STERILIZATION OF SKIN ALLOGRAFTS BY IONIZING RADIATION

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Abstract - The skin has a fundamental role in the viability of human body. In the case of extensive wounds, skin allografts provide an alternative to cover temporarily the damaged areas. After donor screening and preservation in glycerol 85%, the skin can be stored in a Skin Bank. Glycerol at this concentration has a bacteriostatic effect after certain time of preservation. On the other hand, skin sterilization by ionizing radiation may reduce the quarantine period for transplantation in patients. The objective of this work was to evaluate allograft sterilization using two sources of ionizing radiation. Through the analysis of stress-strain, it was intended to verify possible effects of the radiation on the structure of preserved grafts. Three groups of skin samples were selected. The first group was maintained in the initial conditions, not irradiated. The second was exposed to cobalt-60, while the third one was irradiated using an Dynamitron Accelerator JOB188 electron beam. The irradiation dose was 25 kGy for both tests. Both irradiation sources, and the Instron Universal Machine used for biomechanical experiments, are installed at the Centro de Tecnologia das Radiações / Instituto de Pesquisas Energéticas e Nucleares (São Paulo, Brazil). According to the preliminary results, biomechanical characteristics of the samples irradiated seem to be maintained with regard to the non irradiated group.

Key words: Ionizing radiation, allograft, skin, biomechanical properties, Young's modulus

INTRODUCTION

A tenuous layer covers our body and isolates it from the surrounding environment. Covering the totality of the organism surface, the skin corresponds to 16% of the body weight and, although it is not more than 2.0 mm thick, it is an efficient barrier against a wide variety of physical, chemical and biological agents, resisting to mechanical forces, extreme temperatures, toxins and ionizing radiation at low doses. The skin is also responsible for the organism homeostatic maintenance process, the body temperature regulation, the hydric and osmotic equilibrium (22). It possesses also a complex antioxidantive system that includes enzymatic and non-enzymatic components, which consists in the first defense against free radicals originated either by environment or by endogenous processes (17). The protection against the harmful effects of sunrays, especially ultraviolet, is provided by the skin pigment melanin produced by melanocytes which gives maximum protection against UV light.

Due the important role the skin performs, it is comprehensible that vast injuries compromise patient survival (16). It is the case, for example, of serious, extensive and deep burns of full thickness, that destroy the epidermis, dermis and subcutaneous tissue making impossible the spontaneous regeneration of the skin. The decrease of mortality rate is thus related to the efficiency to provide a protection to the burned region in the way to diminish substantially the dehydration and to allow the infection control (9). Besides, this cover allows or promotes regeneration more adequately, avoiding the adjacent tissue retraction and the development of deformed scars.

When donor areas are available, it is possible to proceed with autografting, which means the use of tissue from the same person. In immunologic aspect, this is the best option, because the risk of rejection is inexistent. Nevertheless, such procedure is not always possible when the damaged region represent a large surface of the body and the donor surface is not enough. The bioengineering research of dermis-epidermis replacement synthesis (polymeric or biologic) has brought to the market products that can replace the original tissue (2,18).

Tissue Banks in several parts of the world provide allografts (skin grafts from individuals of the same species but
of different genetic constitution), which are preferentially used as temporary cover for serious burns due to their relatively easy preservation and storage (3,7,13). Many authors refer to skin allografts as being the best, most used and efficient wound coverage alternative for burn patients, with documented data of efficacy (5,14,19,20).

Skin allograft rejection is more complex than that which occurs with transplanted internal organs because the skin, as an organ that interfaces with the external environment, is extremely competent in inducing an immune rejection response (4,10). The epidermis has among the epithelial cells, special cells of the immune system, the Langerhans cells, which have immunoglobulin receptors and that can process and accumulate in their surface the cutaneous antigens and later present them to lymphocytes.

Cadaveric skin grafts, even submitted to preservation processes and posterior sterilization, preserve their antigenicity. Therefore, within three weeks the grafts tend to be rejected, necrosis is produced and should be removed (11). The graft disruption seems to be related more to the invasion of mononuclear cells (lymphocytes and macrophages) than the surrounding antibody action into the grafted cells (21).

