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BIOCOMPATIBLE AND FUNCTIONAL PROPERTIES OF A MICRODISPERSED TISSUE-SPECIFIC 3D MATRIX FROM DECELLULARIZED PORCINE CARTILAGE

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In contrast to decellularization of soft tissues for use as tissue-specific matrices in the creation of tissue-engineered constructs, decellularization of cartilage tissue requires several processing techniques, which can negatively affect the biocompatibility and functional properties of the native extracellular matrix (ECM). **Objective:** to study the biocompatible and functional properties of microdispersed tissue-specific 3D matrix from a porcine cartilage that is decellularized by sequential use of chemical, physical and enzymatic techniques. **Materials and methods.** For decellularization, microdispersed cartilage particles (MCPs), obtained by cryomilling, were incubated in detergent solutions (sodium dodecyl sulfate and Triton X-100), then treated with supercritical carbon dioxide ($scCO_2$) with 10% ethanol and DNase I. The Ames test (*Salmonella typhimurium* reverse mutation assay) was used to determine the genotoxicity of decellularized microdispersed cartilage particles (dMCPs). Local and general toxic effects, as well as resorption of dMCPs were studied *in vivo* on sexually mature outbred rats. Decellularized MCP specimens (10 mg) were implanted into the thigh muscle tissue. Viability of human adipose-derived mesenchymal stem/stromal cells (hAdMSCs), when cultured on dMCPs, was analyzed by *in vivo* microscopy, stained with fluorescent Calcein AM dye. Cell metabolic activity was assessed using PrestoBlue™ Cell Viability Reagent. **Results.** It has been proven that porcine dMCPs implanted in rat muscle after treatment with $scCO_2$ do not exhibit local and general toxic effects, and do not show genotoxicity and negative effects on the reproductive system of animals. After 6 months of *in vivo* experiment, most (87%) of the implanted decellularized cartilage was resorbed. It was shown that the resulting matrices are able to support adhesion and proliferation of hAdMSCs. **Conclusion.** Porcine dMCP specimens are suitable for biocompatible medical products in terms of local and general toxic effects, genotoxicity and reproductive toxicity, and can be used as a matrix for creating cell- and tissue-engineered cartilage constructs.

Keywords: articular cartilage, decellularization, 3D matrix, resorption, biocompatibility, adipose-derived MSCs, adhesion, proliferation, tissue-engineered construct.

INTRODUCTION

A number of biocompatible 3D matrices (also called scaffolds or carriers) made of resorbable synthetic [1, 2] and natural polymers [1, 3, 4], which more or less simulate the properties of native ECM, have been developed to create biomedical products for tissue engineering and regenerative medicine. However, 3D matrices made of polymeric materials do not possess tissue-specific properties characteristic of ECM, which can perform not only scaffold functions but also selectively support adhesion, proliferation and differentiation of cells of a particular tissue or organ [5]. In this regard, there is great interest in 3D matrices from decellularized allogenic or xenogeneic tissues [6–8]. The main problem of decellularization is the need for the most complete removal of cellular material with the highest possible degree of preservation of morphology, composition, biochemical and mechanical properties of native ECM. Due to the fact that the completeness of decellularization is influenced by many factors, such as donor type, organ size and integrity, tissue type, its structure and density, etc., there cannot be a universal decellularization protocol [9, 10]. To date, a wide range of decellularization protocols have been developed, using physical, chemical, enzymatic treatment methods and their combination [11–13].

Among the physical methods, the use of supercritical fluids that have zero surface tension, low viscosity, increased solubility and can penetrate deeply into the bulk of materials seems promising [14–16]. The most interesting is $scCO_2$ [17] – it reaches its critical point

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at a sufficiently low temperature (31.1 °C), it can be easily removed by simple pressure relief after treatment and it is non-toxic. At the same time, extraction of toxic compounds soluble in $scCO_2$ can occur, which leads to increased biocompatibility of the obtained materials decellularized using cytotoxic detergents [18]. Since $scCO_2$ is a non-polar compound, treatment is carried out in the presence of a polar agent, such as ethanol, to improve efficiency. Addition of ethanol increases both the efficiency of high-density tissue decellularization [19, 20] and the preservation of such important ECM components as collagens, glycosaminoglycans, adhesive proteins (fibronectin, laminin and others) and angiogenic factors [21].

The high density of cartilage makes it difficult to achieve complete decellularization, making it hard to diffuse surfactants and remove cellular detritus. Previous studies have shown that to achieve complete cartilage decellularization, it is necessary to use a protocol that includes both chemical (treatment with surfactants) and physical (supercritical fluids, cyclic freeze-thaw) treatment methods or enzymatic (DNase, RNase) methods of tissue exposure [22, 23].

The objective of this work was to investigate the biocompatibility and matrix properties of a microdispersed 3D matrix from porcine cartilage decellularized by sequential treatment with chemical, physical, and enzymatic methods.

MATERIALS AND METHODS

Porcine thigh and knee joints were obtained at slaughterhouse Promagro in Stary Oskol, Russia, after the slaughter of healthy animals (weighing about 120 kg) in accordance with the European Directive 64/433/EEC. After transportation in a refrigerated form, the cartilage was cut off from the articular surfaces with a scalpel, cut into 0.5 × 0.5 × 0.1 cm fragments, frozen at –80 °C and stored at this temperature until cryomilling began.

