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Effect of low temperatures and ionizing irradiation upon physical-mechanical properties and connective-tissue structures of porcine fibrous pericardium and aortic valve leaflets

IRINA MIKHAILOVA
BORIS SANDOMIRSKY
ANNA GORLENKO

Department of Experimental Cryomedicine
Institute for Problems of Cryobiology
and Cryomedicine
of the National Academy of Sciences of Ukraine 23
Pereyaslavskaya str., Kharkov, Ukraine 61015

Correspondence:
Anna Gorlenko
Department of Experimental Cryomedicine
Ukraine, 61145 Kharkov
Klochkovskaya Street 105a, #23
E-mail: anna.gorlenko@gmail.com

Key words: fibrous pericardium, aortic valve leaflets, physical and mechanical properties, morphological structure, cell-free xenogeneic materials, freeze, ionizing irradiation, devitalization

Abbreviations:

AVL	– aortic valve leaflet
CF	– collagen fibers
E	– stretch modulus
EF	– elastin fibers
F	– fibroblasts
FP	– fibrous pericardium
h	– thickness
H&E	– hematoxylin and eosin preparation
L	– relative elongation
λ	– limit strength
δ	– reserve of deformability

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Abstract

Xenogeneic tissue devitalization is one of the creating methods of the tissue-replacing the biocompatible cell-free shells for the regenerative surgery. The work describes the possibility of applying the complex approach based on the continuous usage of cryo and radioactive (electron irradiation exposure) biological tissue damage effects. The pre-implant treatment provides sterilization and a possibility for the low temperature preservation of xenografts. After the transplantation such a cell-free xenoscaffold can be gradually replaced with the autogenic extracellular matrix from the recipient's cells and forms a stable long-term structure of the biological prosthesis.

Fibrous pericardium (FP) and aortic valve leaflets (AVLs) were extracted from the mature pig. The prepared tissues were rinsed with the sterile normal saline solution and frozen down to the liquid-nitrogen temperature. After one time placing on water-bath (37°C) they were exposed to electron irradiation within dosage range of 25-30 kGray and submerged into the liquid nitrogen vapors. After influence of low temperature and ionizing radiation, tissue morphological structure was assessed using the optical microscopy. Deformations, i.e. longitudinal and transverse monoaxial strength were performed to calculate the physical and mechanical properties of FP and AVLs.

Such a devitalization method of the FP and AVLs causes significant destructive changes in cell elements, however the spatial arrangement and structural integrity of the connective tissue fiber are preserved. Joint impact of low temperatures and ionizing radiation gives the synergetic effect, increasing the strength and elastic tissue properties. Freezing down to –196 °C and electron irradiation initiate formation of the intra- and intermolecular transverse cross-linking due to the binding activity of fibrous proteins. It leads to a more dense arrangement of the collagen fiber, adds strength to the implant and provides the structural tissue stabilization. The authors believe that during the remodeling in the recipient organism, the biomaterial structure modified in such a manner can successfully prevent physiological tension.

INTRODUCTION

Current by the problem state of reconstructive and restorative surgery imposes the necessity of the further search for more adequate grafts. In recent decade the cell-free bovine and porcine xenopericardial flap

treated with various preservatives have been used by surgeons engaged in various reconstructive surgery studies. Currently the tissue-replacing biocompatible materials have been used in abdominal surgery, traumatology and orthopedics, pediatric surgery, urology, gynecology, ophthalmology, stomatology and herniology. In recent decades intensive clinical and experimental searches show that the decisive influence on the xenografts is caused by their preservation method. Glutaraldehyde of various exposure is used as a prime preservative agent in the production of modern models of biological prostheses. Some authors believe that despite high elastic and mechanic properties and low porosity of the glutaric-aldehyde-preserved xenopericardium, it possesses residual antigenicity and inclines to calcification causing the restrained attitude to its usage. Nowdays apart from the glutaraldehyde the widely differing chemical compounds have been used, such as diphosphonates, glycosaminoglycans, surface-active materials, etc. But despite the above there is no optimal method of pre-implantation treatment of the xenogeneic biological prostheses that would provide their mineralization-resistance and prolong their maximum functioning. The extensive scientific information has been collected on the efficiency, quantity of early and late complications, comfort and security when applying the widely used xenomaterials *(1-4).* Xenoprosthetics can find a wide clinical application once the new methods of biological tissue modification and new ways of graft genotype adaptation to the recipient tissues are developed.

The problem of obtaining efficient grafts of xenogeneic origin is primarily related to the necessity of overcoming the immune conflict. To increase the biocompatibility of the grafts it is suggested to remove (destroy) the donated cells in them prior to the implantation, i.e. to conduct tissue decellularization (devitalization), thus decreasing the recipient immune response to the graft. The cell-free scaffolds devoid of cell constituents are used as prostheses to implant to the recipient *(5-12)*. After the transplantation the cell-free xenoscaffolds is gradually replaced with the autogenic extracellular matrix from the recipient's cells and forms the stable long-term structure. Meanwhile the connective-tissue fiber of the graft is step by step lysed with macrophages, ensuring the complete graft integration into the recipient organism *(13-14).* The results of the reparative surgeries using the cell-free xenogeneic grafts mostly depend on their type and quality. Therefore for efficient process of xenoprosthetics the pre-implant biological tissue treatment shall include the following tasks: reduction of the material immunizing power; tissue structure stabilization; preservation of the adequate mechanical properties while keeping the biological material sterile.

