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Creating A Three-Dimensional Biocompatible Matrix For Use In Reconstructive Surgery.

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ABSTRACT

Our study describes the development of a bioengineering design for application in organ reconstruction surgery. The selection of appropriate material and development of a basis for a spatial structure, preparation of said structure, bioavailability and cytotoxicity testing in vivo were studied. A three-dimensional volumetric weave was developed from titanium wire and titanium nickelide as a framework for the intracellular matrix. An original method for cleaning and spraying of bioactive coating onto the surface of the 3D matrix was developed. Additionally, a prototype of the intercellular matrix with reinforced frame function was developed using a purified titanium mesh with a collagen filler. In order to test the design in vivo, prototypes for tubular bioconstructions with cultivated laboratory rat stem-cells on the surface were developed and prepared. Microscopical examination showed an absence of toxic effect of the bioconstruction base material. A laboratory experiment was performed, in which hollow tubular implants were created from the bioconstruction prototypes, which were consequently implanted into the body of Wistar rats. Extracted samples were subjected to morphological, cytological and histological examination. Morphological examination evaluated the strength, framework-supporting, deformation characteristics of the obtained tubular bioengineered constructions.

Keywords: bioengineering construction, tubular implant, collagen matrix, titanium nanoconstruction.

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INTRODUCTION

Primarily we performed a literature analysis on the topic of biocompatible nano-composite material use, in order to understand suitable frame components. According to the project's goals and objectives, we analyzed over 80 studies from SCOPUS, ScienceDirect, PubMed, Web of Science, Google Scholar, Cyberleninka and other data bases, from which we concluded the high relevance of biocompatible matrix material use in bioengineering.

According to the analyzed data, research continues to focus on the study of the properties of various modifications of nitinol, related both to the composition of the material itself and to subsequent processing of end-products from this alloy, widely used for intravascular stents and suture material. An analysis of international literature shows that in most cases titanium nickelide is used as a memory-shape material for the manufacture of intravascular and, according to authors Singh C, Wang X (2015) as an external component for braided stents, successfully used to prevent overstrain of the venous graft vascular wall in coronary bypass surgery [1]. The development of artificial heart valves based on nitinol as a framework for the valve walls is being widely studied. Initially, Alavi SH (2013), showed that the introduction of a metal structure preliminarily covered with a cellular layer into the body increases the biocompatibility of the reconstructive material without actively inducing an immune response [2]. Ricardo Moreira (2015) and others describe the development of an artificial heart valve, whose lobes were made of polyethylene terephthalate fibers with cultivated fibroblasts, the supporting structure of which is a self-expanding stent made of nitinol [3]. S.Hamed Alavi et al. (2015) created an artificial tricuspid heart valve based on a titanium nickelide mesh covered with three layers of cells - fibroblasts, myofibroblasts and vascular endothelial cells, with a thickness of 25 microns, thereby most accurately recreating the structure of the native heart valve. During their experimental study, the group of authors managed to show that the bioengineered valve was able to withstand the physiological pressure of blood inside the chambers of the heart [4]. K.Loger et al. demonstrated the effectiveness and complete applicability of microstructured nickel-titanium plates prepared by magnetron sputtering with smooth muscle cells grown on their surface, for use as a heart valve base [5]. Despite the amount of research on nitinol, scientists continue to work to increase both bioavailability and strength properties of the material by modifying the surface of the implant. This is required in order to limit exposure to immune system, which may result in increased corrosion of the implants, with subsequent accumulation of metal alloy ions in surrounding tissue, and overall deterioration of the implant [6-8].

Along with the development of the supporting structure, the most suitable cellular technologies were overviewed in order to create a reconstructive material suitable for the restoration of hollow organs. This problem affects different branches of medicine.

The anatomical deformities of the vaginal area are a highly actual problem. This applies as well to patients with oncological pathology of the uterus, cervix, ovaries, rectum and bladder. These conditions may require partial or total resection of the vaginal cavity, which negatively impacts the quality of life and psychosocial adaptation of the patient. In order to prevent negative aspects of postoperative rehabilitation, it is recommended to reconstruct the resected organs with complex tissue structures, including rectal segments, bladder wall, skin, which have proven to be anatomically and functionally fit to replace lost vaginal tissue [9, 10]. Using autologous tissue for reconstruction in this area often causes complications such as strictures, infection, unwanted hair growth, diverticulosis, and even cases of reconstructive component malignization [11, 12].

The research performed by Dr. Roger E. De Filippo et al. in 2018 was one of the first studies to prove the possibility of tissue engineered structure use in gynecology [13]. In their experimental study, they demonstrated a tubular bioengineered scaffold-based tissue made up of polyglycolate acid fibers, with a width of 15mkm and cellular demensions of 100 to 200mkm. The biopsy material of the vaginal wall of white New-Zealand rabbits was extracted. Muscular and serous tissue were extracted and cultivated from the bioptic material, and where later implanted into the tubular scaffold. The fused structure was then implanted into the area of resected vaginal tissue. 6 months after surgery, the results of the transfer were analyzed and full polyglycolide scaffold resorption was documented with retainment of complete tubular cylindric structure of the reconstructed vaginal wall, which in cellular and structural components resembled normal vaginal tissue. In 2013, authors from China, Lan Zhu et al. published a five year study on a method of tissue engineered reconstruction of patients with vaginal atresia (Mayer-Rokitansky-Kuster-Hauser syndrome) [14]. Acellular