MCPs were obtained in a CryoMill (Retch GmBH, Germany). Grinding was done with the milling pot constantly contacting liquid nitrogen, at 25 Hz frequency for 4 minutes. The fraction of 30–100 μm particles was isolated using sieves with the appropriate mesh size.

MCPs were decellularized according to the previously developed technique [22]. The MCPs were treated at room temperature and periodically stirred on a magnetic stirrer (3 times per day, 1 hour, 200 rpm) in three changes of phosphate-buffered saline (PBS) (pH = 7.4) containing sodium dodecyl sulfate (SDS) and an increasing concentration of Triton X-100 (1, 2, and 3%, respectively).

The MCPs were then thoroughly washed of residual detergents in three changes of PBS containing an antibiotic (ampicillin, 20 μg/ml) and an antimycotic (amphotericin B, 2 μg/ml) for 72 hours at room temperature.

In the second stage, MCPs were treated in an $scCO_2$ atmosphere on a Speed SFE unit (Applied Separations, USA) at 300 bar, +35 °C, and $scCO_2$ flow rate of 2.5 ± 0.5 mL/min for 8–24 hours. 10% ethanol was used as a polarity modifier.

DNase I (New England Biolabs Inc., USA) at a concentration of 50 U/ml in 10 mM Tris-HCl buffer solution (pH 7.6) containing 2.5 mM $MgCl_2$, 0.5 mMol $CaCl_2$ was used to enhance DNA removal from porcine MCPs. The treatment time at 37 °C was 48 hours.

Specimens of decellularized MCPs (dMCPs) were sterilized by radiation at a dose of 1.5 Mrad.

Before the study, dMCP specimens were stored at –20 °C.

Determining DNA content

DNA extraction from MCPs (n = 3) and dMCPs (n = 3) specimens weighing 25 mg was performed using the DNeasy Blood & Tissue Kit (QIAGEN, Germany) according to the manufacturer's instructions. A bacteriophage λ DNA calibration curve (Invitrogen, USA) (0 ng/mL to 1000 ng/mL) was used to determine the absolute amount of DNA.

Scanning electron microscopy of specimen surfaces

MCPs and dMCP specimens were dehydrated in ethanol solutions of increasing concentrations according to the following scheme: 50% alcohol for 3 minutes, 50% alcohol for 3 minutes, 70% alcohol for 3 minutes, 90% alcohol for 6 minutes, and 96% alcohol for 6 minutes; they were air-dried and adhered to electron microscopy tables using double-sided adhesive tape.

Conductive coating was obtained by gold ion sputtering for 40 seconds at a constant current of 5–7 mA on JFC-1600 device (JEOL, Japan). The morphology of MCPs was studied using a JSM-6360LA scanning electron microscope (JEOL, Japan) at an accelerating voltage of 5 kV.

METHODS OF STUDYING THE BIOCOMPATIBLE PROPERTIES OF DMCPS

A. In vitro studies

Genotoxicity

Since the decellularization process uses potentially genotoxic substances (detergents) and fails to completely remove DNA from MCPs, it is possible that the decellularized cartilage may affect the patient's genetic material when clinically used as part of it.

The genotoxicity of dMCP specimens was assessed by the Ames test (*Salmonella typhimurium* reverse mutation assay), which is a bacterial test system for recording mutations to prototrophic histidine by the action of tested samples and (or) their metabolites inducing base replacement or frameshift type mutations in the genome of that organism.

We used a set of indicator strains of *Salmonella typhimurium* obtained from the Russian National Collection of Industrial Microorganisms under state research institute Genetika, having the appropriate certificates.

To prepare the extract, 25 mg of dMCPs were incubated for 3 days at 37 °C in 1 mL of sterile 0.9% sodium chloride solution (NaCl). Five concentrations of the extract were tested: stock solution and four successive 5-fold dilutions in 0.9% NaCl. The experiment was accompanied by positive controls, which were substances inducing mutations in the corresponding test strains.

For metabolic activation, we used the liver S9 fraction of male Wistar rats, which, 5 days before slaughter, were injected with microsomal enzyme inducer Sovol, 300 mg/kg, once intraperitoneally.

After incubation for 48 hours, the number of reverted colonies was counted in the groups of indicator strains. The mean number of revertant colonies for the drug-treated groups were compared simultaneously with the corresponding negative control groups.

If the substance and/or its metabolites have mutagenic activity, they will induce reverse mutations from auxotrophy to histidine prototrophy in histidine-dependent strains of *Salmonella typhimurium*. According to generally accepted approaches, the mutagenic effect was considered to be significant if the average number of revertant colonies per plate in the experimental variant exceeded that in the control variant by 2 or more times.

B. In vivo studies of reproductive toxicity, local and general toxic effects

The experiments were carried out on sexually mature outbred rats. Maintenance and all manipulations with the animals were performed according to the rules adopted in the European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes (ETS 123), Strasbourg, 1986), and in accordance with GOST 33216-2014 Interstate Standard "Guidelines for the Maintenance and Care of Laboratory Animals".

