Research Square

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Enhancing Esophageal Repair With Bioactive Bilayer Mesh Containing FGF

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Abstract

We aimed to prepare a bioactive and biodegradable bilayer mesh formed by fibroblast growth factor (FGF) loaded gelatin film layer, and poly ε-caprolactone (PCL) film layer, and to investigate its treatment efficacy on esophageal anastomosis. It is envisaged that the bioactive mesh in *in vivo* model would improve tissue regeneration in rats. The full thickness semicircular defects of 0.5x0.5 cm^2 were created in anterior walls of abdominal esophagus. The control group had abdominal esophagus isolated with distal esophageal blunt dissection, and sham group had primer anastomosis. In the test groups, the defects were covered with bilayer polymeric meshes containing FGF (5µg/2 cm^2), or not. All rats were sacrificed for histopathology investigation after 7 or 28 days of operation. The groups are coded as FGF(-)-7th d, FGF(+)-7th d, and FGF(+)-28th d, based on their content and operation day. Highest burst pressures were obtained for FGF(+)-7th d, and FGF(+)-28th d groups ($p < 0.005$) and decreased inflammation grades were observed. Submucosal and muscular collagen deposition scores were markedly increased in these groups compared to sham and FGF(-)-7th d groups having no FGF ($p = 0.002$, $p = 0.001$, respectively). It was proved that FGF loaded bioactive bilayer mesh provided effective repair, reinforcement and tissue regeneration of esophageal defects.

Introduction

The primary anastomosis generally applied for repairing congenital esophageal pathologies is not possible if the case is long range esophageal atresia. Esophageal replacement may be required in anastomosis leakage after a primary repair, recurrent esophageal fistulas, refractory esophageal reflux, and severe stenosis unresponsive to dilatation and corrosive stenosis[1]. After the primary surgical repairment was applied as the first choice in childhood esophageal pathologies, problems such as stenosis, leakage, infection and fistula recurrence in the anastomosis area may occur and may require a second surgery. The applicability of bioactive meshes to deal with these difficulties is a challenging and highly investigated area[2]. Bioactive meshes are generally used for tissue engineering applications if they also contain cells besides bioactive molecules as growth factors. These scaffolds are used for tissue regeneration and for maintaining the functions of organs[3]. This field of science is based on multidisciplinary studies with cellular biology, materials engineering, physiology and gene therapy, using engineering principles[4, 5]. Since esophageal pathologies have different anatomical components and tissue types, mucosal regeneration studies gain weight in injuries and non invasive neoplastic diseases. All esophageal replacement studies come to the fore in invasive neoplastic diseases, congenital pathologies and transmural caustic injuries. Therefore, preclinical animal experimental studies are required to determine new strategies in tissue repair and tissue regeneration. Animal models for esophageal repair, especially the use of small species such as murine species, provide strong statistical results and cost efficiency for determining regenerative medicine strategies[6]. In tissue regeneration research, formation of extracellular matrix (ECM), that is present in all tissues and been essential for life, is very important factor for the treatment of wounds. ECM is a complex three dimensional acellular fibrous structure which creates a protective and supportive dynamic scaffolding and mutual signaling

platform for pragmatically moving cells in the wound area. Each stage of wound healing is inextricably linked with the ECM[7]. Hydrogels made of natural polymers such as collagen, alginate, chitosan or gelatin can be used to mimic the natural 3-dimentional (3D) network structure of ECM. This type of hydrogels consisting of one or more of the purified ECM proteins have promising applications in tissue repair studies[8]. In our previous studies we have shown that protein based polymers such as collagen, gelatin and fibrin have the advantage of creating suitable ECM environment, enhancing cell proliferation and tissue regeneration[9,10,11,12].

Collagen, an important component of extracellular matrix (ECM), provides fibrogenesis and integrin mediated mechanical bonding. Cellular mobility and translocation ability are modulated by collagen. It creates stiffness and porosity that allows the cells to migrate together. This structural feature of the ECM provides a model for treatments to improve tissue repair results [13]. Gelatin is a natural polymer obtained by hydrolysis of collagen which is the most widely found protein in the body. It is attractive for tissue engineering applications due to its biodegradable, biocompatible and absorbable. Growth factors (GFs) are signaling molecules directing cell development by providing biochemical cues for stem cell proliferation, migration and differentiation[14]. They are effectively used in the treatments of tissues as well as in tissue engineering applications due to their influence on cell signaling pathways[15,16,17]. GFs can be added directly into the polymeric matrices or can be loaded to micro or nano particles and then added into the scaffold materials[18,19,20]. Growth factors (GFs) have a strong effect on the tissue repair process. Fibroblast growth factor (FGF) stimulates the growth and differentiation of many cells from skeletal muscle cells to smooth muscle cells, including endothelial, chondrocyte, keratinocyte, melanocyte and glial cells[21]. It also plays a role as a potent angiogenic factor by stimulating reperfusion and angiogenesis. Therefore, in the studies on wound healing, the effects of exogenous are frequently investigated. Esophageal repair studies are aimed to increase the formation of submucosal collagen, which is the most important factor affecting wound healing as in all anastomosis healing studies[18,22]. In a study on FGF, its exogenous use has been shown to have a positive effect on esophageal wound healing[18].

Natural and synthetic polymers have been used for preparing scaffolds used as supports for tissue regeneration and healing. Both types of materials have several advantages and disadvantages. Therefore, it is not easy to obtain one suitable construct fulfilling all requirements necessary for a successful scaffold. In some studies, instead of a single layer construct, bilayer systems as a combination of natural and synthetic polymers were developed for tissue regeneration to benefit from the required properties of both materials[23].