dermal matrix (ADM) was used as the main component in a neovaginal structure, prefabricated to form a cylinder. This construction was then implanted into the prepared neovaginal cavity recipient zone, which was initially lined with mucous micrografts from the vaginal antrum in order to stimulate neovaginal epitheliazation. Intraoperatively and during the early postoperative period, the structural integrity of the hollow organ was maintained via tamponade, and later silicone dilators up to 3 months after surgery. The results of the study showed a close anatomical and physiological conformity of the neovaginal transplant to native vaginal tissue. No significant complications occurred, including absence of donor site defect, stricture, local inflammation, immune response towards transplant.

Despite positive results of published studies on methods of neovaginal reconstruction, the creation of replacement bioengineered tissue structures remains an actual problem. Primarily, the rigidness of the foundation structure requires to be perfected in order to reduce the amount of stricture formation and reduce the time of hospitalization and overall patient rehabilitation. Cellular composition of the bioengineered structure also requires further development, since tissue reorganization with granulomatous polyposis formation often develops in current bioengineered transplants.

Different pathological conditions of the urinary tract require reconstructive surgical intervention. These conditions include urethral stenosis (stricture), obliteration of the ureter, ureteral fistulas, tumors of the ureter and others. Iatrogenic pathology is the most common reason for ureteral lesions [15]. Iatrogenic ureteral complications can occur during gynecological, surgical, cardiovascular, urologic and other interventions, as well as radiation therapy, on the pelvic cavity. Over a third of all stricture processes of the anterior urethra are iatrogenic, and about 30% are idiopathic, 19% - post traumatic, 15% are infectious strictures [16]. This stimulates an influx of research towards a method for reconstruction of circular defects of the urinary tract. Different methods of urethroplasty, both local and with transplant and flap use, are preferred.

Since the end of the 20th century, buccal urethroplasty has been widely acknowledged as the method of choice over cutaneous transplants. Buccal urethroplasty is used for reconstruction of short strictures, due to relative simplicity of the method and consistent positive results. A buccal transplant can be used as a tissue patch, and in tubular form. The most promising method of urinary tract reconstruction is bioengineered tissue transplantation [17]. The development of such bioengineered structures is aimed at preventing specific complications in the donor-site, as well as addressing the issue of tissue deficiency for reconstruction.

Bioengineered tissue designs created from natural or artificial matrix and autologous cells of the patient can be used to treat various urethral defects. The matrix is used both without cells and with different types of autologous cells. International literature shows cases of collagen tubes, tubularized Gore-Tex material, decellularized fragments of a canine ureter, submucosal base of a swine small intestine and other subtracts used for creation of a matrix. In most experimental animal studies, the epithelization of the matrix was accompanied with scar tissue formation and obstruction of the neouretera. A.El-Hakim et al. (2005) [18] conducted a large study, in which different types of matrix substrates were evaluated with or without cells. A positive result was seen only when using a matrix based on a decellularized intestinal fragment, coated with autologous cells.

A group or Russian scientists under the guidance of Glibochko P.V., published an experimental study for matrix formation from an acellular vascular wall. The decellularized structure was used for reconstructive urethroplasty, and showed to be a suitable material acquired from a cadaver artery. The material proved to have a low biological activity, positive biodegradation rate and suitable framework functionality required for hollow organ reconstruction [19]. Glibochko P.V. et al, also described a method of replacement urethroplasty with the use of a bioengineered decellularized matrix infused with autologous cells from the patients buccal mucous membrane, for treatment of a 2,5cm proximal bulbous urethra stricture [20]. The method results showed a high effectiveness of the bioengineered tissue construction, which proved to be a stable framwork, which can be safely applied in clinical practice. Despite positive primary results, the authors indicate the necessity for further clinical and experimental studies of the effectiveness of bioengineered tissue transplants in reconstructive surgery of the urinary tract.

A database of foreign studies analysis shows that research is focused on finding the most suitable bioengineered material for reconstructive surgery. A team of scientists from China (Yu X et al.) in 2017

published a study on the creation of a tracheal cartilage matrix made of a gelatin-chondroitin-sulfate-hyaluronan-polyvinyl alcohol (GCH-PVA) framework. The scaffolds showed strength-bearing characteristics similar to native cartilage during the experimental study [21]. A group of American researchers (Al-Ayoubi A.M. et al) in 2017 performed an experimental study on the development of a bioengineering trachea transplant to restore extended lesions. This transplant was made of decellularized dermis of a cow and human mesenchymal stem cells (hMSCs). During the experiment, the ability of the graft to facilitate chondrogenesis, neovascularization, and epithelialization was noted [22]. A team of Korean scientists (Jung S.Y. et al, 2016) proposed the creation of a polyurethane tracheal transplant with a microscale architecture, created using the 3D printing method, the results of an in vivo experiment show repeated epithelization for 4 weeks of implantation and the appearance of ciliated epithelium for 8 weeks [23]. The Dutch scientists (de Jonge P, etc.) (2016) conducted an experimental study on pigs using the developed tubular collagen-vikril scaffolds of the ureter, which concluded that the material was suitable for ureteral reconstruction, but further optimization of the design was necessary to prevention of neuritis strictures [24].