Reproductive toxicity

When genotoxic effects act not only on somatic cells but also on germ cells, changes can become hereditary. To study the effects on generative function and embryotoxic effects in the antenatal and postnatal periods of development of the genotoxic effect on germ cells, the reproductive toxicity of dMCPs was studied.

The animals were divided into groups of 6 males and 12 females per group: control males, control females, males with dMCP specimens, and females with dMCP specimens. Sham-operated rats served as controls.

Fourteen days after implantation, males and females were paired in a 1 : 2 ratio (one male and two females) for 14 days. Every morning, vaginal swabs were taken from females and the presence of spermatozoa in them was determined. If spermatozoa were detected, the rat was weighed, placed in a separate cage, and the gestation period was counted, taking the day of spermatozoa detection as the first day of pregnancy.

During the study, general clinical observation was performed, body weight, feed and water consumption were determined.

Some pregnant females were euthanized on day 20 of pregnancy; fetuses were examined, weighed, and the cranio-caudal size was measured. The number of corpus luteum in the ovaries, implantation sites, and the number of live and dead fetuses were counted. Pre- and post-implantation fetal mortality rates were determined based on the data obtained. The other half of the females were left until delivery to monitor the development of the offspring for 5 days. At the end of the study, adult animals were euthanized. After death, the ovaries were removed from the females and the testes and testicular appendages from the males. These reproductive organs were examined macroscopically for abnormal or pathological changes and weighed on VIBRA AJ-1200CE scale (ShinkoDenshiCo., Ltd., Japan), with further calculation of their relative weight (percentage ratio of organ weight to animal body weight). The ovaries and testes were fixed in 10–15% neutral formalin. After fixation on histoprocessor TLP-144 (MedTechnikaPoint LLC, Russia), histological processing was performed. After that, paraffin blocks were prepared on an ESD-2800 pouring station (MedTechPoint, Russia). Then, using rotary microtome RMD-4000 (MedTechnicaPoint, Russia), we obtained 3–4 nm thick sections that were glued to a slide, deparaffinized, and stained with hematoxylin and eosin. Histological specimens were examined by light microscopy using biological microscope Leica DM1000 (Leica Microsystems GmbH, Germany).

Local and general toxic effects

The specimen (10 mg weight) was implanted into the thigh muscle tissue under sterile conditions under the anesthetic Zoletil 100 (Virbak, France) at a dose of 15 mg/kg. Sham-operated animals (underwent surgery without specimen implantation) served as controls.

To study local effects, the animals were euthanized by carbon dioxide inhalation at day 28, months 2, 3, and 6 after dMCP specimens had been implanted. The results were evaluated macroscopically and by histological methods. According to semi-quantitative assessment results [24], the reactivity class of the studied material in comparison with the control sample was determined (in points): no response or minimal response (0.0 to 2.9); mild response (3.0 to 8.9); moderate response (9.0 to 15.0); severe response (≥15.1).

General toxic effects were investigated at up to 2 months of implantation simultaneously with assessment of their local effects. Analysis of animal condition

included: survival and appearance, animal weight, behavior (excitability, aggressiveness), reaction to external stimuli, pain response, feed and water consumption. At month 2, blood was collected from the animals for clinical (hematological analyzer Mindray BC-2800-vet (Mindray, China) and biochemical (biochemical photometer Stat Fax 4500+ (Awareness Technology Inc., USA) studies and urine (URISCAN Optima, YD Diagnostics Corporation, Korea). The following organs were taken and weighed: heart, lungs, thymus, liver, spleen, kidneys, and brain. These organs were weighed using Shinko scales, AJ-1200CE (Shinko Denshi Co., Ltd., Japan). H&E-stained histological preparations of the heart, lungs, thymus, liver, spleen, intestines, kidney, brain, and regional lymph nodes were examined using biological microscope LeicaDM 1000 (Leica Microsystems GmbH, Germany).

The degree of resorption of the implanted specimens was determined morphometrically using LAS Interactive Measurement Module, a computer morphometric program.

The excised sections of organs and tissues were fixed in 10–15% neutral formalin solution with subsequent embedding in paraffin. 5–7μm-thick sections were prepared. Histological preparations were examined by light microscopy using biological microscope LeicaDM 1000 (Leica Microsystems GmbH, Germany).

Investigation of the ability of dMCPs to support cell adhesion and proliferation (functional properties)

Third-passage hAdMSCs were isolated and cultured in complete cell culture medium (CCCM) according to the standard technique [25].

Adhesion and proliferation experiments were performed using a hAdMSCs culture. These cells are capable of differentiating in the chondrogenic direction with subsequent formation of cartilage tissue. To prevent cell growth outside the matrix surface, the experiment was performed in polypropylene tubes. 5 mg of dMCPs were placed in sterile tubes under aseptic conditions, CCCM was added and incubated for 24 hours at +37 °C to saturate the medium. A day later, hAdMSC suspension was prepared with a concentration of 500,000 cells/mL; excess medium was removed from the tubes, and 1 mL of cell suspension was added to each tube. The tubes were placed in a rack and shaken on laboratory shaker MultiBio 3D (Biosan, Latvia) for 2 hours at room temperature, after which the tubes were placed in a CO_2 incubator and cultured at +37 °C in a humid atmosphere containing (5 ± 1)% CO_2.

