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Use of polyglactin mesh coated with platelet-rich plasma for diaphragmatic defect healing in rabbits. Protocol for a Randomised Controlled Trial

Elissavet Anestiadou[1], Efstathios Kotidis[1], Theodosios Papavramidis[2], Dimitrios Tatsis[3], Chryssa Bekiari[4,5], Stavros Stamiris[6], Orestis Ioannidis[1], Alexandros Theodoridis[7], Ioanna Abba Deka[8], Stamatios Angelopoulos[1], Angeliki Cheva[8]

[1]4th Department of Surgery, General Hospital "George Papanikolaou", Aristotle University of Thessaloniki, Exochi, Greece
[2]1st Propaedeutic Department of Surgery, Medical School, Aristotle University of Thessaloniki, Greece
[3]Department of Oral and Maxillofacial Surgery, General Hospital "George Papanikolaou", Aristotle University of Thessaloniki, Exochi, Greece
[4]Experimental and Research Center, Papageorgiou General Hospital of Thessaloniki, Thessaloniki, Greece
[5]Laboratory of Anatomy and Histology, Veterinary School, Aristotle University of Thessaloniki, Thessaloniki, Greece
[6]Orthopaedic Department, 424 General Military Hospital, Ring Road West 56429, Nea Efkarpia, Thessaloniki, Greece
[7]Laboratory of Animal Production Economics, School of Veterinary Medicine, Aristotle University of Thessaloniki, Greece
[8]Pathology Department, Faculty of Medicine, Aristotle University of Thessaloniki, Thessaloniki Greece

ABSTRACT

Absorbable, biosynthetic scaffolds are a promising option for repair of diaphragmatic defects, since they are associated with less mesh-related complications than non-absorbable synthetic meshes. Coating of absorbable synthetic meshes with anti-inflammatory and healing agents, such as platelet-rich plasma (PRP), is a widely studied technique for improving mesh-tissue integration and reducing recurrence rate. The aim of this study is to investigate the synergic effect of a PRP-coated synthetic absorbable polyglactin mesh 2cmx2cm in healing of a diaphragmatic defect in an experimental rabbit model. Animals will be equally randomised in the PGM-PRP group (absorbable polyglactin mesh enriched with PRP) or the PGM group (use of absorbable polyglactin mesh only). Rabbits will undergo laparotomy under general anaesthesia and resection of a portion of the diaphragmatic central tendon 1cmx1cm. Samples will be euthanised on the 91th postoperative day and samples of 3cmx3cm will be harvested. The mesh-tissue integration will be assessed based on macroscopic, microscopic, immunohistochemical and morphometric parameters. The study is indesign stage and results

Corresponding author:
Elissavet Anestiadou
4th Department of Surgery, General Hospital
"George Papanikolaou", Aristotle University of Thessaloniki,
Exochi, Thessaloniki 57010, Greece
Tel.: +30 6980481017, e-mail: elissavetxatz@gmail.com

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are expected to be available by the end of the following year. High-quality evidence is expected regarding the synergic effect of PRP and synthetic absorbable polyglactin meshes in diaphragm repair.

Key Words: *Platelet-rich plasma; wound healing; mesh; diaphragm; synergistic effect; biomaterials; mesh integration; polyglactin mesh; mesh-augmented surgery*

BACKGROUND

Literature contains growing evidence regarding the role of minimally invasive surgery in management of diaphragmatic defects [1]. However, recurrence rates after laparoscopic hiatal hernia repair are variable, ranging from 1.2% to 66%, based on literature data [2]. The recurrence rate of hiatal hernia after laparoscopic approach is relatively high, ranging from 25 to 42% [3]. To address this need, reinforcement with prosthetic mesh in cases of large defects and/or poor tissue status has emerged as a promising strategy and a wide variety of implants have been proposed [4].