Analysis of published data in Russia, as well as abroad, shows scientific interest in the search for the best basis material for reconstructive surgery transplant construction. M.V. Kisilevskiy et al. in 2016 published experimental data on a biosynthetic tracheal matrix, developed from material acquired via special separation methods from canine bone-marrow donor material. Analysis of in vivo heterotopic implantation results of the developed design showed the preservation of physical properties (size, structural features, integrity), functional characteristics (carcass, strength, elasticity) and initial structure (ordered matrix fibers reinforced with semirings) and absence of local and systemic signs of rejection within 1 month after implantation [25,26]. Samples of the matrix also showed preserved integrity and lumen, colonization by recipient cells, absence of toxic effect and were not biodegraded under the influence of the recipient organism internal environment. A group of researchers from St. Petersburg under the leadership of Orlova N.V. (2016) published work on the reconstruction of the bladder of a rabbit using allogeneic cells of various origin [27]. As a scaffold base, we used a polymer lactic acid material-poly-L,L-lactide, reinforced with fibroin silk in a ratio of 1:1. During the experiment, acellular matrixes were tranplanted, as well as allogeneic smooth muscle cells of the bladder, fibroblasts and allogeneic MSCs (rabbit bone marrow). The results of the experiment showed the expressed regenerative abilities of MSC-containing scaffolds, represented by the absence of a significant inflammatory reaction or signs of implant rejection, and also in the histologically identified initial stages of repair and angiogenesis.

Therefore, current research on the creation of prototypes for organs and tissues using frame structures and cellular material is extremely relevant. The most controversial issue remains the choice of optimal material for the creation of a implant-framework, as well as the study of its physical properties and biocompatibility. The task of creating a new three-dimensional biocompatible matrix formed the basis of our study.

MATERIALS AND METHODS

Based on the analysis of scientific literature, it was decided to use a wire made of titanium and titanium nickelide with a diameter of 20-40 microns to create a biocompatible nanostructure. The known properties of the material and the task of creating a tissue structure with properties of a tubular organ determined our choice.

The topography and the elemental composition of the fiber surface were examined via scanning electron microscopy (SEM) using a "Hitachi S-3400N" microscope. The diameter of the initial fibers, determined from SEM images, was 32 ± 1 μm. The visually visible fiber color was close to black, which is probably due to the presence of carbon on the surface. Purification of fibers in isopropyl alcohol using an ultrasonic device "USPN-2T" did not lead to a decrease in the amount of impurities on the surface.

A submicron topographic contrast on the surface, a large number of inclusions differing in elemental composition, embedded in the surface or "smeared" over the surface were visualized. Carbon predominates in the composition of impurities, but the presence of a large amount of oxygen, as well as sulfur and calcium, indicates the possible presence of organic compounds.

Thus, the analysis of the structure and chemical composition of the fiber surface indicates the need for compulsory postprocessing. As a solution to this problem, it is proposed to use ion-cleaning of the initial material.

A vacuum unit equipped with two planar balanced magnetrons and an ion source with an anode layer were used for ion purification.

All sources were oriented in one technological zone to provide simultaneous surface treatment. Frames with fibers were fixed on a rotating holder using a titanium wire bracket. For the primary cleaning of the substrate surface from organic contaminants, ultrasonic cleaning was performed in isopropyl alcohol.

Ionic cleaning (etching) of the fiber surface was performed by a beam of argon ions using a slot-type ion source for 10 min at an ion energy of 2 keV and an ion current density 2-3 mA / cm2 substrates.

After carrying out ionic cleaning of the fibers, their color had changed from black to a light metalical hue. Visible on SEM images submicron topographic contrast is preserved and more profound. After ionic treatment, the fiber diameter was reduced by 3 μm. SEM imaging showedan absence of contrast associated with the different elemental composition in reflected electrons. Taking into account the sufficiently large thickness of the sprayed material, it can be stated that all impurities from the exposed surface were completely removed.

In order to increase initial material bioavailability and affinity to surrounding tissue surface, a bioactive film is introduced onto the surface of cleaned titanium filament.

TiCaPCON was used as a bioactive cover, which was derived from magnetron scattering of composite target TiC05+10%Ca3(PO4)2, which was achieved via self-sufficient high-temperature synthesis. Scattering of target material was performed in an gas solution of argon and 15% ozone with the following parameters: 0.1Pa pressure, scattering temperature 20-30 minutes, magnetron current 2A, distance to membrane 100mm, at 50V.

A double-axis rotation system over the magnetron was used in order to achieve uniformity of treatment.

After acquiring the prototypes for the base structure, which underwent etching and bioactive layering, the modified structure was tested for adhesion and cellular proliferation modalities with the use of different cellular cultures.

The cultivation of linear cellular colonies was performed in standard conditions in a CO2 incubator at 5% CO2 concentration (95% air), and in conditions with increased humidity in a cellular-growth environment DMEM/F12 (Invitrogen, USA), containing 10% calf fetal serum (“HyClone Defined”, HyClone, USA), insulin 0,4mkM, 0,25mg/l hentamycine at a level of 2,0-2,5 million cells/ml per 175cm2 cultivation tube (Corning, USA). The cultivation growth environment was renewed every 72 hours. Transgression was performed by addition of standard tripsine substitute Tryple (Invitrogen, USA), which does not require inactivation after fermentation, and also via microscopic control of cellular detachment. The cellular suspension was centrifuged at 1500RPM (Eppendorf) for 5 minutes until a cellular precipitate was acquired for subsequent dilution and resuspension.

