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«KARAGANDA MEDICAL UNIVERSITY»

.

**ANNOTATION**

of dissertation research for Doctor of Philosophy degree

on the specialty: 6D110100 «Medicine»

**Theme: «Experimental substantiation of the use**

**of decellularized xenoperitoneum matrix at myringoplasty»**

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**Relevance of the theme:**

The cause of permanent hearing loss is both chronic otitis media and traumatic damage of the eardrum, which occupy a significant place in the overall structure of middle ear diseases. At the same time, mechanical damage of the eardrum occupies a leading place and, according to various authors, accounts for 32-70% of all traumatic injuries [1, 2, 3].

It is claimed that acute post-traumatic perforation of the tympanic membrane closes independently within 7-10 days, but nevertheless the perforations of the tympanic membrane, which occupy 25% or more of the area of the tympanic membrane (1 or more quadrant), do not close for 30 days after injury [1, 3, 4, 5, 6, 7, 8]. Incorrect treatment tactics of posttraumatic middle ear otitis leads to persistent perforation, which is one of the main causes of chronic otitis leading to loss of hearing [1, 9]. To date, there are no reliable prognostic signs for a doctor to predict whether a tympanic membrane defect will close without surgery or not. Perforation of the eardrum for longer than 3 months is considered as chronic otitis [10, 11].

Currently, there are many ways to close tympanic membrane defects in acute injuries using auto- and allografts [5]. It should be noted that the problem of surgical restoration of the eardrum integrity is still relevant. One of the unresolved issues in otosurgery is the search for the optimal material for the tympanic membrane reconstruction [12]. The main reason for the problem is that the eardrum does not have homogenous tissues for autoplasty in the human body [13].

It is known that various plastic materials are used in clinical practice: fascia of the temporal muscle, cartilage and perichondrium, periosteum, cheek mucosa, small intestine mucosa, nasal septum, vein wall, dura mater, amnion, sclera, human allofibroblast culture, polymer implants, 2- and 3-layer grafts of various composition [6, 14, 15, 16, 17, 18, 19, 20]. However, these transplants, along with sufficient effectiveness, are not without significant drawbacks identified during long-term clinical trials. Possible complications include suppuration, displacement of the flap or rejection of the graft, incomplete closure of the tympanic membrane defect due to resorption of collagen fibers during scarring, recurrence of the tympanic membrane defect, fusion with the medial wall of the tympanic cavity. Such possible complications significantly reduce the function of the eardrum and cause the loss of hearing [21, 22, 23]. There is a need for additional surgery, the need for general anesthesia, and the duration of the patient's stay in the hospital increases. Therefore, otosurgeons continue to search and develop new highly effective materials for restoring the integrity of the eardrum, which determines the relevance of this problem [24, 25].

The search for alternative materials that would reduce the risks of postoperative complications, would improve the functionality of the middle ear and, as a result, would improve the quality of life of the patient led to the study of the use of implants with biological origin. Biological implants, as a rule, consist of extracellular collagen matrix, which is obtained from human donor material (allograft) or animal (xenograft of pig or bovine). These materials are able to integrate into the chain of physiological metabolism, which determines the balance of reparative processes without inflammatory reactions, while avoiding the development of immunological rejection [26].

To date, there are many different biological materials that differ both in primary material and processing methods – decellularization and sterilization processes [27].

However, the existence of these bioimplants does not solve a number of issues related to their use in otosurgery, namely, there is no consensus on how and in what cases to use biological implants, there is not enough information about the long-term results of their use, and, importantly, the high cost. The question remains unresolved, which primary raw materials are better used to obtain biological implants. It is believed that different methods of manufacturing bioimplants determine the endogenous properties for each material individually and may cause different biological responses after implantation in vivo. Based on these circumstances, the search for high-tech, biologically «similar» to the human body and at the same time inexpensive implants that can be used in the clinic does not stop at the moment.