Synthetic meshes constitute a durable, cost-effective implant that has long been used for diaphragm reconstruction [5]. However, their use has been associated with a series of complications, including late dysphagia, encountered up to 13% of patients after mesh hiatoplasty, oesophageal stenosis, dense periesophageal fibrosis and adhesion formation, erosion of surrounding structures, infections and need for subsequent explantation. The aforementioned drawbacks lead to significant morbidity rates and often to the need for revisional fundoplication [6]. On the other hand, biological, absorbable implants induce limited inflammatory response and reduce postoperative adhesions. However, biological scaffolds are characterised by rapid absorption, high cost and possible immune reactions [7]. Absorbable, biosynthetic implants are hybrid meshes that provide the advantages of the aforementioned meshes while limiting the weaknesses regarding erosion, infection and adhesion formation. Their mechanism is based on the presence of a substrate for dense fibrous tissue formation through cellular infiltration and vascularisation, ingrowth of native tissue, and collagen deposition. As a result, new-formed tissue acquires mechanical strength after meshes complete absorption due to hydrolysis or enzymatic reactions [4]. However, the long-term safety and effectiveness of absorbable synthetic meshes are still debated [8].

Wound healing is a four-stage process, including haemostasis, inflammatory phase, proliferative phase, and remodeling phase in a consecutive fashion. Numerous agents have been used in the experimental stage to regulate the aforementioned process aiming at better wound healing outcome [9]. Among them is also platelet-rich plasma (PRP), a blood product with platelet concentration 3-5 times higher than the normal count, which is obtained through centrifugation of autologous blood samples. Various uses of PRP have been reported since the 1990s in regenerative medicine. In addition, numerous studies have addressed the effect of PRP on coating meshes, leading to improved mesh incorporation, increased neovascularisation, and decreased recurrence and adhesion rate [10]. However, applications of autologous PRP have been scarcely studied in diaphragmatic defect repair, both as an adjuvant after cruroplasty and as a mesh-coating agent [4].

This protocol is designed to investigate the changes in tissue formation after absorption of polyglactin mesh coated with PRP in an experimental rabbit model.

MATERIALS AND METHODS

Study Design and Population

The present study is a randomised controlled animal trial (RCT) that aims to investigate the effect of PRP-coated synthetic absorbable polyglactin mesh in mesh-tissue integration, after implantation in a central tendon defect of the diaphragm in rabbits. The study process flowchart is presented in Figure 1. The study protocol is prepared according to the ARRIVE guidelines 2.0 (Animal Research Reporting In Vivo Experiments) for reporting an animal study [11]. Thirty white male New Zealand adult rabbits, aging 3-6 months old and weighing 2-2.5 kg, will be used. This particular animal model has already been used for similar studies, since it presents significant similarities with human anatomy [12]. Rabbits will be randomised into two groups by a computer algorithm (Google computer random number generator). An overview of the experimental protocol is presented in Figure 2.

New Zealand adult rabbits included in the study originate from an authorised provider. The present study will be held at the Laboratory of Anatomy, Histology and Embryology of Domestic Animals of the School of Veterinary Medicine, in Aristotle University of Thessaloniki, an accredited facility for experimentations in small and large animals (accreditation numbers EL-54-BIOexp-23 and EL-54-BIOexp-24, respectively). Animals will be single-housed in appropriate