Most devitalization methods are based on the long-term treatment of the xenotissue with different detergent-enzyme and preservative solutions, which functioning is related to the destruction of the immunogenic components, tannage of the tissue and its structural stabilization *(15).* Chemical treatment methods of allow to efficiently decrease the antigenic tissue properties and prevent their bacterial contamination due to the antiseptic properties of the compounds in use. However the chemical treatment deteriorates the quality of the devitalized xenografts and their biomechanical properties get lost. Alongside with this the possibility of the additional tissue mineralization processes rises due to the increase in the quantity of calcinosis nucleation centers, as the chemical composition and physical properties of the biological tissue influence the calcification degree. After the enzyme treatment and binding with glutaraldehyde or epoxy compounds the peculiarities of calcium accumulation by the biological tissue have been noted. The biological tissue devoid of cells, proteoglycans and glycoproteins is quite a loose and porous structure made of collagen fiber capable of calcium-binding *(16-17).* Forming of calcium-containing depositions on the graft's surface or depth results in reduction in its functioning term and necessity of reoperation. The effects mentioned above significantly limit the introduction of such "chemical" procedures into the practice.

In our study we used a new approach to the creation of cell-free xenogeneic materials (tissue implants) using with physical factors (freeze-thawing and ionizing irradiation) *(18).*The aim of this study is a comprehensive assessment of devitalized porcine pericardium tissue and cardiac leaflets by cryo-irradiation as well as the justification of the possibility of their use in regenerative surgery.

Low temperatures were used in the work as a damaging factor for the cell constituents of the tissues under research and for the purpose of the long-term preservation of the devitalized tissue-replacing plastic material. It has been established that deep freezing followed by warming up partially reduces the immunizing power of the biological tissues because the superficial cellular antigen expression is lowered (*19-20).* In the literature there are described the results of using gamma-irradiation to devitalize cardiac valves. The cell death caused by irradiation is explained by the direct radiation and chemical damage of the DNA and cytotoxic influence of the formed free radicals primarily on the cellular membrane phospholipids. Under the influence of the ionizing radiation the cellular mechanisms of interphase cell death are initiated *(21-22).* Therefore the combined usage of the mentioned above physical factors can ensure the reduction of antigenic xenotissue properties by means of damaging the main immunizing power targets, cell elements. Freezing followed by radiation initiates the forming of intra- and intermolecular crosslinking due to the binding activity of fibrous proteins resulting in denser arrangement of the collagen fiber and their structural stabilization *(23-26).* Cryopreservation of biological material ensures the structural integrity of the connective-tissue fiber that significantly defines its biomechanical properties *(27-32).* In addition the ionizing irrradiation provides the complete viral and bacterial sterility of the biological tissue, while

freezing in liquid nitrogen does the low-temperature preservation of the devitalized xenotissue.

The investigation task was to study of stress-strain properties of the pericardium tissue and aortic valve leaflets at devitalization stages of using with low temperature and ionizing irradiation.

MATERIALS AND METHODS

The pericardium and aortic valve leaflets of 6-8 months old outbred pig were used as a material for xenobiografts. In 20 minutes after slaughtering the tissues were aseptically and atraumatically extracted. The pericardium was thoroughly prepared, epiploic appendages and excessive connective tissue were removed and the fibrous membrane was extracted under laboratory conditions. The aortic leaflets remained unexposed. The tissues were three-fold rinsed in cooled down to 4°C sterile normal saline solution with antibiotics and placed into the sterile cryogenic resistant containers ("Eurotubo, Deltalab", Spain). Afterwards that the containers were submerged into the liquid nitrogen and stored at –196°C until the next stage of treatment. The time interval between extraction and freezing did not exceed 5 hours. Then the vials were placed on water-bath at 37°C and transported to the National Science Center "Kharkov Institute of Physics and Technology" of the National Academy of Sciences of Ukraine, where they were electron-irradiated (absorbed radiation dose of 25 kGray) using the LEA-2000 linear electron accelerator. The dose of 25 kGray is minimal for sterility of the medical materials and acceptable for preservation of the fibrous protein of the extracellular matrix. To prevent thermal denaturation of the connective tissue, during the irradiation the samples' temperature was continuously verified (no more than 25°C) and the irradiation itself was performed discretely with dosage distribution over time. The sterile containers containing the irradiated samples was stored in the liquid nitrogen vapors at the temperature from –150 to –170°C.