To assess the interaction between cells and dMCP specimens using in vivo microscopy, we used fluorescent Calcein AM dye (Thermo Fisher Scientific, USA).

The metabolic activity of cells was determined using reagent test PrestoBlue™ (Invitrogen, USA) according to the manufacturer's instructions. Spectrophotometric analysis was carried out on plate reader Tecan Spark 10M (Tecan Trading AG, Switzerland). Cell count was measured according to the calibration curve constructed.

Statistical data analysis

The obtained data were statistically processed using Microsoft Office Excel 2010 software. The group arithmetic mean (M) and standard error of the mean (m) were calculated. Statistical significance of differences was assessed by Student's t-test. Differences between the groups were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Decellularization reduced the amount of DNA from 367 ± 53 ng/mg to 7 ± 1 ng/mg of tissue, which is less than 2% of the initial value and shows the decellularization process to be highly efficient [26]. At the same time, there are significant differences in the surface morphology of native and decellularized cartilage (Fig. 1).

The surface structure of the original cartilage tissue is smooth (Fig. 1). Cell lacunae characteristic of articular cartilage tissue, in which cell outlines were visualized, are detected. For decellularized cartilage, there were no cells in the lacunae on the surface of the microparticles, which also shows the treatment to be effective.

Genotoxicity

The number of revertant colonies in the control (solvent) was within the spontaneous variation for these strains. Strain response to standard mutagens was within normal.

Decellularized tissue-specific matrices from porcine MCPs at concentrations of 0.1, 0.5, 1, 5, and 25 mg/ml did not induce gene mutations in *Salmonella typhimurium* test strains TA 100, TA 98, and TA 97 with and without metabolic activation. In all variants of the experiment, the multiplicity of excess of the average number of revertant colonies per cup in the experiments over that in the control was less than 2-fold.

Thus, experimental samples of porcine dMCPs obtained by sequential treatment with detergents and $scCO_2$ are not genotoxic.

Local effect

Healing of surgical wounds in animals of the experimental and control groups occurred by primary intention. None of the animals showed symptoms of implant rejection, suppuration, suture divergence, and other postoperative complications. There were no necroses, hemorrhages, granulomas and severe edema around all specimens along the periphery in the surrounding muscle tissue. There were no signs of inflammation in the matrix

implantation area. Macroscopic examination revealed no signs of tissue irritation and inflammation, no scar tissue was detected.

Tissue response to surgical intervention and subsequent implantation of porcine decellularized cartilage specimens followed the usual pattern that is characteristic of the wound process and reaction to a foreign body, including traumatic inflammation and connective tissue formation stages.

The microscopic picture on day 28 of dMCPs implantation (Fig. 2) was characterized by the presence of a thin capsule around the implant consisting of dense fibrous connective tissue. No infiltration of the connective tissue into the implants was observed. No necrosis, hemorrhages, lymphohistiocytic infiltration and edema were observed in the surrounding fibrous and muscular tissue. Lymphomacrophage infiltration was noted in the capsule and surrounding tissues, as well as the presence of single giant cells, indicating the initial stages of resorption. Among connective tissue cells, fibroblastic cells prevailed: mesenchymal cells, fibroblasts and fibrocytes. A small number of neutrophils was detected in the surrounding tissues. A scanty vascular pattern was noted.

On day 28 after implantation, there was mild resorption of dMCP specimens. The average area of one implanted dMCPs particle per group of animals was 13867 ± 964 μm^2. The central part of the implant not populated with cells was clearly distinguished. Active infiltration by macrophages was observed at the periphery.

After 2 months (Fig. 2), the implantation site was covered with hair and was not visually identified. Signs of regeneration in the form of muscle-joint tissue were observed. The regenerate was in the form of thin bundles of collagen fibers infiltrated by fibroblasts and fibrocytes. In the stroma, there were small vessels, myocytes forming growth buds and strands of newly formed thin muscle fibers.

At month 3 (Fig. 2), the implant was an oval, with no capsule around it. There was intensive resorption of the matrix by macrophages in the periphery. Compared to 30 days of implantation, the material was resorbed by ~44%. The average implant area was 7644 ± 155 μm^2. The structure of the non-resorbed dMCPs was not altered. There was a macrophage reaction at the periphery of the implant with the presence of lymphocytes.