The basis of the works aimed to prepare structures to replace the esophagus is based on the use of biological and decellularized or synthetic scaffolds. If these studies are exemplified, a biological scaffold derived from extracellular matrix (ECM), which eliminates the need for esophagectomy, has been used in esophageal neoplasia[6]. It has been experimentally demonstrated that the healing of esophageal ulcers is possible with the cellular layer formed by the expansion of the esophageal epithelium. Synthetic

scaffolds were acted as a natural stent allowing anastomosis again, causing fibrosis in the esophagus. It was reported that successful clinical results were not obtained in the use of very soft and permeable scaffolds. Adequate muscle regeneration and vascularization have not been observed in experimental use for esophageal repair of pig dermal collagen scaffold as naturally produced scaffold[24]. Although many interesting achievements have been made about the natural (biological) biomaterials proposed to create esophageal tissue, in some vitro or vivo experiments, it has been reported that there are problems such as poor mechanical strength and rapid degradation[24]. Although in these studies the use of various synthetic and biosynthetic materials that will replace the esophagus on esophageal tissue regeneration has been empirically studied, there is no sufficient information about its long term results[6]. There is no consensus or comparative study on the superiority of synthetic or biological materials in esophageal repair in the literature.

Synthetic scaffolds are widely used in esophageal repair studies because they are easily obtained with reproducible and reliable processes and are xeno-free and low cost. It has been reported that there are difficulties that have to be overcome, such as anastomosis leakage, local infection, graft failure and optimal graft degradation, in the use of meshes either have synthetic or natural origin[25]. To achieve better results, combination of both synthetic polymers and natural ones can be combined so that the construct would have good biomechanical properties, and also better biocompatibility. Also various growth factors (GFs) can be added into the construct to promote tissue regeneration[26]. It has been shown that synthetic materials can create a bioactive surface sufficient to attract the extracellular matrix (ECM) proteins such as fibronectin, laminin and collagen, and provide cell adhesion[26]. Loading tissue-specific GFs and antibodies to biodegradable intelligent synthetic nanofibers can provide functional results by improving wound healing[27]. Smart polymers can replace biological scaffolds, and offer a ready approach to esophageal tissue engineering. Intelligent matrices containing factors that promote tissue regeneration may allow for required cell seeding and obtain a standard in vivo biological response[28].

Poly ε-caprolactone (PCL) is one of the aliphatic polyesters approved by the USA Food and Drug Administration (FDA) and widely used in tissue engineering constructs due to its biocompatibility, mechanical strength, and suitability for modification and low cost[29,30,31]. PCL is prepared in various forms such as films, mats, nanofibers, nano/micro particles or scaffolds[31]. PCL is used with natural polymers like gelatin to provide better cell adhesion and proliferation due to its integrin binding the tripeptide *Arg-Gly-Asp (RGD)* motif[32,33]. PCL is one of the biocompatible polyesters that can be used in the production of scaffolds due to its neutral, natural and long term biodegradability. While the scaffold based on PCL decays, the new tissue takes its place, leaving no synthetic material behind. The degradation products that arise during their biological degradation are also noteworthy because they do not cause any harm to the body[31]. Various techniques are used in the preparation of poly ε-caprolactone (PCL) scaffolds. Modifying the hydrophobic nature of PCL with hydrophilic and bioactive molecules enhances the healing effect of the wound healing process[33].

As defined previously, gelatin is derived from collagen by controlled hydrolysis, and it contains glycine, proline and hydroxyproline, which are residues that help cell adhesion and differentiation. It was reported that it can be used for tissue regeneration applications as it offers additional useful properties such as biological origin, biodegradability and biocompatibility[19,20,34]. Meanwhile, human esophageal epithelial cell proliferation was higher in the PCL-gelatin nanofiber scaffold, and therefore, the PCL-gelatin nanofiber scaffolds are presented as potential candidates for the regeneration of functional esophagu [35]. The main purpose of the study was, to prepare a bilayer mesh based on fibroblast growth factor (FGF) loaded gelatin as one layer and poly ε-caprolactone (PCL) as the second layer which would provide the desired tissue repair with healing cell epithelization for esophageal damages. Cell adhesion can be activated by GFs and protein based gelatin while PCL will provide the mechanical strength to the mesh.

In esophagial repairs, no studies have been conducted on the effect of esophagial wound healing by supporting with a suitable mesh structure having growth factors (GF) in the composition. In our study, it was aimed to search the healing effect a bilayer mesh structure made of gelatin and poly ε-caprolactone (PCL) where fibroblast growth factor (FGF) was loaded in the gelatin which has a porous structure mimicking the extracellular matrix (ECM). It was aimed to show the positive effect of the mesh on the reinforcement of esophageal defects and healing of esophageal anastomoses. To the best of our knowledge, our study is the first one using a bilayer mesh made of FGF for reinforcement of esophageal defects. There are studies for the treatment of esophageal defects where either FGF was used in one-layer gelatin film, or epithelial growth factor (EGF) was used as a local single dose[17,18].

Materials And Methods

Preparation of bilayer meshes

Polymeric bioactive bilayer meshes made of poly ε-caprolactone (PCL) (Mn:80.000 Da, Aldrich, UK) film and gelatin (Scharlau, Spain) film were prepared. As a first layer, PCL films were prepared by solvent casting technique. For this purpose, 5% (w/v) PCL solution was prepared in a dichloromethane (Across) and poured into 10 mm diameter glass petri dishes as molds. Dry PCL films were obtained after the evaporation of the solvent, and films were immersed in 10% hexane diamine-isopropanol solution for 1 h at 37°C for aminolization. These PCL films were washed with deionized water for 24 h at room temperature to remove the remaining free 1,6 hexane diamine. In a separate beaker, gelatin solutions were prepared with addition of FGF (Sigma). The effective dose of FGF was given as 120 ng per 100 g/d[18]. By taking this as a reference, gelatin films containing 2.5 µg FGF per cm^2 of films were prepared. For this purpose, aqueous gelatin solution (5% w/v) was prepared in distilled water, glutaraldehyde (0.05% w/v) was added as crosslinker to stabilize gelatin. After mixing this solution for 1 min, FGF solution was added and the total solution was poured on to aminolyzed PCL films. Gelatin solution having no FGF was also prepared and added on PCL to compare the effect of FGF. The bilayer mesh structures were obtained after the films were dried at room temperature. (Figure 1). The ones having FGF and no FGF are coded as FGF(+) and FGF(-), respectively (Table 1).

