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MECHANICAL PROPERTIES OF NATIVE AND DECELLULARIZED AORTIC WALL AFTER LONG-TERM STORAGE IN BIOCIDE SOLUTIONS

M.B. Vasilyeva[1], E.V. Kuznetsova[2], Ya.L. Rusakova[2], E.V. Chepeleva[2], D.S. Sergeevichev[2], I.Yu. Juravleva[2]

[1] Zelman Institute of Medicine and Psychology, Novosibirsk State University, Novosibirsk, Russian Federation

[2] Meshalkin National Medical Research Center, Novosibirsk, Russian Federation

Objective: to determine the optimal method for long-term wet storage of donor material (50 days after collection), with maximum ability to preserve the original mechanical characteristics. **Materials and methods.** Porcine aortic wall fragments were used as objects of study. Half of the original material underwent detergent-based decellularization. The entire material (native and processed) was placed for 50 days in biocidal solutions: complex alcohol solution; ethanol and glycerol mixture; antibiotics mixture. Then the tests for mechanical strength of native and decellularized samples were carried out by the method of uniaxial longitudinal and circumferential stress.

Results. Storage of native material in all media resulted in a significant increase in tensile strength. In the "complex alcohol solution", "ethanol and glycerol mixture", and "antibiotic mixture" group, tensile strength increased by 1.38-, 1.72- and 1.62-fold compared to the native control in circumferential tension. Also, in the "complex alcohol solution" group, the decellularized material was 1.57-fold stronger than the native in circumferential tension. In the "antibiotic mixture" group, the decellularized material was 1.33-fold less strong than the native in longitudinal tension. According to elongation to rupture data, significantly greater plasticity was noted in the "ethanol-glycerol" storage group for the decellularized aortic wall compared to the control group (1.5-fold). Young's modulus did not reliably differ from those of control in all experimental groups regardless of the stress direction. Notably, decellularized specimens clearly tended to be stiffer under circumferential stress. **Conclusion.** Detergent-based decellularization of the porcine aortic wall and subsequent storage of these samples in our chosen experimental solutions for 50 days does not significantly affect the elastic properties of the material. Our proposed treatment methods partially increase the stiffness of the material after storage in alcohol-containing solutions.

Keywords: Young's modulus, tensile strength, xenografts, bioprosthetics.

INTRODUCTION

Currently, one of the widely used methods of surgical treatment of valve and aortic trunk and pulmonary artery conditions is prosthetics with various valve-containing conduits [1–3] aAllogeneic donor material is the gold standard for replacing damaged elements of the cardiovascular system. It has similar hemodynamic characteristics to native valves, low thrombogenicity and is resistant to potential infection [4, 5]. Besides, this type of implants makes re-intervention due to structural degradation less likely [1, 5–7].

Vascular conduits by tissue-engineering methods are widely used to solve allogeneic material deficiency issues. The main types of vascular and valve scaffolds for tissue engineering are natural scaffolds (biologic decellularized tissue and materials) and synthetic structures made of biodegradable polymers [5, 8]. Each type has both advantages and disadvantages. One of the main advantages of decellularized biological matrices is that they preserve the mechanical anisotropy of native valves and the vascular wall structure, can replace the connective tissue scaffold of the affected tissues and promote their recellularization by cells of the recipient itself later [6]. This approach makes it possible to restore the correct structure and adequate function of the replaced element of the cardiovascular system.

Modern approaches to the development of methods for extending the lifespan of donor valves are being implemented in several directions. They include improving the structural characteristics, optimizing preimplantation treatment methods, studying the factors that influence preservation of structural organization of connective tissue scaffold (CTS) and its initial physical and mechanical properties [6, 9].

At the preparatory stage (before implantation in the patient), the allograft is usually either cryopreserved or stored in a solution of antibacterial and fungicidal preparations. However, analysis of the current state of

Corresponding author: Maria Vasilyeva. Address: 1, Pirogova str., Novosibirsk, 630090, Russian Federation. Phone: (913) 930-15-18. E-mail: vasilievam@yandex.ru

the problem shows the lack of a unified approach to the method of long-term preservation of prosthetic material. If the technique of freezing and/or thawing of the material is violated, zones of micro- and macro-damage to the allograft structure may appear, which can be detected only at the surgical stage or will affect the rate of CTS degradation and graft calcification after it has been installed [10–12]. The use of wet storage in an antibiotic cocktail without cryopreservation avoids this type of damage, which helps to reduce the frequency of reoperation associated with early prosthesis damage [7]. However, in practice, wet storage of allografts in an antibiotic solution is used only for a short period of time – 2–14 days [13, 14]. It is also known that residual antibiotics in tissue allografts may be the cause of allergic postimplantation reactions or mask contamination of the allograft by microorganisms in the postoperative period [15].