For the research the porcine fibrous pericardium (FP) and aortic valve leaflets (AVLs) were devided into 4 groups: group 1 – native tissues (control group); group 2 – irradiated tissues (dose of 25 kGray); group 3 – tissues after freeze (–196°C)-thawing; group 4 – tissues after freeze-thawing and the following irradiation at the dose of 25 kGray. The optical microscopy and H&E staining (for cellular and connective-tissue structures) were used to assess the structure of the pericardium and aortic leaflets after different types of exposure. The material analysis has been conducted using the microscope "Meiji Techno" (Japan) with digital screening.

The studies of the physical and mechanical properties of the FP and AVL were based at the Department of Strength of Materials of the National Technical University "Kharkiv Polytechnical Institute" (NTU "KhPI"). The tests have been conducted using the universal deformation device FP 100/1 (VEB TIW Rauenstein, Germany). Examining the physical and mechanical properties included: determination of thickness (h), stretch modulus (E), strength limit (λ), relative elongation (L), reserve of deformability (δ). The samples thickness was measured using the thickness tester TR-10-60. The tensile strength was defined by the following formula (in MPa): $\lambda=F/S$, where F being the maximum tension force on disorder of the material integrity, S – the sample cross-section area.

The modulus of elasticity was defined by the following formula (in MPa):

$E=(F_2-F_1)\ L_o/S(L_2-L_1)$, where F_1 being the initial tension force in the elastic deformation zone, F_2 – the final tension force in elasticity zone, L_1 – the sample length corresponding to F_1, L_2 – the sample length corresponding to F_2, L_o – initial sample length.

Relative tissue elongation was calculated by the following formula: $L= (L_2-L_1)/L_1\times100\%$, where L_1 being the initial sample length and L_2 being the sample length at the beginning of the rupture.

The deformation capacity margin δ was defined by the following formula: $\delta = L_2/L_1$, where L_1 being the initial sample length and L_2 being the sample length at the beginning of the rupture. The calculations of all the indices are represented as the diagrams.

For the tests the 60 mm long and 9 mm wide segments were extracted from the pericardium tissue, the aortic leaflets remained untouched. The samples of each type of biomaterial were statistically and significantly fixed with the abrasive coats to the device specially designed for the given research. During the tensile and strength tests the tissue material under research was considered anisotropic. The lengthwise and transverse deformation was performed depending on the fiber direction at a rate of 60 mm per minute at a pressure limit of F – 4.0 kg. The monoaxial strength test was carried out until the tissue integrity was damaged while registering the critical load applied and ultimate tensile index. The obtained data were digitally displayed on the PC. Deformation curves were processed and the key indices calculated. All the indices conform to the international standards ISO 5840:2005 «Cardiovascular implants – Cardiac valve prostheses», NEQ.

The statistical data were evaluated against the Mann-Whitney test of significance using the SPSS Statistics 17.0 application. The differences were considered as statistically significant $p<0,05$ Diagrams and graphs were designed and processes using Origin Pro 8 and Microsoft Excel.

RESULTS

The physical and mechanical parameters of biological grafts have a significant impact on physiological aspects

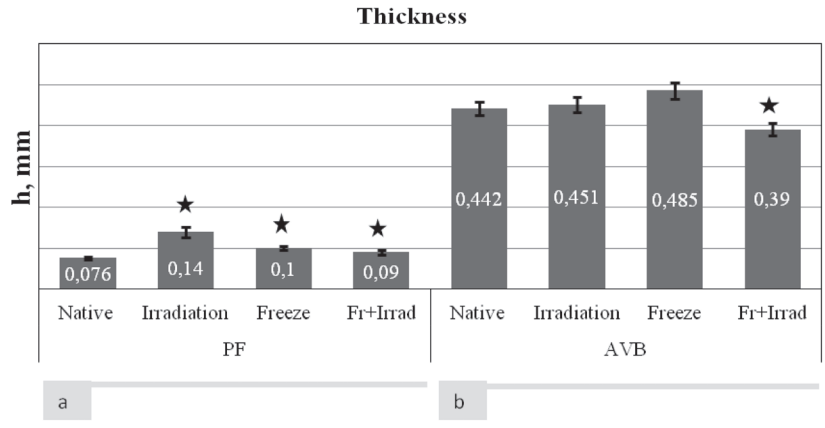

Figure 1. *Thickness (h) in longitudinal and transverse directions of fibers: a – FP, b – AVL; ★ – differences are statistically significant if compared with control, p < 0,05, n=160.*