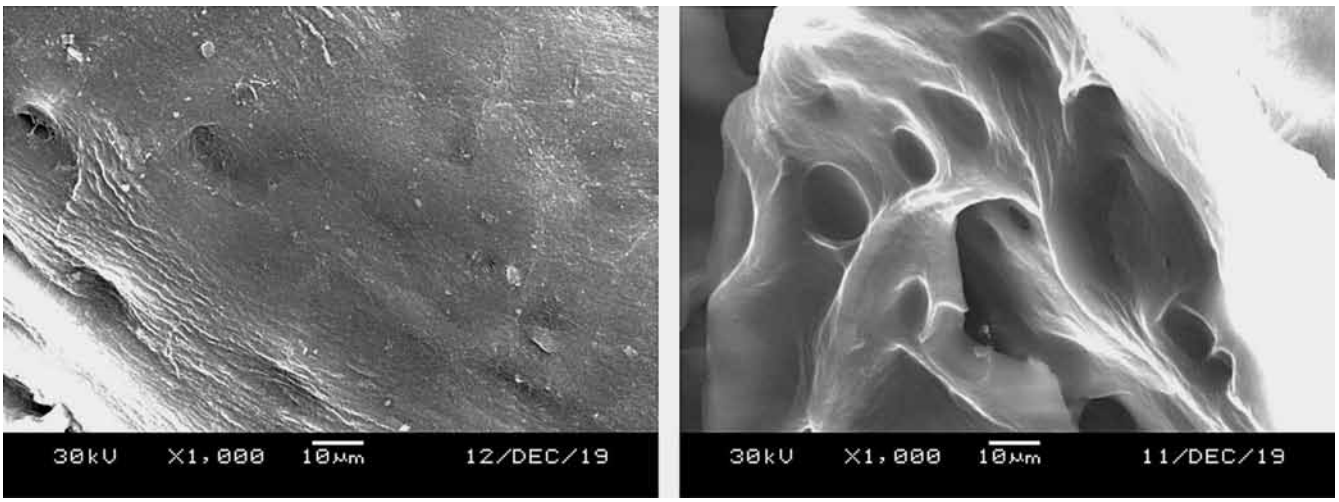
At month 6 of implantation (Fig. 2), a thin capsule of collagen fibers was formed around the dMCP specimens, which is an indicator of high biocompatibility. At the same time, the number of active macrophages in the tissues surrounding the implants still remained high. At the periphery of the implanted particles resorbed by ~87% (average implant area was 1759 ± 1482 μm^2), we observed formation of mature connective tissue in the form of collagen fiber bundles and surrounding fibrocytes.

The slow resorption may be due to the high density of cartilage tissue, making it difficult for cells to penetrate and substances to diffuse into the dMCPs. Data obtained indicate the possibility of using dMCPs as a matrix for in vitro and in vivo formation of cartilage tissue equivalent.

Fig. 3 shows the results of a semi-quantitative assessment of the biological effect of the implanted specimens.

From the data presented in Fig. 3, we can see that the local effect of dMCPs on the surrounding tissues decreases during the six months of implantation from "mild tissue response" (3.0 to 8.9 points) to "no reaction or minimal response" (0.0 to 2.9 points). Note that the change in the nature of the tissue response to dMCPs occurs during the time of resorption of the main mass of implanted particles.

Thus, microdispersed particles of decellularized porcine cartilage and their resorption products show no



The original cartilage **Decellularized cartilage**

Fig. 1. Changes in the structure of porcine cartilage microparticles as a result of decellularization

negative biological effect on the tissues surrounding the implant.

General toxic effect

The animals in the control and experimental groups were healthy for 2 months of the experiment. No changes in the appearance and behavior toward oppression or agitation were observed in any of the rats. The body weight of the rats during the experiment had positive dynamics and did not differ between the groups.

Implantation of dMCP specimens did not affect hematopoiesis in the rats. There were no significant differences in the average values of clinical blood analysis between the control and experimental rats.

To identify the possible damaging effects of the test specimens on the liver, heart, and bone tissue, the activity of alkaline phosphatase, aspartate and alanine aminotransferases was determined in the blood serum. Analysis of data obtained did not reveal any differences in the activity of the studied enzymes and the content of total bilirubin, as well as in the indicators of protein metabolism in the body between the groups.

Introduction of the studied specimens in rats did not affect the urine parameters in comparison with the control group. Protein, glucose, bilirubin, urobilinogen, nitrites, ketone bodies, leukocytes and ascorbic acid were absent in the urine of animals or were below the first value determined by the Uriscan Optima device as positive.

Macroscopic study did not establish any clear effect of the studied specimens on the state of the internal organs of the rats. No differences between the control and experimental groups were found.

Histological examination did not reveal any distinct effect of the studied specimens on the state of the internal organs of the rats. No differences between the control and experimental groups were found.

Consequently, specimens of tissue-specific matrices of decellularized porcine cartilage do not exhibit systemic toxic effects on the body of the animals.

