RECONSTRUCTION OF THE ABDOMINAL WALL DEFECTS USING GELATIN-COATED DECELLULARIZED AND LYOPHILIZED HUMAN AMNIOTIC MEMBRANE

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Ventral hernias, with 50% reoccurrence rate, still remain to be a real problem for many surgeons around the world. European Hernia Society (EHS) classifies ventral hernias as primary and incisional [1]. Primary ventral hernias include epigastric, umbilical, lumbar and spigelian regions. Incisional hernias, which include suprabubic, iliac, suprapubic sites on the abdominal wall, may be caused by obstetrical surgical procedures, trauma, surgical interventions and operations for other indications. If left untreated, incisional hernias may cause the reduction in the strength and integrity of the anterior abdominal wall, as well as the incarceration of the intestines [2]. It is reported that the use of mesh in the repair of abdominal wall defects reduces the incidence of reherniation; however, the dispute between surgeons still exist about the ventral hernia defect reconstruction approach and the selection of the most suitable mesh type in different circumstances [3-8]. The development of meshes has evolved and advanced through the years. Meshes can be made from either synthetic or biologic materials [9,10]. Despite the popularity of non-absorbable mesh (For example Teflon, Dacron, Polypropylene, Marlex), its application may lead to certain complications like - adhesions, seroma formation, infection, chronic inflammation, fibrosis, voiding difficulty, pain [11-13]. The usage of absorbable mesh (polyglactin, polyglicolic acid) may have several drawbacks like - lack of mesh strength, high recurrence rates [14,15]. Postoperative complications following abdominal wall hernia repair with prosthetic mesh may include abscess, hematoma, bowel obstruction, mesh retraction, granuloma formation and erosion into adjacent structures including the intestine, enterocutaneous fistula and recurrent hernia. However, these complications are quite rare and depend both on the material of which the mesh is constructed and on the location of the prosthetic mesh, which can be located in the extrafascial, subfascial, or intraperitoneal position. Biological materials, compared to synthetic ones provide better neovascularization, fibroblast proliferation, is less prone to formation of fistula and adhesion formation [10,14,16]. Despite the favorable outcomes of the biologic materials, after the application of biological prostheses several complications like infection, seroma formation, and evisceration, low mechanical strength of the mesh can also be reported [9,17,18].

The hypothesis for this study was that gelatin-coated decellularized and lyophilized human amniotic membrane grafts (GCDLHAM) may contribute to the effective reconstruction of the abdominal wall defects, prevent complications, as well as adhesions of organs and tissues in the abdominal cavity. The aim of the study was to develop a method for producing GCDLHAM graft and to determine its effectiveness in the reconstruction of the anterior abdominal wall defects in rats.

Material and methods. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of the Institutional Animal Care Committee. The protocol #358 was approved by the Committee on the Ethics of the Tbilisi State Medical University in Tbilisi, Georgia.

Experiments were conducted on 40 Lewis white laboratory rats aged 8–10 weeks, weighing 200–250g, which were obtained from the breeding facility of the Tbilisi State Medical University (Georgia). The animals were housed in standard laboratory conditions under 12-hour day-night cycles with provision of pelleted rodent diet and water ad libitum.

All surgical procedures were conducted under anesthesia with 0.1 ml / 100g of ketamine (Ketalar ®) and 0.05 ml / 100g of xylazine (Xilazin ®), intraperitoneally.

Preparation of decellularized and lyophilized human amniotic membrane. Before the fabrication procedure of biological membrane from human chorion amnion, five placentas were obtained from patients who delivered newborn babies ranging from 38 to 42 weeks of gestation. These donors signed a form of informed consent in advance before giving birth. All patients have undergone adequate pregnancy period and the newborns were delivered healthy with normal weights varying from 2700 to 3700 grams.