To date, a universal protocol for long-term storage of allografts has not yet been found. According to global trends, most developers prefer freezing the material [6, 12]. However, coming up with a reliable long-term wet storage method would allow to avoid the negative effects of the processes associated with cryopreservation. As part of the study of this topic, we previously selected biocidal solutions suitable for long-term wet storage of experimental material [16].

The purpose of this work was to evaluate the effect of decellularization and subsequent long-term (50 days) storage in biocidal solutions of different composition on the mechanical properties of aortic tissues.

MATERIALS AND METHODS

Composition of test solutions:

I – Complex alcohol solution (CSR): mixture containing 1,2-octanediol, phenoxyethanol, sorbic acid (1%) and ethanol (20%) [17].

II – Antibiotics mixture (AM): culture medium RPMI-1640 (Biolot, Russia), metronidazole 0.27 mg/ml, gentamicin 0.53 mg/mL, cefazolin 6.66 mg/mL, ampicillin 2.22 mg/ml, oxacillin 1.11 mg/ml, fluconazole 0.027 mg/mL. After the first 48 hours, the solution was replaced with a similar fresh solution.

III – Ethanol and glycerol mixture (E-G): ethanol (10%) and glycerol (20%) in RPMI-1640 culture medium (BioloT, Russia).

Deriving and decellularization of aortic tissues

Porcine aortic wall fragments (arch and descending section), 25–30 cm long, were taken in the slaughterhouse of a meat processing plant. The obtained samples were placed in cooled sterile 0.9% sodium chloride solution and transported to the laboratory.

The aortic wall was cleaned of the remaining surrounding tissues and cut into several 6–7 cm long tubular fragments. Half of the obtained fragments were subjected to detergent-based decellularization.

The entire cycle of cell removal and subsequent washing was performed at 37 °C in an orbital thermoshaker (Heidolf, Germany). The biomaterial was immersed for 24 hours in a sterile phosphate-buffered saline (pH = 7.4) containing 0.5% sodium dodecyl sulfate and 0.5% sodium deoxycholate (Sigma, USA). This was followed by 6-fold washing of the samples for 12 hours in sterile phosphate-buffered saline (pH = 7.4), according to one of the common protocols [18]. The treatment and washing lasted for a total of 4 days, during which the second half of the samples (native samples) was stored at +4–8 °C in sterile 0.9% sodium chloride solution with the addition of an antimicrobial cocktail (metronidazole 0.27 mg/mL, gentamicin 0.53 mg/mL, cefazolin 6.66 mg/mL, ampicillin 2.22 mg/mL, oxacillin 1.11 mg/mL, fluconazole 0.027 mg/mL).

After decellularization, 20 native and decellularized aortic tubular fragments each were used for mechanical tests under uniaxial longitudinal and circumferential stress. The remaining material from each series – native (n = 60) and decellularized (n = 60) – was randomly divided into three groups and stored in the test biocide solutions for 50 days in sterile conditions at +4–8 °C (in AM and E-G solutions), and in CAS solution at room temperature. Eight groups of specimens were examined (Table). Before mechanical testing, the specimens were washed in sterile 0.9% sodium chloride solution for 20 minutes at room temperature.

Table

List of all experimental groups

S/N	Abbreviation	Storage method	Subgroups
1	Contr	Control samples of porcine aorta, fresh material (to determine the basic mechanical properties)	Contr, N – native material without additional treatment
2			Contr, D – decellularized material without additional treatment
3	CAS	Complex alcohol solution (50 days)	CAS, N – CAS native form
4			CAS, D – decellularized form
5	AM	Antibiotics mixture (50 days)	AM, N – AM native form
6			AM, D – AM decellularized form
7	E-G	Ethanol and glycerol mixture (50 days)	E-G, N – E-G native form
8			E-G, D – decellularized form

Study of mechanical properties

Mechanical tests were performed by subjecting the samples to uniaxial stress after 50 days of storage in the test biocidal solutions. To obtain control values, we used porcine aortic wall material from the native group (*Contr, N*) and decellularized material (*Contr, D*) before storage in the appropriate solutions.

Tubular aortic fragments from each of the 8 subgroups were cut lengthwise and test specimens were carved using a standard cutting matrix in the longitudinal (n = 10) and circumferential (n = 10) directions. The width and length of the working part of the specimen corresponded to the matrix dimensions (9 mm and 28 mm, respectively). The sample thickness was measured three times with an electronic digital thickness gauge (Mitutoyo 547-500S, Japan).

The tests were performed on pull tester ESM301 (MARK-10, USA). The tensile speed of the fabric was 30 mm/min. If break occurred at the place where the specimens were fixed in the clamps, the corresponding measurement data was excluded from analysis.

The strength of the materials was evaluated by tensile stress (σ, MPa):

$$\sigma = \frac{F}{h \times w}$$

where F is the force at the moment of break (N), h is the average thickness of the specimen (mm), and w is the width (9 mm) of the specimen.