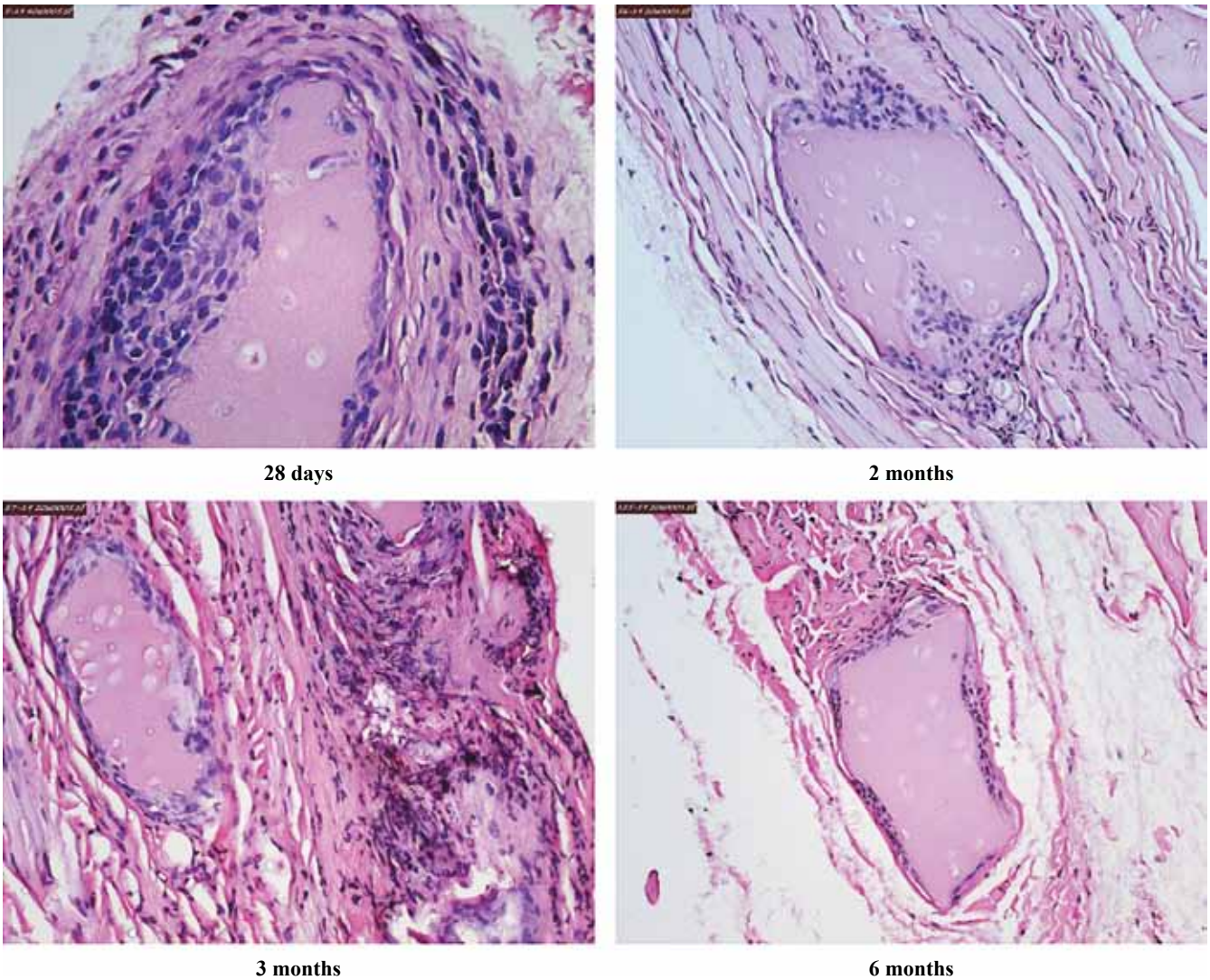


Fig. 2. Tissue response to implantation of decellularized porcine cartilage particles. H&E staining. ×400 magnification

Reproductive toxicity

When observing the sexual behavior of animals after pairing, it was noted that the latent period and duration of sexual activity in experimental males (with implanted dMCP specimens) corresponded to the control males (sham-operated).

During gestation, the behavior of female experimental groups was similar to that of control ones. During gestation, the weight gain of pregnant rats in the experimental groups did not differ from that of the control group.

Some pregnant females were euthanized on day 20 of pregnancy. At autopsy, the number of corpus luteum in the ovaries, implantation sites in the uterus, the number of live and dead fetuses, and the number of resorptions were counted. External examination revealed no developmental abnormalities in any embryo.

Some of the pregnant females were left until delivery to monitor the survival and development of the offspring for 5 days. Duration of pregnancy, number of born rats, their weight and cranio-caudal size did not statistically differ between the experimental and control