The process of decellularization was conducted according to the reports mentioned by Z. Kakabadze et al [19-22]. Upon delivering the placenta to the laboratory, the catheterization of placental umbilical vein and artery was performed via polyethylene catheters which were attached to the vessels with the help of sutures. After insertion and fixation of catheters 0,9% saline solution and heparin were used to irrigate placenta under physiological pressure at 37°C in order to avoid clotting of blood during drainage. After irrigation, placentas were placed in the refrigerator at -80°C for 24 hours and then thawed at room temperature. Then, the placenta was being flushed overnight with Phosphate Buffered Saline (PBS, Sigma) solution via the catheter in the umbilical artery. Afterwards, the process of 72 hours decellularization was performed. In the first 24 hours, placentas were flushed with the mixture of Sodium Dodecyl Sulfate (SDS, Sigma) and distilled water with the SDS concentration of 0,01%. For the following 24 hours the perfusion was performed with the SDS concentration of 0,1% and ultimately, with 1% SDS for the last 24 hours. Finally, in order to free the placenta from the SDS residues, placentas were washed with distilled water for fifteen minutes and afterwards, with 1% Triton X-100 (Sigma) solution for 30 minutes. Decellularized chorion amnion was then irrigated for 1 hour via Phosphate Buffered Saline (PBS) solution. After all the steps of decellularization, amniotic membranes were isolated from placenta, were cut into 5x5 cm pieces and ultimately, fixated on glass frames. Power Dry PL 6,000 Freeze Dryers were used for the lyophilization of these grafts. Until use, decellelurized and lyophilized amniotic membranes (Fig. 1) were kept in aseptic conditions at room temperature.

Creation of gelatin-coated decellularized and lyophilized human amniotic membrane grafts (GCDLHAM). The GCDLHAM was prepared through the chemical cross-linking of gelatin solution with glutaraldehyde according to the method described previously [23,24]. For this, the DLHAM was immersed into a mixed solution of gelatin (5.0%) and glutaraldehyde (0.1%), left at 4ºC for 15min (repeated three times), and then left at 4ºC for 12h. Afterwards, GCDLHAM was placed in 100mM glycine aqueous solution at 37ºC for 1h, and then washed three times with double-distilled water. Finally, the GCDLHAM was freeze-dried and sterilized with ethylene oxide gas, stored at -80ºC, and thawed as needed.

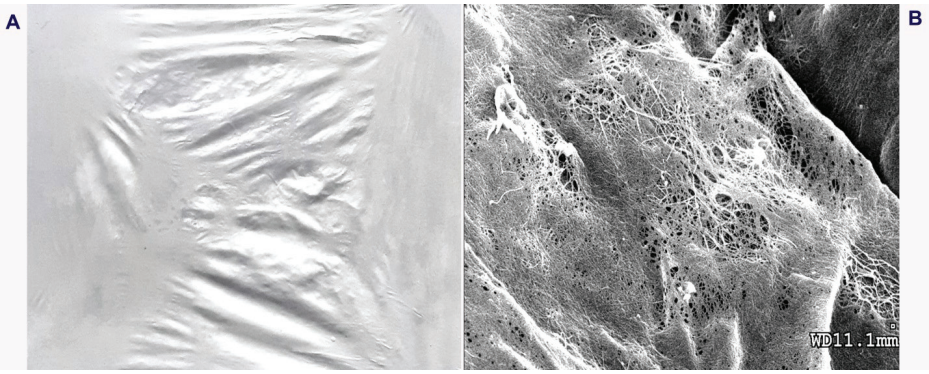

Fig. 1. Human amniotic membrane graft. A) Human amniotic membrane after decellulalization and lyophilization; B) Scanning electronic microscopy of decellularized and lyophilized human amniotic membrane

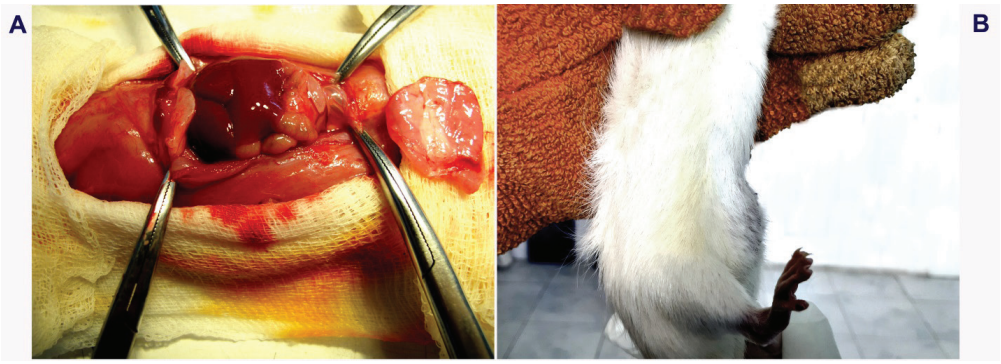

Fig. 2. The creation of abdominal wall defects in rats. A) Defect of the abdominal wall created in the mesogastric region; B) Three weeks after the creation of the anterior abdominal wall defect model

