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Structural and physical characteristics of the dermal decellularized structures evaluation

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Abstract

Introduction: Decellularized biomaterials derived from the biological tissues are ideal for tissue engineering applications because they mimic the biochemical composition of the native tissue. The physical and structural properties of the scaffold are important in the fields of tissue engineering and regenerative medicine.
Material and methods: Study material was 20 decellularized dermal grafts. 10 samples were obtained from piglets slaughtered in the slaughterhouse. Other tissues (n=10) were received from the donor from the Human Tissue and Cell Bank of the Republic of Moldova. Extracellular matrices were obtained by decellularization with 0.5% sodium dodecyl sulfate/0.1% EDTA solution. The evaluation of the structural characteristics was carried out by the histological examination with hematoxylin and eosin, scanning electron microscopy and the quantification of the amount of deoxyribonucleic acids. Assessment of the physical characteristics included analysis of extracellular matrix volume porosity, density, and swelling rate.
Results: Histological examination revealed fewer cells in decellularized tissues compared to non-decellularized ones. More than 80.5% of nucleic acids were removed from porcine matrix and 82.5% of genetic material – from decellularized human dermal structures. A mean correlation and inverse dependence of -0.43 was shown between porosity and swelling rate of decellularized dermis.
Conclusions: The decellularization process significantly ($P<0.05$) removed the cellular components while preserving the connective three-dimensional structure of the dermal matrices clearly shown by quantification of the amount of DNA and microscopic examination of the structures.
Key words: dermis, decellularization, tissue engineering, dermal grafts.

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Introduction

The application of human skin allograft for the wound coverage is widely used worldwide [1]. However, complications have been reported in the use of allogeneic skin associated with the immunogenicity of the graft due to major histocompatibility complex antigens [2], the thickness and availability of the grafted skin, which will prolong the duration of the wound healing and increase the risk of graft failure, scar formation in the donor and transplanted region [3].

Decellularized biomaterials derived from biological tissues are ideal for the tissue engineering applications because they mimic the biochemical composition of native tissue [4]. The extracellular matrix (ECM) is composed of structural and regulatory proteins and polysaccharides and is generated and maintained by cells.

Many cellular functions, such as proliferation, migration or differentiation are controlled by the extracellular matrix [5]. Each organ and tissue is composed of an extracellular matrix distinct in its biochemical composition and structural organization. The physical and structural properties of the scaffold are important in the fields of tissue engineering and regenerative medicine. These materials can be used as films, sponges, hydrogels for the encapsulation, delivery of cells and medicinal agents, being transplanted directly into the wound as a biological dressing [6]. The antigenic individuality of the graft recipient requires tissue engineers to follow rules in choosing decellularization agents and techniques to create a valid biomaterial [7].

The methods used for tissue decellularization must contribute to the preservation of the ECM and avoid disruption of the ultrastructure, which may cause an immunogenic response. Preservation of native structural and biomechanical cues is required. The antigenicity of the biomaterial has the effect on the immune response in terms of the interaction between the decellularized extracellular matrix and macrophages with the subsequent influence on the activation of T cells and the occurrence of graft rejection [7]. Modified skin substitutes are developed

from the acellular materials or can be synthesized from autologous, allogeneic, xenogeneic, or synthetic grafts. Each of these engineered skin substitutes have their pros and cons. However, a fully functional skin substitute is not available, and research continues to develop a skin substitute product that can rapidly vascularize.

There is also a need to redesign currently available substitutes to make them user-friendly, commercially accessible and viable with a longer shelf life [2]. The present study focuses on the evaluation of structural and physical characteristics of decellularized dermal structures. The use of decellularized grafts is a way to help the body recover its damaged skin in cases where the healing process is impossible, such as severe wounds [8].

Material and methods

Skin preparation. To achieve the goal, were studied 20 decellularized dermal grafts. 10 samples were obtained from the piglet weighing up to 10 kg euthanized by blunt trauma. Other tissues (n=10) were received from a 40-year-old male donor, obtained from the Human Tissue and Cell Bank of the Republic of Moldova following the recommendations of the university ethics committee. As a result, were obtained 20 samples with an average surface area of 3.06±0.05 cm2 and a weight of 929±0.09 mg.

Separation method. By treating the tissues with 0.3% trypsin solution at 37° C, was obtained the dermal-epidermal separation of the grafts, according to the protocol [9].