Experimental animals

In the study, 34 male adult wistar-albino rats, the body weight ranging from 200-250 g, were provided by Husnu Sakal Experimental and Practice Center, Ankara Training and Research Hospital, Health Sciences University, Ankara, Turkey. Rats were kept in special cages at a standard room temperature (24 ºC) in a 12 h light/dark circadian rhythms. Animals were fed with standard pellet feed and free access via city water. Institutional Ethical Approval was obtained from the Animal Experiments Local Ethics Committee**,** Ankara Health Research Application Center, Health Sciences University, Ankara, Turkey (Ethical approval number: 17/09/2019-0055).

Study design

In the study, 34 male wistar-albino rats were randomly divided into five groups, having 6 rats in Control Group, and 7 rats in each of the other test Groups. After 12 h of fasting, 10 mg/kg xylazine (Rompun; Bayer AG, Leverkusen, Germany) and 50 mg/kg ketamine hydrochloride (Ketalar; Parke Davis, Eczacibasi, Istanbul, Turkey) were given to rats under anesthesia via intramuscular injection. All procedures were performed in sterile environment. Before the abdominal incision, a silastic 8 F orogastric feeding catheter (Bicakcilar, Turkey) was placed into the stomach via the oral route. Under aseptic and antiseptic conditions, 3 cm midline laparotomy was performed. The distal esophagus was visualized and mobilized for 4 cm length using blunt dissection. After midline laparotomy, a full thickness semicircular defect of 0.5x0.5 cm^2 was created via cutting in the anterior wall of the abdominal esophagus. The defect was either repaired with primer anastomosis without mesh, or with the prepared bilayer mesh using an interrupted absorbable suture of 6-0 polyglactin (Vicryl; Ethicon, USA). (Figure 1). The Control Group had only distal esophageal blunt dissection, and was isolated. The Sham Group had primer anastomosis without mesh application. Bilayer mesh without FGF were applied to animals and these animals were sacrified after 7 days (FGF(-)-7^{th} d Group) for examination. Bioactive bilayer meshes (2 cm^2 having 5 µg FGF) were applied other two groups and these animals were sacrified after 7 days (FGF(+)-7^{th} d Group), and 28 days (FGF(+)-28^{th} d Group) for examination. The abdominal wall and skin were closed by layer with continuous 3-0 silk (Silk; Ethicon, USA) suture. For all group animals, before the abdominal incision closure, 5 mL of 0.9% NaCl was administered intraperitoneally. After the first 24 h, the rats were allowed free access to food and water. The Control, Sham, FGF(-)-7^{th} d, FGF(+)-7^{th} d and FGF(+)-28^{th} d Groups animals all were sacrificed exsanguination under deep anesthesia at the end of experiments. **Lack of movement, absence of heart beat, pupillary response to light and respiratory pattern were** confirming death in all experimental animals. For investigation 4 cm distal esophagus including anastomotic site was resected, and bursting pressure, inflammation and collagen deposition values were determined by histopathological tests.

Burst pressure measurement

Burst pressure was measured infusionally with indeflator system, which have pressure transducer, pressure channel, and sphingomanometry (BIG60 Inflation Device, Merit Medical System Inc., Utah, USA).

After 4 cm distal esophagial segment included anastomotic area was resected, pressure channel catheters were placed within the proximal end. The distal end was tied with a 2/0 silk suture. Monitoring intraluminal pressure with this device, the point of leakage from the anastomosis occurred was recorded as the bursting pressure (Figure 1).

Histopathological examination of the esophagus

Tissue samples of 4x2 cm^2 size of the esophagus obtained from rats were embedded in paraffin after gradual ethanol dehydration (50%, 75%, 96% and 100%, respectively) and xylene translucency following fixation in 10% formaldehyde solution for 2 days. 4 µm sections were taken with Leica RM 2125 RT microtome from paraffin-embedded tissues. Tissue sections were examined by staining with Hematoxylin&Eosin (H&E) and Mason Trichrome Stain. Histopathological examination was evaluated microscopically using OLYMPUS brand, BX51TF model, x4, x10, x20, x40 lenses. The esophageal collagen density, epithelialization and polymorphonuclear leukocytes (PMNL) were evaluated semiquantitatively with the histopathological scoring system using Abramov's Histologic Scoring Syste [36] (Table 2).

Statistical analysis

Statistical analysis of the study was performed with SPSS 20.0 Program (IBM Inc., IL, USA). Descriptive features were presented as frequency and percentage. The differences of histopathological evaluations according to the study groups were determined by the Chi-Square Analysis Method. $P<0.05$ value was considered statistically significant (Table 3).

Results

In our study, on post procedural course animals were survived in all groups. For collecting of the post operative information, weight loss, analgesics administrated, wound healing, activity and feeding was recorded at the animal’s cage card.

When groups were sacrified, local abscess formation was observed macroscopically in Sham Group and FGF(-) Group, but not in FGF(+) Groups.