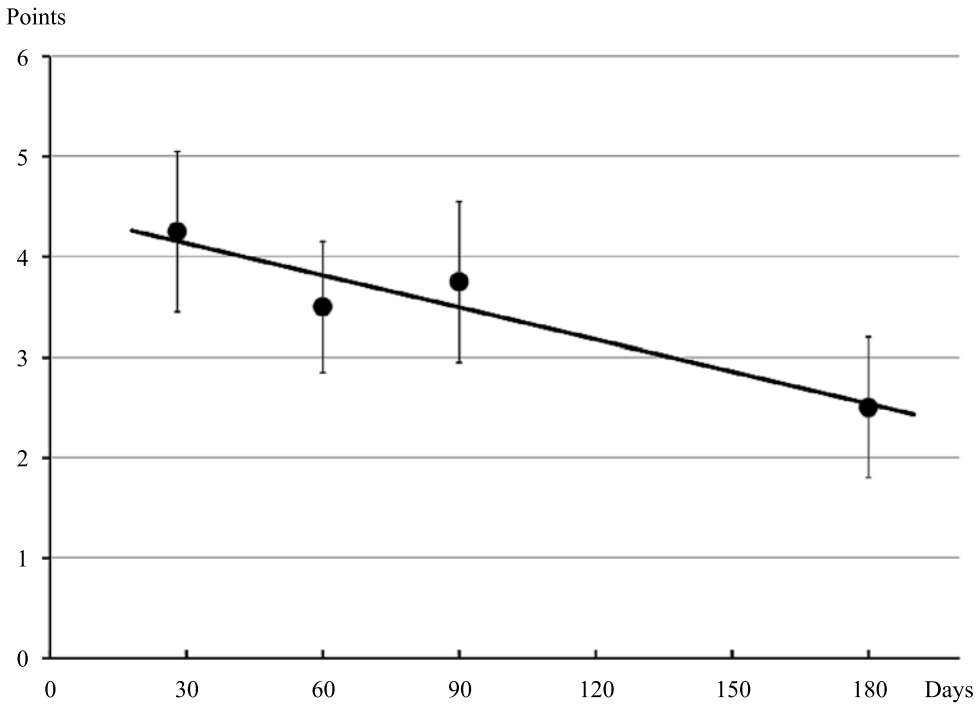
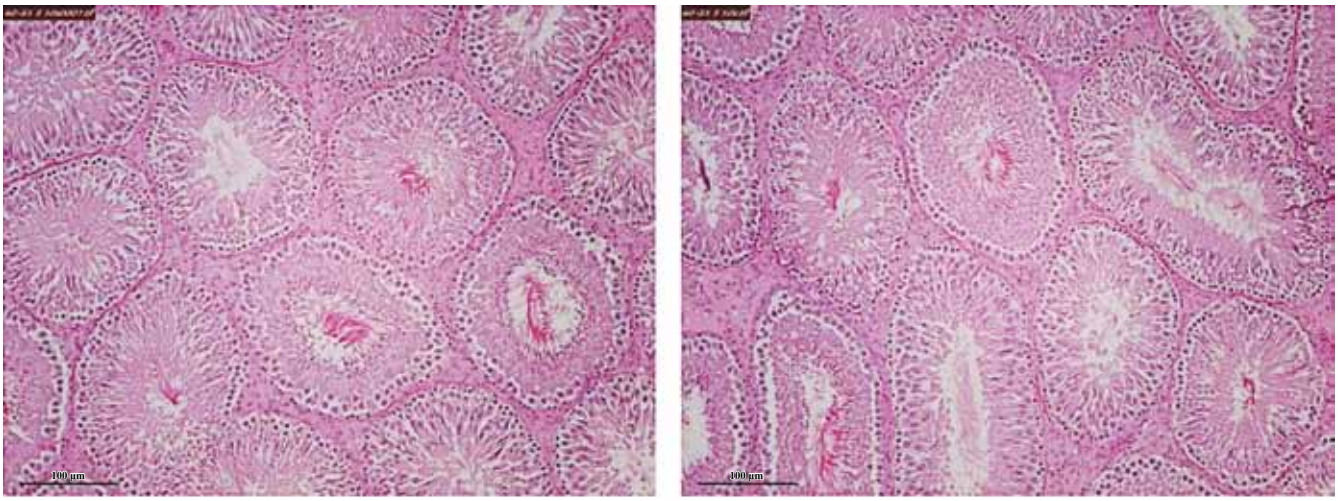


Fig. 3. Semi-quantitative assessment of tissue response to implantation of porcine decellularized cartilage particles



Control group **Decellularized cartilage group**

Fig. 4. Histological section of rat testis. H&E staining. ×100 magnification

groups. There were no stillbirths in any of the females. After delivery, all females exhibited maternal instinct. Five-day offspring survival was 100% in all groups. In this period, the rats developed without abnormalities.

Histological examination of testes (Fig. 4) showed no changes in the morphological structure of the organ and no damage to the spermatogenic epithelium in any case. A similar study of rat ovaries showed no morphological changes in the organ (Fig. 5).

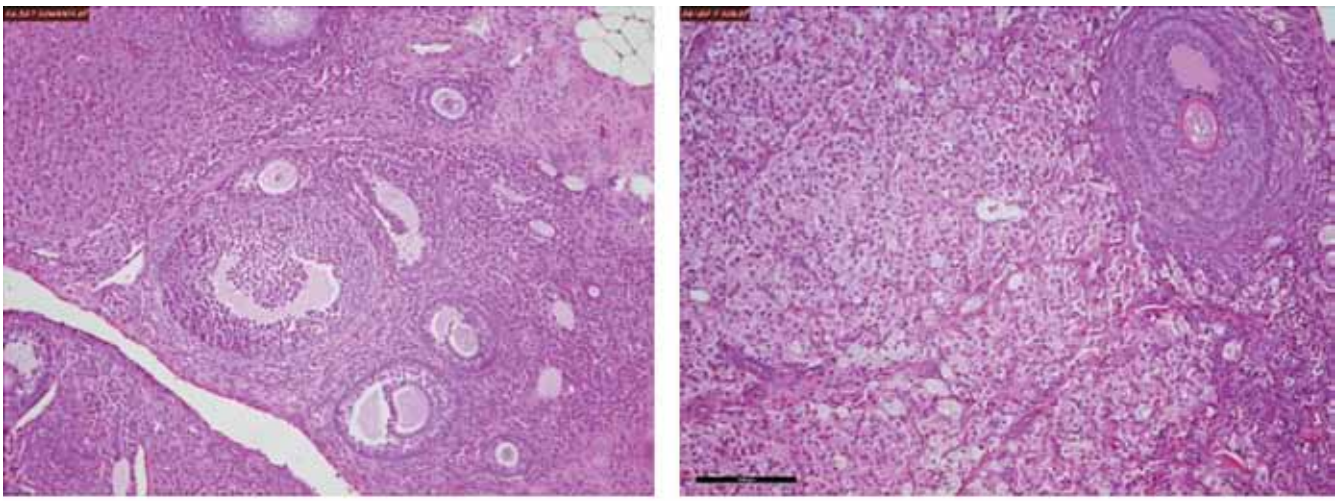
It follows from the results obtained that the dMCP specimens implanted in the muscle tissue, both before mating the animals and during gestation, do not have negative effects on the reproductive system of the animals.

