Original Article

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Tissue-Specific Decellularization Protocols: A Comparative Histological Analysis

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ABSTRACT

Background: Obtaining tissue-engineered constructs by decellularization can become an alternative method for restoring and regenerating tissues and organs with impaired functions. The quality of the extracellular matrix depends on the protocol used.

Objective: The objective of this study is to identify a quick and cost-effective protocol for decellularization of organs containing muscular and epithelial tissues.

Methods: This paper describes two methods of decellularization: the classical method of immersing the tissue in a solution and stirring it until the cells are completely removed from the tissue. This method is only suitable for flat tissues up to 5 mm thick, so we used it to clean the diaphragms. To decellularize larger tissues with the preservation of their structures, we used the native vascular system for the perfusion of solutions inside by cannulating the artery. For decellularization, a modified detergent method was used using sodium deoxycholate, sodium dodecyl sulfate, EDTA, and Triton X-100. For morphological studies, sections were stained using three methods: hematoxylin-eosin, Van Gieson, and Masson's trichrome staining. Histological sections were studied under a Leica DM 1000 microscope with microphotography using a Leica ICC50 HD digital camera.

Results: Using this method, we successfully decellularized kidneys while preserving the vasculature. Such organs can be called cell-free vascularized bioscaffolds. Morphological studies confirmed the preservation of the tissue architecture, the absence of cells, and nuclear material. The main result of this work is the development of two simple methods for creating biodegradable, biocompatible, vascularized tissue (organ) scaffolds with the same degree of complexity as in nature. The main result of this work is the development of two simple methods for creating biodegradable, biocompatible, vascularized tissue (organ) scaffolds with the same degree of complexity as in nature. Using a peristaltic pump, the kidney was flushed with SDS. Using this method, we successfully decellularized kidneys while preserving the vasculature. Such organs can be called cell-free vascularized bioscaffolds—the rat diaphragm decellularization protocol, perfusion with SD. Morphological studies confirmed the preservation of the tissue architecture, the absence of cells, and nuclear material.

Conclusion: The obtained results proved that the method of decellularization depends on the origin of the tissue. To achieve the best results on different tissue structures, it is necessary to use different protocols with specific detergents.

KEYWORDS: Decellularization; Diaphragm; Kidney; Regenerative medicine; Biofabrication; Tissue engineering

INTRODUCTION

The kidney is one of the most frequently transplanted organs; 85% of people on the organ transplant waiting list need a kidney [1]. The first step toward bioengineering renal tissue using patient-derived cells is to develop a scaffold that will serve as a supportive substrate for renal parenchyma, stromal fibroblasts, and vascular cell growth. Biomaterial scaffolds derived from extracellular matrices (ECM) of donor organs have several characteristics that make them ideal for use in tissue engineering: their natural biological composition; appropriate macro- and microstructure to ensure physiological function; cellular biocompatibility promoting adhesion, migration, and construc-

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tive tissue remodeling. A promising method for creating scaffolds for renal tissue regeneration is the decellularization of allogeneic or xenogeneic kidneys, which retains much of the complex natural protein composition of the renal ECM and the internal architectural complexity. Also, it provides a vascular supply to developing cells after the recellularization of the scaffold, which can be repopulated [2, 3].

There is a gap in current knowledge and medical capabilities in research and knowledge that can be applied to create a highly suitable organ for a living organism that provides internal architectural complexity. Decellularization techniques can help make such organs.

Decellularization is a process in which allogeneic or xenogeneic cells are extracted from immunologically incompatible tissues or organs to produce cell-free, bioactive ECM that is directly implanted or used as a scaffold for growing cells to engineer complex tissues or organs [4-6]. For volumetric organs, perfusion decellularization is used, in which detergents, enzymes, or other cell-disrupting solutions uniformly flush the entire organ through the vasculature. To find a suitable and effective decellularization protocol for a specific tissue type, it is necessary to compare the effectiveness of different protocols [7-9]. In addition to proving the complete removal of cells, the structure of the resulting ECM is also analyzed [10, 11].

Although the potential clinical outcome implications are fascinating, there are a number of fundamental technical factors and challenges before the clinical application of such scaffolds can be considered. The quality of the extracellular matrix depends not only on each donor but also on each protocol used. Studies have begun to evaluate and compare the effects of perfusion decellularization protocols more systematically, e.g., different decellularizing agents and their duration of exposure. There can be significant discrepancies in the literature between decellularization protocols, even for the same organs/species [12, 13], strongly suggesting the need for a more widely accepted evidence-based approach. Consideration should be given to the choice of decellularizing agent, concentration, and duration of perfusion, additional steps in the protocol (e.g., freezing, use of biological agents), perfusion pressure, perfusion rates, and post-processing sterilization methods [7]. Based on this, we aimed to optimize two main parameters (decellularizing agent concentration and perfusion duration) to evaluate the decellularization of the extracellular matrix of the rat diaphragm and rat kidney. Optimization was determined concerning the structural and functional characteristics of the ECM bioscaffold assessed by histology. We used a flat organ (rat diaphragm) and decellularized it using a unique technique using a patented device. We also used a voluminous, dense organ (rat kidney). We decellularized it using the method of cannulation and washing it from the inside with detergents through the circulatory system to create whole organ scaffolds with intact and perfused vascular, glomerular, and tubular compartments. Morphological analysis evaluated stained sections of the resulting scaffolds to confirm the absence of cells and the integrity of the matrix structure. We have found that to achieve the best results on different tissue structures, it is necessary to use various protocols with specific detergents.

The purpose of the study was to develop new protocols for the decellularization of various shapes and structures of organs using a small laboratory animal model, followed by multifactorial histological evaluation of the resulting scaffold to maintain structural integrity and confirm complete clearance of cells.

MATERIALS AND METHODS

Experimental Animals

Twelve male Wistar rats (300-350 g) were purchased from the A. Tsyb Medical Radiological Research Center. Animals were littermates of similar size and age (12 weeks old). After preliminary euthanasia, the diaphragms and kidneys were removed from them (Protocol of the Local ethics committee, permit number AR-153334, issue date 07/01/2020).