No histological changes were observed in Control Group representing the normal esophagus histological findings. Sham Group and FGF(-) Group showed that epithelial regeneration did not occured. However, intense inflammation in the submucosal and muscular layer, and mildly increased collagen content were determined (Figure 2, Figure 3).

In bioactive groups having FGF, there was the surface epithelium regeneration as well as significantly increased collagen, which was replaced by the submucosal and muscular layer. Moderate decrease on the 7th day and significant decrease on the 28th day were observed in the inflammatory cell density between the collagen bundles. Significantly increased collagen (blue color) between the submucosal and

muscular layers was observed with Mason Trichrome Stained tissues. When FGF(+) Groups were compared with the FGF(-) Group and Sham Group, the epithelial regeneration and collagen deposition in layers were significantly accelarated and inflammatory cells were decreased ($p<0.05$) in 7th and 28th days (Table 3, Figure 3).

The burst pressures of the esophagial segments demonstrated variations. The ones which treated with FGF containing bioactive meshes (FGF(+) Groups) showed statistically significant higher burst pressure values with respect to the Sham and FGF(-) Groups (Table 3, Figure 4).

As demonstrated by these results obtained in our study, in esophageal tissue regeneration growth factors have an important role. Presence of FGF added bioactivity to the meshes which were prepared from gelatin (that mimic natural extracellular matrix, ECM), and poly-ε-caprolactone (PCL) (which have tissue compatibility and has strength advantages), and significantly affected epithelial regeneration and collagen accumulation.

Discussion

Tissue regeneration technologically combines artificial scaffolds, cell-matrix and growth factors (GFs) to provide double or triple matrix products that can be applied in tissue repair and remodeling. It has been confirmed by Zhu et al. that these scaffolds can provide functional esophagus substitution well enough to support the growth of pig esophageal cells such as epithelial, fibroblast and muscle cells[37]. The effects of growth factors (GFs) on wound healing have been the subject of many studies[9,19,17]. Of these, fibroblast growth factor (FGF) is characterized as a strong mitogen for fibroblasts, capillary endothelial cells and mesenchymal cells. Therefore, it has been suggested that FGF plays a key role in accelerating wound healing, activating fibroblasts and inducing neovascularizatio [38]. With the electrospinning technique, it is possible to design double layer matrices with nanofibers consisting of poly ε-caprolactone (PCL)/poly-L-lactic acid (PLLA) for the lower layer and PCL/gelatin for the upper layer and gelatin microspheres are included in the middle of the two layers for controlled growth factor transmission[39]. Also it was reported that the sandwich scaffolding system have anti-leak and cell-binding support properties[40].

In our study, it was aimed that it is the first study in terms of evaluating the short and long term results of mesh and growth factor (GF) that will create a barrier for sealing in esophageal anastomoses and increase cell migration and proliferation. When poly-ε-caprolactone (PCL) nanofiber layer was used in rabbit esophageal repair, an improvement in both epithelial and smooth muscle cells was provided. When polyvinylidene fluoride (PVDF) and absorbable vicryl surgical layer were used, morbidity and mortality rates were higher compared to the PCL nanofiber layer[41,42].

In esophageal repair studies using poly-e-caprolactone (PCL) material, it has been reported that epithelial and smooth muscle cells can be observed within a postoperative month. Investigating the efficacy of random PCL and PCL-gelatin nanofibrous scaffold using human esophageal epithelial cells, Kuppan et

al. found that epithelial cells were completely covered with epithelial cells after showing rapid adhesion and spread on PCL and PCL-gelatin nanofibrous scaffolds. They reported that PCL-based scaffolds help epithelial cell adhesion, showing the characteristics of living and cobblestone, enabling these cells to grow and proliferate[35].

There is vivo model study in the literature that Senyucel et al. reported that local and sustained release of fibroblast growth factor (FGF) increased wound healing in esophageal anastomoses[18]. In this study, 24 male wistar-albino rats were used by dividing the animals into 3 groups. They performed abdominal esophageal resection and then end-to-end anastomosis to a 1 cm segment in all groups. In the control group, they performed a primary anastomosis, one group made an FGF-free gelatin film, the other group anastomosis supported with FGF and gelatin film. In all groups sacrificed on the postoperative 7th day, bursting pressures and histopathologically collagen deposition and tissue hydroxyproline concentrations in the anastomosis area were examined. They reported that local and continuous FGF release significantly increased burst pressure, and tissue OHP level in the anastomosis line, and the submucosal and muscle collagen concentration was higher than control groups. They showed that FGF provides remodeling by increasing fibroblastic proliferation and collagen maturation. They also reported an increase in epithelial healing. They observed that this epithelial cell restitution is highly advantageous in increasing water/airtightness in esophageal wound healing. They also suggested that fibroblast growth factor (FGF) may induce recovery by inducing angiogenesis induction and esophageal anastomosis, but more studies are needed to demonstrate the effect of neovascularization due to immunohistochemistry and capillary density. Although the increased collagen accumulation effect in the anastomosis region was seen to increase the healing effect in the short term, they could not give any results on whether it could lead to potential stenosis in the long term.

In our study, it was seen that until the 28th day when the rats were sacrified, no feeding difficulties were observed, and also no fibrosis was detected in the immunopathological examination. However, since our experimental animals are very small, there is no physical examination for possible stenosis. But, when the esophagus was catheterized and excised before sacrification, any stenosis was detected.