Decellularization method. Tissue decellularization was performed by processing the grafts with a 0.5% sodium dodecyl sulfate solution in a 1:4 ratio with 0.1% EDTA for 72 hours. Renewal of the decellularizing solution was done every 24 hours [10].

Evaluation of the structural characteristics. The morphological assessment of the decellularized grafts was performed by histological examination with hematoxylin and eosin, scanning electron microscopy of freeze-dried tissues. Three representative tissue samples were used to determine total DNA content.

Evaluation of the physical characteristics. The analysis of the porosity of the grafts was performed by the method of moving 96% ethanol through the tissue:

$$Porosity\ (\%) = \frac{V_1 - V_3}{V_2 - V_3} \times 100$$

where, V_1 – the known volume of ethanol in which the graft was immersed, V_2 – the volume of ethanol and liquid-soaked tissue, V_3 – the volume of liquid when the soaked tissue was removed [11-14].

The swelling rate (%) of the decellularized tissues was estimated by the ratio of the difference of the final weight of the extracellular matrix soaked in phosphate buffer solution (Ws) and the initial weight of the dry-to-immersion structure (Wd) to the weight of the dry tissue scaffold (Wd) [15]:

$$SR\ (\%) = \frac{W - W}{W_d^s} \times 100$$

The analysis of the density of decellularized grafts was performed according to the equation:

$$d = W/(V2 - V3)$$

where W – weight of fluid-soaked graft, V2 – volume of fluid and fluid-soaked tissue, V3 – volume of fluid when the soaked tissue was removed, (V2 – V3) was the total volume of the scaffold. A minimum of three samples were analyzed for each tissue [16, 17].

Statistical evaluation. Material collection and data estimation were done in Microsoft Excel Worksheet using basic concepts and statistical methods. The interdependence of decellularized tissue characteristics was estimated by Bravais-Pearson correlation. Values of $p<0.05$ were considered statistically significant.

Results

As a result of the decellularization of human and porcine dermis (fig. 1) with sodium dodecyl sulfate 0.5% / EDTA 0.1% solution, were obtained acellular structures.

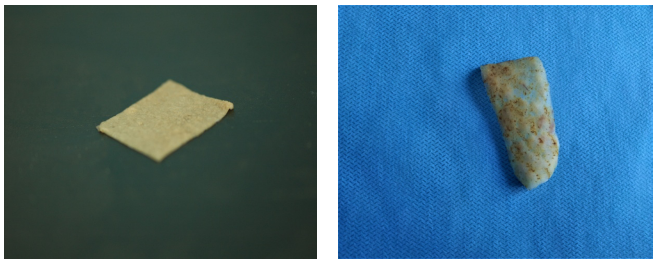

Fig. 1. Native human (a) and porcine dermis (b)

Histologically, in structures decellularized with 0.5% sodium dodecyl sulfate / 0.1% EDTA solution, fewer cells were determined in treated human and porcine dermis compared to non-decellularized structures (fig. 2, 3)

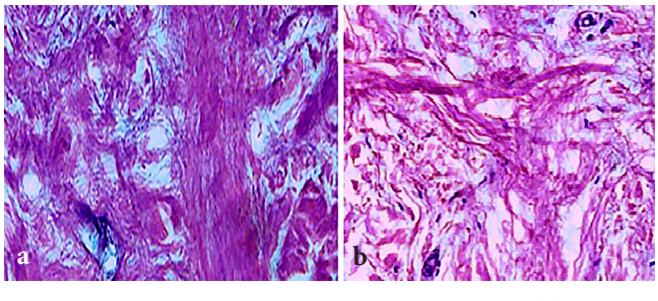

Fig. 2. Histological image of decellularized (a) and intact human dermis (b)

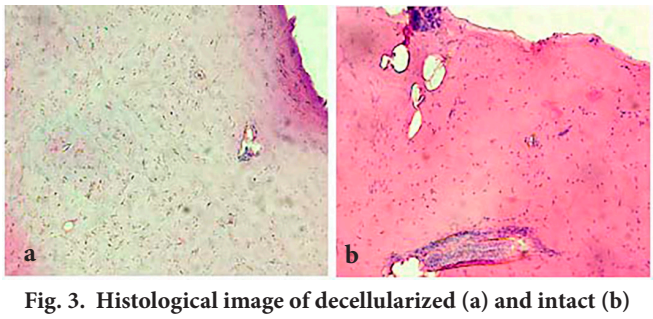

Fig. 3. Histological image of decellularized (a) and intact (b) porcine dermis

Scanning electron microscopy (SEM) shows the three-dimensional (3D) porous image of the human dermis (fig. 4)