Organ Preparation

Animals were euthanized by administering a lethal dose of Anestofol 5% (VIC, Russia). During the operation, 100 units of heparin were administered, after which the diaphragms were removed, avoiding adjacent tissues. To isolate the kidney with all adjacent vessels, we developed a scheme for applying ligatures to close the blood supply system, which is necessary for washing the vascular network with a heparin solution (for 15 minutes). Using this scheme, we eliminated the need for blood and prevented the formation of blood clots. After washing, we cut off the kidneys in the places indicated in the diagram in Fig. 5A. The organocomplex of the kidneys was connected as a single block; the artery was cannulated to wash away blood cells, and then washing solutions were injected through it. The kidney was fixed in a bioreactor (RU No. 199321, dated 08/26/2020) and decellularized using a modified protocol.

Reagents

Anestofol 5% (VIC, Russia), heparin (VIC, Russia), deionized water (DW), sodium deoxycholate (SD) 4% (Product No. D6750, Sigma-Aldrich, USA), phosphate buffer solution (PBS) (Product No. 934B-06, BioVitrum, Russia, pH=7.4), ethylenediaminetetraacetic acid (EDTA) (Product No. V900106, Sigma-Aldrich, USA), sodium dodecyl sulfate (SDS) 1% (Product No. L6026, Sigma-Aldrich, USA), Triton X-100 3% (Product No. X100, Sigma-Aldrich, USA), penicillin-streptomycin (10,000 units/ml) (Product No. 15140122, Thermo FS, USA), formalin solution neutral buffered 10% (Product No. B06-001/S, BioVitrum, Russia), hematoxylin and eosin kit (Product No. HK-G0-D250, BioVitrum, Russia), Masson's trichrome kit (Product No. HK-MS-AQ00, BioVitrum, Russia), Van Gieson trichrome kit (Product No. HK-VG-AQ00, BioVitrum, Russia).

Equipments

Medical cannulation needles (24G), polyglecaprone-25 suture (Ethicon-Johnson Johnson), surgical animal experiment kit, Shenzhen Lab2015 (China) peristaltic pump (Fig. 1C); silicone tubing size (#13, #19), our patented device (No. RU 199321 dated 08/26/2020). This flat biological material attachment device allows the organ to be securely attached to prevent movement in laboratory glassware and ensure complete penetration of detergents to the entire tissue surface.

Experimental Design

In the experiment with decellularization of diaphragms, the animals were divided into three groups: 1. native control group (control 1) (n= 4), the data obtained from which were used for further standardization of the morphological studies being carried out; 2. experimental group (n= 4), whose organs were subjected to decellularization; 3. PBS control group (control 2) (n= 4), whose organs were in a PBS solution under the same environmental conditions as the decellularized samples.

For kidney decellularization, the animals were divided into two groups: 1. native control group (n= 6), the data obtained from which were used for further standardization of the morphological studies performed; 2. experimental group (n= 6), whose organs were subjected to decellularization.

Diaphragm Decellularization Protocol

The original protocol for rat diaphragm decellularization using the detergent-enzymatic method [14] was modified in this study by changing the exposure time, composition, and order of perfusion of solutions to obtain an acellular matrix of the diaphragm. Decellularization includes two cycles of treatment using a method that provides perfusion with DW (30 min), then 4% SD (3 h), after washing with PBS (10 min), then using DW with the addition of 800 µl EDTA (30 min), after which the diaphragm was washed with a PBS with the addition of a 1% penicillin-streptomycin solution (12 h). All solutions were sterile by filtering at room temperature. The solution supply rate was 4 ml/min, with a total exposure time of 32 hours.

Kidney Decellularization Protocol

The second part of the experiment consisted of rat kidney decellularization. For the decellularization process, it is necessary to cannu-

late the kidney through the ascending aorta. For this purpose, a needle was used to fix the aorta using a suture surgical thread. The isolated organ was connected to the tube system using adapters. The flow rate of detergent solutions is essential (we have 2 ml/min), which does not affect the mechanical properties of the matrix. An optimized SDS-based decellularization protocol produces natural rat kidney scaffolds with preserved extracellular matrix. The original protocol for decellularization of the rat diaphragm [7] was modified by the authors by changing the exposure time, composition, and order of perfusion of solutions to obtain the acellular matrix of the kidney. Using a peristaltic pump, the kidney was flushed with the following solutions in sequence: heparinized PBS (15 min), 1% SDS in DW (12 hours), DW (15 min), 1% Triton X-100 in DW (30 min), PBS with the addition of a 1% penicillin-streptomycin solution (48 hours). Total exposure time 61 hours.

Morphological Analysis

The tissues and organs obtained after the experiment were fixed in 10% buffered formalin for 24 hours. After standard histological processing on a Leica TPI020 histoprocessor, oriented tissue fragments were embedded in Histomix paraffin medium at a HistoStar embedding station (Thermo Scientific). For morphological studies, sections 5 μm thick, obtained on a Leica RM2235 microtome, after dewaxing, were stained using three methods: hematoxylin-eosin (H&E), Van Gieson, and Masson's trichrome staining. Histological sections were studied under a Leica DM 1000 microscope with microphotography using a Leica ICC50 HD digital camera.

Hematoxylin and eosin staining were used to visualize any nuclei remaining after decellularization: cell nuclei (DNA and RNA contained in the nuclei) are stained deep blue, and the cytoplasm and intercellular substance are stained pink. Masson's trichrome staining is used primarily to differentiate connective tissue elements: elastin and muscle fibers are red-brown, collagen is green to blue, cell cytoplasm is light red or pink, and nuclei are dark brown to black. Van Gieson staining is designed to study the structure of connective tissue. This staining is a simple method to differentiate collagen fibers from other connective and smooth muscle tissue components. Collagen fibers are painted red (dark pink), epithelial cells are colored brown, and cell nuclei are black.