There is another vivo model study using epithelial growth factor (EGF) as a growth factor (GF) in esophageal repair. Adam et al. suggested that by applying a local single dose (100 pictogram) EGF to the anastomosis line, EGF can benefit from cell proliferation and migration of any epithelial cell during the esophageal wound healing process[43]. They did not find any difference in inflammatory scoring between the groups. However, they detected that collagen accumulation was increased in the esophageal layers in the group treated with EGF, which was sacrificed after 21 days, and EGF strengthened the anastomosis line. They reported that long-term effects of fibroblast growth factor (FGF)-gelatin film application with the local sustained release were not reported. Unlike the study of Senyucel et al., they reported that single-dose EGF application did not cause fibrosis on the 21st day of stenosis. Therefore, they reported that the application of the GF, which is planned to be applied to the esophagus anastomosis line, will not cause problems such as stenosis and leakage in esophageal wound healing.

The strength of our study that when the esophageal anastomosis was performed with fibroblast growth factor (FGF) loaded poly-e-caprolactone (PCL)-gelatin mesh, we not only showed that the esophagus healed with fibrosis in the 28-day long-term, but also that in the FGF unloaded PCL-gelatin group, PCL-gelatin played a role like ECM, supporting the esophageal anastomosis line and contributing positively to wound healing. Besides, according to the results obtained in our PCL-gelatin experimental Group unloaded FGF, we found that the PCL-gelatin bilayer mesh played an extracellular matrix (ECM) role in the esophageal anastomosis line and positively contributed to wound healing. According to epithelial growth factor (EGF), the effect of FGF on acute and chronic wound healing has been observed promising.

In conclusion, it has been proved that it is possible to strengthen the anastomotic line by repairing the esophageal defect using a double-layer mesh design based gelatin and poly ε-caprolactone (PCL) loaded fibroblast growth factor (FGF).

In our study, we experimentally evaluated the therapeutic effect of esophageal wound healing on the healing of esophageal anastomoses using of the bilayer mesh design based fibroblast growth factor (FGF) enhanced with gelatin and poly ε-caprolactone (PCL), in rat esophagus (Fig. 5). The results achieved showed that using of this two-layer mesh based FGF enhanced with gelatin and PCL in rate esophagus has made a difference between the 7th and 28th. It was observed that the decrease in inflammation started on the 7th day was resulted in complete decrease in inflammatory cells, significant increase in collagen formation and completion of epithelization on the 28th day.

It is mentioned that all available findings will be considered as a preliminary report. Finally, to achieve full scientific validity, using other research methods and advanced techniques (tube mesh with 3D software) and trying in larger animals such as pigs will fully clarify whether it is recommended for clinical use.

Declarations

Compliance with Ethical Statements

All authors declare that this manuscirpt is orginal, has not been published before and is not currently being considere for publication elsewhere. The authors declare no conflicts of interest. And there has been no significant financial support for this work that could have influenced its outcomes.

Conflict of Interest

The authors declare no conflicts of interest. And there has been no significant financial support for this work that could have influenced its outcomes.

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Informed Consent (Ethical Approval)

The care and use of laboratory animals is approved by the Animal Experiments Local Ethics Committee**,** Ankara Health Research Application Center, Health Sciences University, Ankara, Turkey (Ethical approval number: 17/09/2019-0055). According to the study, 34 male adult wistar-albino rats, weighing between 200 and 250 g, were in good health and had no diseases. The procedures performed in studies involving animals followed the ethical guidelines outlined in the Declaration of Helsinki from 1964.

Husnu Sakal Experimental and Practice Center, Ankara Training and Research Hospital, Health Sciences University, Ankara, Turkey provided the animals. All of the animals were addressed and used in accordance with the Animal Research: Reporting of in Vivo Experiments (ARRIVE) quality assessment criteria including ethical statement, study design, experimental procedure, experimental animals, housing and husbandry, simple size, allocating animals to experimental groups, experimental outcomes, statistical methods, baseline data, number of analysed, outcomes and estimation and adverse events.

Footnotes

Author Contributions

Conception and design of study, manuscript writing, coordination of the study, database management, data analysis, contribution to the concept: Ozkan Cesur, Tugba Endogan Tanir, Pinar Celepli, Fatma Ozarslan, Sema Hucumenoglu, Adnan Karaibrahimoglu, Nesrin Hasirci;

Acquisition of data: Pinar Celepli, Fatma Ozarslan, Sema Hucumenoglu, Adnan Karaibrahimoglu; Revising the manuscript critically for important intellectual content: Tugba Endogan Tanir, Nesrin Hasirci.

The authors approval of the final version of the manuscript to be published: Ozkan Cesur, Tugba Endogan Tanir, Pinar Celepli, Fatma Ozarslan, Sema Hucumenoglu, Adnan Karaibrahimoglu, Nesrin Hasirci.

Consent to Publish

Not applicable. This article does not contain any studies involving human participants.

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Tables

Table 1. Contents of bilayer meshes applied to animals

Bilayer Mesh	Content
FGF(-)	Form from gelatin film and PCL film as second layer (has no FGF). Films of 1x2 cm^2 were applied to rats.
FGF(+)	Contains 2.5µg FGF per cm^2 of gelatin film and, PCL film as second layer. Films of 1x2 cm^2 having 5µg FGF were applied to rats.