Deformability was determined by the maximum elongation at break (ε, %):

$$\varepsilon = \frac{\Delta L}{L} \times 100$$

where ΔL is the maximum elongation of the specimen (mm) and L is the initial length of the specimen (mm) equal to the distance between the clamps (28 mm).

Stiffness was determined by the value of Young's modulus (E, MPa):

$$E = \frac{\sigma}{\varepsilon}$$

where E is Young's modulus (MPa), σ is the tensile stress; ε is the elongation of the fragment (mm) at the break of the specimen.

Statistical analysis

Results were processed by variation statistics using the *Statistica 13.0* software (TIBCO Software, USA). Normality of distribution of the obtained data and homogeneity of variance were checked using the Shapiro–Wilk test. Mann–Whitney U Test was used to judge the significance of differences. Data on the graphs are presented as median and interquartile range (25th and 75th percentiles).

RESULTS

Sample thickness

The thickness of fragments of decellularized aorta from the control group was 1.22 times higher than that of native aorta from the control group ($p < 0.01$). Among the decellularized specimens after storage, a lower thickness was found in the alcohol-containing "*CAS, D*" and "*E-G, D*" subgroups by 0.82 and 0.87 times, respectively, compared to the corresponding controls ($p < 0.05$) (Fig. 1).

Stress-strain properties of the biomaterial

The **tensile strength** for non-decellularized (native) specimens increased significantly after storage in all the test solutions in comparison to the control ($p < 0.05$): 1.38 times in the "*CAS, N*" subgroup, 1.72 times in the "*E-G, N*" subgroup and 1.62 times in the "*AM, N*" subgroup. Among the decellularized samples, we observed an insignificant tendency towards increased tensile stress in the "*CAS, D*" subgroup compared to the control. In the "*E-G, D*" subgroup, there was a significant increase in tensile stress by 1.5 times ($p < 0.05$). When comparing the strength of native and decellularized specimens, significant ($p < 0.05$) differences were found in the *Contr* and *CAS* groups, where the decellularized material showed a 1.47- and 1.57-fold higher tensile strength, respectively (Fig. 2, a).

The longitudinal tensile strength for both native and decellularized specimens did not change after storage in all types of solutions compared to the control. When comparing within each experimental group between native and decellularized specimens, a significant difference was found only in the AM group (decellularized material was 1.33 times less strong than native ($p < 0.05$) (Fig. 2, b).

The **elongation at break** for both native and decellularized samples did not change after storage in all types

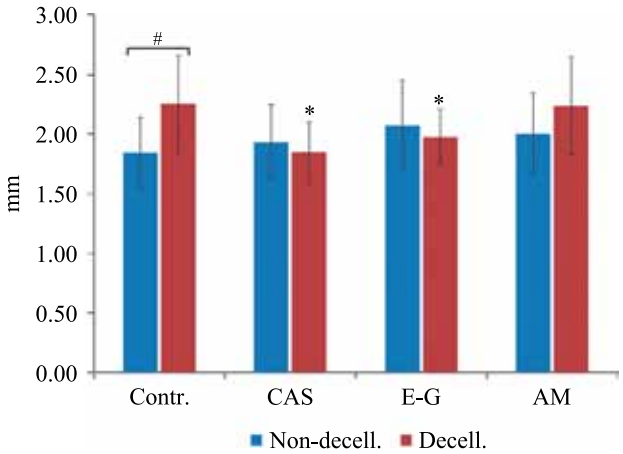

Fig. 1. Thicknesses of the test specimens;
*, $p < 0.05$ in comparison with the corresponding control group; #, $p < 0.05$ in comparison between non-decellularized and decellularized samples within the same group

of solutions compared to the control, regardless of the tension direction (Fig. 3). The exception was the "*E-G, D*" subgroup, where the highest elongation was detected under circumferential stress compared to all other subgroups ("*Contr, D*"/"*E-G, D*" by a factor of 1.5, $p < 0.01$) (Fig. 3, a).

Regardless of the tension direction, there were no significant differences between the **Young's modulus** in native and decellularized tissues both within one group and when compared with the corresponding control values (Fig. 4).

DISCUSSION

Currently, one of the common methods of long-term storage of allogeneic material is cryopreservation, as this method allows creating a bank of cardiac valve allografts for really long storage for subsequent implantation in humans. This method of preservation requires careful compliance with all freezing and thawing stages, increased technical reliability of equipment, and, possibly, search for new cryopreservation media [12, 19].