General morphology and absence of cells were examined by H&E staining, and tests were performed in triplicate. For morphological assessment, 20 random fields at 40× magnification were captured by a mounted microscope camera and objectively analyzed by examining the remaining cell nuclei/whole cells, and the ECM structure. For the same 20 random fields, the ECM was visually analyzed for integrity.

Ethical Considerations

Animal experiments in this work were carried out in strict accordance with the Guide for the Care and Use of Laboratory Animals. The animal experimental protocols used were approved by the Commission for Bioethical Control of the Care and Use of Laboratory Animals for Scientific Purposes of the National Medical Research Radiological Center (permit number: AR-153334, issue date 07/01/2020).

RESULTS

Diaphragm

The effectiveness of the protocol for the rat diaphragm decellularization in the study was determined qualitatively, visually (Fig. 1A, 1B). The number of preserved nuclei in the studied tissue samples and the integrity of the extracellular matrix were assessed in comparison with the control. It can be seen that the tissue samples, after decellularization, changed their color and became transparent, which indicates a visible absence of the cellular component. Further evaluation of the samples was carried out histologically using three staining methods.

H&E staining showed complete removal of nuclear and cellular materials after decellularization compared to native samples. H&E

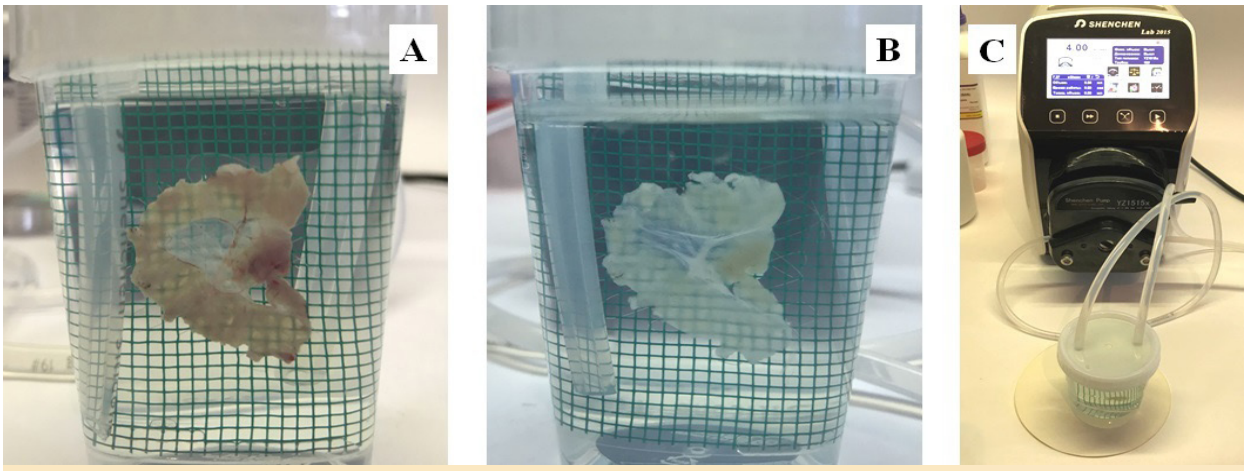

Figure 1: (**A**) Rat diaphragm before decellularization is fixed in the author's patented device for decellularization of flat tissues, (**B**) Rat diaphragm after decellularization, and (**C**) Author's installation for diaphragm decellularization.

staining of decellularized tissue sections revealed the expected pink eosinophilic staining for collagen without basophilic staining of the cellular material. It was observed that the extracellular matrix remained intact after decellularization (Fig. 2). The results obtained after staining the diaphragm tissue with Masson's trichrome (Fig. 3) and picric acid and fuchsin of van Gieson staining (Fig. 4) showed that the collagen and elastic components of the extracellular matrix were well preserved. Histological analysis confirmed that decellularized rat diaphragm tissues retained ECM components without residual cellular and nuclear material.

Hematoxylin and eosin (H & E) Staining

In intact tissue (control 1), cells, their internal contents, and nuclei with distributed chromatin are observed (Fig. 2) — the structure of myofibrillar cells with normal striations. On the histophotograph of decellularized tissue, there is a noticeable absence of myofibrillar cells with cross-striations, destruction of myofibrils has occurred, and no cell nuclei. The integrity of the vessels is preserved. In control 2 (PBS control group), there are practically no differences from intact tissue, except for dead cells and nuclear pyknosis due to hypoxia. The structural organization of the tissue is preserved; in the upper part of Fig. 2, there is a connective tissue membrane with the visible presence of cells. A myofibrillar cell structure with striations is present.

Masson's trichrome staining

With this type of staining, connective tissue (collagen) is blue, elastin and muscle fibers are stained bright red, the cytoplasm of cells is light red or pink, and the nuclei are dark brown. In the intact tissue sample (Fig. 3), the connective pleura of the diaphragmatic tissue is noticeable in a distinct blue color at the upper edge of the section. Red blood cells are visible in a section of the vessels. After decellularization, the vessel walls and other elements of the extracellular matrix are preserved: loose collagen fibers are blue, and elastin fibers are brown. It can be seen that after decellularization, the muscle fibers are destroyed, leaving only the collagen framework. In control 2, it is noticeable that the tinctorial properties of the protein are changed, and vacuolization of the myocyte cytoplasm is observed.