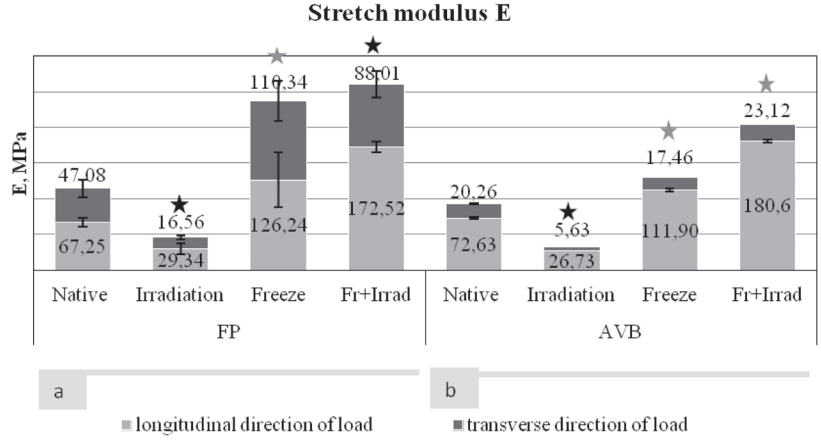

Figure 2. *Stretch modulus (E) in longitudinal and transverse directions of fibers: a – FP, b – AVL; ★ – differences are statistically significant if compared with control, p < 0,05.*

of their functioning in a organism, including such processes as phagocytosis, hemo- and lymphatic circulation and cytoadherence.

To calculate the key indices the thickness of pericardium tissue side and valve leaflets of all the groups under research was measured. The measurements were carried out on three points of the sample and the average value was calculated. As shown in the bar chart (Fig. 1), after all the impacts this value for the peridcardium tissue is firmly increasing in relation to the native one, thus indicating the samples thickening. For the valve leaflets this index is increased for groups 2 and 3, but for the tissue under joint impact of freezing and radiation (group 4) it is statistically and significantly decreasing. The authors believe that the reason for this is partial dehydration of the extracellular matrix.

Fig. 2 shows the values of the modulus of elasticity of pericardium and valve leaflets after all the types of exposure. The given index E is defined solely by the elastic properties of the material and is responsible for the tissue firmness that is directly proportional to the modulus of elasticity E. The modulus of elasticity value for the pericardium tissue exposed to freeze-thawing (group 3) and freezing-irradiation (group 4) significantly increases for the lengthwise tension by 51% and 61% respectively and decreases for irradiation by 57% (group 2). The same trend is observed for the transverse tension (Fig. 2a). For the lengthwise valve tissue deformation the E value is also statistically and significantly higher comparing to the control, by 46% and 58% for group 3 and 4 respectively. In the tissues exposed to radiation (group 2) E is statistically and significantly decreased by 64%. In the radial direction the modulus of elasticity is almost unchanged and remains on native tissue level, except for the samples exposed to radiation, where this index is decreased (Fig. 2b).

The next most important property of the material under deformation is strength, i.e. its capability to resist destruction under exposure (Fig. 2). The tensile strength is the key standard indicator of tissue mechanical proper-

ties. For the quantitative assessment of strength the tensile strength (λ) is used as a disruptive mechanical tension value, i.e. the ratio of disruptive load value to the cross-section area on the point of disruption. Under the lengthwise deformation the pericardium strength (λ) is slightly reduced after freeze-thawing (by 18%), but remains at the control group's level after devitalization. In the transverse load direction the strength is reduced in all the groups under test, but the most notable decrease in strength parameters is observed in the pericardium tissues after ionizing irradiation, by 75% (Fig. 3a). For the valves disruptive load value (λ) is higher only for the tissue exposed to the combined impact, by 27% in the lengthwise and by 20% in the transverse direction of tension. The rest of the groups demonstrate similar values and not statistically significant.

Relative elongation (L) and deformation capacity margin (δ) define the elastic biological tissue properties. Relative elongation describes the material plasticity and tensile deformation capability of the tissue. The elongation is affected by the structure, texture and tissue fiber composition. Figure 4a shows the diagrams for the L value for the pericardium tissue, these indices statistically and significantly decreased for all the groups. For group 4 the L value is almost 3 times reduced for the lengthwise direction and 3.5 times for the transverse. For the valves the relative elongation (L) value is fairly higher in the group of irradiated FP and AVL (group 2), by 54%, and in groups 3 and 4 lower by 33% and 47% respectively. For the group of devitalized valves group (Fig. 4b) under the radial deformation the L value remains on the same level as for the control group.

Reserve of deformability defined the elastic properties of the material and its capability of deforming in plastic range until the integrity rupture. The reserve of deformability indices (δ) are shown in Figure 5. Pericardium deformation capacity is statistically and significantly reduced for all the groups, but remains quite a high (Fig.

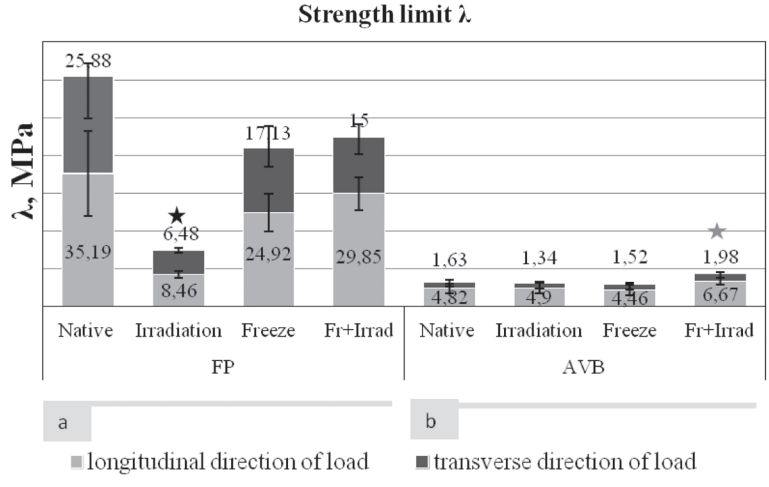

Figure 3. *Strength limit (λ) in longitudinal and transverse directions of fibers: a – FP, b – AVL; ★ – differences are statistically significant if compared with control, $p < 0,05$.*