Interaction of tissue-specific matrix obtained from decellularized porcine cartilage with cells

By day 3 of the experiment, the number of proliferating adherent hAdMSCs was $112 \pm 10 \times 10^3$ cells/mm^2; by day 7 of the experiment, the number had doubled.

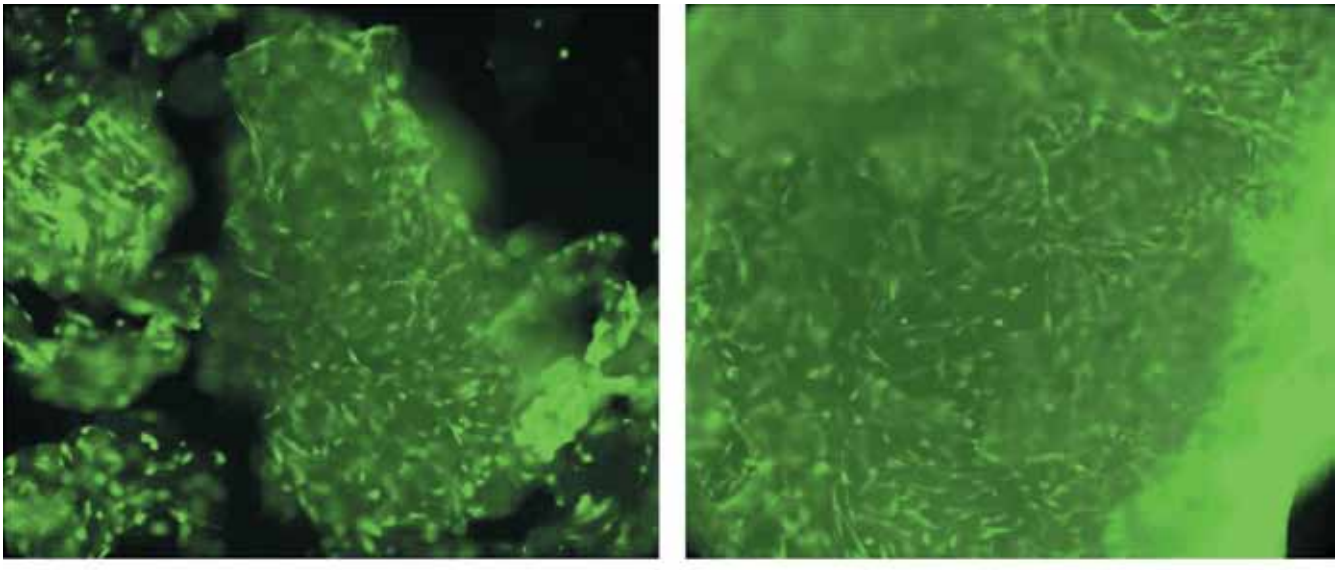
The study of cell viability using Calcein AM dye showed that by day 3 of the experiment, proliferation of cells, evenly distributed over the matrix surface, was visualized (Fig. 6).

Cell morphology is normal and fibroblast-like. By day 7, the number of cells increased, the attached cells were evenly distributed over the surface of dMCP matrices, had a flattened fibroblast-like morphology, maintained viability and normal morphology.



Control group **Decellularized cartilage group**

Fig. 5. Histological section of rat ovary. H&E staining. ×100 magnification



3 days **7 days**

Fig. 6. hAdMSCs cultured on decellularized microdispersed porcine cartilage particles. Calcein AM staining. ×100 magnification

CONCLUSION

The effectiveness of sequential treatment with surfactants, $scCO_2$ and DNase to obtain a biocompatible tissue-specific matrix from xenogeneic material, decellularized porcine articular cartilage, has been proven.

The experiments showed that the specimens of microdispersed decellularized porcine cartilage tissue do not exhibit adverse biological effects in terms of local and general toxicity, genotoxicity and reproductive toxicity. The specimens are capable of maintaining hAdMSC adhesion and proliferation.

So, the obtained xenogeneic tissue-specific microdispersed matrix can be recommended for the creation of tissue equivalents of human cartilage tissue.

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The authors declare no conflict of interest.

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