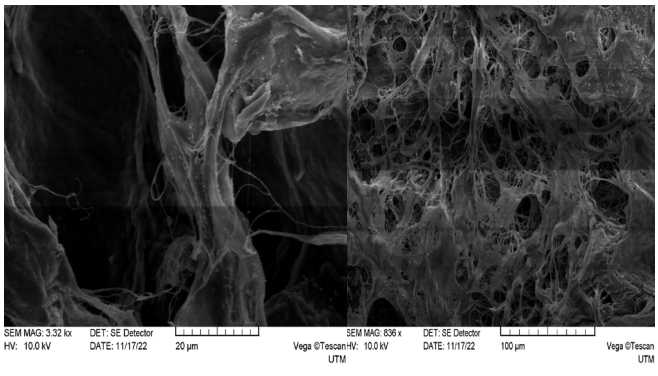

Fig. 4. SEM image shows reticular acellular human dermis

Through the spectrophotometric method, it was determined that in the human dermis decellularized with sodium dodecyl sulfate 0.5%, the amount of deoxyribonucleic acids was 1 ± 0.35 ng/µl compared to the non-cellularized human tissue 13.9 ± 0.4 ng/µl. As a result, it became possible to remove 82.5% of genetic material from human dermal structures. Respectively, 2.43 ± 0.5 ng/µl was determined in the porcine dermis compared to 17.43 ± 3.4 ng/µl in the intact sample, thus 80.5% of the nucleic acids were removed from the porcine matrix.

Evaluating the physical properties of the grafts (surface, volume, density, porosity, swelling rate), were collected the average values of the characteristics (fig. 5, tab. 1).

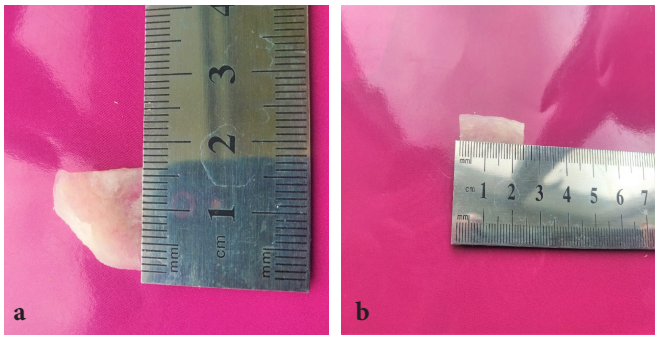

Fig. 5. Estimation of the surface and volume changes of the decellularized porcine dermis following the passage of liquids through the tissue (a, b)

Table 1. Distribution of the physical characteristics of decellularized structures

Characteristics	Human dermis (n=10)	Porcine dermis (n=10)
Surface (cm^2)	3.3±0.1	3.06±0.05
Volume (ml)	4.56±0.05	1.05±0.05
Density (mg/ml)	360±0.83	379.9±0.83
Porosity (%)	35±0.003	53±0.031
Swelling rate (%)	320.4±0.052	650.4±0.65

The significant difference ($P<0.05$) of the characteristics of decellularized dermal structures we observed. Calculating the Bravais-Pearson index showed a medium and weak direct and inverse correlation between density and swelling rate of decellularized porcine dermis 0.33, which means that tissues with significant swelling rate are more or less dense. A mean correlation and inverse dependence of -0.43 was shown between porosity and swelling rate of decellularized porcine dermis, indicating that increasing porosity will decrease the swelling rate of the sponge non-significantly ($P>0.05$).

Discussion

Grafts from xenogeneic dermal substitutes are often applied to extensive skin defects. Although human acellular dermal matrices (ADMs) have shown satisfactory effects in the treatment of large wounds when xenogeneic ADMs do not show immunogenicity due to major histocompatibility complex antigens [18]. An extensive list of animal ADM products is currently available, most of which are achieving success in clinical use. Since the 1960s, pigs have served as primary donors for xenografts in the United States due to their accessibility and histological structural similarities to human skin [19].