As the review of specialized literature shows, features and patterns of decellularization, preservation of allograft and xenograft tissues are of considerable research interest [20–22]. Of particular interest is the issue of sufficiently long-term wet storage of obtained donor tissues. In some works, where it was important to preserve cell viability, culture nutrient media were used [23, 24]. In other works, where the principal goal was to preserve connective tissue scaffold without taking into consideration the cellular component, the suitability of saline solution with modifications was investigated [25]. For instance, Wollmann et al. showed that preservation of mechanical and structural properties of the decellularized tissue matrix is not significantly affected during long-term wet storage in sterile saline solution (up to

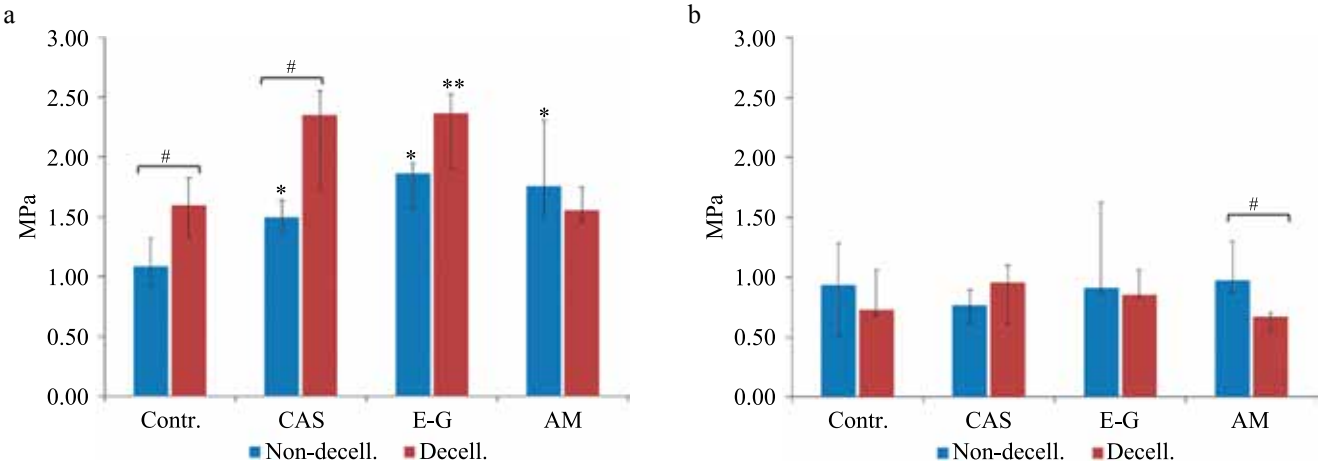

Fig. 2. a) Circumferential tensile strength for the test specimens; b) Longitudinal tensile strength for the test specimens; *, $p < 0.05$ in comparison with native control; **, $p < 0.05$ in comparison with decellularized control; #, $p < 0.05$ compared to non-decellularized and decellularized samples within the same group

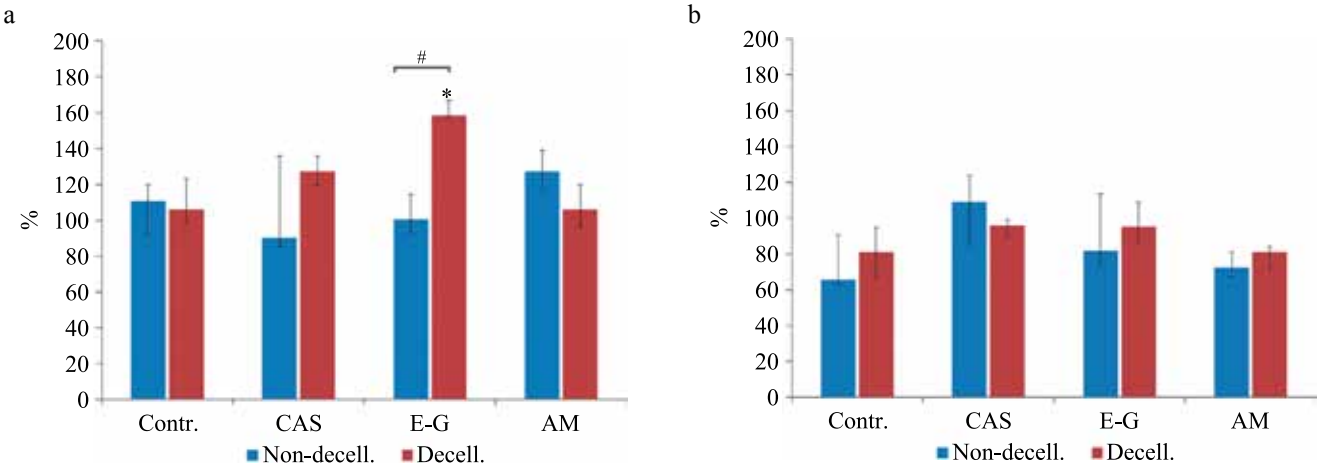

Fig. 3. a) Elongation at break for the test specimens under circumferential stress; b) Elongation at break for the test specimens under stress;
*, $p < 0.05$ in comparison with control; #, $p < 0.05$ in comparison between non-decellularized and decellularized samples within the same group

12 months) [26]. In this experimental work, we chose RPMI-1640 nutrient medium as the basis for storage media II (AM) and III (E-G). The excellent multicomponent buffer medium ensured that the pH of the solutions was maintained throughout the experiment. Performing all manipulations in laminar boxes with abacterial air medium during preparation of working solutions and throughout the experiment allowed us to avoid contaminating the material with microbial and other negative agents.