Despite a sufficient amount of work on the problem of the use of xenoimplants in world science, it is necessary to recognize the ambiguity of the data provided by different scientists, and the lack of research on the use of a decellularized xenoperitoneum matrix for tympanic membrane defects closing also determines the relevance of the planned clinical study.

The conducted study is a continuation of two experimental researches on the study of the extracellular xenoperitoneum matrix for anterior abdominal wall hernia repair (research on grant funding, State registration No. 0115RK00305) and the study of the extracellular xenoperitoneum matrix at nephropexy (research on grant funding, State registration No. 0115RK00306) [28].

The analysis of the data obtained during the experiment showed that when using the decellularized extracellular xenoperitoneum matrix, a stage-specific change of tissue reactions characterizing the normal course of the regenerative process occurs. This material, along with high mechanical strength, demonstrates adequate biocompatibility with the macroorganism in the experiment, with the formation of mature, consistent contact with tissues and reliably minimal tissue inflammatory reactions. The results allowed continuing the further study of the decellularized xenoperitoneum matrix in the framework of the conducted study [29].

 **Working hypothesis:**

 The decellularized xenoperitoneum matrix demonstrates adequate biocompatibility with the tissues of the tympanic membrane in an experiment with the formation of a consistent contact with minimal tissue inflammatory reactions.

**The objective of the study** was to conduct an experimental substantiation of the use of a new biological material decellularized xenoperitoneum matrix for myringoplasty at perforation of the tympanic membrane.

**Research tasks:**

1. To develop an experimental model of eardrum plastic surgery using decellularized xenoperitoneum matrix on laboratory rabbits;
2. To evaluate the reaction of the immune system of the macroorganism in response to the decellularized xenoperitoneum matrix implantation and the conserved dura mater through the study of circulating immune complexes of various molecular weights;
3. To characterize the audiometric data after myringoplasty with the use of decellularized xenoperitoneum matrix and conserved dura mater in the experiment;
4. To give a morphological characterization of the histostructure of the implantation zones of the decellularized xenoperitoneum matrix and the conserved dura mater with the tissues of the tympanic membrane after myringoplasty in an experiment with the assessment of morphometric data.

**Scientific novelty:**

For the first time, a comprehensive assessment of the possibility of decellularized xenoperitoneum matrix using for eardrum plastic surgery with morphological justification is given considering the morphometry data, immune response to implantation and based on a functional assessment of the state of the eardrum hearing after implantation in order to close the defect.

**The main provisions submitted for the defense of the dissertation:**

1. The method of the proposed tympanoplasty allowed us to obtain adequate access to the middle ear structures in experimental conditions.
2. Evaluation of the reaction of the immune system of the macroorganism in response to the implantation of a biological implant by studying circulating immune complexes of different molecular weights revealed no differences between the experimental groups and the control group.
3. The obtained quantitative audiometric data after myringoplasty using decellularized xenoperitoneum matrix in the experiment demonstrated that the total value of the audiogram peaks had statistically significant differences with the comparison group (p=0.045).
4. Comparative morphological analysis showed positive dynamics of the reparative process at all periods of the experiment. Morphometric indicators of the stage-phase process of tissue healing were regressed on day 30 in a representative section of the implantation zone after myringoplasty using decellularized xenoperitoneum matrix.

**Practical significance:**

The results of the experimental study can be the scientific basis for potential possibility substantiating of decellularized xenoperitoneum matrix using in clinical practice as an alternative material for myringoplasty.

The knowledge gained in the course of the study about the features of the reparative process in the implantation zone of the decellularized xenoperitoneum matrix with tympanic membrane tissues based on an assessment of the state of the histostructure of the wound process considering the results of morphometry indicators in a representative section of the implantation zone will allow, after clinical studies, to cover the necessity of biological implant for the treatment of patients with chronic otitis.

**Implementation into practice:**

The certificate of State registration of the rights to the copyright object dated 31.12.2020 No. 14256 «Method of myringoplasty using extracellular xenoperitoneum matrix on rabbits» was obtained.