Table 2. Description of the histopathological scores of the semi-quantitative evaluation

Histological Parameter	Criteria	Score
Submucosal collagen deposition	None	0
	Mild (submucosal collagen<muscularis mucosa thickness x2)	+1
	Marked (submucosal collagen>muscularis mucosa thickness x2)	+2
Muscular layer collagen deposition	None	0
	Mild (collagen deposition around the smooth muscles	+1
	Marked (collagen deposition around the smooth muscles and replacement of muscles with collagen)	+2
Epithelialization	Thickness of cut edges	0
	Migration of epithelial cells	1
	Moderate	2
	Bridging of the excision complete regeneration	3
PMNL=Polymorphonuclear leukocyte	Minimum	0
	Mild	1
	Moderate	2
	Marked	3

Table 3. Results of the semi-quantitative histopathological evaluation and bursting pressure, presented by its mean and standard deviation (mean±sd)

Group	Submucosal Collagen Deposition	Muscular Layer Collagen Deposition	Epithelisation	PMNL	Bursting Pressure (mmHG)
Sham	0,85±069	1,14±0,37	0,42±0,53	2,85±0,37	20,84±2,98
FGF(-)-7th d	0,42±053	0,71±048	1,28±0,75	2,57±0,53	29,94±4,55
FGF(+)-7th d	1,6±06	1,75±0,46	2,12±0,83	1,25±0,46	53,52±1,85
FGF(+)-28th d	2±0*	2±0*	2,6±0,89*	0,6±0,54*	60,15±7,46*

M=Mean, SD=Standard-deviation, *Chi-suare test, significant at 0,05 level, (p<0.05)

Figures

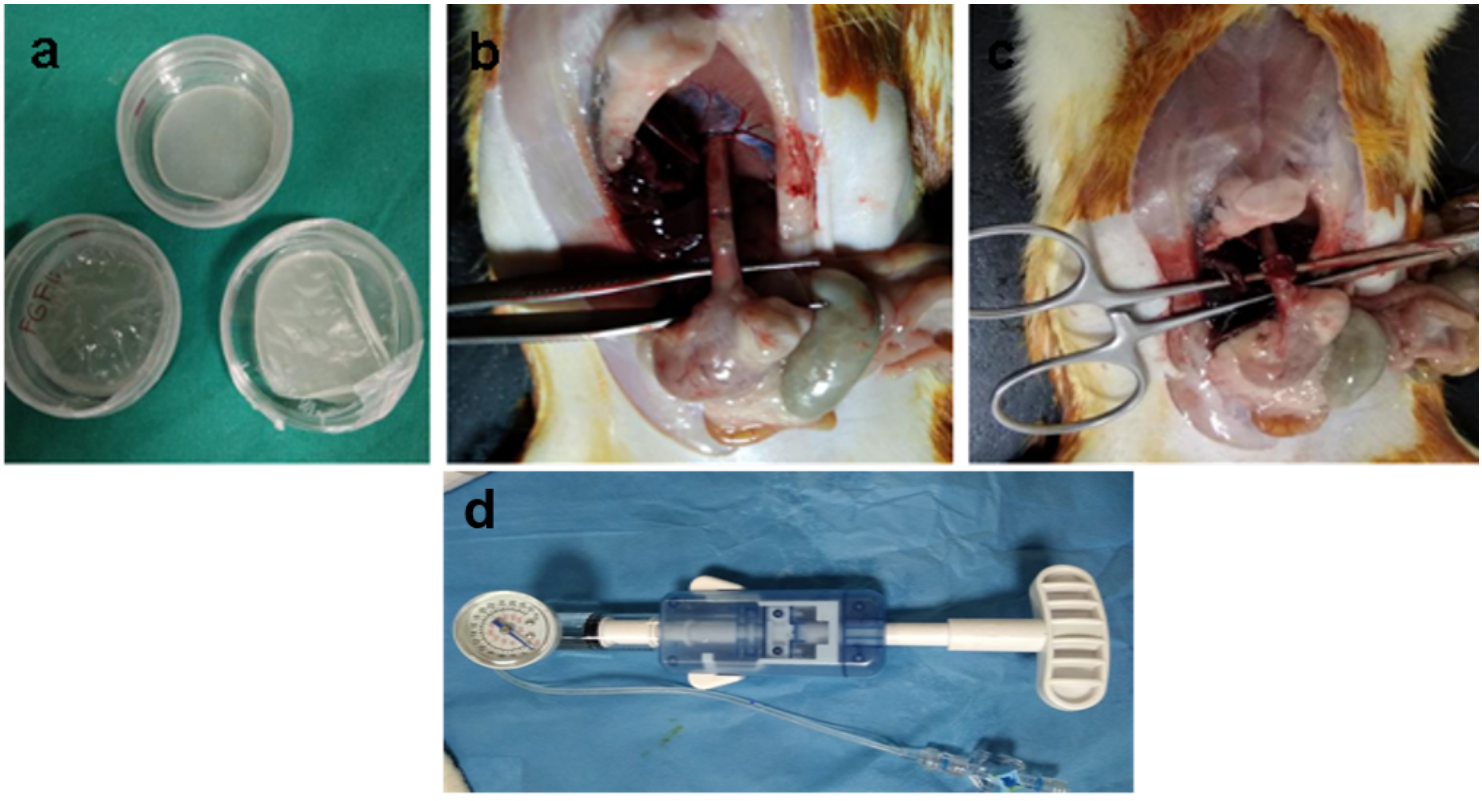

Figure 1

Design of study a) Bilayer meshes, b) Preparation of esophagial defect, c) Placement of bilayer meshes on the wound area, d) Syhngomanometer device