The success of decellularized heart valve transplantation largely depends on the components of the cell removal method itself (enzymatic, detergent, etc.) and the potential immune response after implantation [27, 28]. The decellularization protocol we used [18] and its variations are also used by other researchers, which testifies to the adequacy of this method for further use in creation of tissue-engineered materials based on donor tissues [20, 21].

Our study found that the thickness of samples in the control group after decellularization increased by 10–30% from the initial state of the native material. This may possibly be due to the appearance of a loosening of the intercellular spaces in the connective-tissue scaffold when the cells were removed and small intercellular molecules (proteoglycans, glucosaminoglycans and other non-collagenous proteins) were partially washed out under the effect of detergents, which were replaced by water molecules [29].

An important property of a deformable material is strength, understood as the ability to resist destruction by external forces. Tensile strength is one of the main indicators characterizing the mechanical properties of a tissue, and a tissue is quantified using tensile stress [30]. We found that decellularization of the aortic material significantly increases circumferential tensile stress. At the same time, the Young's modulus also increases, but without a significant difference from those of the native control group. After long-term storage in alcohol-containing (*CAS* and *E-G*) and alcohol-free (*AM*) solutions, the main changes in the mechanical properties were also evident in the circumferential tensile strength of the aortic wall material. Almost all data on mechanical properties in longitudinal stress remained at the level of the control values of the corresponding subgroups (decellularized and non-decellularized). Such anisotropy of biomechanical properties in the connective tissue scaffold of the aortic wall is down to the peculiarities of three-dimensional orientation of CTS fibers, which provides effective compensation of intravascular fluid pressure for further active blood promotion. It was shown earlier that the main mass of elastin fibers in the aortic wall is located in the media as part of elastin lamellae, which are located predominantly in the circumferential direction. Orientation of collagen fibers in the media is directed to the vessel axis at about 45° angle; the direction is more longitudinal in the intima and adventitia [31–33].

In uniaxial tension tests, we obtained a stress-strain curve for each specimen. The graph of such a curve consists of 3 parts: the low elastic modulus portion, the linear portion, and the yield or break portion. There is a biomechanical interpretation of this phenomenon. The portion with low elasticity modulus occurs due to straightening of spiral collagen fibers according to the direction of the applied force. The linear portion (consisting of two parts) appears as a result of direct stretching of collagen fibrils in the tissue. Breaking area of the stress-strain curve is connected with direct damage to the fibrils [33]. When determining the tensile strength, the maximum applied force, after which the specimen breaks, is initially fixed. In fact, it depends on how many and mainly of what quality collagen are the fibers present in the tissue.

Most likely, the significant increase in the strength of materials during storage in alcohol-containing solutions is associated with a change in the structure of the main CTS proteins – collagen and elastin. It is known that the triple stranded structure of collagen, as well as

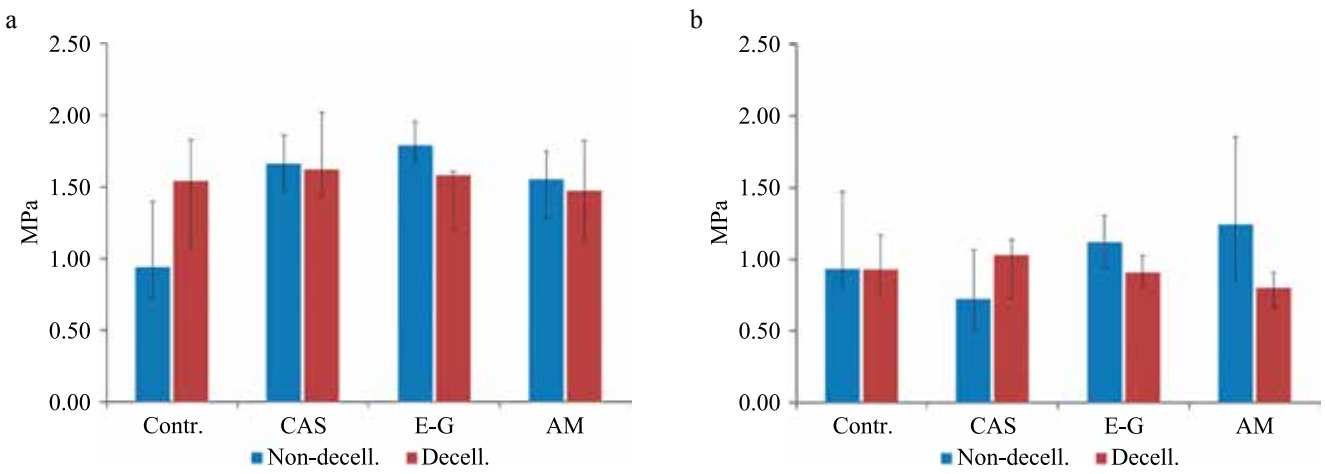

Fig. 4. Young's modulus for the test specimens. a, circumferential stress; b, longitudinal stress

the ratio of hydrophilic and hydrophobic sites of protein molecules, change under the influence of alcohols [34].