In case of very serious lesions, during which the dermis is also damaged, allografts can be composed by dermis and epidermis. Herson (10) describes the definitive incorporation of allogenic dermis grafted in burned subjects. Some days after using partial thickness skin allotransplants, for covering full-thickness burned areas, only the epidermis was removed and replaced by epithelial tissue form the same patient. The follow-up of the cases showed that not only the allogermis was incorporated but also that the autoepidermis kept its functional characteristics.

The possibility of human skin preservation for further use as a graft is not new. Since the beginning of the 20th Century, several studies have been reported about skin conservation in refrigeration and about developing different methods of preservation (10).

Glycerol at high concentrations (85%) can be used as a biological tissue preservation medium. When the skin cells are dehydrated, glycerol maintains their structure because it replaces the water extracted from the cells and distributes the remaining water throughout the tissue (15,19). Furthermore, it does not affect the collagen and elastin fibers present in the dermis (19). Together with being a simple technique of preservation, glycerol presents confirmed bacteriostatic and anti-viral action. To obtain the maximal bacteriostatic effect, a minimum quarantine period of 3 to 4 weeks exposure to glycerol is recommended before clinical use (1,15).

Sterilization by ionizing radiation

The use of a sterilization method together with the preservation in glycerol contributes to shorten the time of graft quarantine and assure sterility.

The Instituto Central do Hospital das Clínicas (ICHC) of the Faculdade de Medicina da Universidade de São Paulo in collaboration with the Instituto de Pesquisas Energéticas e Nucleares’(IPEN-CNEN / São Paulo, Brazil are operating since 2000, the first tissue bank in Brazil that uses tissue sterilization by ionizing radiation. Large scale use of ionizing radiation to sterilize hospital products was started in the 1950’s. This practice was increased between the 70’s and 80’s, when the use of polymeric products sensitive to heat treatment was introduced in the market. In a similar fashion, biological tissues cannot be autoclaved or sterilized by heat, while the use of ethylene oxide produces toxic residues which require a period of days before the tissues can be used safely.

Ionizing radiation does not produce radioactive residues or toxic residues, does not present contamination risks and the materials can be processed both at room temperature or frozen. Gamma radiation and high energy electrons have high penetrating power so that products can be irradiated already wrapped. Nevertheless, it is necessary to note that these sources generate heat; therefore, high energy electrons doses must be cumulative to reach the total established levels so as to avoid damage of the tissue.

The effect of ionizing radiation on living tissues is well known. Specifically, the collagen I of the dermis presents alterations in its molecular organization, with the reduction of the diameter of the fibers and the increase in frequency of abnormal fiber bundles formation (23). Tissue Bank allografts processed in glycerol have minimal water available as target for ionizing radiation and the formation of free radicals, which brought foward the possibility of a reduced direct radiation effect on the grafts. The aim of this study was to evaluate possible interferences of the direct radiation on the structure and biomechanical characteristics of these skin grafts through the analysis of changes in tensile strength and histological observations.

Skin structure and biomechanical characterization

Two layers, epidermis and dermis, with distinct embryonic characteristics and origin, form the skin but have a great adhesion and interactivity (10).

Biomechanical features of the skin are determined mainly by the direction and the resistance presented by the network of collagen and elastin from the dermis. From the bioengineering point of view, the skin is not composed of a homogeneous material. Analyzing histologically a skin sample that was fixed in the relaxed state, its collagen fibers are apparently randomly arranged. Nevertheless, if the skin is kept stretched during fixation, an amount of fibers are orientated in the direction of the traction; therefore, the higher the force applied to the skin, the higher the amount of lined up collagen fibers. Concerning the elastic fibers, those present in
the relaxed skin, are arranged in spirals around collagen fibers. When the skin is distended, the elastic fibers are bound by molecular bridges to collagen fibers and also align in the direction of the stretch (8).