24 hours later MCF-7 with 3T3 cellular adhesion was visualized on all nanostructure models. During the first 24-hour period, minimal adhesion was visible, by the fourth day adhesion of cellular groups was noted (cellular proliferation was present on all nanostructure surfaces). Complete cellular coverage of both cellular subtypes was achieved by the 7th day of cultivation. Cellular infiltration of the area between nanostructure fibers was achieved only when 3T3 cells were used for cultivation. Separately, the conditions of cellular cultivation of the base of the cultural cell were evaluated.

It was noted that the in cultivation cell with specimen #1, during cellular cultivation of MCF-7 cells, the cellular monolayer was compromised, this is the main sign of cytotoxicity. 3T3 cells did not shows signs of cellular monolayer compromise.

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Thusly, the most optimal and resultative method of material preparation for increased bioavailability and decreased cytotoxicity was found.

A decision was made to use a titanium wire as the main structural element for a intercellular matrix carcas, as it is the most useful material for the creation of a spatial integrant structure. As a result, a titanium composition net was developed from a prepared titanium fibers with a diameter of 30-40mkm for the purpose of the experimental study. (Fig. 1).

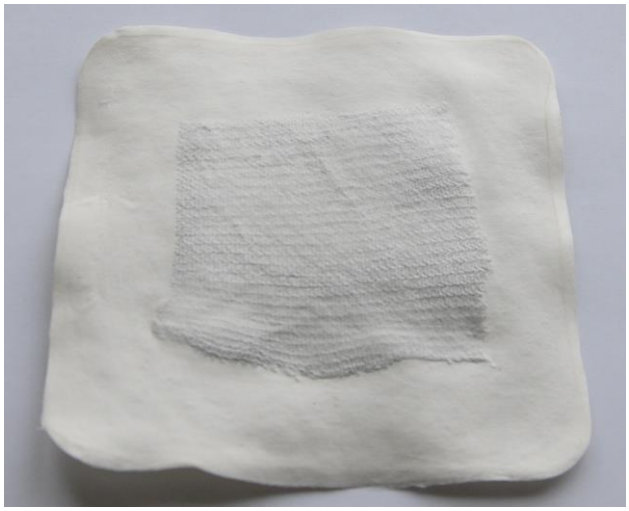

Figure 1. - A titanium composition net developed from prepared titanium fibers.

Topographic visualization and the evaluation of elemental composition of mesh fiber surfaces was performed using the scanning electron microscopy (SEM) with the "Hitachi S-3400N" microscope equipped with an X-ray energy dispersive NORAN spectrometer. It showed a large number of impurities accounting for cytotoxicity, overviewed at the previous stages of the study. Due to this fact, the technological preparation of the mesh was carried out according to the previously developed protocol, which includes ionic cleaning and application of TiCaPON bioactive coating.

The development of a filler for the framework structure was carried out. A collagen matrix was used as a filler for the study. This matrix was used as a filler for the prototype of the structure with a reinforced purified titanium mesh frame with bioactive coating, inserted between two layers of the collagen matrix. (Fig. 2).

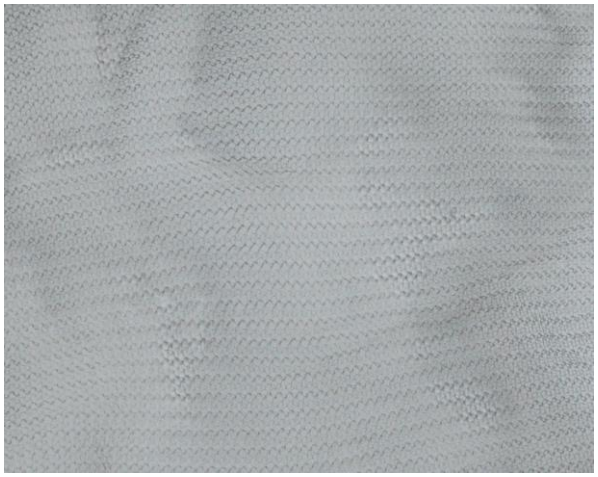

Figure 2. - Reinforced purified titanium mesh frame with bioactive coating, inserted between two layers of the collagen matrix.

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Analysis of cytotoxicity was performed as the next stage of the study. The degree of tropism and adhesive function of two types of developed nanostructures were also analyzed in vitro. In order to make possible the use of bioconstructions populated with cellular material in experiments in vivo, stem cells of Wistar line laboratory rat were cultured.

MSC KM's of the 4th passage were used, aquired from the bank of the Center for Collective Use "Regenerative Medicine" of I.M.Sechenov First Moscow State Medical University. Cultivation was carried out under standard conditions in a CO2 incubator with a CO2 concentration of 5%, atmospheric air of 95% and high humidity. The replacement of the culture medium with fresh medium was performed every 72 hours. The composition of the complete growth medium: DMEM / F12 (Invitrogen, USA) containing 10% calf fetal serum ("HyClone Defined", HyClone, USA), insulin 0.4 µM, 0.25 mg / l gentamicin. MSC KM was applied at a concentration of 5 × 105 cells / cm2.