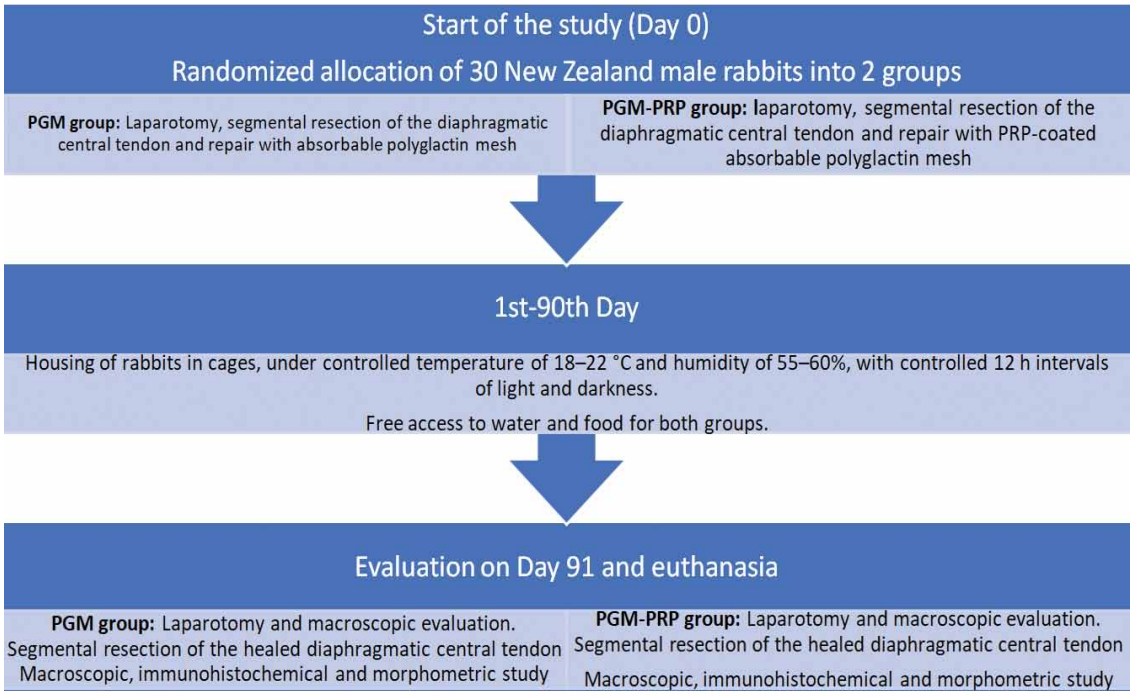

FIGURE 1. Study process flowchart.

cages, under a controlled temperature of 22-24 °C and humidity of 55–60%, with controlled 12 h intervals of light and darkness and free access to water and food.

All experimental procedures will be performed by an academic general surgeon with technical expertise on experimental surgery, as well as long experience regarding diaphragm surgery in experimental models. Anaesthesia, analgesia management and euthanasia will be performed

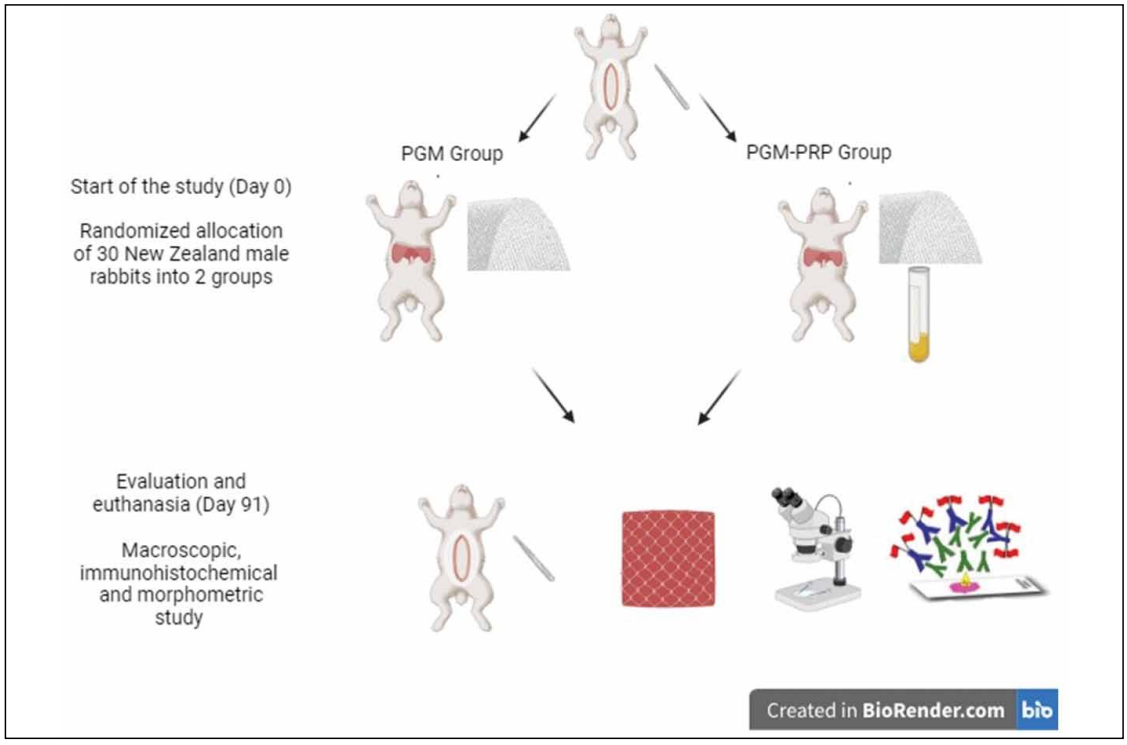

FIGURE 2. An overview of the experimental design. This figure was created with BioRender.com.

by an experienced veterinarian. An experienced pathologist was responsible for evaluating the slides.

