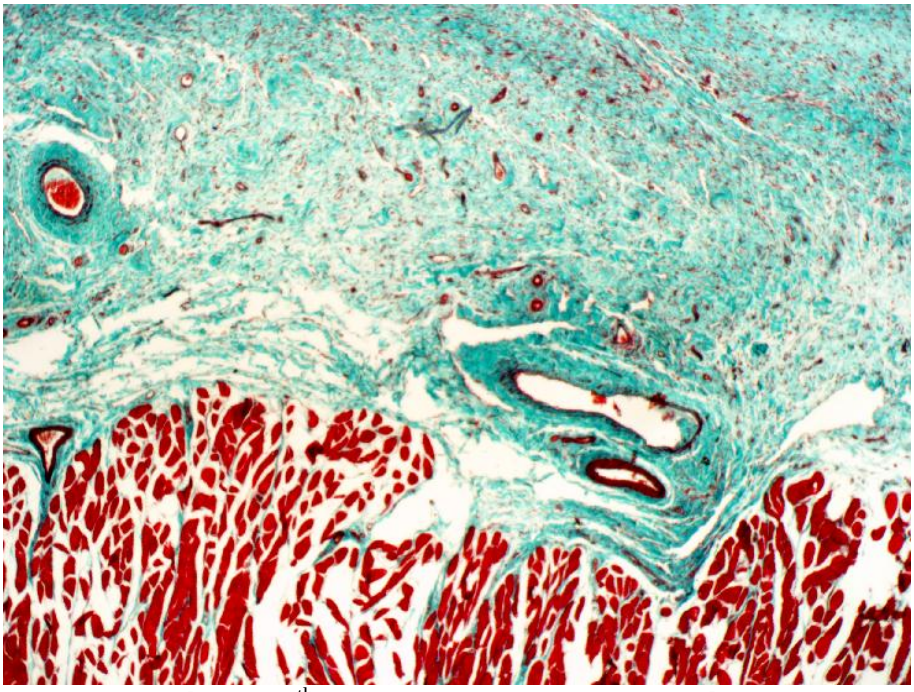

Figure 68: Micrograph at 15th P.O.Ds in MgO NPs group showing increase in the amount of collagen fibers that appear as bright green color. Masson's trichrome, 100x.

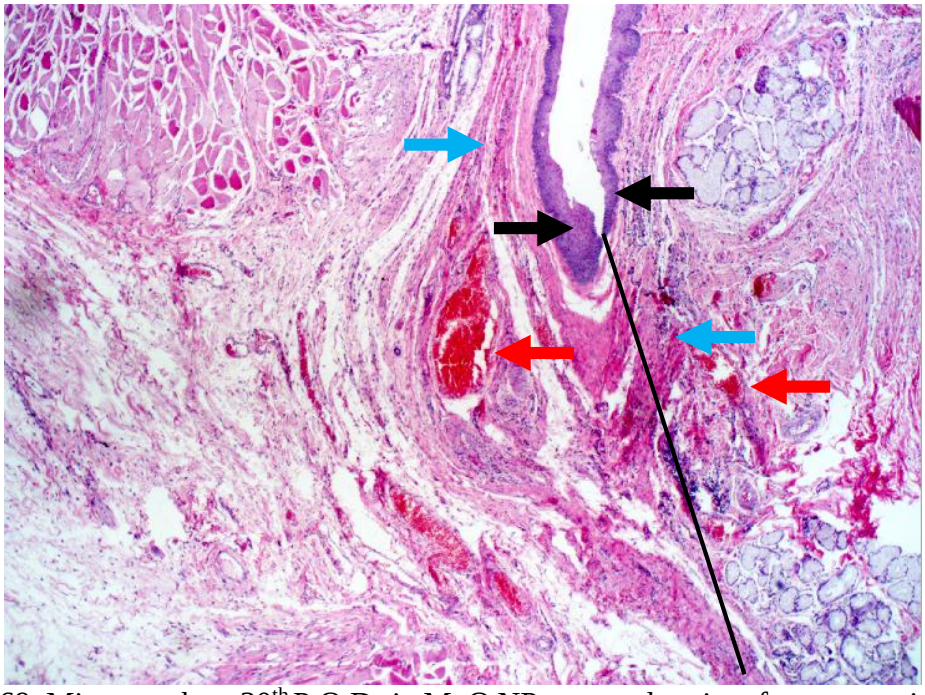

Figure 69: Micrograph at 30th P.O.Ds in MgO NPs group the site of anastomosis (**line**), revealed re-epithelization process (**arrow**) increase in collagen fibers deposition (**arrow**) with hemorrhage and congestion (**arrow**). H&E, 40x.

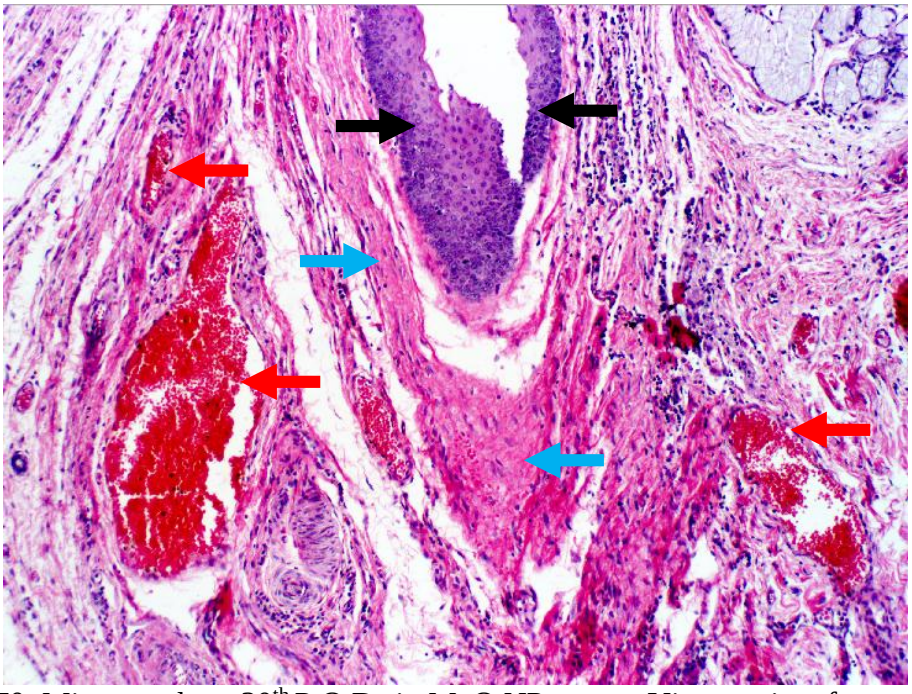

Figure 70: Micrograph at 30th P.O.Ds in MgO NPs group View to site of anastomosis, showing re-epithelization process (**arrow**) immature collagen fibers (**arrow**) with congestion of blood vessels (**arrow**). H&E, 100x.

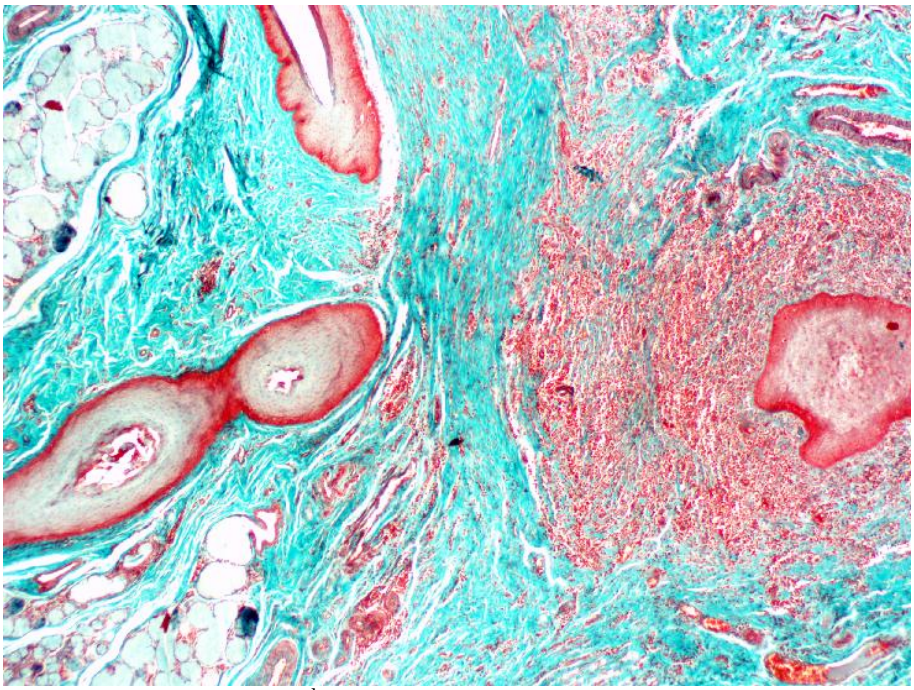

Figure 71: Micrograph at 30th P.O.Ds in MgO NPs group showing deposition of collagen fibers that take a bright green color. Masson's trichrome, 100x.

