RADIATION STABILITY OF FIBROUS CONNECTIVE-TISSUE BIOMATERIALS

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Abstract

During the recent years methods of treatment based on a tissue transplantation have spread widely in a medical practice. Banked connective-tissue grafts have a considerable proportion in a surgery of tissue grafting. One of the most difficult stages of graft making is its sterilization. It is known that some grafts after standard gamma - sterilization (2,5 Mrad) lose their plastic properties. At the same time structural changes of biomaterials at various regimens and doses of radiation influence are unexplored.

For our study we took the material of 84 female and male corpses and chose 3 kinds of allotransplants - tendons, derma and dura mater of brain (DMB). We used gamma and electronic irradiation at the doses of 1,5 and 2,5 Mrad for the radiation sterilization of the specified grafts.

Our studies showed that changes in the structure of a fibrous framework of connective-tissue grafts after the radiation sterilization depend on a fibroarchitectonics of donor tissues. On the basis of received data the concept of connective-tissue structures stability to the radiation sterilization was developed. We distinguished three levels of structural stabilization of connective-tissue biomaterials and determined their role at the various types of radiation sterilization. At the comparison of two types of radiation we revealed more expressed changes at the irradiation by electrons. Radiation (gamma) sterilization keeps the framework of grafts almost constant.

Key words: radiation sterilization, biomaterials, grafts.

During the recent years methods of treatment based on a tissue transplantation have spread widely in a medical practice. Banked connective-tissue grafts have a considerable proportion in a surgery of tissue grafting. Thus, in the All-Russian center of eye and plastic surgery the grafts with relatively low antigenic properties are developed and widely used, they stimulate directional regeneration of a fibrous connective tissue and have the trade mark Alloplant (E. R. Muldashev and co-authors, 1974 — 2002.). At the present time 73 types of the specified grafts have the state registration and are serially produced. Taking into account that the framework of the various types of alloplant contains a complex of components of extracellular connective tissue matrix with different histochemical structure, we consider the performed grafts among biomaterials.

One of the most difficult stages of graft making is its sterilization. It is known that some grafts after standard gamma - sterilization (2,5 Mrad) lose their plastic properties. At the same time structural changes of biomaterials at the various regimens and doses of radiation influence are unexplored. Thereupon, investigations on a morphological reorganization of grafts with various fibroarchitectonics after a radiation sterilization are also interesting. We suppose that the received results will allow us to predict possible destructive changes of biomaterials and choose the adequate regimen of sterilization. The stated problems are the aim of the present study.

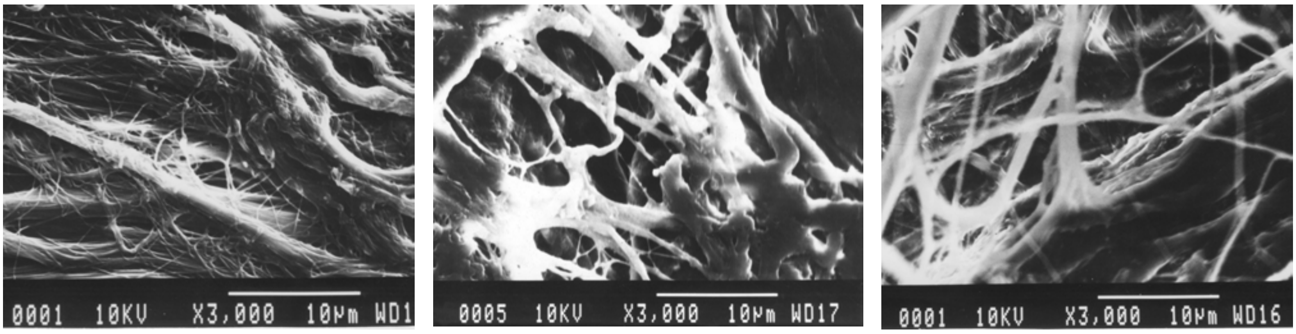
MATERIAL AND METHODS OF STUDY

For our study we took the material of 84 female and male corpses and chose 3 kinds of allotransplants - tendons, derma and dura mater of brain (DMB), which were treated according to the requirements of ТУ-42-2-537-93. We used gamma and electronic irradiation at the doses of 1,5 and 2,5 Mrad for the radiation sterilization of the specified grafts. Data of the bacteriological control verified the efficiency of radial sterilization of the grafts at all modes and doses of radiation irradiation under study. The graft samples before and after irradiation were analyzed using a complex of histological methods (van Gieson's stain, polarizing microscopy of uncolored sections, scanning electron microscopy).