PRP preparation

Three New Zealand rabbits were used for the isolation of PRP, since their highly inbred nature allows for pooling of donor blood. After anaesthesia induction, 5 ml of blood was received via ear central vein aspiration and mixed with 3.8% sodium as anticoagulant. This results in a lower fraction, the blood cell component (BCC), and in an upper fraction, which is called the serum component (SEC). Centrifugation of the SEC and the upper 6 to 8 mm of BCC at 2000 rpm for 5 minutes, and removal of the top platelet poor plasma results in PRP isolation [13]. A platelet count was performed before and after PRP preparation to ensure that the platelet count from each animal was standardised.

Mesh Implantation

Rabbits will undergo midline laparotomy under general anaesthesia and aseptic conditions and a full thickness part of the tendon center of the diaphragm, 1 cmx1 cm will be resected. The defect will be repaired with absorbable polyglactin mesh (VICRYL™, Ethicon) of 2cmx2cm, while in PGM-PRP group, 200 μl of activated PRP will be applied directly to the abdominal surface of the mesh after implantation. Mesh fixation will be performed using a 5mm overlap on each side using polypropylene 4-0 (PROLENE™ Ethicon) sutures.

Euthanasia and tissue sampling

On the 91th postoperative day, a re-laparotomy and resection of a specimen of 3cm x 3cm, including the part of the healed defect will be performed. Rabbits will be euthanised on the 91th postoperative day by high-dose intravenous sodium pentobarbital, following all relevant bioethical rules. Selection of euthanasia day was made because polyglactin mesh is totally resorbed after 60 to 90 days in vivo [14].

Macroscopic examination

Macroscopic examination of the implanted area will be performed, and presence of infection, hernia recurrence, adhesions, purulent discharge, hematoma, oedema or necrosis will be recorded.

Histologic and Immunohistochemistry Analysis

After fixed in 4% formalin, specimen will be examined for the following parameters:

Inflammatory response (number of white blood cells, and especially lymphocytes, macrophages and plasma cells quantitatively and based on a scale from 0 to 4): records will be made according to Ehrlich/Hunt scale (Philips modification) [15].

Fibroblast and collagen production: the recording will be made according to Ehrlich/Hunt scale (Philips modification) quantitatively with a scale from 0 to 4 as follows: 0=no evidence, 1=occasional evidence, 2=slightly increased, 3=frequently, 4=confluent cells or fibers) [15].

Neoangiogenesis: CD31 antibodies (WM59; Genetex) will be used to detect CD31 levels in tissue samples.

Expression of VEGF and TGFb3 growth factors, by immunohistochemical staining.

Morphometric study

Evaluation of the maximum diameter and maximum thickness of the implanted sections among two groups, as well as comparison with adjacent healthy tissue will be performed. Images will be processed using the ImageJ program (ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA).

Statistical Analysis

The confidence interval will be set at 95% which means that differences between groups will be considered statistically significant when $p<0.05$. The statistical analysis of the results will be performed using the statistical program Jamovi Version 1. 2. 27.0. Based on PowerAnalysis performed, each group should include 13 rabbits. After calculating a dropout rate of 15% for each group, 15 rabbits will be included in each study group.