4-4-2: Immunohistochemistry (IHC) study:

The Immunohistochemistry study includes Vascular endothelial growth factor A- expression (VEGF-A) at 7th and 15th P.O.Ds and also include interleukin- 6 (IL-6) at 15th P.O.Ds. and interleukin- 12 (IL-12) at 30th P.O.Ds in all groups

4-4-2-1: Vascular endothelial growth factor A- expression (VEGF-A)

At 7th P.O.Ds the vascular endothelial growth factor –A expression (VEGF-A) in the epithelial cell of newly blood vessels which appear as cytoplasmic golden brown granules was a positive expression in control, bone marrow and hyaluronic acid groups (Figures 72, 73 ,74), while in MgO NPs group was a strong positive (Figure 75).

At 15th P.ODs the vascular endothelial growth factor –A expression was weak positive in control group(Figure 76) and in bone marrow and hyaluronic acid groups was positive expression (Figures 77,78), while ,in MgO NPs group was a strong positive expression (Figure 79).

4-4-2-2: Interleukin- 6 (IL-6)

At 15th P.O.Ds interleukin 6 in control group was weak positive expression in the cytoplasm of granulation tissue which appear as golden brown patches(Figure 80) , in bone marrow group was Positive expression in the cytoplasm of granulation tissue which appear as golden brown fimbria (Figure 81) and in hyaluronic acid also was weak positive expression in the cytoplasm of granulation tissue which appear as golden brown patches(Figure 82). In the other context ,in MgO NPs group IL -6 was strong positive expression in the cytoplasm of granulation tissue which appear as golden brown fimbria (Figure 83).

4-4-2-3: Interleukin -12 (IL-12)

At the 30th P.O.Ds the interleukin -12 expression in the cytoplasm of epithelial cell which appear as golden brown patches was weak positive in control group (Figure 84) and was a positive expression in bone marrow and hyaluronic acid (Figures 85, 86) whereas , in MgO NPs group was strong positive expression (Figure 87).

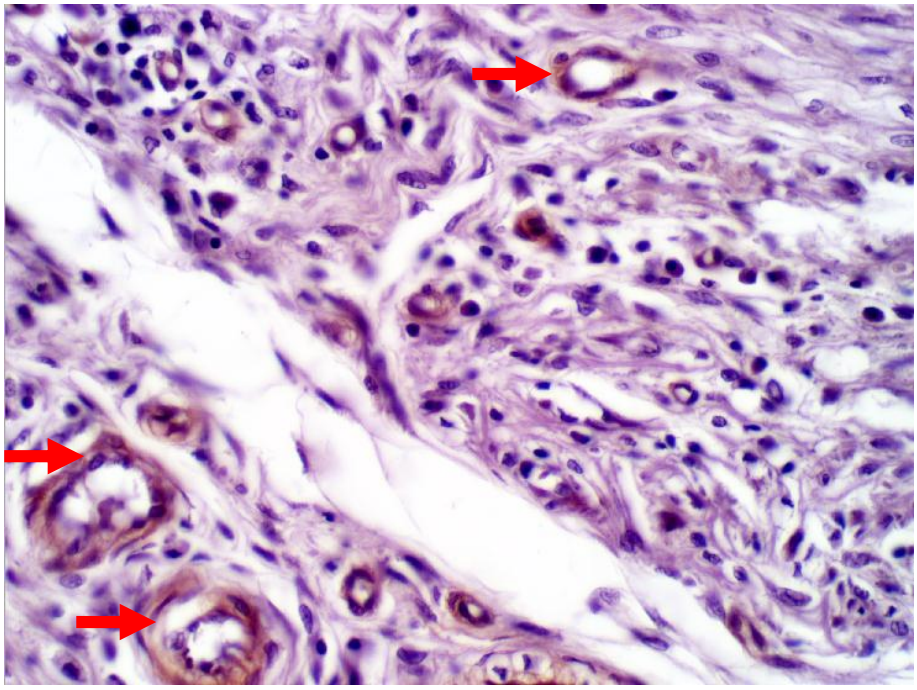

Figure 72: Micrograph at 7th P.O.Ds in control group showing Positive expression of VEGF-A in the endothelial cell of newly blood vessels which appear as cytoplasmic golden brown granules (arrow). VEGF-A IHC, 400x.

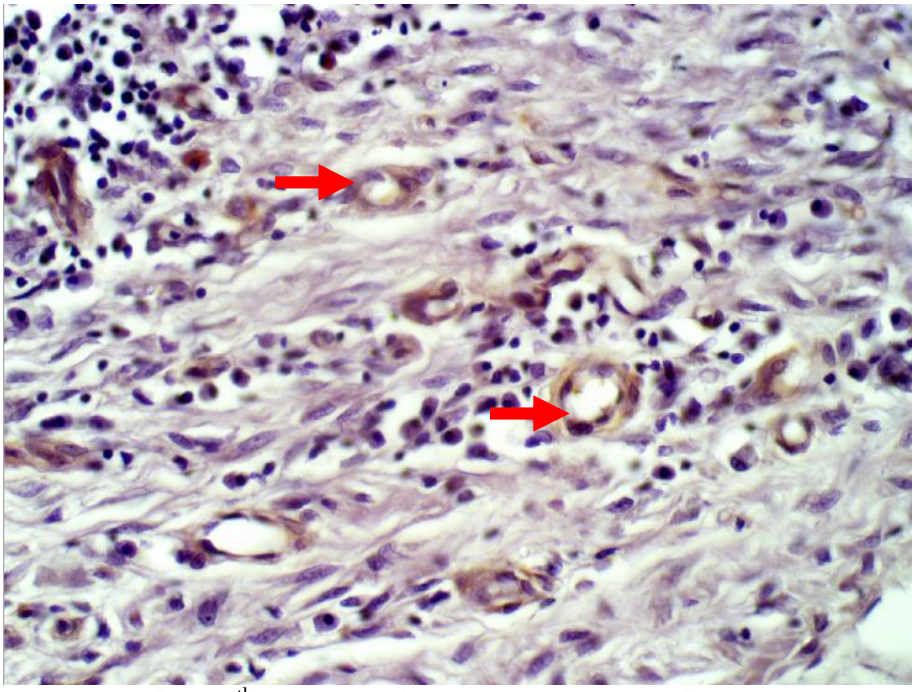

Figure 73: Micrograph at 7th P.O.Ds in bone marrow group showing Positive expression of VEGF-A in the endothelial cell of newly blood vessels which appear as cytoplasmic golden brown granules (arrow). VEGF-A IHC, 400x.

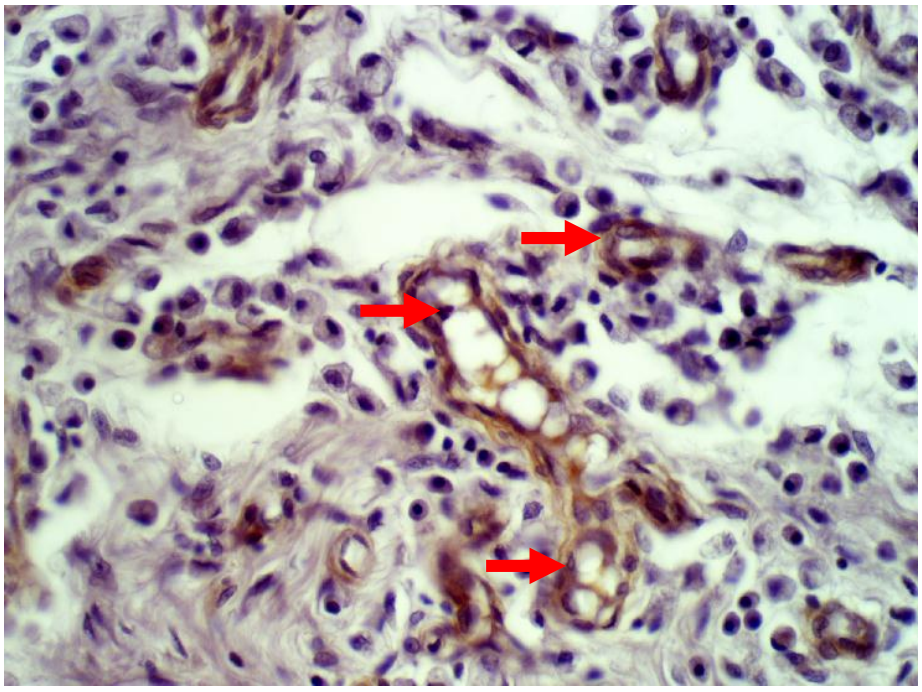

Figure 74: Micrograph at 7th P.O.Ds in hyaluronic acid group showing Positive expression of VEGF-A in the endothelial cell of newly blood vessels which appear as cytoplasmic golden brown granules (arrow). VEGF-A IHC, 400x.