Surgical procedures. The creation of abdominal wall defects in rats. After anesthesia, defect of the abdominal wall was created in the mesogastric region in all animals, through the resection of a 1.0 cm diameter fragment of muscle-aponeurotic layer and the parietal peritoneum (Fig. 2A). Three weeks after the creation of the anterior abdominal wall defect model (Fig. 2B). Reconstruction was performed in all experimental animals.

Reconstruction of the abdominal wall. Animals were divided into four equivalent groups. In first group (n=10), the defects of the abdominal wall were repaired using ULTRAPRO™ mesh placed in intra-abdominal position. In second group (n=10), defects of the abdominal wall were reconstructed with ULTRAPRO™ mesh located in intra-abdominal position which was covered by DLHAM from both sides. In third group (n=10), defects of the abdominal wall were reconstructed with biological mesh from GCDLHAM placed in intra-abdominal position. In fourth group (n=10), defects of the abdominal wall were repaired with biological surgical mesh XI-S+® (Colorado Therapeutics Denver,USA) placed in intra-abdominal position. XI-S+® represents a product derived from xenogenic (porcine) pericardium that goes through cross-linking procedure which is produced by Colorado Therapeutics providing biocompatibility, durability of the material and consists of significantly low DNA and glutaraldehyde (GA) residuals.

All implants were fixed to the edges of the defect of the abdominal wall with the help of 7/0 monofilament polypropylene sutures (Prolene®, Ethicon). Further, the skin and subcutaneous fatty tissue were sutured tightly using 4/0 monofilament polypropylene sutures (Prolene®, Ethicon).

After surgical operations, all animals were kept under standard vivarium conditions. The animals were taken out of the experiment on 3rd, 5th, 7th, 14th, 30th, 60th and 90 days after surgery by an intra peritoneal injection of a lethal dose of a 0.5% sodium thiopental solution.

During autopsy, the abdominal cavity was subjected to a U-shaped laparotomy around the sides and bottom edges of the prosthesis. The abdominal cavity was macroscopically inspected and the presence of suture dehiscence, the occurrence and quality of adhesions, fistulas and intra-abdominal complications were determined.

The transplanted mesh fragments with surrounding abdominal tissue were removed and fixed in 10% formalin and subjected to histological preparation, with dehydration in alcohol and xylene, and embedded in paraffin blocks. Histological samples were made on microtome and slides were prepared with standard hematoxylin and eosin (H/E), Masson's Trichrome stains. These slides were submitted to pathological examination to verify the type and degree of inflammation, inflammatory cells, fibroblasts, collagen, and neovascularization in the regions.

Results and discussion. In the first group, on the twentieth day after implantation, one case of skin suture stratification was observed. In other cases, skin wounds were successfully closed without any macroscopic signs of inflammatory and infectious processes in the soft tissues of animals (Fig. 3).

Three months after implantation, in the animals of the second group, we observed adhesions involving only the omentum, which were easily separated. In the animals of the first group, the adhesions between the implant, omentum and intestines were denser and stronger (Fig. 4 A-B). In order to free the intestines and omentum from adhesions, they had to be dissected. One case of mesh retraction was observed in the animal of the fourth group (Fig. 4C). It should be noted that animals of the first

group had more newly formed blood vessels (Fig. 4D) compared to other groups of animals. The animals treated with GCDL-HAM and XI-S+® grafts had nearly 100% adhesion reduction, compared to the animals of the first group that were treated with ULTRAPRO™ mesh.

Two weeks after implantation, histological studies showed inflammatory cell infiltrations in all groups (Fig. 5 A-H). Significant infiltrations of the inflammatory cells were mainly expressed in the first and second groups. Three weeks later, in animals of the second and third group, the onset of remodeling processes were noted, which consisted of a gradual degradation of the amniotic membrane, the formation of new blood vessels and the deposition of new collagen. A month after implantation, inflammatory reactions gradually decreased in the animals of the first group and was completely absent in other animal groups. At the same time, in the animals of the second and third group, a large number of ordered collagen fibers were observed that were incorporated in the host tissue (Fig. 5 I-L). Three months after implantation, GCDLHAM graft was integrated with the host tissues so that it was difficult to distinguish it from the surrounding tissues. In the second group, ULTRAPRO™ mesh was still detectable through the decellularized amniotic membrane. In animals of the fourth group, the XI-S+® graft was surrounded by a well-defined connective tissue capsule and was tightly fixed to the host tissues.