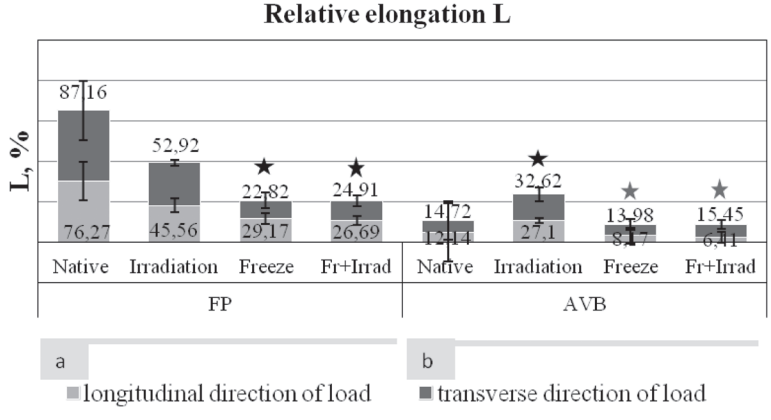

Figure 4. *Relative elongation (L) in longitudinal and transverse directions of fibers: a – FP, b – AVL; ★ – statistically significant if compared with control, $p < 0,05$.*

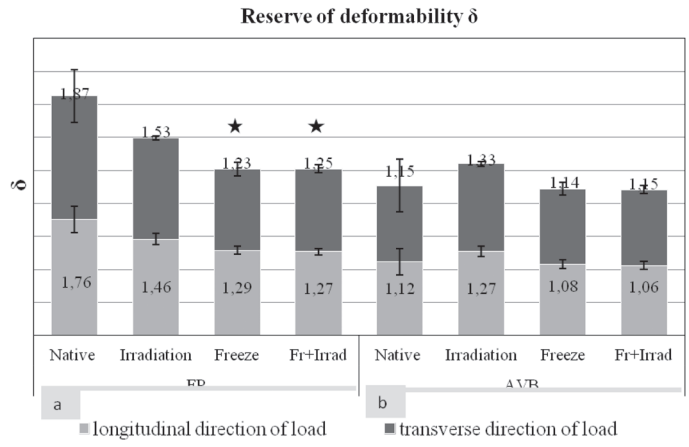

Figure 5. *Reserve of deformability (δ) in longitudinal and transverse directions of fibers: a – FP, b – AVL; ★ – differences are statistically significant if compared with control, p < 0,05.*

5a). The valve leaflets (Fig. 5b) preserve their elastic properties after all types of exposure. Hence after the devitalization (group 4) the deformation capacity of FP and AVL is preserved for all the samples.

Morphological investigation allows to evaluate extent of damaged cellular components and connective-tissue structure and also it allows to characterize their behavior under the influence of physical factors *(33).* Fibrous pericardium consists of dense regular connective tissue represented by thick collagen fibers (CF) and thin elastic fibers (EF). CF and arranged by crimped bundles and densely packed in a parallel array to provide maximum strength. These location of fibers provides resistance to mechanical stress. In the thickness of tissue between the CF fibroblasts are determined (Fig. 6a, single arrows) which are located on a white background of the ground substance *(34).* Aortic valve leaflets (AVLs) are covered by endothelium, the basis is dense irregular connective tissue (Fig. 6b) consist of CF and significantly fewer of thin EF. EF form three-dimensional net and they intertwist with CF (Fig. 6b, double arrows). These structure bundles are variously. This property limits the extensibility of the tissue, prevents the formation of cracks after excessive deformation and contributes to tissue's recovery. Fibroblasts are located in the area of the ground substance (Fig. 6b, single arrows).

Histological studies have shown that uniform seperation into fibers CF and EF occurs after ionizing irradiation at a dose of 25 kGray comparing to native tissue (Fig. 7), this is expressed advent of large cavities between them (Fig. 7 a, b, stars). Collagen bundles of FP (Fig. 7a, double arrows) do not have oriented array, their tortuosity is changed. Fibroblasts of FP and AVL are deformed – they have unusual elongated shape (Fig. 7,8 a, b, single arrows). Segmented and deformed nuclei of fibroblasts were noted in this group, this indicates an interphase cell death that neutralize antigenic effect of tissue (Fig. 7). Also there was noted partial desquamation of endothelium (Fig. 7b).