Van Gieson staining

With this type of staining, the cell nuclei become black, collagen, as well as any connective tissue, red or dark pink, other tissue elements (muscle fibers and red blood cells) - yellow or pale orange, fibrin - yellow or orange. The diaphragmatic pleura is a single-layer epithelium (mesothelium), a connective tissue layer, clearly visible in the upper parts of all photos of this type of staining. In intact tissue (Fig. 4), it can be noted that the tinctorial ability to react to the dye is normal. After decellularization, there is intact collagen, an absence of cells in the pleura, an absence of myocyte

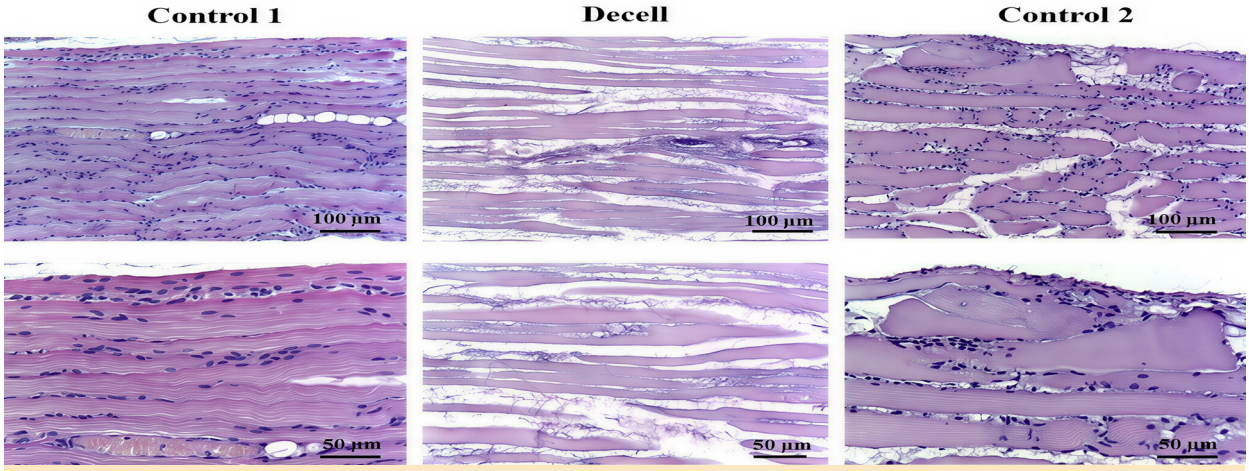

Figure 2: Histology of the rat diaphragm, H&E staining: intact tissue (control 1), decellularized tissue, PBS control group. On the histophotograph of decellularized tissue, there is a noticeable absence of myofibrillar cells with cross-striations, destruction of myofibrils has occurred, and no cell nuclei. The integrity of the vessels is preserved.

cell nuclei, and an absence of cross-striations of muscle fibers. The inner membrane of the vessel is preserved, and a collagen layer is present around the vessel. It can be seen that only the collagen framework remained after decellularization. In control 2, the tissue can be characterized as dead, the nuclei are pyknotic, the transverse striation of the muscle fibers is preserved, and tissue swelling has occurred. Histological data, along with staining of tissue samples, demonstrated the complete removal of cells in decellularized samples and the preservation of connective tissue fiber structures (collagen and elastic fibers). These results were found to be consistent across all samples tested.

Kidney

Comparison of trichrome staining of native and decellularized rat kidney sections demonstrates a complete and efficient decellularization process. After specific staining for elastin and collagen, sections of decellularized kidney tissue confirm the preservation of proteins and extracellular matrix structure in the absence of cells.

Hematoxylin and eosin (H & E) Staining

In intact tissue samples (Fig. 6A-6C), one can see the presence of normal cells, their internal contents, and nuclei with distributed chromatin. In the cortex zone (Fig. 6A, 6B), the normal structures of the kidney are

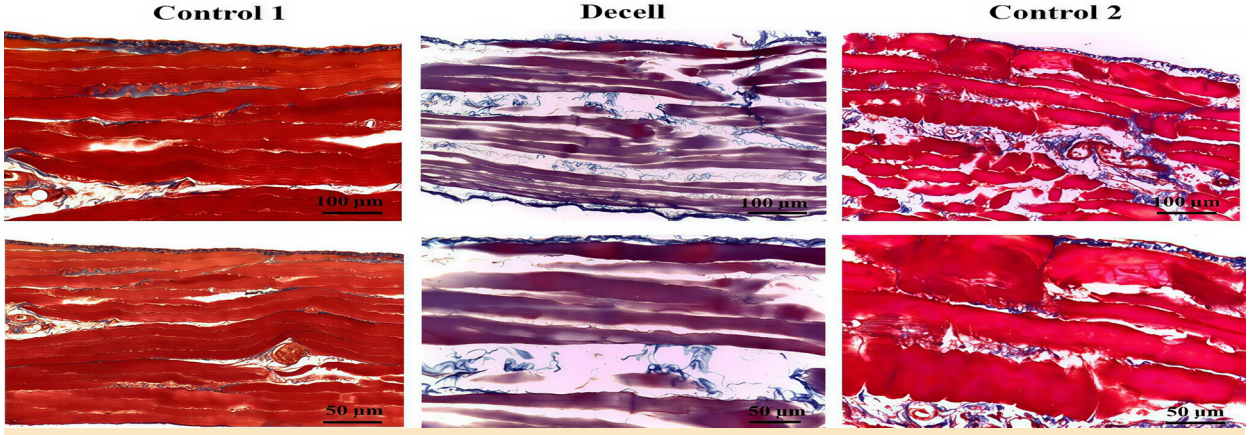

Figure 3: Histology of the rat diaphragm, Masson's trichrome staining: intact tissue (control 1), decellularized tissue, PBS control group. It can be seen that after decellularization, the muscle fibers are destroyed, leaving only the collagen framework.