RESULTS AND THEIR DISCUSSION

The tendinous graft, in which structure the unidirectional fascicles of collagen fibers prevail (fig. 1 A), is exposed to the expressed structural changes at all investigated regimens and doses of irradiation. The irradiation of the tendinous graft by gamma-rays at the dose of 1,5 Mrad leads to the changes in the structure of fascicles of I and II orders. In the fascicles of I order there is a longitudinal splitting and fragmentation up to the separate collagen fibers. The longitudinal splitting becomes expressed due to the deep destruction of the collagen fibers, surrounding the fascicles of I order. A disorganization of the specified network of fibers results in a broadening of interfascicular intervals. The similar changes occur at the level of II order fascicles. The longitudinal fragmentation and broadening of interfascicular spaces are also observed here. The processes of destruction of loose connective tissue, surrounding the fascicles of II order, result in the longitudinal splitting of fascicles, that is revealed by scanning electron microscopy. At increase of the dose of gamma-irradiation up to 2,5 Mrad the described changes are more expressed. The collagen fibers of surrounding network at the level of fascicles of I and II orders are exposed to the deep destruction: the sharpness of contours disappears, the fibers become amorphous, it seems to be connected with their physicochemical disorganization (fig. 1 B) (Liu B., Harrell R., Davis R. 1989).

Sterilization of the tendinous graft by the electron irradiation also results in the expressed structural changes of its fibrous framework. At the dose of 1,5 Mrad the contours of fascicles of I order disappear, the fascicles are fragmented. The separate collagen fibers and their fragments are appeared from the fascicles of I order by their longitudinal splitting. At the dose of 2,5 Mrad a homogenization of the graft happens, at that it becomes impossible to distinguish the fascicles of I and II orders. In this connection the tendinous graft represents similar fascicles of 2 microns in diameter, they are loose and connected by isolated deformed collagen fibers. The network of fibers, surrounding fascicles, disintegrates completely (fig. 1 C).



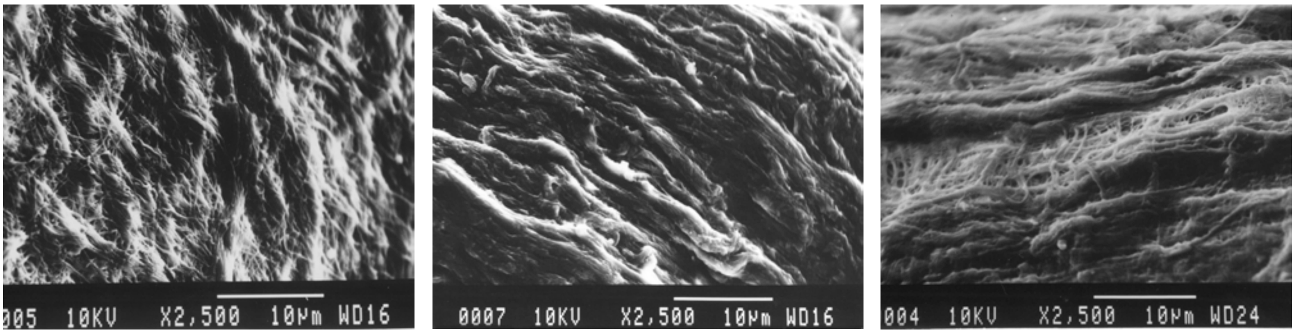
A B C

Fig. 1. The tendinous graft: A - control; B - deep destruction of fibers after γ-rays sterilization, the dose - 2,5 Mrad; C - complete destruction of fibers after sterilization by electron irradiation, the dose - 2,5 Mrad. Scanning electron microscopy x3000.

Our data correspond with the results of study of Salehpour A., Butler D.L., Proch F.S. (1995), who have revealed the destruction of collagen fibers of the tendinous graft at the gamma - sterilization by the doses of 4, 6, 8 Mrad.

Examination of the DMB graft (fig. 2 A) after the radiation sterilization has revealed its higher stability to the irradiation. Thus, at the gamma-irradiation by the doses of 1,5 and 2,5 Mrad the structure of grafts keeps safe in whole (fig. 2 B). Low expressed local changes in the form of fascicles splitting to smaller structures and separate fibers do not result in changes of a general fibroarchitectonics of the described graft.