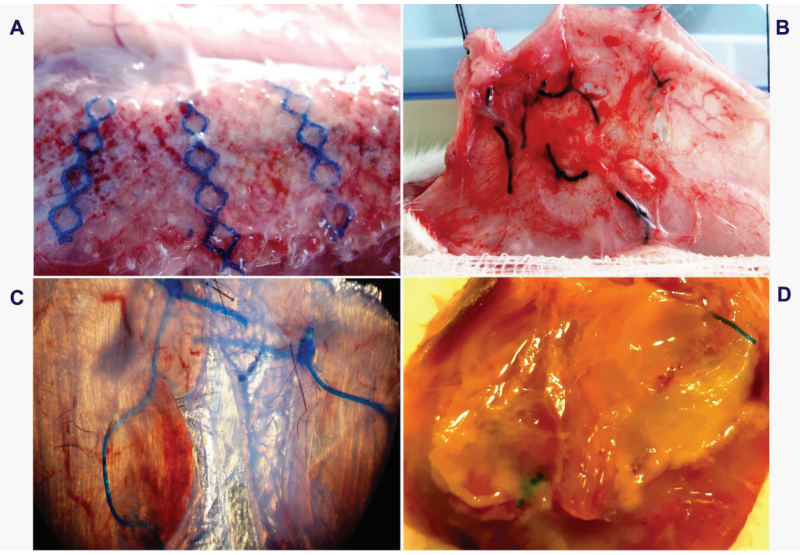

Fig. 3. Macroscopic samples. A) ULTRAPRO™; B) ULTRAPRO™ mesh covered by decellularized and lyophilized human amniotic membrane; C) Gelatin-coated decellularized and lyophilized human amniotic membrane; D) Biological surgical mesh XI-S+®. All grafts are surrounded by host tissues. Three weeks after implantation

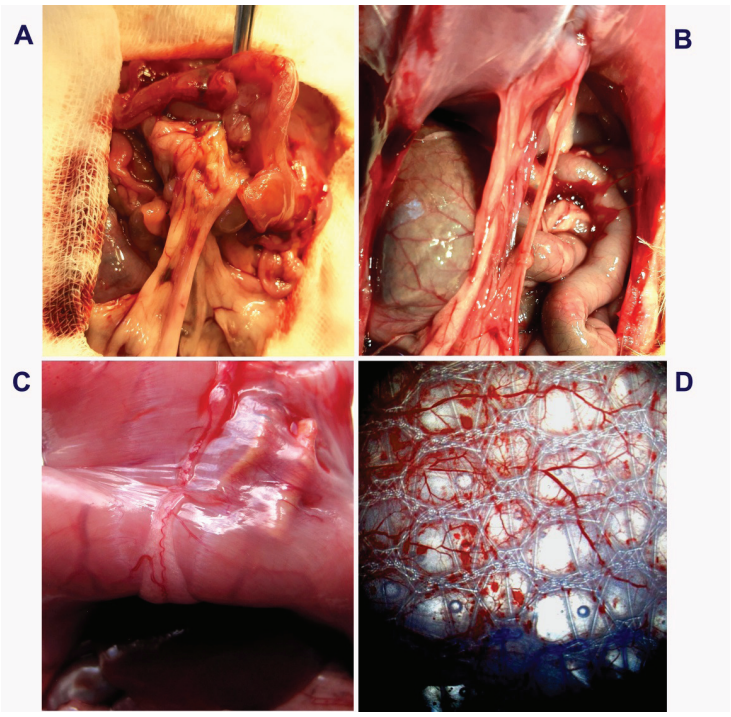

Fig. 4. Postoperative findings. A) Adhesions between the implant, omentum and intestines in the animals of the first group; B) Adhesions involving only the omentum in the animals of the second group. tC) Mesh retraction in the animal of the fourth group D) Newly formed blood vessels in the animals of the first group