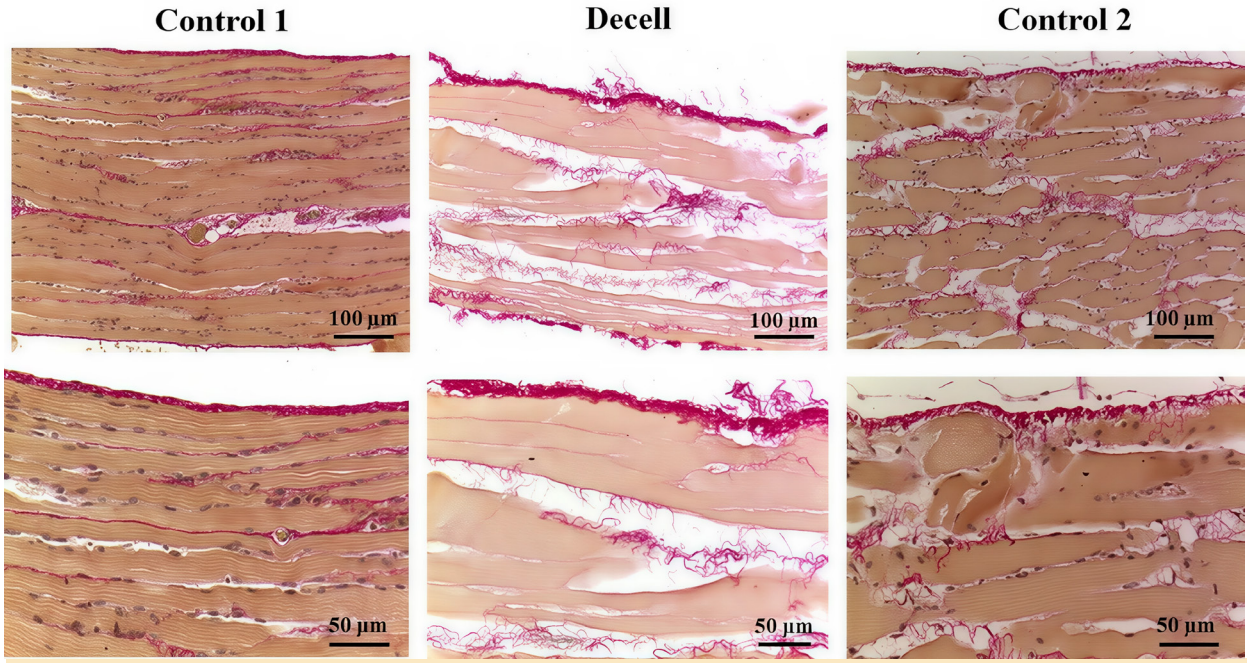

Figure 4: Histology of the rat diaphragm, Van Gieson staining: intact tissue (control 1), decellularized tissue, PBS control group. After decellularization, there is intact collagen, an absence of cells in the pleura, an absence of myocyte cell nuclei, and an absence of cross-striations of muscle fibers. The inner membrane of the vessel is preserved, and a collagen layer is present around the vessel.

visible - glomeruli with capsules surrounded by convoluted tubules. The juxtaglomerular zone at the border of the cortex and medulla (Fig. 6C) also shows a normal structure with tubules forming the loops of Henle and collecting ducts. A histophotograph of decellularized tissue in the outer cortex (Fig. 6D, 6E) shows glomerular structures without cells surrounded by tubules. In the juxtaglomerular zone at the border of the cortex and medulla (Fig. 6F), a glomerular structure is also visible in the upper zone of the photo. Still, most of it is represented by tubular organizations of tubules, forming loops of Henle, and collecting ducts. There is an apparent absence of cellular debris and nuclear particles in the cortex and medulla of the acellular matrix. The mesh structures of the extracellular matrix of the glomerular tubules remain intact, and the integrity of the extracellular structure of the vessels is preserved.

Masson's trichrome staining

In intact tissue (Fig. 7A, 7B), at three different magnifications, one can observe both a general picture of the glomerular structure and tubular organization of the organ and a detailed composition of the renal glomerulus, inside of which capillaries filled with yellow-red blood cells are visible. The nuclei of cells of all types are clear, and the structure is normal. After decellularization (Fig. 7C, 7D), only the collagen framework is observed; all cells in the entire kidney volume are completely removed, and the structure of all zones of the kidney and vessel walls remains unchanged. Collagen fibers are colored blue, elastin fibers are brown, and no endothelium exists. The black arrow in the figure indicates preserved elastin fibers in the arteriolar membranes.

Van Gieson staining

In intact cortical tissue (Fig. 8A, 8B), normal glomerular structure and tubular organization are observed, and cell nuclei are clear. The photo also shows a vessel with contents. After decellularization (Fig. 8C, 8D), the absence of any cellular content is observed, the integrity of the architecture of the glomerular and tubular structures within the framework, and the preservation of collagen are visible. Cells and their nuclei are absent. The inner membrane of the vessel can be identified by the presence of a brightly colored collagen layer.