The deformation capacity of the material is reflected in the indices of relative elongation during tensile strain. This index is influenced by the structure and fiber composition of the fabric [35]. In our study, it was shown that the circumferential tensile elongation in all experimental groups remains at the level of the control values except for the "*E-G*" subgroup. In this case, the deformation capacity index is 1.58-fold higher than that of the non-decellularized counterpart ("*E-G, N*"). In this situation, we can assume the influence of E-G solution components (glycerol as a plasticizer) on the structural organization of CTS fibers of the aortic wall. Some researchers have noted similar changes in the biomechanics of materials stored in glycerol solutions [36].

Young's modulus characterizes the stiffness of a material [37]. Regardless of the stress direction, the Young's modulus in all experimental groups after storage in the test solutions does not significantly differ from those of native tissue. At the same time, the values have a clear tendency to increase, which indicates increased stiffness. Comparison of Young's modulus values in the circumferential and longitudinal direction within groups with the same type of tissue treatment demonstrates significant differences in average values. This fact is also explained by the specific location of different CTS fibers in the aortic wall [31].

CONCLUSION

Detergent-based decellularization of porcine aortic wall followed by wet storage of the samples in the test solutions for 50 days does not significantly worsen the elastic properties of the material. There is a slight increase in the circumferential tensile stiffness of the test material.

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The authors declare no conflict of interest.

REFERENCES

1. *Horke A, Tudorache I, Laufer G, Andreas M, Pomar JL, Pereda D et al.* Early results from a prospective, single-arm European trial on decellularized allografts for aortic valve replacement: the ARISE study and ARISE Registry data. *Eur J Cardiothorac Surg.* 2020; 58 (5): 1045–1053. doi: 10.1093/ejcts/ezaa100. PMID: 32386409; PMCID: PMC7577293.
2. *Arabkhani B, Bekkers JA, Andrinopoulou ER, Roos-Hesselink JW, Takkenberg JJM, Bogers AJJC*. Allografts in aortic position: Insights from a 27-year, single-center prospective study. *J Thorac Cardiovasc Surg*. 2016; 152 (6): 1572–1579. doi: 10.1016/j.jtcvs.2016.08.013; PMID: 27842683.
3. *Demidov DP, Astaspov DA, Bogachev-Prokophiev AV, Zheleznev SI*. Quality of life after aortic valve replacement with biological prostheses in elderly patients. *Patologiya krovoobrashcheniya i kardiokhirurgiya = Circulation Pathology and Cardiac Surgery*. 2017; 21 (3): 40–47. [In Russ, English abstract]. doi: 10.21688/1681-3472-2017-3-40-47.
4. *Spirydonov SV, Odintsov VA, Shchetinko NN, Mozgova EA, Hrynchuk II, Ostrovsky YuP*. Aortic allografts in the world cardiac surgery: historical aspects of clinical implementation, and review of implantation outcomes. *Medical Journal*. 2015; 1: 55–67. [In Russ, English abstract].
5. *Fioretta ES, von Boehmer L, Motta SE, Lintas V, Hoerstrup SP, Emmert MY*. Cardiovascular tissue engineering: From basic science to clinical application. *Exp Gerontol*. 2019; 117: 1–12. doi: 10.1016/j.exger.2018.03.022. PMID: 29604404.
6. *VeDepo MC, Detamore MS, Hopkins RA, Converse GL*. Recellularization of decellularized heart valves: Progress toward the tissue-engineered heart valve. *J Tissue Eng*. 2017 Aug 25; 8: 2041731417726327. doi: 10.1177/2041731417726327. PMID: 28890780; PMCID: PMC5574480.
7. *Cebotari S, Tudorache I, Ciubotaru A, Boethig D, Sarikouch S, Goerler A et al.* Use of fresh decellularized allografts for pulmonary valve replacement may reduce the reoperation rate in children and young adults: Early report. *Circulation*. 2011; 124 (11 SUPPL. 1): 115–124. doi: 10.1161/CIRCULATIONAHA.110.012161. PMID: 21911800.
8. *Argento G, Simonet M, Oomens CW et al.* Multi-scale mechanical characterization of scaffolds for heart valve tissue engineering. *J Biomech.* 2012; 45: 2893–2898.
9. *Odarenko YuN, Rutkovskaya NV, Rogulina NV, Stasev AN, Kokorin SG, Kagan ES, Barbarash LS.* Analysis of 23-year experience epoxy treated xenoaortic bioprosthesis in surgery mitral heart disease. Research factors of recipients by positions of influence on the development of calcium degeneration. *Complex Issues of Cardiovascular Diseases*. 2015; (4): 17–25. [In Russ, English abstract] doi: 10.17802/2306-1278-2015-4-17-25.
10. *Lisy M, Kalender G, Schenke-Layland K, Brockbank KGM, Biermann A, Stock UA*. Allograft Heart Valves: Current Aspects and Future Applications. *Biopreserv Biobank*. 2017; 15 (2): 148–157. doi: 10.1089/bio.2016.0070. PMID: 28151005.
11. *Brockbank KGM, Lightfoot FG, Song YC, Taylor MJ.* Interstitial ice formation in cryopreserved homografts: A possible cause of tissue deterioration and calcification *in vivo*. *J Heart Valve Dis*. 2000; 9 (2): 200–206. PMID: 10772037.
12. *Britikov DV, Lauk-Dubitsky SE, Serov RA. Khugaev GA.* Morphological evaluation of new method of valve and vascular allografts cryopreservation. *Russian Annals of Surgery*. 2019; 24 (1): 16–23. [In Russ, English abstract] doi: 10.24022/1560-9502-2019-24-1-16-23.
13. *Hickey E, Langley SM, Allemby-Smith O, Livesey SA, Monro JL*. Subcoronary allograft aortic valve replacement: parametric risk-hazard outcome analysis to a minimum of 20 years. *Ann Thorac Surg*. 2007; 84 (5):