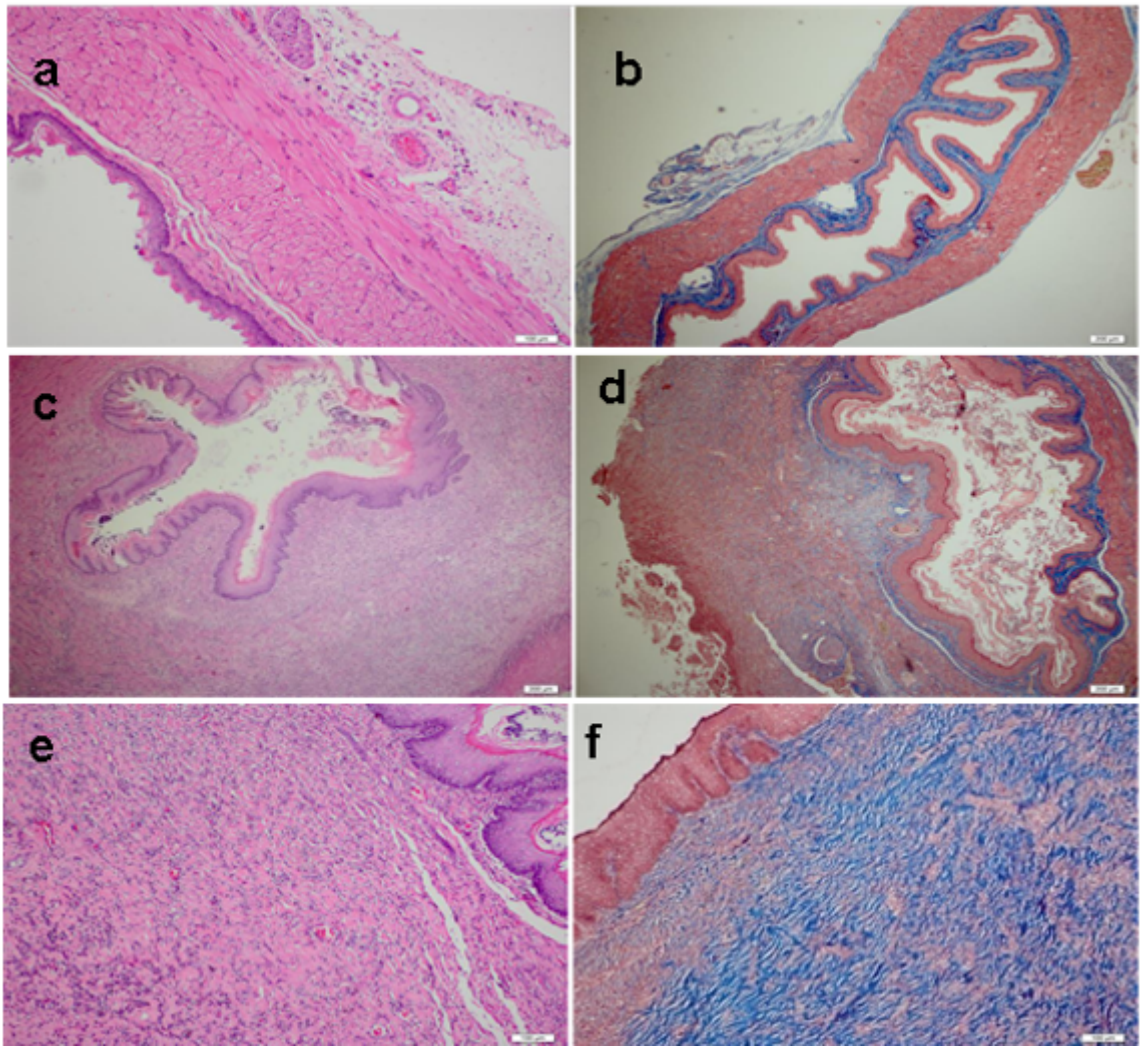

Figure 2

Mean histopathological pictogrammes of the groups studied. a and b Esophageal mucosa belonging to the control group. a) There is normal archetype preserved esophageal full-thickness mucosa sample containing lamina propria and muscularis propria consisting of compact connective tissue observed under the surface ceratinized squamous epithelium, in the Hematoxylin&Eosin (H&E), x100). b) There is no collagen increase in the submucosal and muscular layers, in the Mason Trichrome Stain, x100. c) and d) Esophageal mucosa belonging to the groups of sham and FGF (-). For the Groups of Sham and FGF (-) not received FGF, there are noteworthy epithelial degeneration, intense inflammation in the submucosa and muscular layer, and mildly increased collagen, in the Mason Trichrome Stain, x100 and Hematoxylin&Eosin (H&E), x100. e) and f) Esophageal mucosa belonging to the groups given FGF. e) In the surface epithelium, there is marked regeneration as well as the presence of significantly increased collagen, which is replaced by the submucosal and muscular layer. There is a moderate decrease in the inflammatory cell density observed between the collagen bundles on the 7th day and a significant decrease on the 28th day, in the Hematoxylin&Eosin (H&E), x100. f) There is significantly increased collagen (blue color) between the submucosal and muscular layers observed, in the Mason Trichrome Stain, x100