The results of the experimental study give grounds to recommend conducting clinical trials on the possibility of decellularized xenoperitoneum matrix using during myringoplasty as a clinical study in ENT-practice.

Some of the results of this dissertation work obtained during the study were introduced into the educational process as part of research-based learning (RBL) for students under the postgraduate education program (residency, master's, doctoral studies) of the Department of Surgical Diseases and the Department of Pathology in Karaganda Medical University. The act of implementing the research results dated 08.09.2022.

**Approbation of the study:**

The main provisions of the dissertation were reported and discussed at the following scientific events:

– The 67th International Scientific and Practical Conference in the Tajik State Medical University named after Abuali Ibni Sino (annual) «Medical science of the XXI century – looking towards the future» (November 29, 2019, Dushanbe);

– The I International MED Congress «Man and Health. Multidisciplinary approach in Medicine» (October 18-19, 2022, Semey);

– at an expanded meeting of the Department of Surgical Diseases and the Department of Pathology of NC JSC «Karaganda Medical University».

**Publications:**

Based on the materials of the dissertation, 4 scientific articles were published, including:

– 3 in scientific issues recommended by the Committee for Control in the Field of Education and Science of the Ministry of Education and Science of the Republic of Kazakhstan;

– 1 publication in an international scientific publication included into the Scopus information base:

Yesniyazov D., Tussupbekova M., Abatov N., Yukhnevich Y., Badyrov R. Myringoplasty with Morphological Rationale of Application of Xenoperitoneum Decellularized Matrix in Experiment // Open Access Macedonian Journal of Medical Sciences. – 2021 Oct 05; №9(A): 811-816.

The certificate of State registration of the rights to the copyright object dated 31.12.2020 No. 14256 «Method of myringoplasty using extracellular xenoperitoneum matrix on rabbits» was obtained.

**Materials and methods:**

A comparative experimental study of a new biological material decellularized xenoperitoneum matrix for myringoplasty at perforation of the tympanic membrane on 60 mature rabbits of both sexes with weighing 1500±300 grams was carried out on the basis different structural departments of Non-commercial joint-stock company «Karaganda Medical University»: at the vivarium, at the Department of Pathology, at the Scientific-research center and in the pathology laboratory of university clinic.

**Research design:**

The experiment was conducted according to the developed standard operating procedures. The animals were distributed in 2 groups (group 1 – decellularized xenoperitoneum matrix, group 2 – conserved dura mater) into 3 subgroups, each subgroup having 10 individuals randomly. Each group corresponded to the used bioimplant, each subgroup – to the observation period depending on the time of the animal's withdrawal from the experiment. The observation periods were 7 days, 21 days and 30 days. The experiment was carried out on the left ear of the animals, the right ear remained unoperated. During the evaluation, the presence or absence of hearing in quantitative terms was calculated in both groups on the 30th day after the surgery, the right ear was the control. The distribution of experimental animals, their characteristics and observation periods are presented in Table 1.

Table 1 – The distribution of experimental animals for groups

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| --- | --- | --- | --- | --- | --- |
| Group | Subgroup | Numbern  | Sex | Age, days | Body weightbefore surgery, g |
| Decellularized xenoperitoneum matrix (DeXP) (n=30) | 7 days | 10 | m=2,f=3 | 90±15 | 1500±300 |
| 21 days | 10 | m=2,f=3 | 90±15 | 1500±300 |
| 30 days | 10 | m=2,f=3 | 90±15 | 1500±300 |
| Conserved dura mater (DM)(n=30 ) | 7 days | 10 | m=2,f=3 | 90±15 | 1500±300 |
| 21 days | 10 | m=2,f=3 | 90±15 | 1500±300 |
| 30 days | 10 | m=2,f=3 | 90±15 | 1500±300 |

The criterion of acceptable randomization was the absence of external signs of the disease and the homogeneity of the groups by body weight (±10%). Prior to implantation of the decellularized xenoperitoneum matrix and the conserved dura mater, each rabbit underwent otoscopy with a description of the condition of the external auditory canal and eardrum. Identification of each animal was carried out by assigning an individual number with a dye mark on the left auricle.