DISCUSSION

PRP is a universally available, low-cost, autologous product containing numerous growth factors and chemokines that enhance tissue wound repair by promoting the proliferative wound healing phase, such as platelet-derived growth factor (PDGF), epidermal growth factor (EGF), transforming growth factor (TGF), insulin growth factor 1 and 2 (IGF-1, IGF-2), vascular endothelial growth factor (VEGF), interleukins, and others [16]. Main mechanisms of PRP action on wound healing include decrease of inflammatory response , increase of collagen deposition, promotion of wound contraction, myofibroblast recruitment and increased neovascularisation [17]. Literature contains various reports regarding use of PRP in reconstructive surgery, urogynaecology, orthopaedic surgery [18] and hernia repair surgery [17]. In addition, PRP holds an emerging role in mesh-augmented surgery,

with reports of combined use with propylene meshes and biological scaffolds. Moure et al conclude that PRP coating of cadaveric acellular dermal matrices decreases inflammatory response and leads to increased material stiffness [17]. Similar results presented in an experimental study of Parizzi et al, with PRP enrichment of polypropylene mesh leading to lower inflammatory infiltrate count and increased deposition of collagen III [19]. However, literature lacks trials that investigate the role of combined use of PRP and mesh in diaphragmatic defect repairs.

Our study will investigate the impact of autologous PRP gel application on mesh-tissue integration of a synthetic absorbable polyglactin mesh for diaphragmatic defect repair in a rabbit model. We have hypothesised that PRP application will lead to lower inflammatory response, higher angiogenesis rate, and collagen deposition. It is expected that group treated with PRP-coated mesh will present lower inflammatory response, higher levels of angiogenesis, and increased collagen deposition. Similar studies have compared the use of PRP vs the absorbable Bio-AVR mesh in cruroplasty reinforcement in a laparoscopic experimental porcine model. Boru et al concluded that, although there was no statistically significant difference between the two groups in semiquantitative histological scoring of tissue reaction, PRP represents a promising co-adjuvant to local remodelling and healing in hiatal hernia repair [4]. In addition, Altieri and colleagues analysed three groups of pigs with an iatrogenic hiatal defect, repaired laparoscopically by hiatus repair alone, hiatus repair with acellular dermal matrix and hiatus repair with acellular dermal matrix infiltrated with plasma concentrate (fPC), respectively. Outcomes revealed increased collagen deposition, neovascularisation and mechanical strength with fPC-mesh combination [20]. However, previous studies did not describe the combined use of absorbable synthetic meses with PRP for diaphragm repair. Our study, thus, will be the first to perform PRP coating of polyglactin mesh for diaphragmatic defect repair.

CONCLUSION AND STUDY SIGNIFICANCE

Our study will investigate the effect of a PRP-coated polyglactin mesh on wound remodeling and tissue integration in diaphragmatic deficits. PRP is expected to act as an anti-inflammatory and wound healing accelerator that will improve mesh-tissue integration through growth-factor release. The results of our study will provide a better understanding on the underlying regulatory role of PRP on mesh-tissue integration.

The present study could build a foundation of knowledge for further investigation regarding the use of both PRP and mesh implants in clinical practice, since the synergic effect of PRP and mesh implants has been scarcely examined. More specifically, confirmation of the beneficial role of PRP-enhanced polyglactin meshes in diaphragm repair could extend its use, especially in challenging cases of large defects or recurrences management.