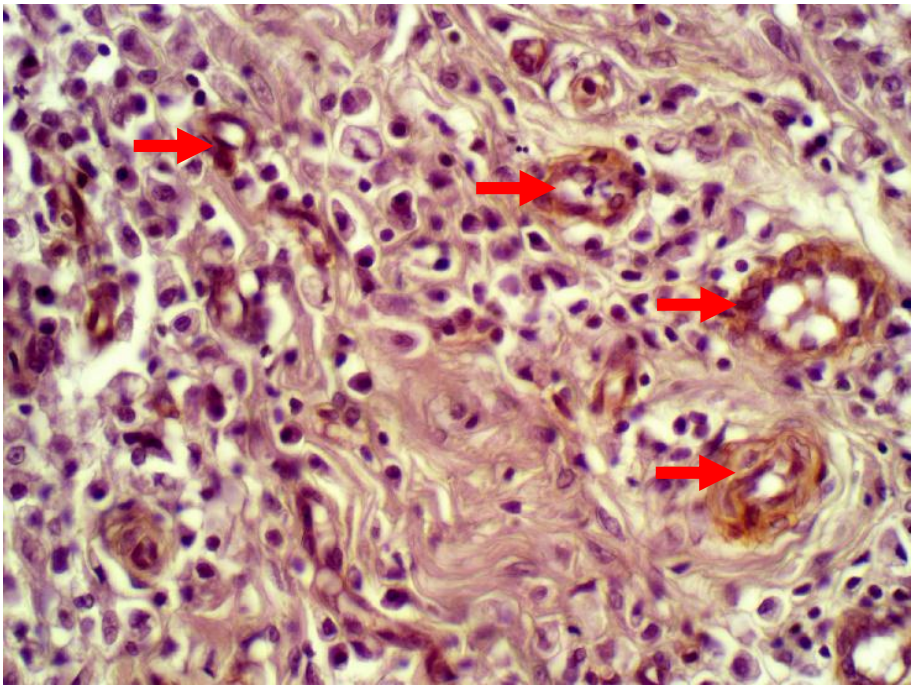

Figure 75: Micrograph at 7th P.O.Ds in MgO NPs group showing strong Positive expression of VEGF-A in the endothelial cell of newly blood vessels which appear as cytoplasmic golden brown granules (arrow). VEGF-A IHC, 400x.

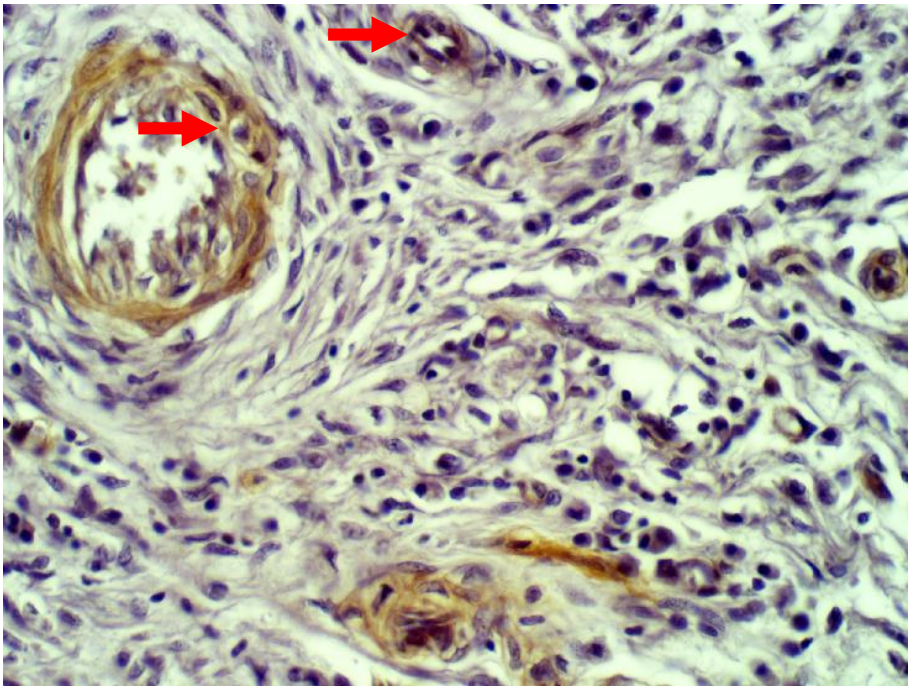

Figure 76: Micrograph at 15th P.O.Ds in control group showing weak Positive expression of VEGF-A in the endothelial cell of newly blood vessels which appear as cytoplasmic golden brown granules (**arrow**). VEGF-A IHC, 400x.

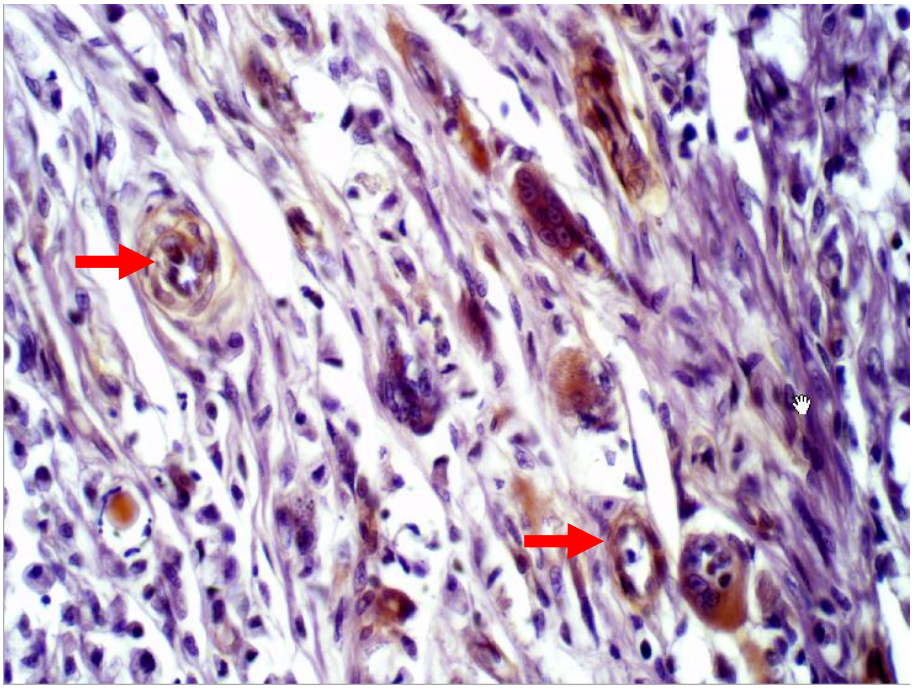

Figure 77: Micrograph at 15th P.O.Ds in bone marrow group showing Positive expression of VEGF-A in the endothelial cell of newly blood vessels which appear as cytoplasmic golden brown granules (**arrow**). VEGF-A IHC, 400x.

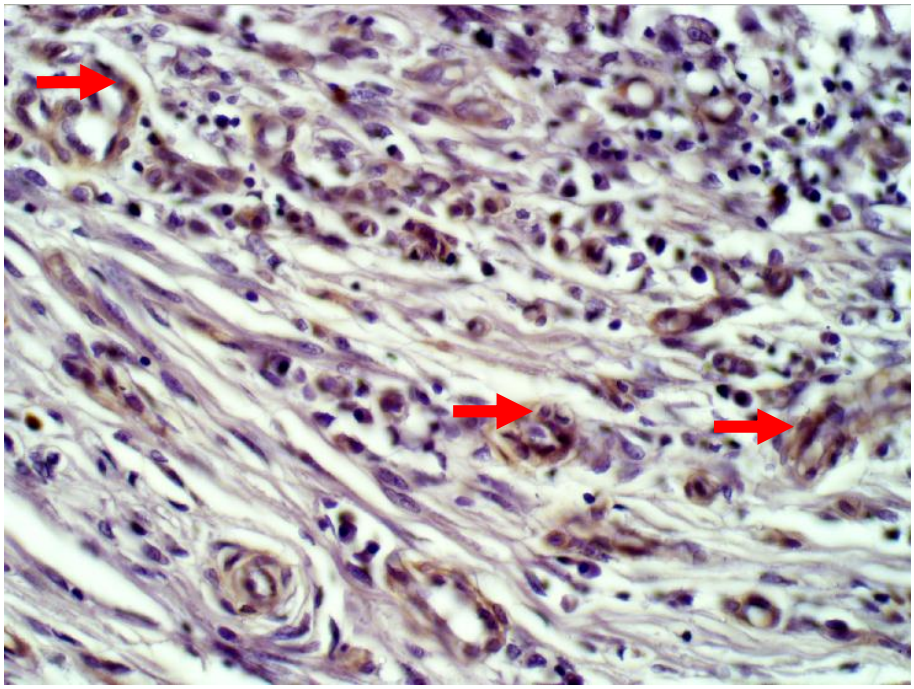

Figure 78: Micrograph at 15th P.O.Ds in hyaluronic acid group showing Positive expression of VEGF-A in the endothelial cell of newly blood vessels which appear as cytoplasmic golden brown granules (**arrow**). VEGF-A IHC, 400x.