Microscopy confirmed the absence of toxic effect of the framework bioconstruction material, which was represented by the active growth of cellular material on the surface of the prototypes. On the 5th day, the formation of individual groups of cells on the surface of grid fibers was noted. By the 11th day - the complete covering of the fibers with a cellular monolayer with the filling of inter-fiber space was documented.

Additionally, the samples of the material were microscopically examined with a laser scanning confocal microscope LSM-710 (Carl Zeiss Microscopy, Jena, Germany). For this purpose, the samples were stained with acridine orange and ethidium bromide. A stock solution of dyes was prepared as follows: 1.5 mg of acridine orange and 5.0 mg of ethidium bromide was dissolved in 0.1 ml of 95% ethanol, then 5 ml of distilled water was added. The working solution of dyes was prepared from the stock by dissolving in a 100X volume of PBS. Previously washed from the culture medium samples were filled with a working solution of dyes for 5 minutes for the purpouse of colouring. As a result, acridine orange stains live cells, and ethidium bromide - dead cells. (Fig. 3).

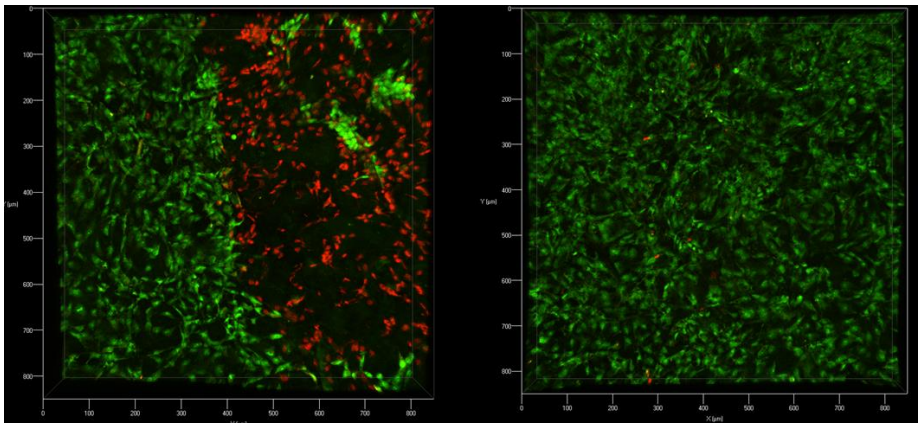

Figure 3. - MSC CM on a hybrid matrix of NiTiCol. Green colouring represents living cells, red - dead cells. Confocal microscopy. Staining: acridine orange and ethidium bromide.

Stained samples from in-vitro cultivation plates were transferred to sterile Petri plates with a thin glass bottom (0.16 mm) in PBS solution. To obtain images, an EC-Plan Neofluar 10x / 0.3 / M27 lens was used. Excitation of fluorescent acridine orange was performed with a laser with a wavelength of 488 nm, fluorescence was recorded in the range of 495 ÷ 545 nm. Excitation of fluorescent ethidium bromide was performed with a laser with a wavelength of 561 nm, fluorescence was detected in the range of 580-690 nm. 3D images were obtained by reconstructing Z-stacks obtained by scanning in 1024×1024 pixel mode (850×850 µm), with a confocal diaphragm of 34µm in diameter, scanning speed of 0.64 µs/pixel. As a result, a superposition of fluorescent images of acridine orange (green), ethidium bromide (red) and the image obtained in transmitted light mode was obtained (in 3D reconstruction, the transmitted light mode was not included in the general superposition) (Fig. 4 A, B).

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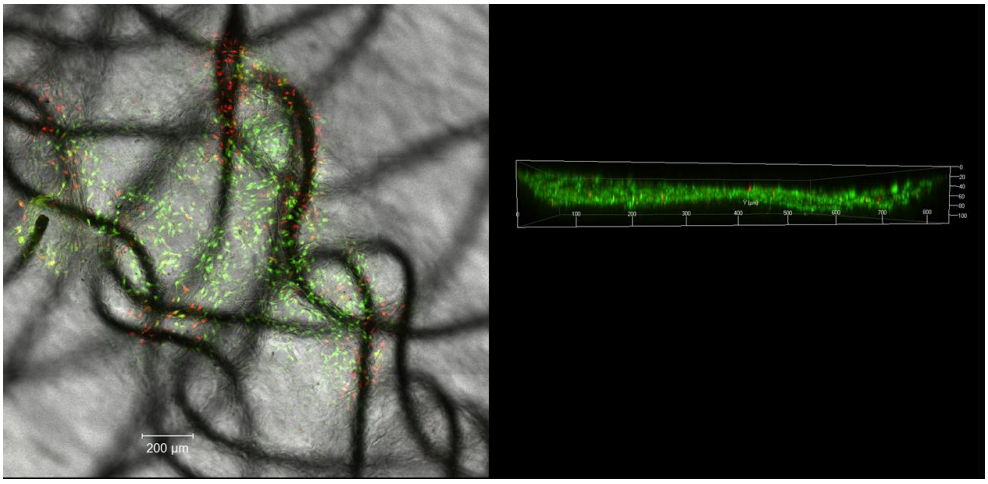

Figure 4 (A, B). A - MSC CM on a hybrid matrix of NiTiCol. Green colouring represents living cells, red - dead cells. Confocal microscopy with transmitted light. Staining: acridine orange and ethidium bromide. B - 3D-reconstruction: MSC CM on a hybrid matrix of NiTiCol forming a continuous layer of cells. Green colouring represents living cells, red - dead cells. Confocal microscopy with transmitted light. Staining: acridine orange and ethidium bromide.