REFERENCES

1. Thoman DS, Hui T, Phillips EH. Laparoscopic diaphragmatic hernia repair. Surg Endosc. 2002 Sep;16(9):1345-9.
2. Braghetto I, Lanzarini E, Musleh M, Korn O, Lasnibat JP. Thinking about hiatal hernia recurrence after laparoscopic repair: When should it be considered a true recurrence? A different point of view. Int Surg. 2018 Jan-Feb;103(1-2):105-15.
3. Akmaz B, Hameleers A, Boerma EG, Vliegen RFA, Greve JWM, Meesters B, et al. Hiatal hernia recurrences after laparoscopic surgery: exploring the optimal technique. Surg Endosc. 2023 Jun;37(6):4431-42.
4. Boru CE, Manolescu N, Ulmeanu DI, Copca N, Constantinica V, Copaescu C, et al. Platelet-rich plasma PRP vs. absorbable mesh as cruroplasty reinforcement: a study on an animal model. Minim Invasive Ther Allied Technol. 2022 Feb;31(2):252-61.
5. Al-Nouri O, Hartman B, Freedman R, Thomas C, Esposito T. Diaphragmatic rupture: Is management with biological mesh feasible? Int J Surg Case Rep. 2012;3(8):349-53.
6. Antoniou SA, Pointner R, Granderath F-A, Köckerling F. The Use of Biological Meshes in Diaphragmatic Defects - An Evidence-Based Review of the Literature. Front Surg. 2015 Oct;2:56.
7. Wang See C, Kim T, Zhu D. Hernia mesh and hernia repair: A Review. Eng Regen. 2020;1:19-33.
8. Asti E, Sironi A, Bonitta G, Lovece A, Milito P, Bonavina L. Crura augmentation with Bio-A(®) mesh for laparoscopic repair of hiatal hernia: Single-institution experience with 100 consecutive patients. Hernia. 2017 Aug;21(4):623-8.
9. Tatsis D, Vasalou V, Kotidis E, Anestiadou E, Grivas I, Cheva A, et al. The combined use of platelet-rich plasma and adipose-derived mesenchymal stem cells promotes healing. A Review of Experimental Models and Future Perspectives. Biomolecules. 2021 Sep;11(10):1403.
10. Belebecha V, Casagrande R, Urbano MR, Crespigio J, Martinez RM, Vale DL, et al. Effect of the platelet-rich plasma covering of polypropylene mesh on oxidative stress, inflammation, and adhesions. Int Urogynecol J. 2020 Jan;31(1):139-47.
11. Percie du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M, et al. The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. PLoS Biol [Internet]. 2020 Jul;18(7):e3000410. Available from: https://pubmed.ncbi.nlm.nih.gov/32663219/ doi:10.1371/journal.pbio.3000410
12. Böhm G, Binnebösel M, Krähling E, Schumpelick V, Steinau G, Stanzel S, et al. Influence of the elasticity module of synthetic and natural polymeric tissue substitutes on the mobility of the diaphragm and healing process in a

rabbit model. J Biomater Appl. 2011 May;25(8):771-93.
13. Manafi A, Kaviani Far K, Moradi M, Manafi A, Manafi F. Effects of platelet-rich plasma on cartilage grafts in rabbits as an animal model. World J Plast Surg. 2012 Jul;1(2):91-8.
14. Pendegrass CJ, Oddy MJ, Sundar S, Cannon SR, Goodship AE, Blunn GW. The novel use of resorbable Vicryl mesh for in vivo tendon reconstruction to a metal prosthesis. J Bone Jt Surg Br Vol. 2006 Sep;88-B(9):1245-51.
15. Tallón-Aguilar L, Lopez-Bernal F, Muntané J, García-Martínez J, Castillo-Sanchez E, Padillo FJ. The use of tachosil as sealant in an experimental model of colonic perforation. Surg Innov. 2015 Feb;22(1):54-60.
16. Van Eps J, Fernandez-Moure J, Cabrera F, Wang X, Karim A, Corradetti B, et al. Decreased hernia recurrence using autologous platelet-rich plasma (PRP) with StratticeTM mesh in a rodent ventral hernia model. Surg Endosc. 2016 Aug;30(8):3239-49.
17. Fernandez-Moure JS, Van Eps JL, Scherba JC, Yazdi IK, Robbins A, Cabrera F, et al. Addition of platelet-rich plasma supports immune modulation and improved mechanical integrity in Alloderm mesh for ventral hernia repair in a rat model. J Tissue Eng Regen Med. 2021 Jan;15(1):3-13.
18. Nazaroff J, Oyadomari S, Brown N, Wang D. Reporting in clinical studies on platelet-rich plasma therapy among all medical specialties: A systematic review of Level I and II studies. PLoS One. 2021 Apr;16(4):e0250007.
19. Parizzi NG, Rubini OÁ, Almeida SHM de, Ireno LC, Tashiro RM, Carvalho VHT de. Effect of platelet-rich plasma on polypropylene meshes implanted in the rabbit vagina: histological analysis. Int Braz J Urol. 2017 Jul-Aug;43(4):746-52.
20. Altieri MS, Pagnotti G, Corthals A, Shroyer K, Pryor AD, Talamini M, et al. Autologous augmentation of hiatal hernia repair with filtered platelet concentrate improves tissue remodeling in a swine model. Surg Endosc. 2017 Apr;31(4):1591-8.