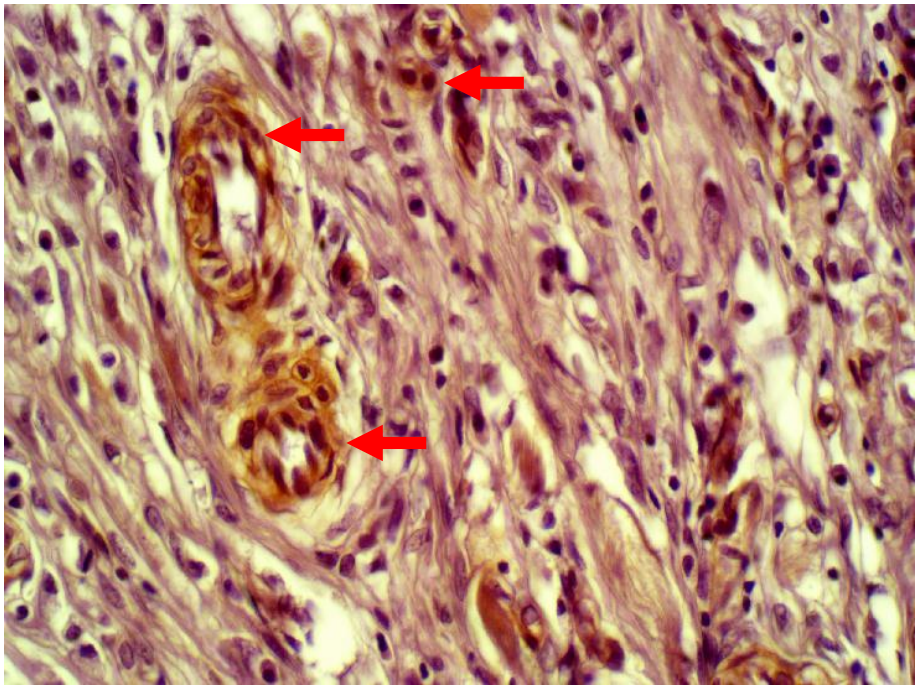

Figure 79: Micrograph at 15th P.O.Ds in MgO NPs group showing strong Positive expression of VEGF-A in the endothelial cell of newly blood vessels which appear as cytoplasmic golden brown granules (**arrow**). VEGF-A IHC, 400x.

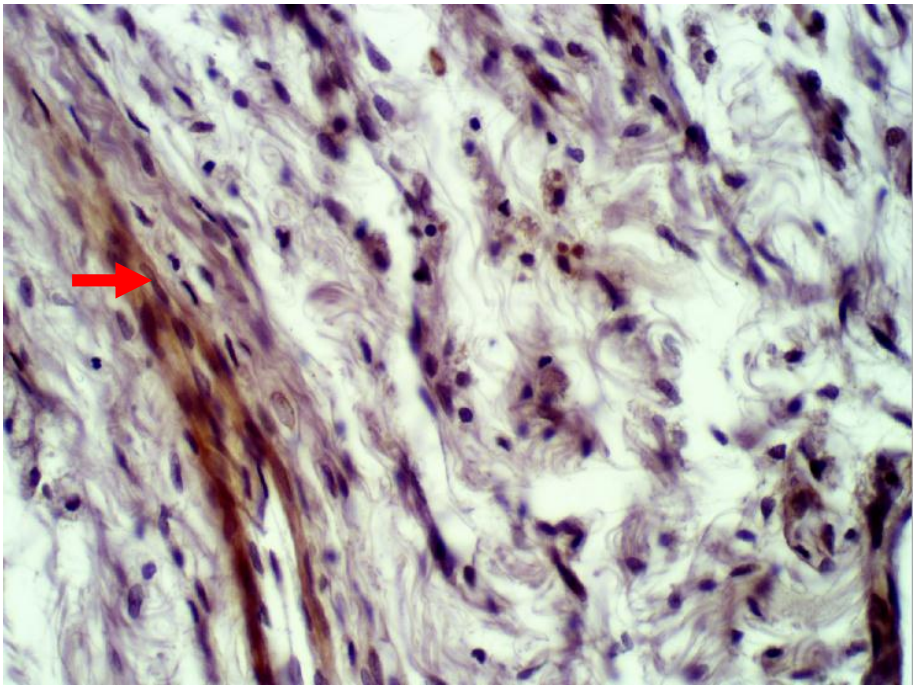

Figure 80: Micrograph at 15th P.O.Ds in control group showing Weak positive expression of IL-6 in the cytoplasm of granulation tissue which appear as golden brown patches (**arrow**). IL-6 IHC, 400x.

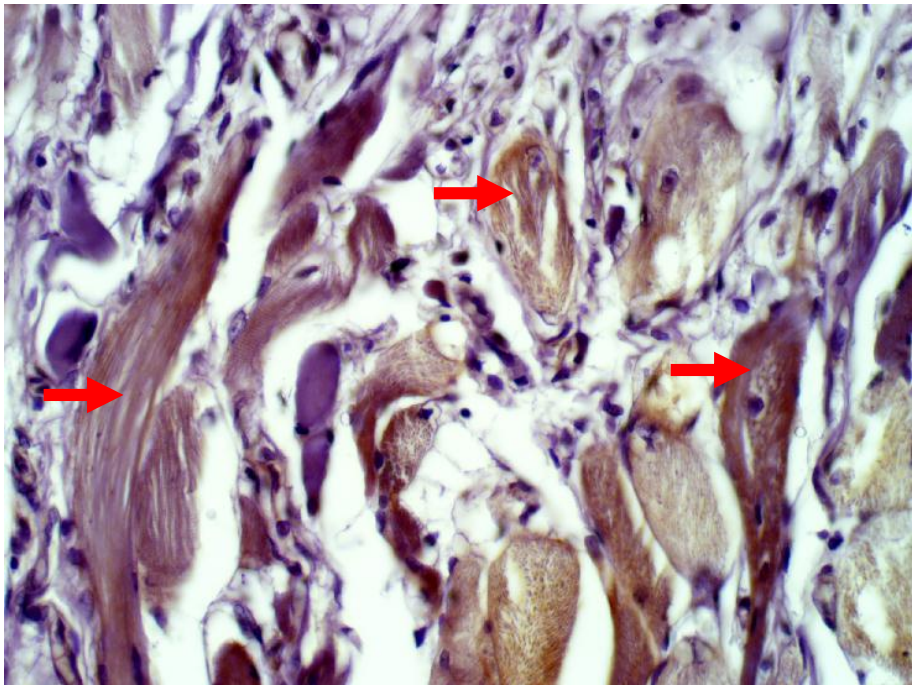

Figure 81: Micrograph at 15th P.O.Ds in bone marrow group showing Positive expression of IL-6 in the cytoplasm of granulation tissue which appear as golden brown fimbria (**arrow**). IL-6 IHC, 400x.

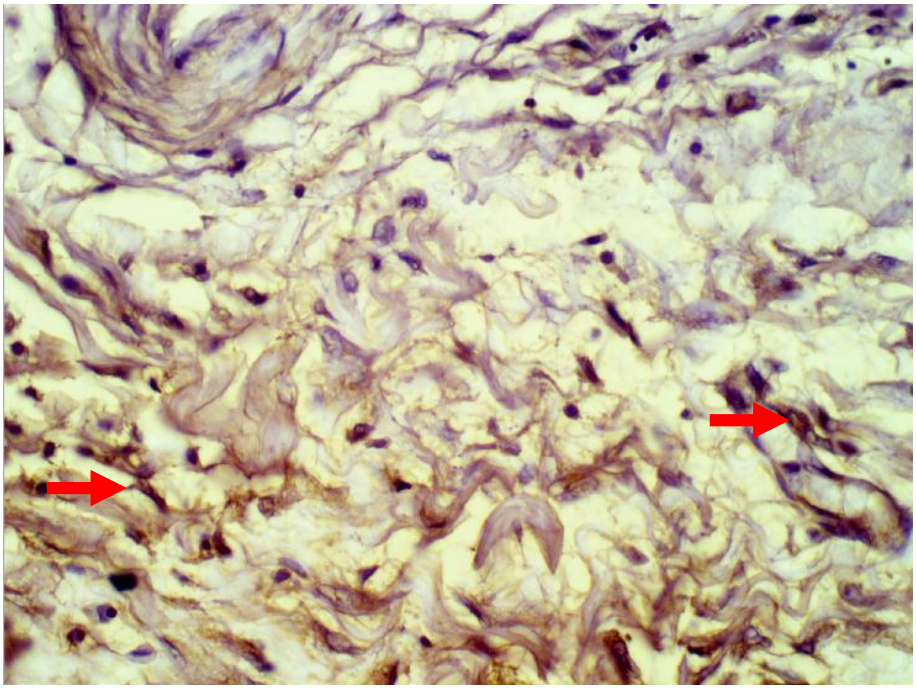

Figure 82: Micrograph at 15th P.O.Ds in hyaluronic acid group showing Weak positive expression of IL-6 in the cytoplasm of granulation tissue which appear as golden brown patches (**arrow**). IL-6 IHC, 400x.

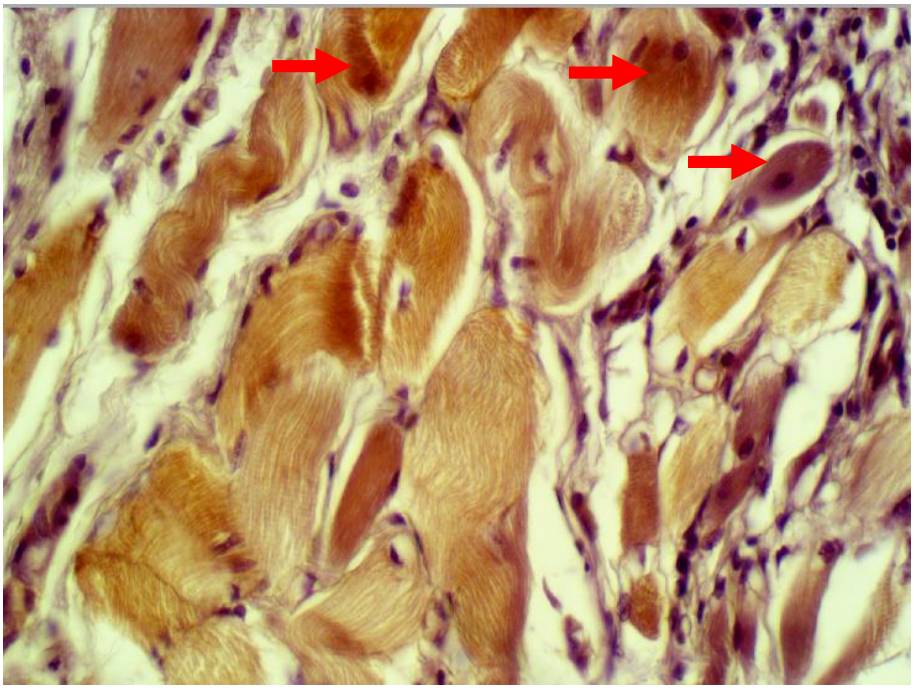

Figure 83: Micrograph at 15th P.O.Ds in MgO NPs group showing Strong positive expression of IL-6 in the cytoplasm of granulation tissue which appear as golden brown fimbria (**arrow**). IL-6 IHC, 400x.