Another component that contributes to the mechanical characterization of the skin is the epidermis. This layer is more superficial, formed by keratinized stratified epithelial tissue, originated by ectodermis, and generally very thin in most parts of the body (0.06-0.12 mm). The resistance of the epidermis is due to several characteristics of this epithelium. It presents a compact structure of contiguous cells and very little extracellular matrix. Among the keratinocytes there are adhering structures (desmosomes) that keep the cells attached to each other. The skeleton responsible for the firmness of the epithelium is formed during the maturation process with the accumulation of intracellular (filamentous) keratin; in the corneal layer the keratin represents 80% of the epidermal proteins. When keratinocytes finally die, they remain adherent to each other and rest of the epithelium thanks to intercellular junctions so that mechanical resistance of the epidermis is observed only when the stretching force is higher than 70% (6). Thanks to the structure of the superficial corneal scales, skin resists to friction forces of moderate intensity, changes in environmental pH, temperature and enzymatic digestion (12).

The adhesion between epidermis and dermis results from a basement membrane which is produced by both layers. The transmembrane proteins of the epidermal cells interact with collagen fibrils synthesized by fibroblasts, ensuring the adhesion and physical continuity between both layers. The interface between them is papillary conformation, increasing the contact surface allowing the skin to stretch without breaking up (10).

MATERIALS AND METHODS

Samples preparation

Skin from cadaveric donors was provided by the ICHC-IPEN Tissue Bank, after approval of the Research Committee. The partial thickness grafts were originally taken with an electric dermatome and later processed in glycerol 85% and kept under 4°C.

The technique of systematic samples was used in the biomechanical tests and with the material thickness around 0.10 mm. The average thickness was measured with a watch pachymeter (Mitutoyo), before and after irradiation, using tree points set within the same distances in each sample.

The samples were flattened and wrapped in 5 layer polyvinylon bags (the same as used in the Tissue Bank) in to which 2 ml of glycerol 85% were added and the maximum air possible was extracted. The bags were sealed thermally and identified.

Three sets of 10 skin grafts samples were established. The first set was kept in the initial conditions, i.e. not irradiated. The second set was submitted to the dose of 25 kGy of gamma radiation in a cobalt-60 irradiator (Gammacel), installed in the Centro de Tecnologia das Radiações (CTR) / IPEN, with an activity of 3.1 x 10^-11 Bq (8.35 kCi) and a dose rate of 5.89 kGy/hr. The third set was exposed to an electron beam in a Dynamitron Accelerator JOB 188, installed in the CTR / IPEN, calibrated with the following parameters: total dose of 25 kGy, energy of 0.73 MeV, beam current 0.4 mA, dose rate of 2.44 kGy/sec, tray speed 3.36 m/min, passage dose 1.0875 kGy in the total of 24 passages of the tray by the beam.

Biomechanical tests

After the irradiation and before having been mechanically tested, the samples were rinsed in sterile saline solution 0.9%, immersed in a fresh solution for 24 hr so as to wash out the glycerol, to re-hydrate the tissue and to recover normal physiologic characteristics such as opacity and flexibility (10), simulating the routine of clinical use.

Each sample was taken out from the saline solution, stretched and fixed in a acrylic plaque with the epidermis facing up. The skin was cut with a special knife assembled in a wooden plate into a pattern form of halters dumbbell with the width of 4 mm in the most narrow position (proof body), a width of 10 mm in the most wide position and 50 mm in length (25).

The knife was supported with the blade turned to the skin in the longitudinal position and submitted to homogenous pressure of 500 kg in a manual press to obtain the cut. The cut test samples were kept in Petri plates with sterile saline solution 0.9% at room temperature until the assay time.

For the tensile strength tests the samples were fixed in claws at a distance of 27 mm in an Instron Universal Machine, installed at CTR / IPEN. This equipment has a computerized system installed which files the data obtained during the assay that can later be later processed and printed into tables and graphs. A force of 1 kN was applied with the module resulting in, a distancing claw speed of 50 mm/min until, the sample rupture.