Later in the laboratory conditions, two types of hollow tubular implants were created from the obtained bioengineering structures. The first type - implants based on purified titanium mesh, populated with a cellular culture, and implants based on a purified titanium mesh with a collagen filler, also populated with a cellular culture. The second type - implants based on a purified titanium mesh and implants based on a purified titanium mesh with collagen filler. Both types of implants were up to 4 cm long and up to 5 mm in diameter.

Finally, laboratory experiment was performed. It required implantation of prepared prototypes of tubular bioengineering structures into laboratory animals in order to evaluate the behavior of various bioengeneered implant structures in vivo. The experiment was approved by Local Ethics Committee of I.M. Sechenov First Moscow State Medical University (Sechenov University) and carried out in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments.

In the experiment, two groups of rats participated, four in each group. In the first group, animals with implanted tubular structures without cultured cellular material on the surface were observed, and in the second group, tubular implants populated with cellular cultures were observed.

All rats were subjected to subcutaneous implantation of bioengeneered tubular prototypes. All animals underwent sedation and aneshtesia, after which incision was performed dorsally on both sides of the spine. Next, a subcutaneous tunnel was bluntly made, into which a prototype of the tubular structure was subsequently placed, and the wounds were sutured.

One month after the surgical procedure, all animals were withdrawn from the experiment. Morphological and cytological evaluation of the obtained material was performed (Fig. 5 A,B; 6 A,B).

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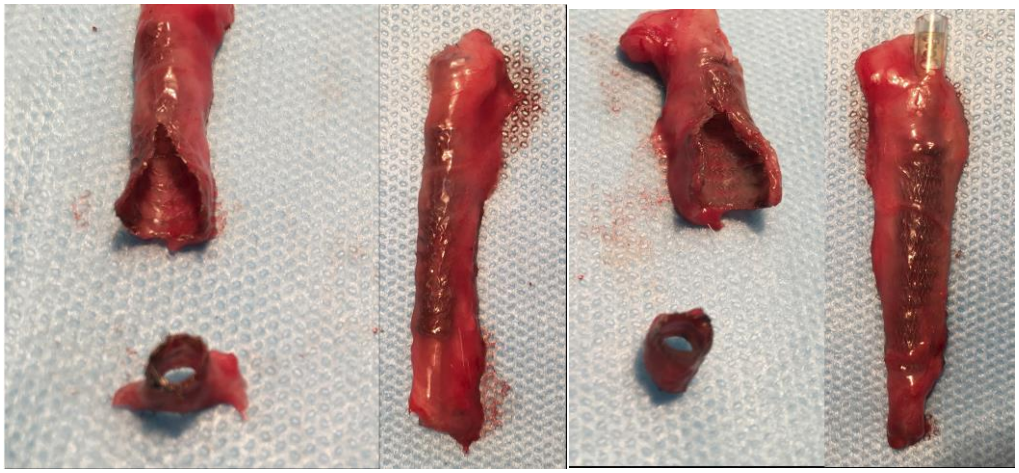

Figure 5 (A, B) - Morphological evaluation of implanted tubular structures 1 month after implantation. Group 1. A – Prepared structural titanium mesh Group 1. B – Prepared structural titanium mesh with collagen filler. Group 1.

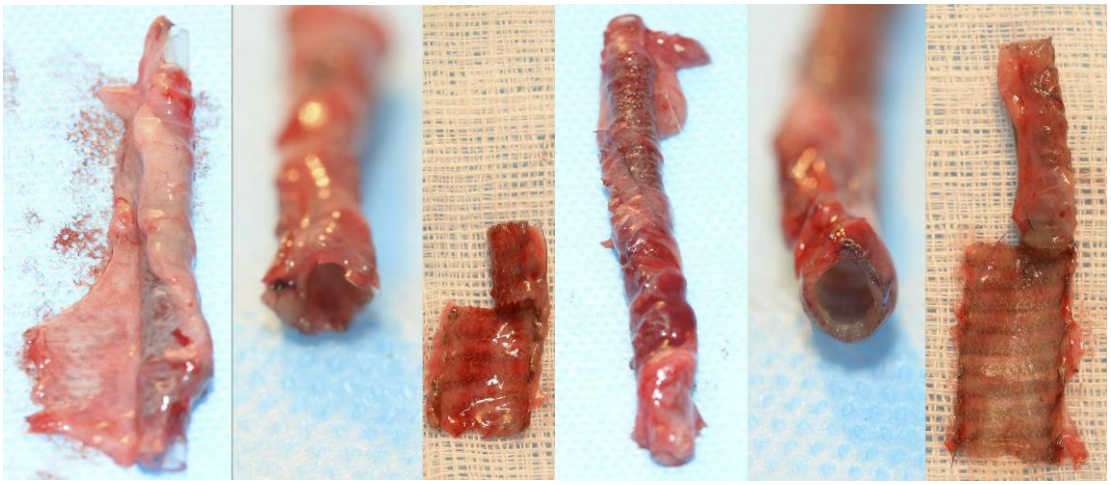

Figure 6 (A, B). - Morphological evaluation of implanted tubular structures 1 month after implantation. Group 2. A – Prepared structural titanium mesh with cellular culture. Group 2. B – prepared structural titanium mesh with collagen filler with cellular culture. Group 2.