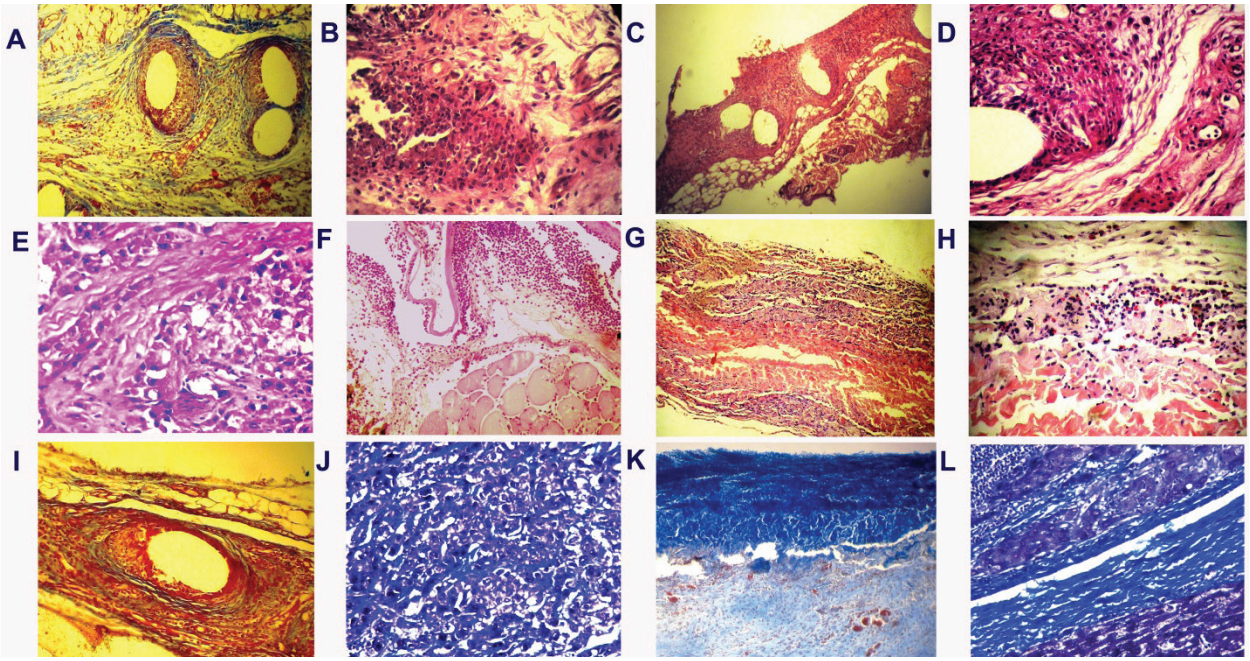

Fig. 5. Evaluation of histological images of the grafts. A and B) ULTRAPRO™; HE staining, one month, ×400 ; C and D) ULTRAPRO™ mesh covered by decellularized and lyophilized human amniotic membrane; HE staining, one month, ×200/400; E and F) Gelatin-coated decellularized and lyophilized human amniotic membrane; HE staining, one month, × 400/200; G and H) Biological surgical mesh XI-S+®; HE staining, one month, ×200/400; I) ULTRAPRO™; Masson's Trichrome staining, one month, ×200; J) ULTRAPRO™ mesh covered by decellularized and lyophilized human amniotic membrane; Masson's Trichrome staining, one month, ×400; K) Gelatin-coated decellularized and lyophilized human amniotic membrane; Masson's Trichrome staining, one month, ×200; L) Biological surgical mesh XI-S+®; Masson's Trichrome staining, one month, ×400

One of the main strategies of tissue engineering is to restore, maintain or improve damaged tissue functions using various biomaterials. In recent years, many works related to the development of potentially applicable scaffold materials for tissue engineering have been presented in the literature. Of particular interest in these works was scaffolding in the form of three-dimensional porous biomaterials. Scaffold plays a significant role in tissue repair and regeneration.

The amniotic membrane and the possibility of its use as a scaffold for reconstruction of the anterior abdominal wall attracted our attention. There are many reports about the usage of amniotic membrane for burns varicose ulcers [25,26-28], urinary bladder reconstructions [25,29], nerve and tendon damage [25,30], adhesions control and early healing of peritoneal lesions [25,31], dural repair and transphenoidal surgeries [32], ophthalmic surgery [33], vestibuloplasty [34], periodontal surgical procedures [35], gastric mucosal defect repairs [35], treatment of meningomyelocele and spinal cord malformations [36].