FP was presented by hyperchromatic bundles of condenced and densed collagen fibrils in the histological preparations after freeze-thawing (group 3) (double ar-

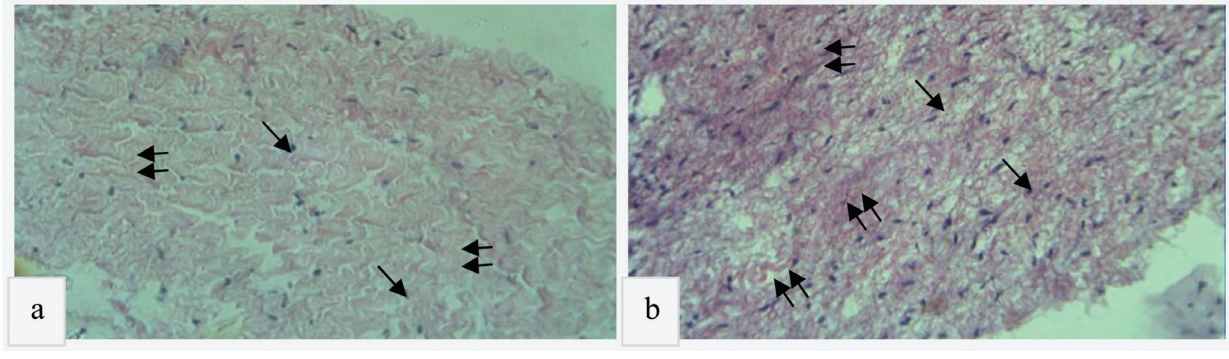

Figure 6. *Morphological structure of native tissue (group 1), hematoxylin and eosin [H&E] preparation, × 40: a – FP, b – AVL. Collagen and elastic fibers marked by double arrows, fibroblasts marked by single arrows.*

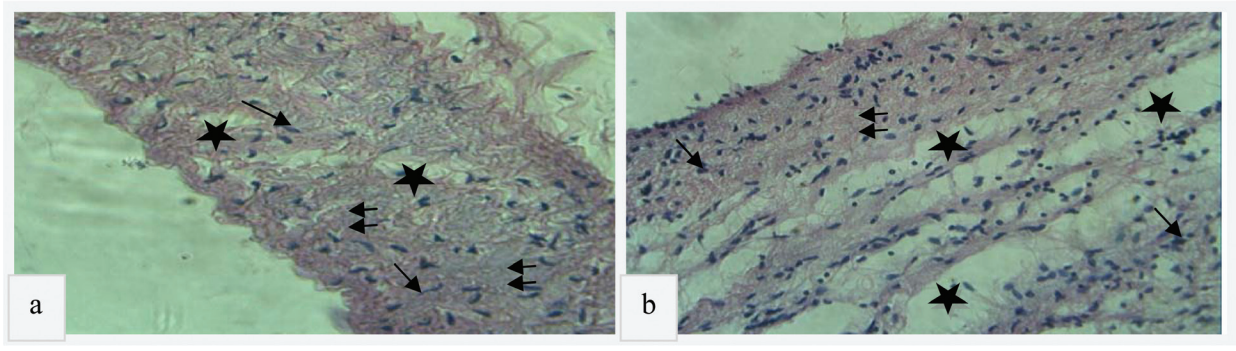

Figure 7. *Morphological structure of tissue after ionizing irradiation in a dose of 25 kGray (group 2), tissue stained with H&E, × 40: a – FP, b – AVL. Collagen and elastic fibers marked by double arrows, fibroblasts marked by single arrows, cavities marked by stars.*

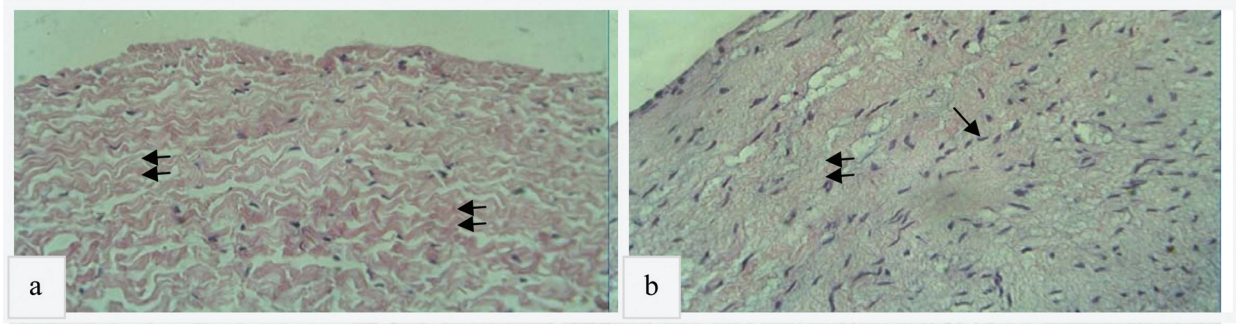

Figure 8. *Morphological structure of tissue after freezing (-196°C) and thawing (+37°C) (group 3), tissue stained with H&E, × 40: a – FP, b – AVL. Collagen and elastic fibers marked by double arrows, fibroblasts marked by single arrows.*