The basic rules for keeping and caring for animals corresponded to the standards given in the Guide for care and use of laboratory animals. Eight edition. ILAR publication, 2012, National Academy Press. All routine animal care procedures were performed in accordance with standard operating procedures.

**Research methods**

Experimental studies were conducted on the basis of the Scientific-research center of the Non-commercial joint-stock company «Karaganda Medical University».

* A defect of the tympanic membrane (acute perforation) in laboratory rabbits was modeled under experimental conditions, followed by plastic surgery of the tympanic membrane (myringoplasty) using a decellularized xenoperitoneum matrix.
* Circulating immune complexes (CIC) of high, medium and low molecular weight were determined by precipitation of antigen-antibody complexes from blood serum in a solution of polyethylene glycol-6000 (PEG-6000) prepared using 0.1 M borate buffer (pH 8.4), followed by photometric determination of the optical density of the precipitate.
* A quantitative assessment of hearing in animals (audiometry) was carried out, which was based on the assessment of the total length of the three most pronounced peaks of the audiogram obtained by the method of evoked auditory potentials during the BAER test using the BAERCOM UFI device, which made it possible to assess the acuity of hearing in digital equivalent after implantation.
* Morphological and morphometric characteristics of structural changes in implantation zones were given with a quantitative assessment of cellular infiltrate (granulocytes, lymphocytes, macrophages, plasma cells, stroma cells) in a representative section of the implantation zone.

**Statistical research methods**

For all quantitative data, the group arithmetic mean (𝑋̅), standard deviation (SD) was calculated, with the calculation of the median value (Me), 25-75% of the interquartile interval (IQR) for each indicator. The reliability of the differences between the studied groups was determined by statistical methods using nonparametric criteria: the Mann – Whitney criterion, comparisons of independent groups («experience – control»). The reliability of intra-group differences was determined by a statistical method using parametric criteria: the Kendall concordance criterion for comparing three independent groups and in pairs by the Wilcoxon criterion.

During the correlation analysis, the nonparametric Spearman Rank correlation (rs) method was used. This coefficient is used to identify and assess the closeness of the relationship between two sets of comparable quantitative indicators. The correlation coefficient takes values from -1 to 1, and at rs = 1 there is a strictly direct relationship, and at rs = -1 – strictly feedback. If the correlation coefficient is zero, then there is practically no relationship between the values. Due to the fact that the coefficient is a method of nonparametric analysis, checking for the normality of the distribution is not required.

The software «Statistica 8.0» and the spreadsheet processor Excel from the Microsoft Office 2012 software package were used for the calculation and design of the material.

**Conclusions**

1. The developed method of myringoplasty in the experiment made it possible to obtain adequate access to the structures of the middle ear in rabbits and to perform surgical intervention.

2. A comparative analysis of the macroorganism immune system reaction in response to the implantation of decellularized xenoperitoneum matrix showed that there were no differences between the experimental groups and the comparison group in both groups. The obtained data did not go beyond the physiological values on days 7 and 21, however, on day 30 there were statistically significant differences with the comparison group for medium-molecular CIC (p=0.007) and for low-molecular CIC (p=0.015).

3. The obtained quantitative audiometric data after myringoplasty with decellularized xenoperitoneum matrix in the experiment showed that with hearing acuity in the DeXP group of 40 [32; 48] mm, statistically significant differences were noted with the comparison group of 32 [30; 35] mm (p=0.045), however, no statistically significant differences were found with the control group of 44.5 [43; 50] (p=0.104).

4. Comparative morphological analysis showed a positive dynamics of the reparative process in different periods of the experiment after myringoplasty with decellularized xenoperitoneum matrix. Thus, morphometric indicators of the stage-phase process of tissue healing regressed from (11 [8.25; 11.5]) on the 7th day, till (0 [0;0]) on day 30.

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