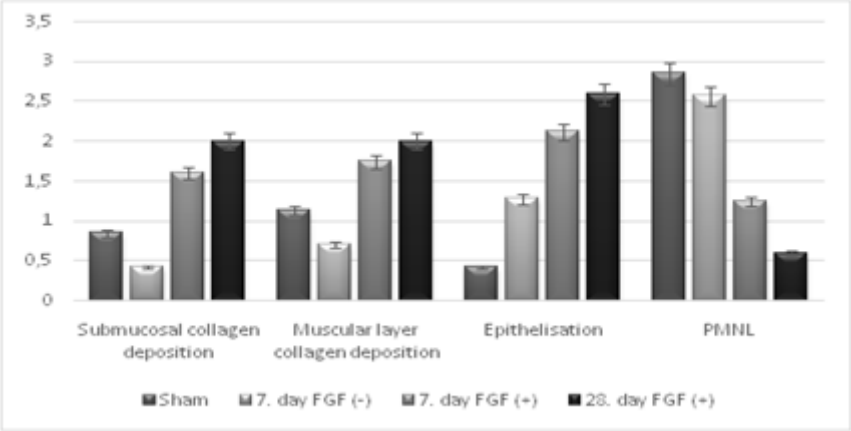

Figure 3

Mean histopathological scores according to groups

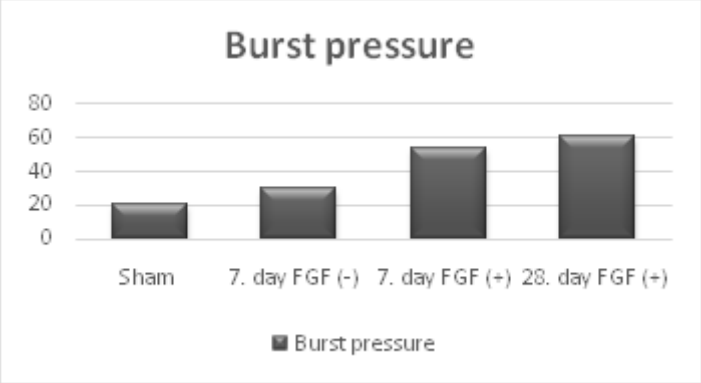

Figure 4

Differences of mean bursting pressure in the groups

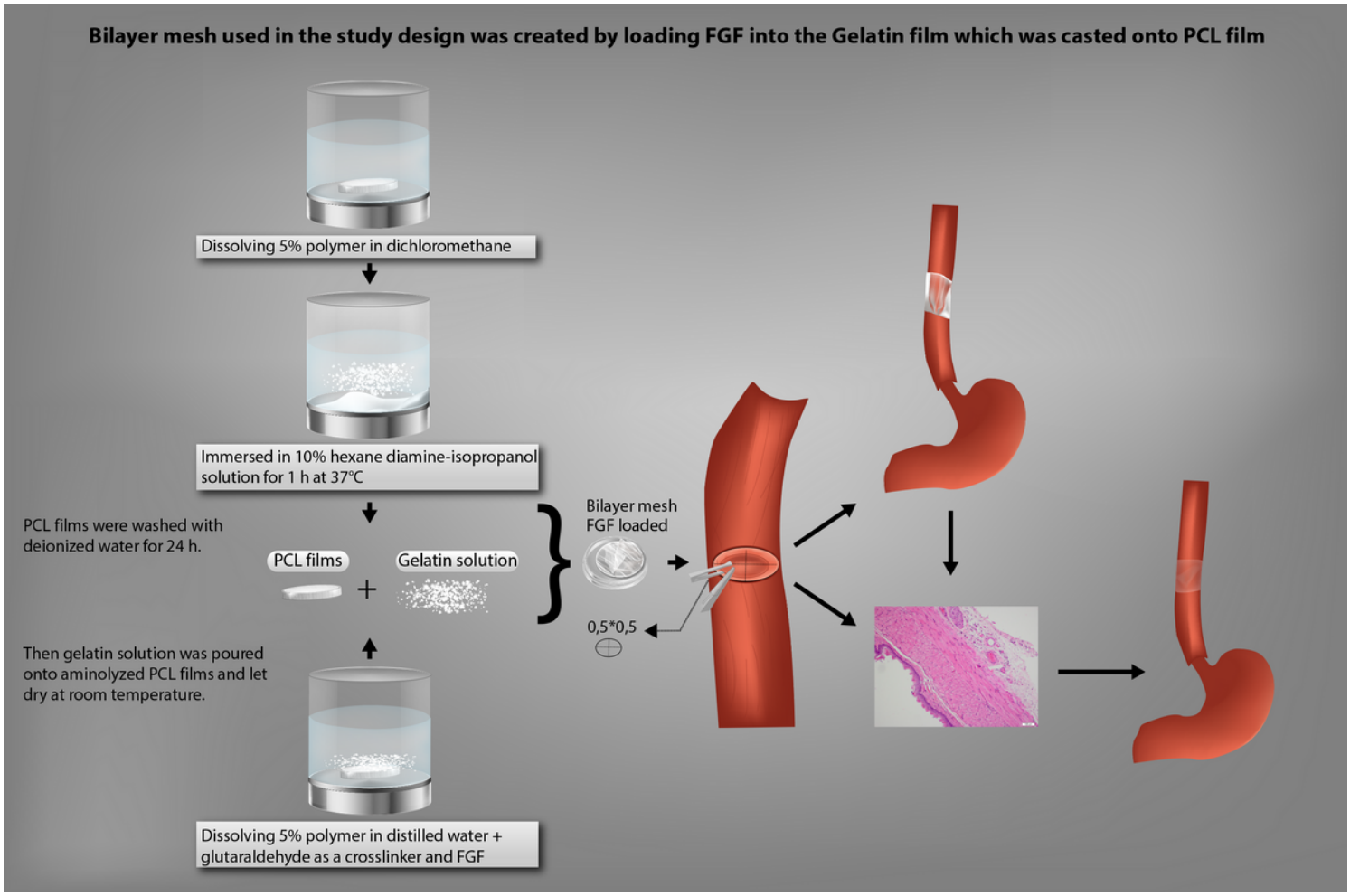

Figure 5

Bilayer mesh used in the study design was created by loading FGF into the Gelatin film which was casted onto PCL film Showing preparation of bilayer meshes; PCL films were prepared by dissolving 5% polymer in dichloromethane. Dried PCL films were immersed in 10% hexane diamine-isopropanol solution for 1 h at 37°C for aminolization. PCL films were washed with deionized water for 24 h. Gelatin solution was prepared by dissolving 5% polymer in distilled water and with addition of glutaraldehyde as crosslinker, and FGF. Gelatin solution was poured onto aminolyzed PCL films and let dry at room temperature. A full thickness semicircular defect of 0.5x0.5 cm2 was created via cutting in the anterior wall of the abdominal esophagus. The defect was repaired with primer anastomosis with the prepared bilayer mesh, using interrupted sutures.