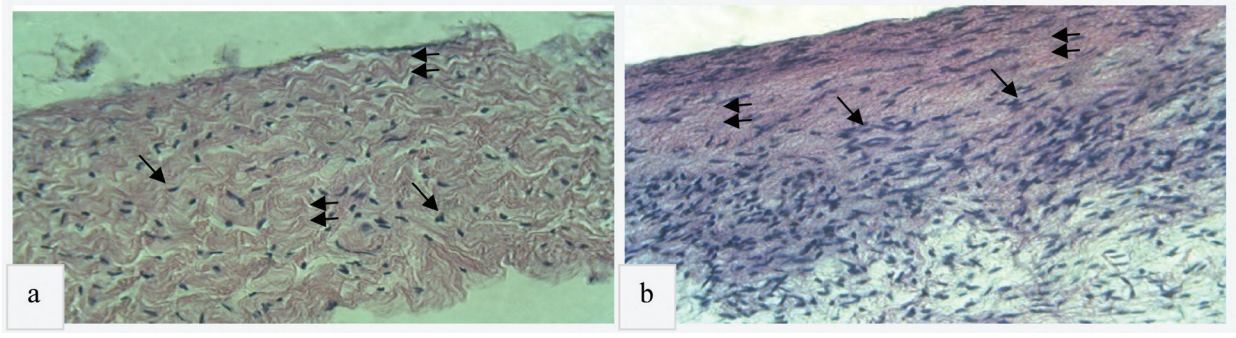

Figure 9. *Morphological structure of tissue after freeze-thawing and subsequent irradiation at a dose of 25 kGray (group 4), tissue stained with H&E, × 40: a – FP, b – AVL. Collagen and elastic fibers marked by double arrows, fibroblasts marked by single arrows.*

rows Fig. 8a). Sizes of fibroblasts did not change but their nuclei were deformed. The structure of EF (Fig. 8b) characterizes by reduction of tortuosity and compact arragment. Continuity, integrity space orientation of EF were not impaired. There were observed the appearance of cavities in the stroma of FP and AVL which associated with mechanical stresses in the tissue due to crystal formation processes. Thus, changes in the structure of the connective tissue are in the consolidation and more compact arrangement of fibers, the structural integrity was not broken.

After freeze-thawing and following ionizing irradiation in a dose of 25 kGray the areas of endothelial desquamation were noted in the tissue of aortic valve leaflets (Fig. 9b). In the tissue FP and AVL the presence of deformed and segmented fibroblast nuclei were noticed (signs of karyorrhexis). The structure of CF and EF was preserved and contoured well with no sites of fibers' seperation. Packing up, thinning of fibers and reduction of tortuosity, all these correspond to changed in the morphology of FP and AVL after freezing (Fig. 9, double arrows).

DISCUSSION

The resulting microscopic data correlate well with the results of biomechanical indexes of the pericardium tissue and aortic valves after the exposure under study.

From our perspective, the strength tissue properties are associated with partial or complete destruction of fibroblasts that in the initial state provide a natural spatial distribution and frame retention. Cell destruction leads to the formation of internal voids within the intermembranous space, enabling approximation of collagen and elastic fibers. Furthermore, disintegration of collagen fasciclesdue to changes in protein-protein interactions may cause a significant influence on the strength tissue properties.

Biological effect of the isolated ionizing radiation is not a direct but an indirect effect of radiolysis products ofwater,which is a part of the cell, and on the biochemical levelleads to creation of the new chemically highly active products that cause additional damage of the biologically important macromolecules. Such damage is associated not only with the nuclear components, but also with the connective tissue extracellular matrix.Under the influence of radiation, active forms of oxygen and various chemical agents the covalent linkage or cross-linking can be created between the bases of two different DNA strings, DNA and protein or two amino acid residues *(35)*.

Thisishowliterature describes the process of the impact of ionizing radiation onto the collagen fiber, during which the collagen fiber dehydration occurs - they shrink thus stimulating the creation of cross-linking. Along with the dehydration the collagen chain splitting may occur that can become a significant side effect leading to denaturation of collagen fiber. The tissue is noticed to shrink thrice during the dehydration. These changes are considerablymanifested in firm decrease of elasticity indexes (L) and tensile strength (λ) in groups of irradiated tissues *(36)*.

Own studies have shown that isolated irradiation has a destructive impact on connective tissue structures of the tissues under study–elastic and strength tissue properties – E and λ are reduced, the connection between the individual fibers of fibrous proteins are destroyed and the tissueis "disintegrated". Preliminary deep freezing of tissue eliminates these negative effects of ionizing radiation.