The graphs obtained with the Instron program present the relationship between the stretching x tension; the tensile strength at the rupture point is represented by tension (σ in MPa) which is the ratio between applied force (F, in N) and the area of the transversal section (A, in mm²):

\[ \sigma = \frac{F}{A} \]  
Eq. 1

The deformation or stretching (ε, in mm/mm) of the sample is the result of the length variation (L) divided by initial length (L1):

\[ \varepsilon = \frac{L}{L_1}; \text{where } L = L_2 - L_1 \]  
Eq. 2

The elasticity modulus (E, in MPa) or Young modulus is the measurement of the resistance to the traction force, thus, the higher the tension applied to the sample at the rupture moment, the higher the elasticity:

\[ E = \frac{\Delta \sigma}{\Delta \varepsilon} \text{ or } E = \frac{\sigma_2 - \sigma_1}{\varepsilon_2 - \varepsilon_1} \]  
Eq. 3

The values of Δσ and Δε are obtained directly from the graph.

Each set of samples received an identification:

PHG: human skin preserved in glycerol not irradiated.
PHGig: human skin preserved in glycerol gamma irradiated.
PHGie: human skin preserved in glycerol submitted to electron beam.

Statistics analysis

The statistical analysis was performed using the average values of elasticity module calculated for the 10 samples in each group of skin samples. For the significance test the Student t-test for independent samples was performed.

Histological analysis

Histological slides stained with hematoxylin-cosin were prepared for each group of samples (Fig. 1).

RESULTS

The Table 1 shows the obtained elasticity average values. The data in Table 1 was used to build the comparative graph

<table>
<thead>
<tr>
<th></th>
<th>PHG</th>
<th>PHGig</th>
<th>PHGie</th>
</tr>
</thead>
<tbody>
<tr>
<td>Media</td>
<td>E</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(MPa)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.88 ± 3.74</td>
<td>6.15 ± 1.77</td>
<td>7.15 ± 2.85</td>
<td></td>
</tr>
</tbody>
</table>

Table 1: Values of elasticity modulus average
shown in the Fig. 2 in order to obtain a better visualization of the correlation between the average values of the elasticity modules, for each sample group.

The calculation for the independent samples t-test (24) (Table 2), show that the PHGic and PHGig measurements not present any significant difference for the significance level of 0.01, when compared to the average of PHG.

The values of calculated \( t \) are smaller than tabled \( t \) (2.88), for the safety level of 0.01 and 18 freedom degrees; therefore, the averages are not significantly different.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Average (X)</th>
<th>Variance (( s^2 ))</th>
<th>n</th>
<th>( t )</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHG</td>
<td>8.875</td>
<td>13.97872</td>
<td>10</td>
<td>-1.15973</td>
</tr>
<tr>
<td>PHGic</td>
<td>7.150</td>
<td>8.14513</td>
<td>10</td>
<td>-2.08428</td>
</tr>
<tr>
<td>PHGig</td>
<td>6.148</td>
<td>3.13955</td>
<td>10</td>
<td>-2.08428</td>
</tr>
</tbody>
</table>

\( n = n^0 \) of samples; \( t = \) Student's test for independent samples

**DISCUSSION**

Human skin samples processed in glycerol and later rehydrated, were used as material for the tests performed on non and irradiated skin treatment following the protocols for processing and clinical use of alloskin grafts at the Tissue Bank from ICHC.

Because it is a biological material, allograft samples are not uniform, presenting pores and microclefs that can compromise the results depending on the test carried out to evaluate material resistance. Thus, it was necessary to proceed with a systematic sampling, which at the same time represented the batch and had homogenous characteristics. The tested samples were selected following the established thickness between 0.15 mm and 0.25 mm and also by the individual checking of the test samples for the absence of microclefs.

The statistical analyses indicate that there is no difference when the tensile strength of the irradiated samples is compared with the control group in this assay. This is an indication that the ionizing radiation may not change significantly skin structure. The observation of the histological preparations supports these results through the preservation of the adhesion between dermis and epidermis and the anatomical integrity of both structural layers. However, complementary experiments should be carried out in order to verify the occurrence of ultra-structural changes.
Fig. 2 Elasticity modulus

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