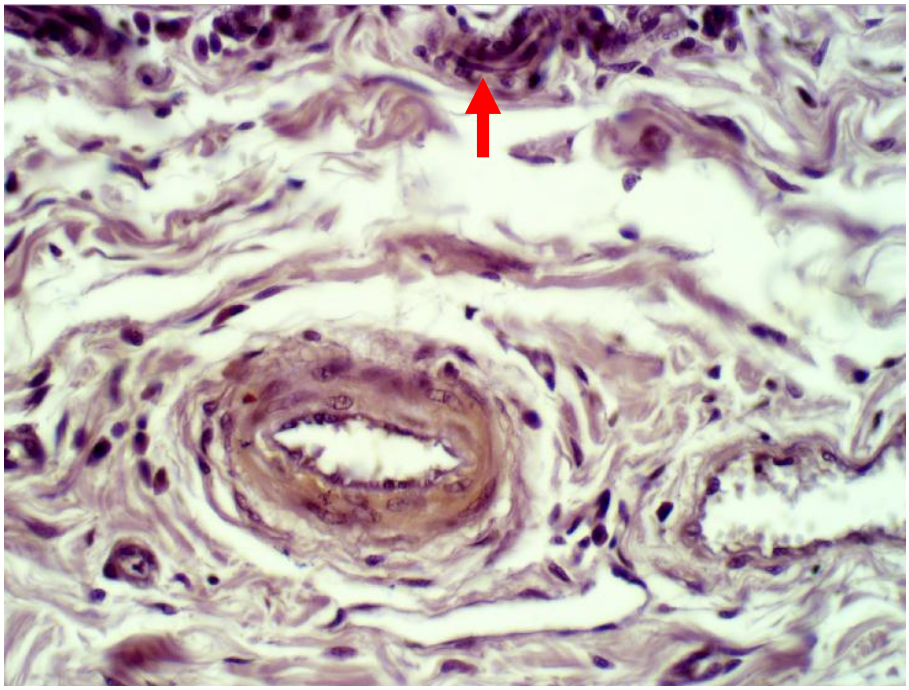

Figure 84: Micrograph at 30th P.O.Ds in control group showing Weak positive expression of IL-12 in the cytoplasm of epithelial cell which appear as golden brown patches (**arrow**). IL-12 IHC, 400x.

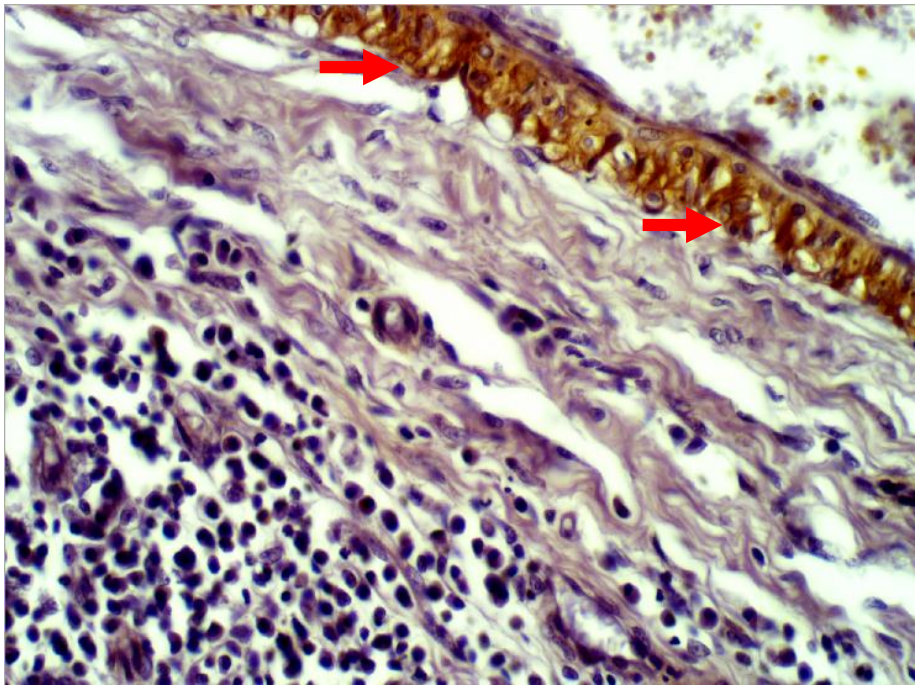

Figure 85: Micrograph at 30th P.O.Ds in bone marrow group showing Positive expression of IL-12 in the cytoplasm of epithelial cell which appear as golden brown patches (**arrow**). IL-12 IHC, 400x.

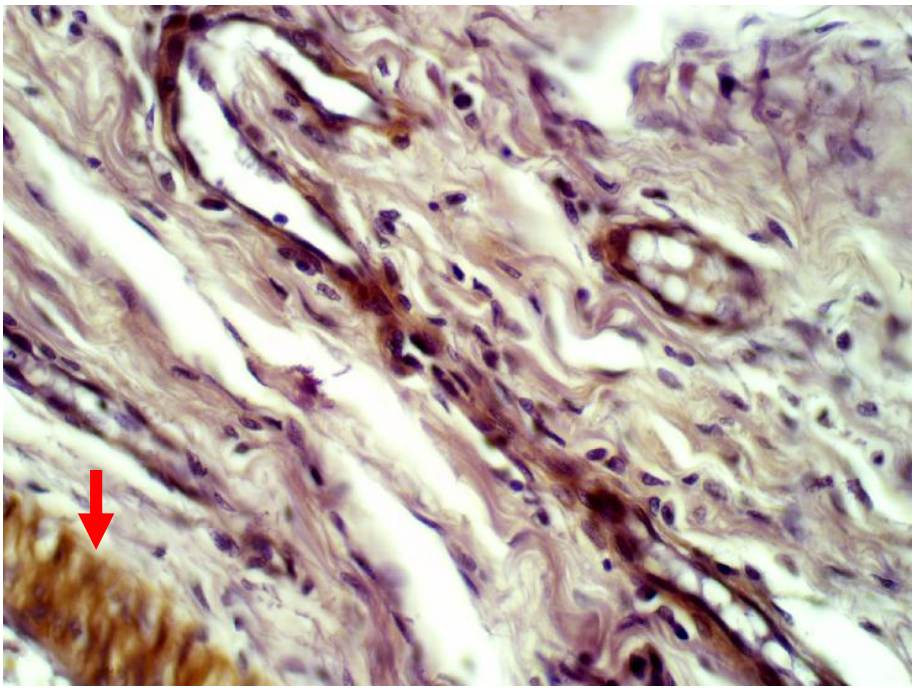

Figure 86: Micrograph at 30th P.O.Ds in hyaluronic acid group showing wed Positive expression of IL-12 in the cytoplasm of epithelial cell which appear as golden brown patches (**arrow**). IL-12 IHC, 400x.

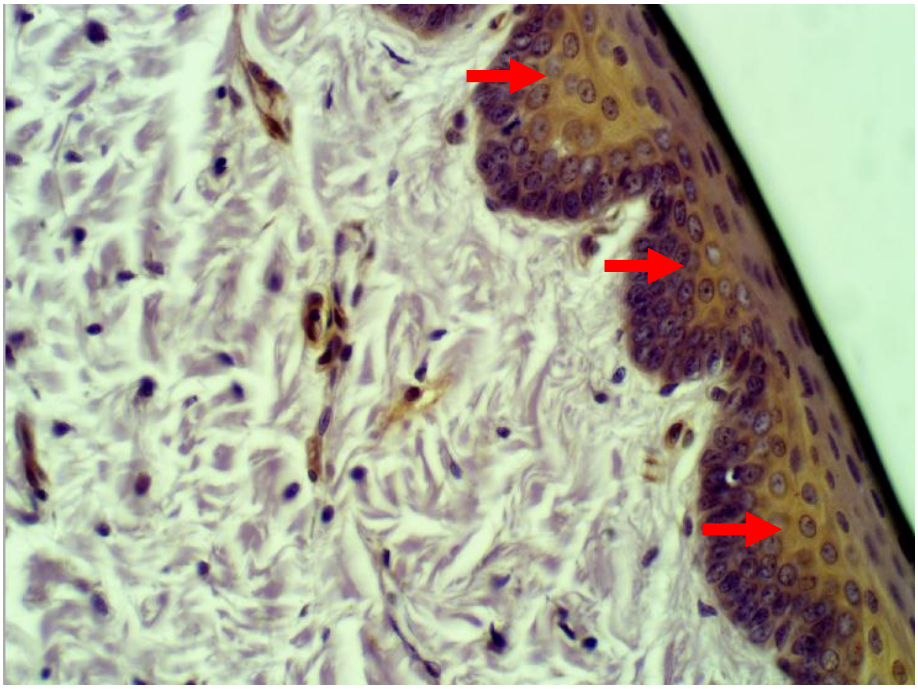

Figure 87: Micrograph at 30th P.O.Ds in MgO NPs group showing Strong positive expression of IL-12 in the cytoplasm of epithelial cell which appear as golden brown patches (**arrow**). IL-12 IHC, 400x.