Freezing processes have considerable impact on the connective tissue structures of the pericardium and aortic valves due to ice crystal formation processes. Incaseoftemperaturereductionthe ice crystallization front distributes perpendicularly to the tissue surface into the interstitial space. This causes pressureincreaseandosmolalityboost of interstitial environment and consequently to the interstitialspace water discharge *(37)*. At the same time the defrosting process has also a considerable effect on appearance of macro- and microscopic damage. Biological tissue damage has been discovered to be associated with intracellular ice formation during warming and not during molecule crystallization while freezing.Collagen fiber on valve and pericardium tissue form a specific space structure that influences the aligned growth of ice crystals that apparently occurs over the tracts created by collagen fiber. Collagen fiberdamage degree varies in borderline and deep layers of connective tissue structure.In deep layers ice crystals cause less prominent structure damage *(38-39)*.

Morphological research demonstrates that collagen fiber thickening occurs after freezing-warming up due to their structure ordering. Crystallization processes cause defects of single fibrils. In the areas of their mechanical damage creation of additional intermolecular cross links is suggested, and their compact arrangement (thickening) initiates creation of intermolecular cross links within the collagen fiber. The mentioned exposure leads to distribution of the deformation load not on single collagen fibrils, but on the formed fibril complexes and fascicles, providing preservation of mechanical strength and elasticity increase.These changes are more expressed in the lengthwise load direction than in the transverse one.

The tissue exposure with the ionizing radiation in the dose of 25 kGy after preliminary freezing-warming up also leads to a firm increase of strength and elasticity properties. Such a result makes it possible to suggest that preliminary freezing exhibits a radioprotective effect for the following ionizing radiation. This radioprotective effect is explained by changing the amount of free-bound water in the tissue after freezing-warming up, as water is the most important structural component.In the dehydrated tissue the processes of radiolysis are exhibited less prominently, however the reactions of dimerization, polymerization and other molecule amplifications and intermolecular changes are preserved.

After the combined exposure the physical and mechanical properties of the aortic valves have been found more preferable in comparison to the ones of the xenopericardium plates. They were found strong, but less stringent than the pericardium tissue, and at the same time more plastic and elastic while being more well-framed. Elasticity indexes have significantly increased for the pericardium tissue, it has become more rigorous while keeping its plastic properties. Thus such a way of preservation provides structural stabilizations of the tissue under research, yet for each tissue type elasticityin the longitudinal and transverse directions, and the resistance to fracture and twisting is preserved.

While analyzing the obtained data it was discovered that the joint impact of low temperatures and ionizing radiation display the synergetic effect, increasing the strength and elastic tissue properties (Fig. 2, 3). This effect virtually confirms the literary data about forming additional cross-linking under the impact of low temperatures, as well as ionizing radiation.

Considering the results of morphological studies and biomechanical properties of the pericardium and the valve

leaflets after the combined effect of low temperatures and ionizing radiation, we conducted the following analogy. If we make the following assumption, we can consider the material of the leaflet and the pericardium as a ternary composite system which is a homogeneous matrix reinforced with collagen and elastic fibers. From the viewpoint of mechanics of composite materials, if such a system is the process of increasing the stiffness (E) and strength (λ) due to reducing of the reserve of deformability (δ), it is equivalent to introducing to a system additional connecting factor *(40-41).* We believe that this analogy is appropriate and suggests, such connecting factor is additional intra-and inter molecular cross-linking, which arise in collagen fibers after the combined effect on tissue.

Effects assessment of the morphological structure and strength properties cannot make a claim for discovering the mechanisms occurring in the system. Additional research is needed for a more in-depth validation of a cascade of structural changes in the connective tissue under the impact of joint physical factors -freezing and ionizing radiation.

CONCLUSIONS

1. The given method of pericardium and valve leaflets tissue devitalization provides a complete destruction of cell elements and preservation of the connective tissue basis. Structural matrix proteins preserve architecture and fulfill tissue shell functions.

2. The calculation measurements showed that after the deep freezing and irradiation the average thickness (h) of the pericardium tissue samples was 0.09±0.01 mm and getting thicker comparing to the native ones (0,076±0,01 mm), while the valve leaflets (0.39±0.01 mm) getting thinner comparing to the control ones (0.44±0.02 mm) due to partial dehydration of the extracellular matrix.

3. The tests demonstrated that, once devitalized, the elasticity E and strength λ of the tissue under research grow significantly thus leading to the firm reduction of their relative elongation L. The damage of the tissue integrity due to cell destruction and cavitation leads to insignificant reduction of reserve of deformability δ.

4. While analyzing the obtained data it was discovered that the joint impact of low temperatures and ionizing radiation manifests the synergetic effect and significantly increases the strength and elastic tissue properties.

5. Impact of the isolated electron irradiation on tissue significantly deteriorates its qualities and demonstrates the drastic reduction of the strength and elastic properties. Prefreezing to -196 °C prevents negative impact of the ionizing radiation.

6. Increase in the strength and elastic properties is related to impact of the low temperatures and ionizing radiation that initiate cross-linking thus leading to denser collagen fiber arrangement and structural tissue stabilization.

7. While comparing the structure and values of the operating tension of the native tissue to the obtained results it can be expected that in case of long-term presence in the recipient organism the given material can successfully prevent physiological tension.

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