Several reports have attested to the benefits of using pigskin in the treatment of extensive wounds. These advantages include reduced healing rates for partial-thickness wounds and granular wounds [19]. Currently available acellular porcine dermal matrices include Permacol (Tissue Sciences Laboratories), Strattice (LifeCell Corp.), Collamend (Bard), Xenoderm (mbp), and XenMatrix (Davol, Inc.) [20]. Researchers have developed protocols for the decellularization of various native tissues [21]. Sodium dodecyl sulfate is the most powerful detergent used to decellularize biological tissues. It is ionic and disrupts all interactions between biomacromolecules. This agent is the most reported in decellularization protocols, and numerous teams have used it to process human skin [22]. SDS treatment is usually performed by immersing the tissue in a 0.5% w/v solution (range of concentrations reported in the literature: 0.1–1%) and takes hours. Thick and complex tissues containing a dense connective layer or a multilayered epithelium, such as the skin, are processed from 12 to 24 hours. All decellularization studies including SDS in concentrations > 0.1% in their protocols reported total removal of cells and cellular debris from native tissue and low levels of remaining DNA [23].

During the given study were found similar results. Porcine skin was decellularized with sodium dodecyl sulfate with the technique used according to the protocol having the amount of DNA of 1 ± 0.35 µg/µl. Sodium dodecyl sulfate is considered an excellent agent for tissue decellularization, but ECM damage has been frequently reported. Loss of ECM density as well as glycosaminoglycan content and collagen network damage have been observed

especially in the matrix produced from fibrillar sheets [24] or in complex tissues such as human skin [25]. Increasing SDS concentration from 0.05% to 0.5% on fibroblast sheets or exposure time from 6 to 12 h was directly correlated with amplification of ECM changes. Scanning electron microscopy studies also suggest rearrangements of ECM fibers and variations in scaffold porosity after SDS treatment. Different combinations of SDS and Triton X-100 were also tested on rat fasciocutaneous flaps, resulting in high DNA removal after perfusion of 1% SDS for 24 or 72 h, followed by Triton X-100 treatment of 1% [26]. Porosity measurement using SEM images is not an accurate method for the porosity analysis of scaffolds.

The ethanol displacement technique was also used by Nokoorani Y.D. et al. in 2021 [8]. Porosity greatly affects the biological and mechanical characteristics of a scaffold. It plays a vital role in cell migration and proliferation and can influence the exchange of gases and nutrients, especially when the vascular system is not functioning sufficiently. Porosity of chitosan and gelatin scaffolds obtained by Nokoorani Y.D. et al. in 2021 was between 27 and 32% using the ethanol displacement technique, which is comparable to the obtained results of 35% ± 0.003 for human tissue and 52% ± 0.031 for porcine tissue. Swellability is an essential property of scaffolds and is also a factor that determines the usability of biomaterials *in vivo* [27]. Traditional dressings are used to keep the wound dry and to defend the body against microorganisms. Therefore, an ideal skin scaffold should absorb wound exudates and keep the wound moist [28, 29]. In the study by Nokoorani Y.D. et al. in 2021 the average swelling rate being 292–377% in the first 15 minutes of the experiment is comparable to the obtained results 320.4±0.052 for human dermis and 650.4±0.65 for porcine matrix [8].

Conclusions

The decellularization process significantly ($P<0.05$) removed the cellular components while preserving the connective three-dimensional structure of the dermal matrices clearly shown by quantification of the amount of deoxyribonucleic acids and microscopic examination of the decellularized dermal structures. Decellularization of the dermis led to the change in density, volume and swelling rate significantly ($P<0.05$). Increasing the porosity of the decellularized dermal structures resulted in a non-significant ($P>0.05$) decrease in the swelling rate of the sponge. Decellularized dermal tissue shows value for regenerative medicine by being readily available without the use of complex chemical syntheses and complicated manufacturing processes.

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Authors' contributions
OM proposed the concept and design of research, selected the literature and contributed to the elaboration and writing of the manuscript. AC and TB performed microscopic images and helped draft the manuscript. VN conceptualized the idea, designed the research and monitored the experiment. All the authors approved the final version of the manuscript.

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Ethics approval and consent to participate
The project was approved by the Research Ethics Committee of *Nicolae Testemitanu* State University of Medicine and Pharmacy (Protocol No 31, 14.12.2016).

Conflict of interests
No competing interests were disclosed.