4-5: Scoring of histological and immunohistochemistry studies

The results of histological and immunohistochemistry studies scoring of all groups are including :

4-5-1: Inflammatory response and infiltration of inflammatory cells

The result of the current study in the 7 day post operation showing that the inflammatory response and infiltration of inflammatory cells were the highest in MgO NPs group, then in bone marrow group, then in hyaluronic acid group and at least significant differences was recorded in control group at $P<0.05$. A similar result was showing in the next 15 and 30 day after operation, these results indicate that the best inflammatory response that associated with infiltration of inflammatory cells were recorded in MgO NPs group in compare with control group in 7, 15 and 30 day post operation (Table 3).

Table 3: Inflammatory response and infiltration of inflammatory cells

Group	7 day	15 day	30 day
Control	0.45±0.04 D	1.77±0.07 D	2.46±0.09 D
MgO NPs	2.21±0.80 A	3.27±0.84 A	3.91±0.94 A
Bone marrow	1.78±0.27 B	2.97±0.74 B	3.74±0.84 B
Hyaluronic acid	0.97±0.11 C	1.87±0.12 C	2.94±0.24 C

Different vertical letters mean presence of significant differences at $P<0.05$.

4-5-2: Granulation tissue formation and maturation

The result of current at the 7th post operation showing that the formation of granulation tissue was the highest in control group, then in hyaluronic acid group, then in bone marrow group and at least significant differences was recorded in MgO NPs group at $P<0.05$. A similar result was showing in the next 15 and 30 day after operation, these results indicate that the lowest amount of granulation tissue formation were recorded in MgO NPs group in compare with control group in 7, 15 and 30 day post operation (Table 4).

Table 4: Granulation tissue formation and maturation

Group	7 day	15 day	30 day
Control	1.61±0.10 A	2.61±0.21 A	3.75±0.20 A
MgO NPs	0.94±0.09 D	1.57±0.10 D	2.71±0.11 D
Bone marrow	1.23±0.11 C	2.01±0.12 C	3.01±0.42 C
Hyaluronic acid	1.41±0.11 B	2.47±0.17 B	3.40±0.71 B

- Different vertical letters mean presence of significant differences at $P<0.05$.

4-5-3: Angiogenesis and newly blood vessels formation

The result of current in the 7 and 15 day post operation showing that the formation of newly blood vessels were the highest in MgO NPs group, then in bone marrow group, then in hyaluronic acid and at least significant differences was recorded in control group at P<0.05. These results indicate that the highest angiogenesis process was recorded in MgO NPs group in compare with control group in 7 and 15 day post operation (Table 5). In contrast and in coordination with fast and perfect healing process the newly blood vessels formation were dimensioned in lowest pattern in MgO NPs group then in bone marrow group then in hyaluronic acid group and at last in control group, this indicate the healing process was finished rapidly in MgO PNs group in compare with control and other groups at P<0.05.

Table 5: Angiogenesis and newly blood vessels formation

Group	7 day	15 day	30 day
Control	2.04±0.12 D	2.94±0.11 D	1.81±0.14 A
MgO NPs	3.54±0.19 A	3.18±0.13 A	0.91±0.09 D
Bone marrow	2.41±0.12 B	3.45±0.15 B	1.21±0.14 C
Hyaluronic acid	2.21±0.14 C	3.14±0.17 C	1.42±0.11 B

- Different vertical letters mean presence of significant differences at P<0.05.

4-5-4: Re-epithelization of esophageal mucosa

The result of current in the 15 and 30 day post operation showing that re-epithelization process were the highest process in MgO NPs group, then in bone marrow group, then in hyaluronic acid and at least significant differences was recorded in control group at P<0.05. in contrast and in compare with the normal healing process this stage was not presence in the 7 days post operation in all groups. These results indicate that the best and fats re-epithelization process was recorded in MgO NPs group in compare with control group in 15 and 30 days post operation (Table 6).

Table 6: Re-epithelization of esophageal mucosa

Group	7 day	15 day	30 day
Control	0.00±0.00 A	1.24±0.11 D	2.91±0.51 D
MgO NPs	0.00±0.00 A	2.87±0.12 A	3.87±0.54 A
Bone marrow	0.00±0.00 A	2.14±0.14 B	3.74±0.47 B
Hyaluronic acid	0.00±0.00 A	1.72±0.10 C	3.11±0.27 C

- Different vertical letters mean presence of significant differences at P<0.05.

4-5-5: Fibrosis and collagen fiber deposition

The result of current in the 15 and 30 day post operation showing that collagen fiber deposition were the lowest in MgO NPs group, then in both bone marrow and hyaluronic acid group and at highest significant differences was recorded in control group at P<0.05. In contrast and in compare with the normal healing process this stage was not presence in the 7 day post operation in all groups. These results indicate that the healing without fibrosis was recorded in MgO NPs group in compare with control group in 15- and 30-days post operation (Table 7).

Table 7: Fibrosis and collagen fiber deposition

Group	7 day	15 day	30 day
Control	0.00±0.00 A	1.09±0.12 A	2.12±0.12 A
MgO NPs	0.00±0.00 A	0.55±0.11 C	0.67±0.10 C
Bone marrow	0.00±0.00 A	0.95±0.14 B	1.21±0.17 B
Hyaluronic acid	0.00±0.00 A	0.97±0.10 B	1.22±0.11 B

- Different vertical letters mean presence of significant differences at P<0.05.

4-5-6: VEGF-A expression

The result of current in the 7 post operation showing that highest express of VEGF-A were recorded in MgO NPs group, then in both bone marrow and hyaluronic group and the lowest VEGF-A expression were recorded in control group at P<0.05, while in 15 and 30 day post operation the lowest VEGF-A express were recorded in MgO NPs group, then in both bone marrow and hyaluronic acid group, and at the highest VEGF-A expression was recorded in control group at P<0.05 (Table 8).

Table 8: VEGF-A expression

Group	7 day	15 day	30 day
Control	2.08±0.14 C	3.67±0.17 A	1.57±0.18 A
MgO NPs	4.18±0.24 A	2.03±0.11 C	0.94±0.10 C
Bone marrow	3.45±0.11 B	2.54±0.18 B	1.24±0.16 B
Hyaluronic acid	3.42±0.21 B	2.67±0.15 B	1.30±0.20 B

- Different vertical letters mean presence of significant differences at P<0.05.

4-5-7: IL-6 expression

The result of current in the 7 and 15 day post operation showing that highest express of IL-6 were recorded in MgO NPs group, then in both hyaluronic and bone marrow group and the lowest IL-6 expression were recorded in control group at P<0.05, while in 30 days post operation the lowest IL-6 express were recorded in MgO NPs group, then in both bone marrow and hyaluronic acid group, and at the highest IL-6 expression was recorded in control group at P<0.05 (Table 9).

Table 9 : IL-6 expression

Group	7 day	15 day	30 day
Control	1.09±0.10 C	2.10±0.10 C	3.81±0.11 A
MgO NPs	2.97±0.54 A	3.71±0.24 A	1.24±0.17 C
Bone marrow	1.81±0.11 B	2.55±0.32 B	1.81±0.27 B
Hyaluronic acid	1.97±0.31 B	2.71±0.10 B	1.99±0.15 B

- Different vertical letters mean presence of significant differences at P<0.05.

4-5-8: IL-12 expression

The result of current in the 7 and 15 day post operation showing that highest express of IL-12 were recorded in Mgo NPs group, then in both hyaluronic and bone marrow group and the lowest IL-12 expression were recorded in control group at P<0.05, while in 30 days post operation the lowest IL-12 express were recorded in MgO NPs group, then in both hyaluronic acid and bone marrow group, and at the highest IL-12 expression was recorded in control group at P<0.05 (Table 10).

Table 10: IL-12 expression

Group	7 day	15 day	30 day
Control	1.12±0.12 C	2.17±0.22 C	2.75±0.18 A
MgO NPs	2.14±0.16 A	3.47±0.21 A	1.07±0.27 C
Bone marrow	1.65±0.28 B	2.57±0.15 B	2.14±0.10 B
Hyaluronic acid	1.74±0.11 B	2.61±0.17 B	2.01±0.14 B

- Different vertical letters mean presence of significant differences at P<0.05.

Chapter five
Discussion

5-1: Post-operative Observations

The swelling appeared at the site of operation may be due to the surgical trauma and post surgical inflammatory process, this change disappeared 5 days after operation and this observation agreed with (Anderson,1980; Jones and Hunt ,1983; Al-Maseeh and Eesa, 2009) . Two animals in group B (bone marrow group) and one in group A (control group) showing hoarseness , which could be due to partial paralysis of the vocal cord resulting from trauma of the recurrent laryngeal nerve during exposure of esophagus (Baba , *et al*.,1999; Al-Maseeh and Eesa, 2009 ; Gooszen, *et al*.,2018) .