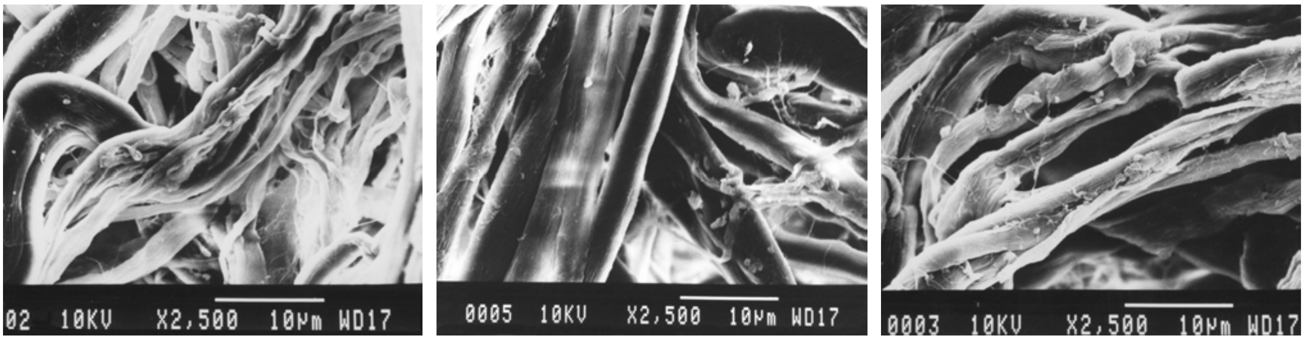
The electron irradiation by the dose of 1,5 Mrad do not lead to the visible structural changes of the DMB graft also. However, an increase of the dose up to 2,5 Mrad results in loosening of connective tissue layers between the fascicles and broadening of interfascicular spaces (fig. 2 C). However, polarization-optical investigations show a conservation of anisotropic properties of the collagen fibers.



A B C

Fig. 2. The DMB graft: A - control; B - γ-rays sterilization, the dose - 2,5 Mrad; C - sterilization by electron irradiation, the dose - 2,5 Mrad. Scanning electron microscopy x2500.

Our study of the structure of the derma graft (fig. 3 A) has shown, that it has the highest stability to the radiation irradiation. At the gamma - sterilization by the doses of 1,5 and 2,5 Mrad the structure of described graft keeps safe (fig. 3 B). A basis of the dermal graft is a complex plexiform fibrous framework with a compact fascicles layout. Thus, the separate fascicles pass from one layer to another, forming differently directed fibroarchitectonics of the graft. The fascicles are connected to each other by an underdeveloped network of collagen and elastic fibers. Apparently, tightly located and twisted fascicles of the dermal graft allow to keep its structure even at more hard electron irradiation by the doses of 1,5 and 2,5 Mrad (fig. 3 C). According to our electron microscopy data, the structure of the dermal graft remains constant.

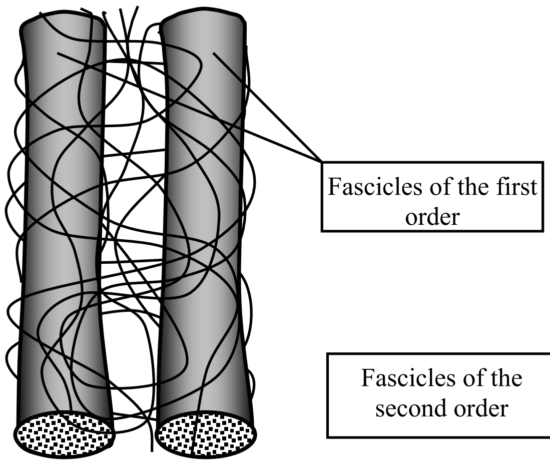


A B C

Fig. 3. The dermal graft: A - control; B - γ-rays sterilization, the dose - 2,5 Mrad; C - sterilization by electron irradiation, the dose - 2,5 Mrad. Scanning electron microscopy x2500.

The results of our morphological studies of different connective tissue grafts after the radiation sterilization allow to elaborate the concept of radiation stability of fibrous structures. The investigated tissues are distributed according the levels of structural stabilization and stability to the radiation sterilization as follows:

Tendon graft ⇒ **DMB graft** ⇒ **Derma graft**



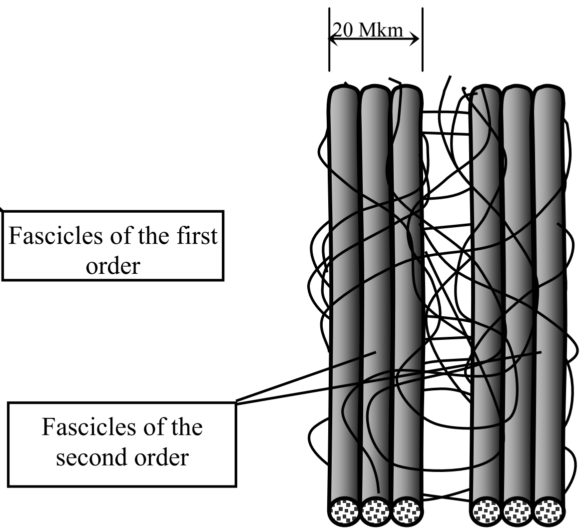


Fig. 4. The first level of structural stabilization of the fibrous framework of connective tissue grafts.