In both groups of animals, propper adhesion of the developed bioengineered structures was achieved. No evidence of immune inflamatory response was found. It is worth noting that neovascularization and complete coverage of implanted structures with a connective tissue was observed in both groups. However, the most noticeable vascular inflitration and connective tissue coverage was noted in group samples containing a collagen filler with a cellular medium on its surface. When carrying out a morphological evaluation of the obtained samples, sufficient compression elasticity and a high support capacity of the developed material were revealed.

RESULTS

When analyzing the aquired data, it can be concluded that the obtained 3D biocompatible nanoconstructions, based on a titanium mesh, which underwent ionic cleaning and bioactive coating with TiCaPON - are not toxic and have sufficient surface adhesion properties in order to produce a cellular monolayer in the cultivation of two different types of standard cellular cultures, as shown in the intermediate stages of the study.

The developed matrix based on titanium is suitable for creating a volumetric fabric of different bioengineering designs. It is important to note the possibility of forcefull transformation of the obtained

structure, which makes it possible to use the developed matrix in various fields of regenerative medicine, such as urology, pulmonology, gynecology, reconstructive surgery and others.

Our team has found ways to increase the strength, structural and adhesive properties of the intercellular matrix. Multistage preparation of the base material and the use of collagen fillers as an additional structural element provide propper functional enhancement of the bioengeneered nanostructures. In order to achieve anatomically correct structures, research is directed towards the developement of diverse constructive elements.

DISCUSSION

The novelty of the obtained results is primarily determined by the selected material for the development of the intercellular matrix - a titanium wire with a diameter of about 20 μm, which provides, both the resilient and elastic properties of the structure, and optimal cultivation conditions for cellular cultures with specified properties. These qualities are extremely important for the creation of hollow organs and other anatomic structures.

For the first time in the field of tissue engineering, the structural components were ionically cleaned and then coated with TiCaPCON, which allowued us to expect a qualitatively new structural component of the three-dimensional biocompatible matrix framework for reconstructive surgery.

As a result of our studies, we found that ionic cleaning is necessary to create three-dimensional biocompatible nanoconstructions based on titanium, which will ensure the optimal adhesion properties of the structural surgaces surfaces and removal of cytotoxic impurities, which result from the technological preperation of the material. Two standard linear cell cultures (epithelial and mesenchymal) can form a monolayer on the surface of the filaments in in-vitro conditions. Purified nanoconstructions based on titanium with a bioactive coating are suitable for the creation of tissue bioengineered designs. This is confirmed by the conducted experimental study and is the basis for further studies of the structural material and other experimental studies.

Data availability

The raw/processed data required to reproduce these findings cannot be shared at this time due to technical or time limitations.

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REFERENCES

[1] C. Singh and X. Wang, A new design concept for knitted external vein-graft support mesh, J Mech Behav Biomed Mater, 48 (2015) 125-133.
[2] S.H. Alavi, Inflammatory response assessment of a hybrid tissue-engineered heart valve leaflet, Ann. Biomed Eng, 41 (2) (2013) 316-326.
[3] R. Moreira, Tissue-Engineered Heart Valve with a Tubular Leaflet Design for Minimally Invasive Transcatheter Implantation, Tissue Eng Part C Methods, 21 (6) (2015) 530–540.
[4] H. Alavi, Hybrid Tissue-Engineered Heart Valve, The annals of thoracic surgery, 99 (2015) 2183–2187.
[5] K. Loger, “Microstructured Nickel-Titanium Thin Film Leaflets for Hybrid Tissue Engineered Heart Valves Fabricated by Magnetron Sputter Deposition,” Cardiovascular Engineering and Technology, 1 (2016) 1-9.
[6] P.K. de Jonge, Recent advances in ureteral tissue engineering, Curr. Urol. Rep, 16 (1) (2015) 465.
[7] L. Zhu, Anatomic and sexual outcomes after vaginoplasty using tissue-engineered biomaterial graft in patients with Mayer-Rokitansky-Küster-Hauser syndrome: a new minimally invasive and effective surgery, J Sex Med, 10 (2013) 1652–1658.
[8] F. Porpiglia, Temporary implantable nitinol device (TIND): a novel, minimally invasive treatment for relief of lower urinary tract symptoms (LUTS) related to benign prostatic hyperplasia (BPH): feasibility, safety and functional results at 1 year of follow-up, BJU International, 116 (2015) 278–287.
[9] W.H. Hendren, Use of bowel for vaginal reconstruction, J Urol. 152 (1994) 752-758.