The signs of regurgitation and dysphagia were seen during solid food feeding on the 6^{th} PODs , which were a result of neck incisions that , can cause damage and weakening of the accessory swallowing muscles in the neck, and this agrees with (Chen, *et al*.2014) Moreover , dysphagia can be resulted from the stenosis due to scar contraction at the site of anastomosis and this was indicated by the radiographic finding , this observation agrees with others researches that have suggested dysphagia occurs due to anastomotic stricture and showed signs of dysphagia within days or weeks after esophageal surgery (Strombeck, 1990 ; Collard , *et al*.,1998 ; Cheryl and Theresa , 2007 ; Al-Maseeh and Eesa, 2009; Chen , *et al*.2014).

We noticed that the severity of dysphagia was decreased with time in each subgroup, these result was a in agreement with (Chen , *et al*.2014) , as well as, reducing in the degree of stenosis with time , this interpretation closely similar to(Gupta, *et al*., 1980). Whereas, the decreasing in the severity of dysphagia of treated groups may be due to effects of additive

agents that helped to speed up the healing of esophagus, that confirmed by gross examination and histological study, this result was in the line with findings of (Oryan, *et al*.,2012; Chou, *et al*., 2014; Hickey and Webster, 2015; Shengkun, *et al*.,2016; Xiang, *et al*.,2019; Lima, *et al* ., 2020), whom were suggested the bone marrow , hyaluronic acid and MgO NPs have ability to accelerate wound healing when applied locally on the wound .

Based on the above , the healing was accelerated and led to decreasing the degree of stenosis, that was confirmed by radiographic examinations , this result agreed with faster healing that leads to a minimal amount of fibrous tissue formation manifested by a decrease in the percentage degrees of stenosis (Al-Maseeh and Eesa, 2009)

At first glance, none of the animals was suffering from anastomotic leakage, in order to interpret this speculation in this work, we confirmed it by clinical observation and endoscopy as adjunctive method for detection on stich dehiscence that causing leakage .The clinical observation did not showed any signs of outflow of saliva from the neck incision and which indicates that there is no anastomotic leakage that occurs as a result to stich dehiscence , depending on (Bruce, *et al* .,2001; chen, *et al*.2014) who have established that saliva flow from the neck is a clear symptom of leakage, as well as, no swelling was noted clinically at the cervical incision after operation , this was used as an indicator to confirm no animal is suffering from esophageal leakage supported by (Collard , *et al*.,1998 ; Al-Maseeh and Eesa, 2009) they have concluded that persistent swelling for more than 10 days post operatively at the cervical incision as a pathognomic signs of the esophageal cervical leakage .

The second confirmation is determined by the endoscopy which is used for confirmation absence of stich dehiscence and leakage . Using of esophageoscopy was safely and effectively without any risk or damage

to the anastomotic site, this is agrees with (Page, *et al* .,2013 ; Maish, *et al* .,2015; Nishikawa, *et al* .,2016; Fujiwara, *et al*., 2016, Fabbi, *et al*., 2021) they have suggested the endoscopy is a useful method for the intactness of the esophagus during detection of the possible leakage at the anastomotic site .

The interpretation of the absence of esophageal leakage that based on clinical finding and endoscopy, which is attributed to the experience of the surgeon ,this conclusion is similar and agrees with (Yuan, *et al*.,2015; Fabbi, *et al*., 2021) they have concluded that the rate of the anastomotic complications are purely related to the surgeons and their experiences . Moreover , the absence of leakage is also attributed to our use of the two layer suturing with end to end anastomosis that agrees with (Zhu, *et al*., 2008; Nederlof, *et al*.,2014; Fabbi, *et al*., 2021).

5-2: Gross pathological examination

The different degrees of the clarity of anastomotic line in each subgroup and Among the groups were that possibly related to good binding or healing between the two edges of anastomotic site that , enhanced and accelerated by the effect of additive agents, this result agrees with (Chou, *et al*., 2014; Hickey and Webster, 2015; Sharma *et al*.,2017 ; Xiang, *et al*.,2019; Nyman, *et al*.,2019, Lima, *et al* ., 2020), whom were suggested the bone marrow , hyaluronic acid and MgO NPs have ability to accelerate wound healing when applied locally.

5-3: The Radiographic Examination

The increasing percentage degree of stenosis in control group at all postoperative days may be due to fibrous(scar) tissue formation at the site of anastomosis resulting in scar around the anastomosis and reducing the width of scar by the wound contraction leads to narrowing the esophageal lumen and increasing degree of stenosis with the time after operation (Kumar, *et al* ., 1994 ; Lecoindre and Cadore , 1996 ; Cheryl and Theresa,2007), while, in the bone marrow , hyaluronic acid and MgO NPs groups the percentage degrees of stenosis in the esophagus were decreased with passing of time after operation , may be due to acceleration of healing process via the effect of additive agents, this result agrees with other studies by (Chou, *et al*., 2014; Hickey and Webster, 2015; Shengkun,*et al*.,2016; Xiang, *et al*.,2019; Nyman, *et al*.,2019), whom were suggested the bone marrow , hyaluronic acid and MgO NPs have ability to accelerate wound healing when applied locally on it by stimulation the endothelial cells to be differentiated and proliferated and help reepithelization faster normal wound healing and reducing the healing time in compare with control group . And the accelerated healing leads to diminish the degree of stenosis through decreasing scar formation around the anastomotic site this result agreed with faster healing that leads to a minimal amount of fibrous tissue formation (Al-Maseeh and Eesa, 2009) that leaves a fixed-size a fibrous tissue around the anastomotic site additional to an inelastic anastomosis (Kim and Takabe, 2010; Chen , *et al*.2014).

In our present study and in a comparison among groups, at any postoperative time, the percentage degrees of stenosis in control group was considerable , at the same time it was diminished in hyaluronic acid group , moreover , in bone marrow group more diminishment was noticed and even more in MgO NPs group, may be related to different

characterization of additive agents (bone marrow ,hyaluronic acid and MgO NPs) and its different effect on wound healing .

5-4: Histological and immunohistochemistry studies

The result of histological examination showed that the healing process were best recorded in the MgO NPs , bone marrow, and hyaluronic acid groups ,respectively, when compared with control group. MgO NPs have a great effect on wound healing and the cellular process a combine it, there was variety of factors play an important role in the progress and regress healing, one of the most important factor is presence of bacterial infection and contamination either during or post surgery (Mingyue, *et al*., 2021), the MgO NPs play as important role in elimination of these infective factors, a wide range of nanoparticle showed to have or exeret anitbacteirla effect such as sliver, zinc, copper and magnesium, in which the MgONPs showed to have antibacterial effect against 36 species of pathogenic bacteria included *Staphylococcus* and other pyogenic bacteria, in addition to that the low risk of cytotoxic effect of MgO NPs to the cell in wound site is very important in compare with sliver and zinc nanoparticle which they have significant effect as cytotoxic to cells, which play an important role in promote the healing process and depress the effect of bacterial contamination (Jiajia, *et al*., 2020). in addition to that the MgO NPs considered as the most biocompatible and degradable nanoparticle and have a great impact on the wound healing, in which at the end of healing process these particles were removed and degraded from the wound site by the effect of phagocytosis, this biological property was not found in other nanoparticle, making MgO NPs most tissue compatible material (Catarina, *et al*.,2020). The pro-angiogenic activity of MgO NPs help in the wound healing, in which MgO NPs will increase the expression of VEGF at the anastomotic site which considered as the primary cytokine that will

increase and promote the angiogenesis process and increase in the formation of newly blood vessels which considered the transporting vessels to bring more nutrients and cells that promote the healing process and lead by the end to finalized the healing process as soon as possible in compare with the normal healing events (Lili, *et al*., 2015). On other hand, the MgO NPs showed that they have a great ability of stimulate the epithelial cells to be differentiated and proliferated and help in reepithelization faster than normal healing process, in which MgO NPs will exert direct effect on the phagocytic cells to increase secretion of IL-12 rapidly and for long duration in compare with normal healing process, these cytokines known to have a direct effect of the endothelial cells proliferation and differentiation which eventually promote the healing process (Cheyann, *et al*., 2016). Antibacterial effect, angiogenic activity and endothelial cell stimulation effect of MgO NPs play an important role in decrease the healing process duration in addition to promote return the tissue to their normal status before surgical intervention, which is obtained in current study in compare with normal healing process as figured in control group.