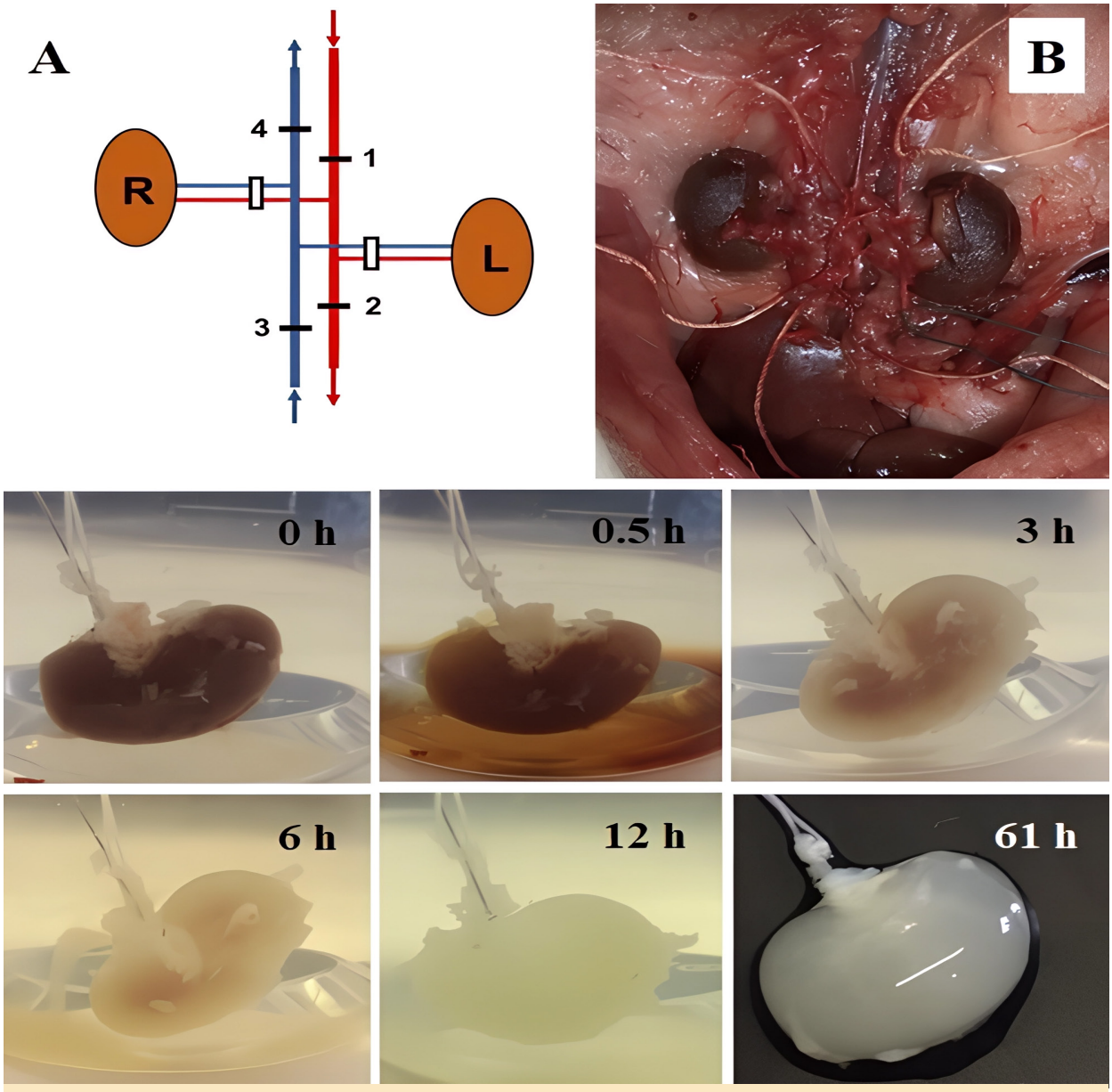

Figure 5: Method of isolation of rat kidneys: A - schematic representation of the location of the ligatures during the procedure for removing the kidneys. The right kidney is marked with the letter R, and the left kidney is marked with the letter L. The black lines indicate the places where the ligatures are applied - 1, 2, 3, 4, and numbers indicate their sequence. The white lines indicate the sites of cut-offs of the kidneys. The aorta is marked in red, the vein is shown in blue, the arrows indicate the direction of blood flow, and B is the photo. Rat kidney decellularization process: from the start (0 h) to a completely decellularized kidney (61 h).

All stains show preservation of the organ's typical structure and geometry of both internal and external areas. We can confidently say that our methods made it possible to decellularize the organ entirely and evenly in all parts.

DISCUSSION

Selecting the ideal scaffold material is an important step in kidney tissue engineering. A perfect material is characterized by the following properties: good biocompatibility, ensuring the regeneration of host tissue without immune rejection, three-dimensional structure shaped like internal organs, cell adhesion

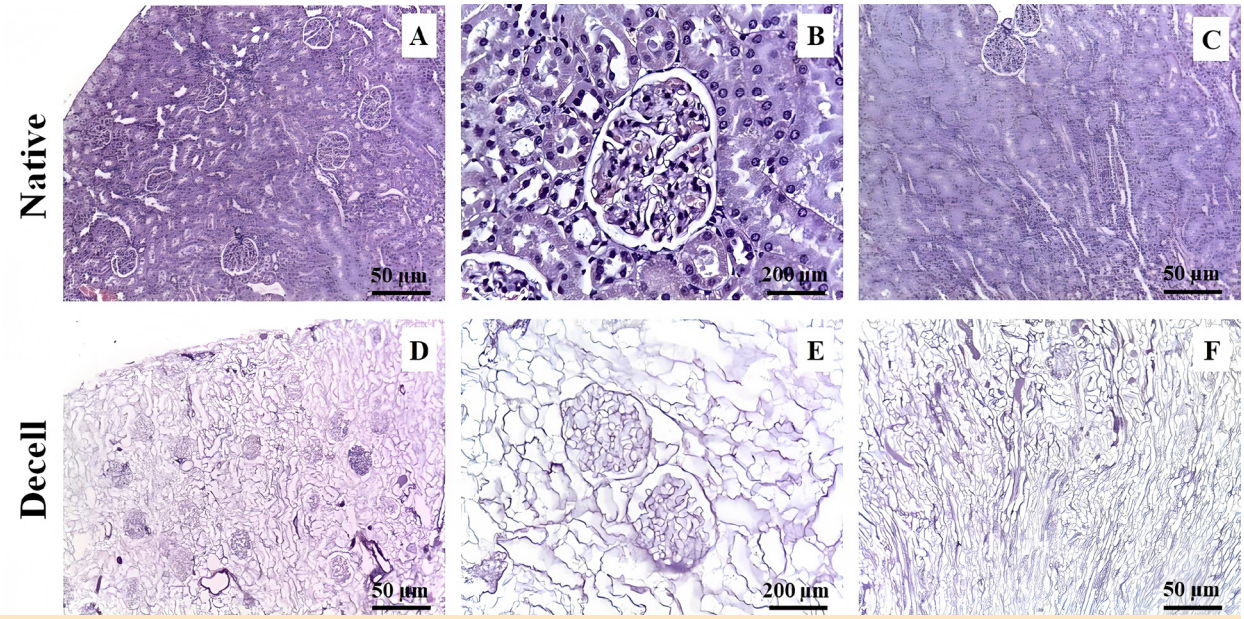

Figure 6: Histology of the rat kidney, H&E staining: control: (**A**, **B**) outer part of the cortex, glomerulus surrounded by tubules, (**C**) juxtaglomerular zone at the border of the cortex and medulla; decellularized tissue: (**D**, **E**) outer part of the cortex, glomerulus surrounded by tubules, (**F**) juxtaglomerular zone at the border of the cortex and medulla. The mesh structures of the extracellular matrix of the glomerular tubules remain intact, and the integrity of the extracellular structure of the vessels is preserved.

and growth, and the ability to biodegrade [2]. The acellular matrix scaffold is an ideal choice because it preserves the natural expression of proteins in the ECM and contains an internal structure that matches the real organ.