[10] W.H. Hendren Repair of the high vagina in girls with severely masculinized anatomy from the adrenogenital syndrome, J Pediatr. Surg. 30 (1995) 91-94.
[11] B. Filipas, The use of isolated bowel segment in complicated vaginal reconstruction, BJU Int., 85 (2000) 715.
[12] A. Seccia, Neovaginal reconstruction with the modified Mc Indoe technique: a review of 32 cases, Ann Plast Surg, 49 (2002) 379.
[13] R.E. De Filippo, Tissue engineering a complete vaginal replacement from a small biopsy of autologous tissue, Transplantation, 86 (2) (2008) 208-214.
[14] Lan Zhu, Anatomic and sexual outcomes after vaginoplasty using tissue-engineered biomaterial graft in patients with Mayer-Rokitansky-Küster-Hauser syndrome: a new minimally invasive and effective surgery, J Sex Med, 10 (2013) 1652–1658.
[15] F.N. Burks, Management of iatrogenic ureteral injury, Ther. Adv. Urol. 6(3) (2014) 115–124.
[16] V. Pansadoro, Iatrogenic prostatic urethral strictures: classification and endoscopic treatment, Urology, 53(4) (1999)784–789.
[17] A.A. Badawy, Buccal mucosa graft for ureteral stricture substitution: initial experience, Urology, 76(4) (2010) 971–975.
[18] A. El-Hakim, First prize: ureteral segmental replacement revisited, J. Endourol., 19(9) (2005) 1069–1074.
[19] P.V. Glybochko, Ju.G. Aljaev, V.N. Nikolenko, A.B. Shehter, A.Z. Vinarov, L.P. Istranov, E.V. Istranova, R.K. Abojanc, A.V. Ljundup, M.I. Danilevskij, A.E. Guller, P.A. Elistratov, D.V. Butnaru, D.F. Kantimerov, G.A. Mashin, A.S. Titov, A.V. Proskura, K.V. Kudrichevskaja, Experimental justification of the development of tissue engeneering construction using matrix based on decellular vascular wall and cells of buccal mucosa for substitution urethroplasty, Urologiia, 6 (2014) 41-46.
[20] P.V. Glybochko, Ju.G. Aljaev, V.N. Nikolenko, A.B. Shehter, A.Z. Vinarov, L.P. Istranov, E.V. Istranova, R.K. Abojanc, A.V. Ljundup, M.I. Danilevskij, A.E. Guller, P.A. Elistratov, D.V. Butnaru, D.F. Kantimerov, G.A. Mashin, A.S. Titov, A.V. Proskura, K.V. Kudrichevskaja, Tissue-Engineered substitution urethroplasty based on decellularized vascular matrix and autologous cells of the buccal mucosa: the first experience, Urologiia, 3 (2015) 4-10.
[21] Yu X, Qian G, Chen S, Xu D, Zhao X, Du C., A tracheal scaffold of gelatin-chondroitin sulfate-hyaluronan-polyvinyl alcohol with orientated porous structure, Carbohydr Polym., 159 (2017) 20-28.
[22] Al-Ayoubi AM, Rehmani SS, Sinclair CF, Lebovics RS, Bhora FY., Reconstruction of anterior tracheal defects using a bioengineered graft in a porcine model, Ann Thorac Surg., 103(2) (2017) 381-389.
[23] Jung SY, Lee SJ, Kim HY, Park HS, Wang Z, Kim HJ, Yoo JJ, Chung SM, Kim HS., 3D printed polyurethane prosthesis for partial tracheal reconstruction: a pilot animal study, Biofabrication 8(4) (2016) Available from URL: http://iopscience.iop.org/article/10.1088/1758-5090/8/4/045015/meta
[24] de Jonge P, Simaioforidis V, Geutjes P, Oosterwijk E, Feitz W., Ureteral reconstruction with reinforced collagen scaffolds in a porcine model, J Tissue Eng Regen Med. (2016) Available from URL: https://onlinelibrary.wiley.com/doi/full/10.1002/term.2366
[25] Kiselevskiy M.V., Anisimova N.Y., Kornushenkov E.A., Shepelev A.D., Chvalun S.N., Polotskiy B.E., Davydov M.I. Biocompatible synthetic tracheal matrices based on polymer ultra-fibrous materials colonized by mesenchymal multipotent cells, Sovremennye tehnologii v medicine, 8(1) (2016) 6-13.
[26] M.V. Kiselevskii, N.Yu. Anisimova, A.D. Shepelev, T.Kh. Tenchurin, V.G. Mamagulashvili, S.V. Krasheninnikov, T.E. Grigoriev, O.V. Lebedinskaya, S.N. Chvalun, M.I. Davydov Mechanical properties of bioengineered tracheal prosthesis based on a synthetic ultrafibrous matrix Russian Journal of Biomechanics 20 (2) (2016) 116–122.
[27] N.V. Orlova, A.N. Murav'ev, T.I. Vinogradova, N.M. Blyum, N.Yu. Semenova, N.M. Yudintseva, Yu.A. Nashchekina, M.I. Blinova, M.A. Shevtsov, M.L. Vitovskaya, N.V. Zabolotnykh, M.G. Sheikhov Experimental reconstruction of rabbit bladder using allogeneic cells of different tissue origin Meditsinskiy Al'yans 1 (2016) 50-52.