Whole bone marrow application in the site of wound healing has a significant impact on wound healing rapidly than that recorded in normally healed wound, this biological material contains steam cells and procurers of inflammatory cells (Evangelos and Vincent., 2003), these inflammatory cells were especially monocytes that produce monocyte colony stimulating factor (MCSF) which found to have a great importance in the acute cellular events at the first stages of healing during the period from 12 hours to 36 hours after incision, in which MCSF will promote margination, pavementation and migration of mononuclear inflammatory cells in the blood vessels and enhance their migration by highly express of integrin receptor family in wall of blood vessels to facilitate their pseudopodia

movement of monocytes, which directly converted into macrophages as soon as they enter the tissue, then they chemotaxis to site of wound healing to start phagocytosis of cellular debris (Yaojiong, *et al*.,2007). On other hand, the steam cells that present in whole bone marrow materials will differentiated and converted into fibrocytes cells and fill the gaps between dead and live cells, this process to converting into fibrocytes will lead to increase in collagen formation and deposition and this will lead eventually to healing by fibrosis (Xiaobing, *et al*.,2006). In addition, the whole bone marrow contains the most important cytokine that effect of conversion of fibrocytes to fibroblast is fibroblast growth factor, which facilitate and enhance transformation of fibrocytes to fibroblast and activation of these cell to knitting arachidonic acid into collagen type II or IV, these two features of enhancing have a great impact healing of chronic wound (Luis, *et al*.,2015). The whole bone marrow materials showed that to have a great impact on the first cellular events in acute wound healing also in collagen deposition and stretching in chronic wound healing, these two events did not have a great impact on the wound healing duration nor intensity, which make this wound dressing materials is less effective in wound healing in compare with MgO NPs, but in the same time bone marrow have a great impact to increase the speed of acute inflammatory process as a first step in wound healing and rapidly and intensively in collagen formation and deposition at the last stage which in have a good effect to reduce the healing time in compare with control group and hyaluronic acid group, and this result was obtained in current study.

Hyaluronic acid has a great impact on wound healing and play a key stone in process related to collagen deposition and wound stretching especially in chronic wound to prevent healing by fibrosis, in which hyaluronic acid have a direct effect of the phagocytic cells to promote their production and secretion of IL-6 and IL-8, these interleukins which have a

great importance in stimulation and activation of fibrocytes to be biogenically converted into fibroblast, this last cell play a major role in knitting the arachidonic acid to form a certain types of collagen fibers, in which these cells in presence of high amount of IL-6 and IL-8 in wound site will knitting the arachidonic acid into collagen fiber type II and IV, which is the most beneficial type of collagen fibers in compare will collagen fibers type III which is presence in case of pathogenic fibrosis and scare tissue formation (V´eronique, *et al*.,2006; Litwiniuk, *et al*., 2016). In addition hyaluronic acid have a great importance in activation of keratinocytes and re-epithelization process, in which these two process control by the IL-12 and IL-6, and the hyaluronic acid known with their a great effect in increase secretion of IL-6 which will promote the re-epithelization process, in addition to that hyaluronic acid have no effect on the healing process especially the cellular events and the angiogenesis process (Nyman *et al*.,2019 ;Yayoi, *et al*.,2021), these properties make this wound dressing material less effective in wound healing in compare with MgO NPs and bone marrow, on other hand it have a great impact on wound healing in compare with normal healing process, these conclusions was recorded by current study.

Chapter six

Conclusions

1- The percentage degree of stenosis was less recorded in MgO NPs group, then in the bone marrow, and in hyaluronic acid group in comparison with control group.

2- Histological and immunohistochemistry studies approved that the best healing process of esophagus was in MgO NPs followed by bone marrow then hyaluronic acid groups respectively in comparison with control group

Recommendations

1- Extending the postoperative periods more than 30 days to follow up the healing process and observation of any post operative complications

2- Studying effect of using MgO NPs , bone marrow and hyaluronic acid on the different esophageal grafts .

3- Using another types of NPs agents and studying its effect on the esophageal healing.

4-Using anastomotic bursting pressure to measuring the mechanical strength of anastomosis which represents the resistance of esophagus to intraluminal pressure

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جامعة الموصل
كلية الطب البيطري

دور نقي نخاع العظم ، حامض الهايلورونك وجزيئات المغنيسيوم النانوية على التئام تفاغر المرئ في الكلاب

رضوان رياض كاظم العجيلي

أطروحة دكتوراه
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بإشراف
الأستاذ الدكتور

عبد الحليم مولود صالح الحسن

الاستاذ الدكتور(المشرف الثاني)
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أطروحة تقدم بها

رضوان رياض كاظم العجيلي

إلى
مجلس كلية الطب البيطري في جامعة الموصل
وهي جزء من متطلبات نيل شهادة الدكتوراه فلسفة
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الخلاصة

هدفت الدراسة الحالية الى تقييم عملية التئام نسيج المرئ بعد اجراء عملية التفاغر للجزء العنقي من المرئ و المحدث تجريبياً في الكلاب ثم معاملة منطقة التفاغر بنخاع العظم وحامض الهايلورانك وجزيئات المغنيسيوم النانوية. اجريت الدراسة على ٣٦ كلباً محلياً كانت سليمة سريرياً ومن كلا الجنسين تراوحت اعمارها ما بين١٨ شهر الى ٣٦ شهر بمعدل (٢٧ ± ١,٥) شهرا وتراوحت اوزانها ما بين ٢٠- ٣٠ كغم بمعدل(٢٥ ± ١,٣) كغم . قسمت الحيوانات عشوائياً الى اربعة مجاميع اذ ضمت كل مجموعة ٩ كلاب . كل كروب رئيسي تم تقسيمه الى ثلاث مجاميع فرعية حسب الوقت بعد العملية الجراحية ٧ ، ١٥، ٣٠ يوم وكل مجموعة مكونة من ثلاثة كلاب . تم نشر نقي نخاع العظم المسحوب ذاتيا من الحيوان و حامض الهايلورونك و اوكسيد المغنيسيوم النانوي على الطبقة المخاطية والعضلية للمرئ عند موضع التفاغر وحسب التالي

مجموعة ١ :مجموعة السيطرة تم ترك موضع التفاغر بدون اضافة اي مادة

مجموعة ٢: مجموعة نقي نخاع العظم

مجموعة ٣ : مجموعة حامض الهايلورونك

مجموعة ٤ : مجوعة اوكسيد المغنيسيوم النانوي

تم تقييم النتائج اعتماداً على خمسة معايير وهي العلامات السريرية من خلال متابعة ومشاهدة الحيوانات بعد العملية للكشف عن اي تغييرات اوظهور علامات غير طبيعية مثل صعوبة البلع او وجود تسريب من خلال الجرح ظاهرياً وكذلك استخدام التنظير الداخلي للمرئ بواسطة (BORESCOPE) للبحث عن اي انفتاح للخيط والذي عادةً ما يحصل نتيجة خطأ جراحي و

كذلك اجراء الفحص العياني المبني على متابعة وضوحية خط التفاغر وتحديد وقت اختفائه بالاضافة الى استخدام التصوير الشعاعي الملون للمرئ لتحديد النسبة المئوية لدرجة التضيق واخيرا اجراء الفحص النسيجي والفحص الكيميائي المناعي لتحديد ومتابعة التئام موضع التفاغر.

اظهرت نتائج الفحص السريري صعوبة في البلع ناتج عن التضيق الحاصل عند موقع االتفاغر وبدرجات متفاوته في حدتها حيث كانت واضحة في اليوم ٧ واقل وضوحاً في اليوم ال١٥ واقل منها في اليوم ال٣٠ مابعد اجراء العملية ، كما واظهرت النتائج السريرية لصعوبة البلع حيث كانت حادة جدا في مجموعة السيطرة ثم اقل منها في مجموعة حامض الهايلورونك واقل في مجموعة نقي نخاع العظم واقل في مجموعة اوكسيد المغنيسيوم النانوي .

كما واظهرت نتائج الفحص العياني لموضع التفاغر وضوحية عالية في خط التفمم وكانت تتناقص تدريجيا ما بعد العملية ب٧و١٥و٣٠ يوم كما وكانت تتناقص ما بين المجاميع الاربع حيث كان خط التفاغر قليل الوضوحية في مجموعة اوكسيد المغنيسيوم واكثر وضوحا منه في مجموعة نقي العظم واكثر منه في مجموعة حامض الهايلورونك واكثر في مجموعة السيطرة .

واظهرت نتائج التصوير الشعاعي ما بعد العملية ب ٧ و١٥ و٣٠ يوم زيادة غير معنوية في النسبة المئوية لدرجة التضيق عند $P<0.05$ في مجموعة السيطرة بينما في المجاميع الاخرى كانت تتناقص تناقصا غير معنوي عند $P<0.05$ في مجموعة نقي العظم وتناقصا معنويا عند$P<0.05$ في مجموعة حامض الهايلورونك ومجموعة اوكسيد المغنيسيوم النانوي ، وكما لوحظ ان النسبة المئوية للتضيق كانت كبيرة في مجموعة السيطرة وفي نفس الوقت كانت اقل منها في مجموعة الهايلورونك واقل منها في نقي العظم واقل منها في مجموعة اوكسيد المغنيسيوم النانوي. كما واظهرت نتائج الفحص النسيجي والكيمياء النسيجي ان عملية الشفاء كانت افضل في مجموعة اوكسيد المغنيسيوم النانوي وتليها مجموعة نقي العظم ومن ثم مجموعة حامض الهايلورونك مقارنة مع مجموعة التحكم.

نستنتج من هذه الدراسة ان نقي نخاع العظم وحامض الهايلورونك وجسيمات المغنيسيوم النانوية ممكن ان تستخدم وبنجاح عند وضعها موضعيا على نسيج المرئ لتسريع عملية التئامه بالاضافة الى ان مجموعة المغنيسيوم كانت افضل وتليها مجموعة نقي نخاع العظم وبعدهما مجموعة حامض الهايلورونك مقارنة مع مجموعة السيطرة من خلال تقليل صعوبة البلع واختفاء خط التفاغر وتقليل نسبة التضيق وتسريع عملية التئام المرئ.