For our experiments, we considered a number of agents for their possible use in tissue decellularization. These are physical, chemical, and enzymatic agents. The advantage of physical decellularization methods, such as freezing and thawing, is that they affect the entire tissue sample uniformly, regardless of diffusion or perfusion. This makes the action of physical methods more predictable compared to chemical or enzymatic agents. Physical methods result in effective cell lysis, but cell debris is not removed from the matrix, which may cause immunological or biological effects. Thus, physical methods must be combined with chemical or enzymatic methods to clear the matrix of cellular debris. Enzymatic agents lead to less reproducible results and pose a risk of significant changes in the composition of the extracellular matrix; their use carries the risk of matrix protein denaturation and thereby disrupts matrix integrity when used at higher concentrations [15]. Moderate protein denaturation may be beneficial because it eliminates antigens and thus reduces the risk of immunogenic side effects. However, we chose to use chemical agents as an alternative decellularization step because they are the most predictable influencing factors. An acellular scaffold of the diaphragm and kidney can be successfully obtained using a microperistaltic perfusion pump, which provides the best access to reagents to the organ to remove all cells and wash away their remains.

Our study and other data [16, 17] indicate that rat kidneys can be decellularized to form an acellular ECM scaffold through a simplified process without the influence of physical and enzymatic factors. By retaining their structural and biochemical components and intact vasculature, these scaffolds can be used as a natural three-dimensional structure for cell growth. When implanted into recipient rats, the vasculature of the regenerated kidneys can maintain blood pressure and produce urine in vivo [16, 17]. Our future goal is to recellularize the obtained decellularized kidney scaffolds to create a functional kidney. As the most natural scaffold biomaterial, the decellularized scaffold retains intact three-dimensional vascular structure, extracellular matrix architecture, and cytokines in the ECM [18-20].

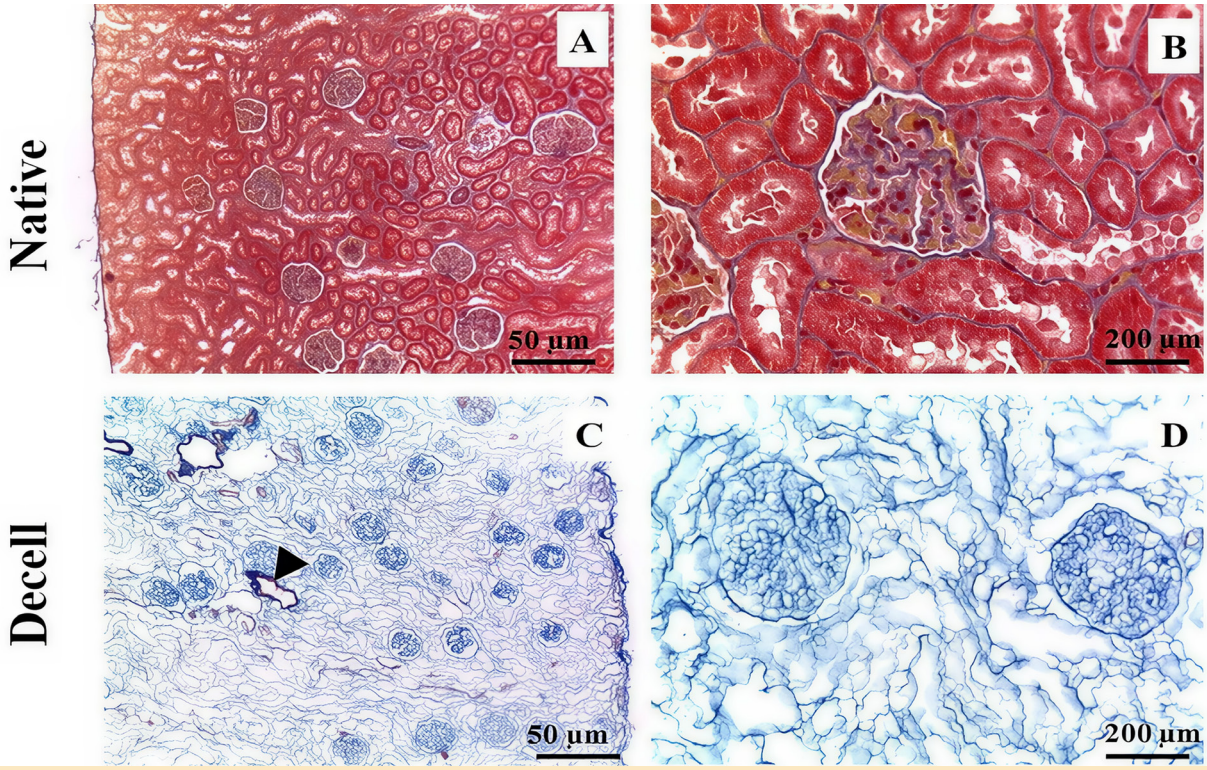

Figure 7: Histology of the rat kidney, Masson's trichrome staining. After decellularization (**C**, **D**), only the collagen framework is observed; all cells in the entire kidney volume are completely removed, and the structure of all zones of the kidney and vessel walls remains unchanged. The black arrow in the figure indicates preserved elastin fibers in the arteriolar membranes.