Fig. 5. The second level of structural stabilization of the fibrous framework of connective tissue grafts.

Least steady to the radiation influence graft is the tendinous graft, in which the fascicles of I order with the diameter about 2 microns are braided and connected with each other by the network of collagen fibers. We consider the presence of the described network and the fascicles of I order as the first level of structural stabilization of the connective tissue grafts (fig. 4). The fascicles of II order with the diameter about 20 microns are also braided by the similar network, which supports a structural integrity of the fascicles and connects them. Accordingly, we consider the presence of the fascicles of II order with a loose network of collagen fibers, which braids them, as the second level of the fibrous structures stabilization (fig. 5). Both mentioned levels of stabilization are characteristic for the described tendon graft. Our studies have shown, that the described two levels of stabilization separately do not provide sufficiently reliable protection of the graft at the radiation sterilization. Destruction of the collagen fibers, which form a felt-like network around the fascicles, happens at the gamma-rays irradiation by the dose of 1,5 Mrad already. Practically, the major stabilization factor for the fascicles of I and II orders disappears, as a consequence of it their fragmentation into the separate fibers and thin fascicles happens.

The study of the DMB grafts has allowed to reveal the third level of structural stabilization, for which the complex plexiform fibrous framework is characteristic, where the fascicles have a spiral course, passing from one layer to another (fig. 6). The third level of stabilization causes a higher stability to the radiation irradiation of the connective tissue grafts. Therefore, the DMB grafts show slight local change of the loose network of collagen fibers at the electron sterilization by the dose of 2,5 Mrad.

In the dermal graft the first and second levels of stabilization are minimally developed. However, the spatial organization of fascicles is represented by the complex plexiform collagen frame, that conforms with the data of Bikmullin R.A. (1996).

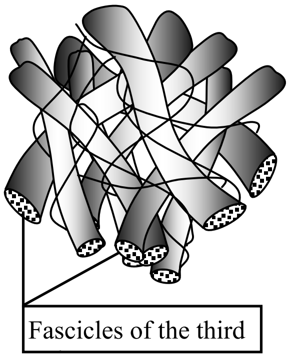


Fig. 6. The third level of structural stabilization of the framework of connective tissue grafts.

On the basis of the received data we made a characteristic of the grafts depending on the features of their fibrous framework (see the table).

The table

Characteristic of the grafts depending on the features of the fibrous framework structure

Types of grafts	Levels of stabilization		
	I	II	III
Tendon graft	++	++	—
DMB graft	++	++	++
Derma graft	+	+	+++

In the given table the grafts under study and appropriate levels of structural stabilization, which are estimated using three-mark system, are given. So, only first two levels of stabilization are expressed in the tendon grafts. In the DMB grafts all three levels of stabilization are equally represented, that, obviously, provides a reliable enough stability to the radiation influence. In the derma grafts first two levels of stabilization are poorly expressed. At the same time, the complex spatial architectonics of the derma graft allows us to estimate a role of the third level of stabilization. The given combination of all three levels, at the leading role of the third level of stabilization, allows to ensure grafts the maximal stability and to keep their fibrous framework at the influence of radiation sterilization at all regimens and doses under study.

The received results and theoretical generalizations, made on their basis, allow to determine the optimum dose and type of radiation sterilization in every case on the basis of study of the fibrous structures of various connective tissue grafts.

CONCLUSION

As is shown in this study, the changes in the structure of the fibrous framework of the connective tissue grafts after the radiation sterilization depend on fibroarchitectonics of the donor tissues. On the basis of the received data the concept of stability of connective tissue structures to the radiation sterilization is developed. We define three levels of structural stabilization of connective tissue biomaterials and determine their role at the various types of radiation sterilization. It is determined, that the various regimens of irradiation in a sterilizing dose do not cause the morphological changes in the biomaterials with a complex mutually plexiform collagen framework, whereas the grafts with the unidirectional orientation of the collagen fascicles are exposed to the deep changes from the longitudinal fragmentation of fascicles up to the destruction of particular collagen fibers.

At the comparison of two types of radiation we revealed more expressed changes at the irradiation by electrons. Radiation (gamma) sterilization keeps the framework of grafts almost constant.

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