1564–1570. doi: 10.1016/j.athoracsur.2007.02.100. PMID: 17954063.
14. *Ostrovskij JuP, Judina OA, Muratov RM, Spiridonov SV.* Tehnologija izgotovlenija i metodika ispol'zovanija kriosohranennyh allografтov v hirurgii porokov aortal'nogo klapana. Minsk: Belaruskaja nauka (2016), 229. ISBN 978-985-08-1955-0.
15. *Gatto C, Giurgola L, D'Amato Tothova J*. A suitable and efficient procedure for the removal of decontaminating antibiotics from tissue allografts. *Cell Tissue Bank*. 2013; 14 (1): 107–115. doi: 10.1007/s10561-012-9305-5. PMID: 22407218.
16. *Vasilyeva MB, Krasilnikova AA, Kuznetsova EV, Lunina MV, Samoylova LM, Rusakova YaL et al.* Alternative biocidal solutions for storage of allogeneic vascular grafts used for the replacement of cardiovascular elements. *Patologiya krovoobrashcheniya i kardiokhirurgiya = Circulation Pathology and Cardiac Surgery*. 2018; 22 (4): 95–102. [In Russ, English abstract] doi: 10.21688/1681-3472-2018-4-95-102.
17. *Zhuravleva IYu*. Biotsidnaya kompozitsiya dlya asepticheskogo khraneniya konservirovannogo proteznogo materiala iz tkaney zhivotnogo proiskhozhdeniya. Patent RF na izobretenie RU 2580621C1. Byull. No 10 (ot 10.04.2016).
18. *Lichtenberg A, Tudorache I, Cebotari S, Ringes-Lichtenberg S, Sturz G, Hoeffler K et al. In vitro* re-endothelialization of detergent decellularized heart valves under simulated physiological dynamic conditions. *Biomaterials*. 2006; 27 (23): 4221–4229. doi: 10.1016/j.biomaterials.2006.03.047. PMID: 16620956.
19. *Cheung DT, Weber PA, Grobe AC, Shomura Y, Choo SJ, Luo HH et al.* A new method for the preservation of aortic valve homografts. *J Heart Valve Dis*. 2001; 10 (6): 728–734. PMID: 11767178.
20. *Yao Q, Zheng YW, Lan QH, Kou L, Xu HL, Zhao YZ.* Recent development and biomedical applications of decellularized extracellular matrix biomaterials. *Mater Sci Eng C Mater Biol Appl*. 2019 Nov; 104: 109942. doi: 10.1016/j.msec.2019.109942. PMID: 31499951.
21. *Taylor DA, Sampaio LC, Ferdous Z, Gobin AS, Taite LJ*. Decellularized matrices in regenerative medicine. *Acta Biomater*. 2018 Jul 1; 74: 74–89. doi: 10.1016/j.actbio.2018.04.044. PMID: 29702289.
22. *Theodoridis K, Müller J, Ramm R, Findeisen K, Andrée B, Korossis S et al.* Effects of combined cryopreservation and decellularization on the biomechanical, structural and biochemical properties of porcine pulmonary heart valves. *Acta Biomater*. 2016; 43: 71–77. doi: 10.1016/j.actbio.2016.07.013. PMID: 27422199.
23. *Boekema BKHL, Boekestijn B, Breederveld RS.* Evaluation of saline, RPMI and DMEM/F12 for storage of split-thickness skin grafts. *Burns*. 2015; 41 (4): 848–852. ISSN 0305-4179, https://doi.org/10.1016/j.burns.2014.10.016.
24. *Ranawat AS, Vidal AF, Chen CT, Zelken JA, Turner AS, Williams RJ* 3rd. Material properties of fresh cold-stored allografts for osteochondral defects at 1 year. *Clin Orthop Relat Res*. 2008; 466 (8): 1826–1836. doi: 10.1007/s11999-008-0311-7. PMCID: PMC2584258.