Artificially created tissues are currently at different stages of application: some of them are already used in the clinic, and others are undergoing preclinical and experimental studies [21-24]. Modern progress in regenerative technologies indicates that artificial tissues may be widely used in the treatment of patients needing tissue and organ replacement. Organ engineering is an innovative field of research that has the potential to overcome some of the challenges of transplantation as well as ensure the availability of transplantable organs. Most whole organ tissue engineering research uses decellularized organs as a scaffold because no other fabrication method to date can provide a scaffold replicating the complex structure and anatomy of a human organ and recreate vasculature similar to that found in primary organs [21-24].

The choice of the active agent, decellularization method, and duration of exposure to the active solutions is determined by taking into account the anatomical and histological features, structure, and properties of the organ or tissue being studied. An unsuccessful choice of decellularizing agent can lead to the destruction of the matrix structure and the loss of its mechanical and biological properties. It must be taken into account that any chemical agent damages the matrix, and only a correctly selected method and duration of exposure can minimize the consequences of this exposure. Therefore, the problem of finding the optimal technology for tissue decellularization while maintaining the extracellular substance as intact as possible remains open [25].

Non-ionic detergents, such as Triton X-100, can strongly break lipid-lipid and lipid-protein bonds but are less effective in protein-protein interactions. Accordingly, the effectiveness of non-ionic detergents depends on the tissue undergoing the decellularization process [19]. Chelating agents, such as EDTA, bind divalent metal ions, thereby disrupting cell adhesion to the ECM. Reyna *et al.* [26] showed their high efficiency in removing skeletal muscle cells by destroying actin and myosin, thereby damaging the cell and reducing DNA content

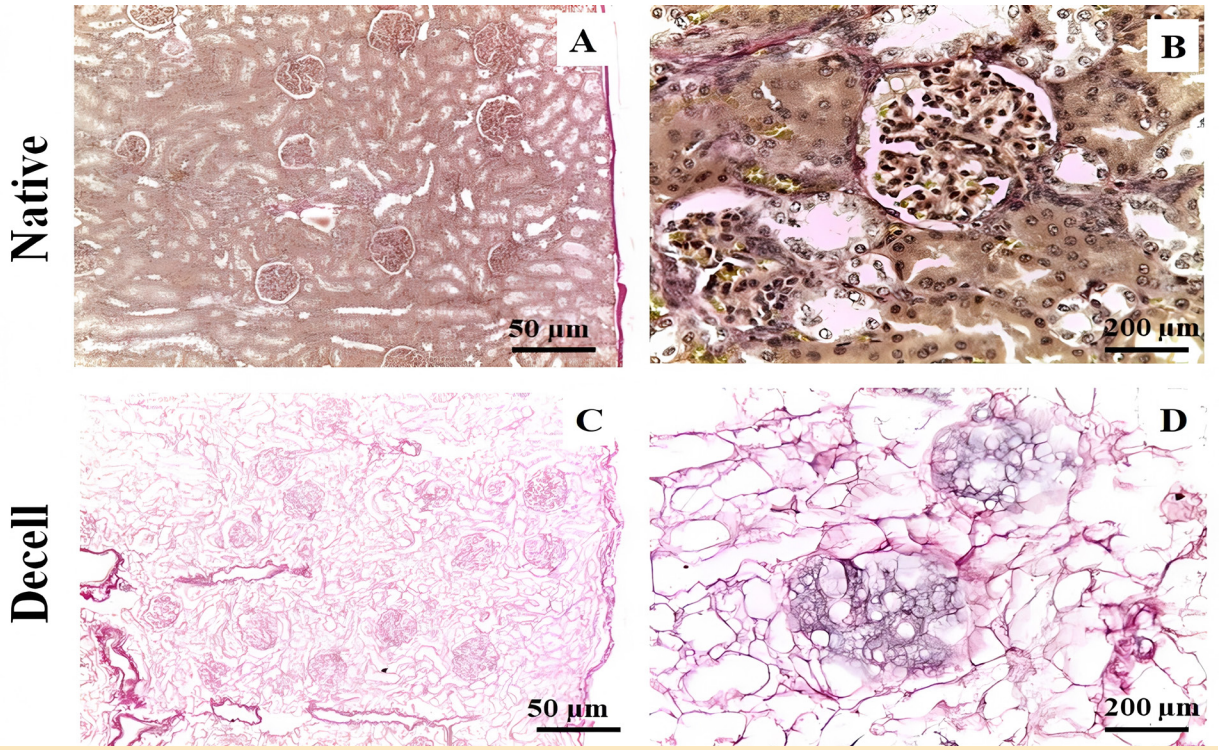

Figure 8: Histology of the rat kidney, Van Gieson staining. After decellularization, the absence of any cellular content is observed, the integrity of the architecture of the glomerular and tubular structures within the framework, and the preservation of collagen are visible. Cells and their nuclei are absent. The inner membrane of the vessel can be identified by the presence of a brightly colored collagen layer.

to less than 10% compared to controls. Based on the presented features of the reagents, we used Triton X-100 specifically for the kidney decellularization protocol to destroy epithelial cell membranes and EDTA in the diaphragm decellularization technique to target muscle proteins.

In conclusion, the main result of this work is the development of two simple methods for creating biodegradable, biocompatible, vascularized tissue (organ) scaffolds with the same degree of complexity as in nature. An acellular scaffold of the diaphragm and kidney can be successfully obtained using a microperistaltic perfusion pump, which provides the best access to reagents to the organ to remove all cells and wash away their remains. In the decellularization methods we used for the diaphragm and kidney of rats, it is possible to decellularize the organ thoroughly and evenly in all parts. To achieve the best results on different tissue structures, it is necessary to use different protocols with specific detergents. To confirm the success of our results, scaling up experiments for the kidneys of large laboratory animals (pigs) is planned, and further testing of the qualitative and quantitative characteristics of the resulting ECM is also necessary. The obtained results prove the success of our decellularization methods and allow us to continue research on the possibility of using the decellularized matrix of diaphragms and kidneys for recellularization with subsequent transplantation.

CONFLICTS OF INTEREST: None declared.

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