Our previously described report [37] has shown that decellularized human amniotic membrane can be effectively used as a non-invasive treatment for pharyngocutaneous fistula after total laryngectomy. Immunohistochemical and histological studies described in report has revealed five distinct layers of the normal human amniotic membrane: epithelium, basement membrane, fibroblast layer, compact layer and intermediate (sponge) layer. The basement layer was formed by glycoproteins such as nidogen, laminin and fibronectin, as well as by type III and IV collagens. Next was the compact layer, forming the main fiber structure of the amnion, which was represented by I, III, IV, and V collagen types and fibronectin. In addition, we detected that after decellularization human amniotic membrane contained numerous growth factors, such as Epidermal Growth Factor (EGF), basic Fibroblast Growth Factor (bFGF), Keratinocyte Growth Factor (KGF), Vascular Endothelial Growth Factor (VEGF), Transforming Growth Factor alpha (TGFa), Transforming Growth Factor beta (TGFb), Platelet-Derived Growth Factor (PDGF) and other.

Reports in recent years recommend the use of human amniotic membrane for the cover of the peritoneal cavity as reinforcement in the reconstruction of the abdominal wall with the help of polypropylene mesh [31]. Authors note that human amniotic membrane, as a biological coverage of the abdominal cavity in the abdominal wall reconstruction using polypropylene prosthesis, can be an alternative in cases where there is no viable peritoneum. They also report that the association of the amniotic membrane with the polypropylene mesh in the treatment of abdominal wall defects of Wistar rats did not alter the formation of adhesions after the first week of operation. However, the amniotic membrane was associated with a marked increased inflammation and angiogenesis activity and the predominance of mature collagen fibers, regardless of the anatomical plane in which it was inserted, accelerating healing.

There are also reports about the usage of Amniotic Membrane-Coated Polypropylene Mesh for the repair of incisional hernia [38]. Authors note that the use of polypropylene mesh coated with fresh amniotic membrane provides the advantage of decreasing postoperative intra-abdominal adhesions along with less inflammation and higher epithelialization after abdominal wall repair.

The positive results obtained by the authors are primarily associated with the fact that human amniotic membrane has a low Immunogenicity. These characteristics of human amniotic membrane reduce the chance of transplant rejection, which

represents an essential advantage when selecting materials for the application in regenerative medicine [39,40]. There are reports according to which we find that human amniotic membrane has anti-inflammatory, antifibrotic, antimicrobial, angiogenic properties, low immunogenicity and can also promote epithelization [41]. While using GCDLHAM graft for the reconstruction of the anterior abdominal wall defects, we found that three weeks after operation, in the animals of the second and third group, the onset of remodeling processes was noted, which consisted of a gradual degradation of the amniotic membrane, the formation of new blood vessels and the deposition of new collagen. Three months after implantation GCDLHAM graft was integrated with host tissues so that it was difficult to distinguish it from surrounding tissues. However, in the second group, ULTRAPRO™ mesh was still detectable through the decellularized amniotic membrane. Encouraging results were also noted when using a XI-S+® graft. Three months after implantation, XI-S+® graft was surrounded by a well-defined connective tissue capsule and was tightly fixed to the host tissues.

Conlclusion. While using GCDLHAM and XI-S+® grafts, all the defects were repaired successfully and none of the rats in these groups showed any evidence of bulging, herniation, development of wound rupture and infection, or fistula formation in postoperative period. Gelatin-Coated decellularized human amniotic membrane can be used as anti-adhesive barrier in abdominal and pelvic surgery, as well as the repair of the abdominal wall hernia.

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SUMMARY

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Ventral hernias, with the incidence of reherniation nearly as high as 50%, still remain to be a real challenge for surgeons worldwide. The use of mesh in the repair of abdominal wall defects reduces the incidence of reherniation; however, using a prosthetic mesh can lead to complications like wound infection, hematoma, seroma, enterocutaneous fistula, small bowel obstruction, recurrent herniation and erosion into adjacent structures including the intestine. The aim of the study was to develop a method for producing gelatin-coated decellularized and lyophilized human amniotic membrane graft and to determine its effectiveness for the reconstruction of the anterior abdominal wall defects.