25. *Ziza V, Canaud L, Gandet T, Molinari N, Alonso W, Chastan R, Branchereau P, Picard E.* Outcomes of cold-stored venous allograft for below-knee bypasses in patients with critical limb ischemia. *J Vasc Surg*. 2015; 62 (4): 974–983. doi: 10.1016/j.jvs.2015.04.437. PMID: 26141692.
26. *Wollmann LC, Suss PH, Kraft L, Ribeiro VS, Noronha L, da Costa FDA, Tuon FF*. Histological and Biomechanical Characteristics of Human Decellularized Allograft Heart Valves After Eighteen Months of Storage in Saline Solution. *Biopreserv Biobank*. 2020; 18 (2): 90–101. doi: 10.1089/bio.2019.0106. PMID: 31990593.
27. *Nam J, Choi SY, Sung SC, Lim HG, Park SS, Kim SH, Kim YJ*. Changes of the Structural and Biomechanical Properties of the Bovine Pericardium after the Removal of α-Gal Epitopes by Decellularization and α-Galactosidase Treatment. *Korean J Thorac Cardiovasc Surg*. 2012; 45 (6): 380–389. doi: 10.5090/kjtcs.2012.45.6.380. PMID: 23275920; PMCID: PMC3530722.
28. *Gilbert TW.* Strategies for tissue and organ decellularization. *J Cell Biochem*. 2012; 113: 2217–2222. doi: 10.1002/jcb.24130. PMID: 22415903.
29. *McKee CT, Last JA, Russell P, Murphy CJ*. Indentation versus tensile measurements of Young's modulus for soft biological tissues. *Tissue Eng Part B Rev*. 2011; 17 (3): 155–164. doi: 10.1089/ten.TEB.2010.0520. PMID: 21303220; PMCID: PMC3099446.
30. *Sokolis DP, Kefaloyannis EM, Kouloukoussa M, Marinos E, Boudoulas H, Karayannacos PE*. A structural basis for the aortic stress – strain relation in uniaxial tension, *Journal of Biomechanics*. 2006; 39 (9): 1651–1662. doi: 10.1016/j.jbiomech.2005.05.003.
31. *Yu X, Wang Y, Zhang Y*. Transmural variation in elastin fiber orientation distribution in the arterial wall. *J Mech Behav Biomed Mater*. 2018; 77: 745–753. doi: 10.1016/j.jmbbm.2017.08.002. PMID: 28838859; PMCID: PMC5696052.
32. *Sugita S, Matsumoto T.* Multiphoton microscopy observations of 3D elastin and collagen fiber microstructure changes during pressurization in aortic media. *Biomech Model Mechanobiol*. 2017; 16 (3): 763–773. doi: 10.1007/s10237-016-0851-9. PMID: 27878400.
33. *Silver FH, Horvath I, Foran DJ*. Viscoelasticity of the vessel wall: the role of collagen and elastic fibers. *Crit Rev Biomed Eng*. 2001; 29 (3): 279–301. doi: 10.1615/critrevbiomedeng.v29.i3.10. PMID: 11730097.
34. *Rezvova MA, Kudrjavceva JuA*. Sovremennye podhody k himicheskoj modifikacii belkov v biologicheskih tkanjah, posledstvija i primenenie. *Bioorganicheskaya khimiya / Russian Journal of Bioorganic Chemistry.* 2018; 44 (1): 22–37. doi: 10.7868/S0132342318010025.
35. *Mikhailova IP, Manchenko AA, Byzov DV, Sandomirsky BP*. Fiziko-mehanicheskie svojstva devitalizirovannyh ksenoimplantatov na osnove perikarda, stvorok aortal'nogo klapana i arterij. *Problemy kriobiologii i kriomediciny.* 2015; 25 (4): 311–328.
36. *van Doormaal TP, Sluijs JH, Vink A, Tulleken CA, van der Zwan A.* Comparing five simple vascular storage protocols. *Journal of Surgical Research*, 2014; 192 (Issue 1): 200–205. doi: 10.1016/j.jss.2014.05.001.

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