Experiments were conducted on 40 Lewis white laboratory rats. Animals were divided into four equivalent groups. Abdominal wall defects were created in all rats and repaired using the ULTRAPRO™ mesh (group I), ULTRAPRO™ mesh which was covered by decellularized and lyophilized human amniotic membrane from both sides (group II), mesh from gelatin-coated decellularized and lyophilized human amniotic membrane (group III) and biological surgical mesh XI-S+® (group IV).

Three months after implantation, meshes from gelatin-coated decellularized and lyophilized human amniotic membrane were integrated with host tissues so that it was difficult to distinguish it from the surrounding tissues. However, in the second group, ULTRAPRO™ mesh was still detectable through the decellularized amniotic membrane. Encouraging results were also observed when using a XI-S+® graft. Three months after implantation, XI-S+® graft was surrounded by a well-defined connective tissue capsule and was tightly fixed to the host tissues.

While using gelatin-coated decellularized and lyophilized human amniotic membrane grafts and XI-S+® grafts, all the defects were repaired successfully and none of the rats in these groups showed any evidence of bulging or herniation, development of wound rupture, wound infection or fistula formation in postoperative period. Gelatin-coated Decellularized human amniotic membrane can be used as anti-adhesive barrier in abdominal and pelvic surgery, as well as for the repair of the abdominal wall hernia.

Keywords: tissue engineering, abdominal wall, decellularized human amniotic membrane, ventral hernia repair.

РЕЗЮМЕ

РЕКОНСТРУКЦИЯ ДЕФЕКТА БРЮШНОЙ СТЕНКИ С ИСПОЛЬЗОВАНИЕМ ДЕЦЕЛЛЮЛЯРИЗОВАННОЙ И ЛИОФИЛИЗИРОВАННОЙ АМНИОТИЧЕСКОЙ МЕМБРАНЫ ЧЕЛОВЕКА, ПОКРЫТОЙ ЖЕЛАТИНОМ

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Послеоперационные вентральные грыжи, резидив которых достигает 50%, по-прежнему остаются серьезной проблемой для хирургов во всем мире. Использование сетки при реконструкции дефектов брюшной стенки снижает частоту рецидива; однако использование протезной сетки может привести к таким осложнениям, как инфекция раны, гематома, серома, кожно-кишечный свищ и непроходимость кишечника.

Цель исследования - разработать эффективный метод лечения вентральных грыж с использованием децеллюляри-

зованной и лиофилизированной амниотической мембраны человека, покрытой желатином.

Эксперименты проведены на 40 белых лабораторных крысах линии Lewis. Животные разделены на четыре эквивалентные группы. Всем животным предварительно создана модель дефекта передней брюшной стенки. Животным первой группы дефект передней брюшной стенки восстанавливали с помощью сетки ULTRAPRO™ (ETHICON™); животным второй группы - с помощью сетки ULTRAPRO™ (ETHICON™), которая предварительно была покрыта децеллюляризованной и лиофилизированной амниотической мембраной человека с обеих сторон; животным третьей группы дефект передней брюшной стенки восстанавливали с помощью децеллюляризованной и лиофилизированной амниотической мембраны человека, покрытой желатином; животным четвертой группы - с помощью биологического трансплантата XI-S + ® (США).

У животных первой группы спустя три месяца после имплантации сетки ULTRAPRO™ в брюшной полости наблюдали спаечный процесс. Сетка была замурована в плотных спайках, в которую были включены сальник и петли тонкого кишечника. Во второй группе животных в эти же сроки спаечный процесс в брюшной полости был незначительным. Однако, сетка ULTRAPRO™ все еще обнаруживалась через децеллюляризованную амниотическую мембрану. У животных третьей группы децеллюляризованная и лиофилизированная амниотическая мембрана человека, покрытая желатином, была интегрирована с тканями хозяина, так что ее трудно было отличить от окружающих тканей. Обнадеживающие результаты наблюдались также при использовании трансплантата XI-S+. Спустя три месяца после реконструкции дефекта передней брюшной стенки трансплантат XI-S+® был окружен соединительнотканной капсулой и плотно прикреплен к тканям хозяина.

При использовании децеллюляризованных и лиофилизированных трансплантатов амниотической мембраны человека с желатиновым покрытием и трансплантатов XI-S+® спаек в брюшной полости, признаков грыжи, раневой инфекции или образования свищей не обнаружено. Децеллюляризованная человеческая амниотическая мембрана может быть использована в качестве антиадгезивного барьера при абдоминальной и тазовой хирургии, а также для восстановления грыжи брюшной стенки.

რეზიუმე

მუცლის კედლის დეფექტების რეკონსტრუქცია ჟელატინით დაფარული დეცელალურიზებული და ლიოფილიზირებული ადამიანის ამნიონური მემბრანის გამოყენებით

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ვენტრალური თიაქრები, რომელთა განვითარების რეციდივი, დაახლოებით, 50%-ია, ქირურგებისათვის დღესაც უდიდეს გამოწვევას წარმოადგენს. მიუხედავად იმისა, რომ ბადეების გამოყენებამ შეამცირა თიაქრის რეციდივის სიხშირე, სინთეტიკური ბადეების გამოყენებამ შესაძლოა მაინც გამოიწვიოს ისეთი გართულებები, როგორიც არის ინფექცია, ჰემატომა, სერომა, ენტეროკუტანური ფისტულა, წვრილი ნაწლავის ობსტრუქცია, თიაქრის რეციდივი და ახლო მდებარე ქსოვილების ეროზია, მათ შორის ნაწლავებისაც.

კვლევის მიზანს წარმოადგენდა ჟელატინით დაფარული დეცელულარიზებული და ლიოფილიზირებული ადამიანის ამნიონური მემბრანის შექმნა და შემდგომ მისი ეფექტურობის განსაზღვრა მუცლის წინა გვერდითი კედლის რეკონსტრუქციის დროს.

ექსპერიმენტები ჩატარდა Lewis-ის ჯიშის 40 თეთრ ლაბორატორიულ ვირთაგვაზე. ცხოველები დაყოფილი იყო 4 ჯგუფად. მას შემდეგ, რაც ყველა ცხოველს შეექმნა მუცლის წინა გვერდითი კედლის დეფექტი, რეკონსტრუქცია ჩატარდა ULTRAPRO™-ის ბადის (ჯგუფი I), დეცელულარიზებული და ლიოფილიზირებული ადამიანის ამნიონური მემბრანით დაფარული ULTRAPRO™-ის ბადის (ჯგუფი II), ჟელატინით დაფარული დეცელულარიზებული და ლიოფილიზირებული ადამიანის ამნიონური მემბრანის (ჯგუფი III) და ბიოლოგიური ქირურგიული XI-S+® ბადის (ჯგუფი IV) დახმარებით.

იმპლანტაციიდან სამი თვის შემდეგ ჟელატინით დაფარული დეცელულარიზებული და ლიოფილიზირებული ადამიანის ამნიონური მემბრანა კარგად იყო ინტეგრირებული ქსოვილებთან და მისი გარჩევა რთული იყო ახლო მდებარე ქსოვილებისაგან. თუმცა, მეორე ჯგუფში, ULTRAPRO™-ის ბადე დეცელულარიზებული ამნიონური მემბრანის საშუალებით კვლავ შესამჩნევი იყო. კარგი შედეგები გამოვლინდა XI-S+® ბადის გამოყენების შემდეგაც. იმპლანტაციიდან სამი თვის შემდეგ XI-S+® ბადე იყო შემოფარგლული კარგად გამოკვეთილი შემაერთებელქსოვილოვანი კაფსულით და მჭიდროდ ფიქსირებული მიმდებარე ქსოვილებთან.

ჟელატინით დაფარული დეცელულარიზებული და ლიოფილიზირებული ადამიანის ამნიონური მემბრანის და XI-S+® ბადის გამოყენების შედეგად ყველა დეფექტის მკურნალობა ეფექტურად დასრულდა; პოსტოპერაციულ პერიოდში არცერთ ვირთაგვას არ აღენიშნა თიაქრის განვითარება, ჭრილობის მიდამოში რუპტურა, ინფექციის და ფისტულის არსებობა. ჟელატინით დაფარული დეცელულარიზებული და ლიოფილიზირებული ადამიანის ამნიონური მემბრანა შესაძლოა გამოყენებულ იყოს როგორც ანტი-ადჰეზიური ბარიერი აბდომინურ ქირურგიაში და თიაქრების რეკონსტრუქციაში.