Medical Engineering and Physics 38 (2016) 862–869

Contents lists available at ScienceDirect

Medical Engineering and Physics

journal homepage: www.elsevier.com/locate/medengphy

# Dermis mechanical behaviour after different cell removal treatments

Mara Terzini[a], Cristina Bignardi[a], Carlotta Castagnoli[b], Irene Cambieri[b], Elisabetta M. Zanetti[c,*], Alberto L. Audenino[a]

[a] DIMEAS, Politecnico di Torino, Cso Duca degli Abruzzi 24, 10129 Torino, Italy
[b] Skin Bank, AOU Città della Salute e della Scienza, Via Zuretti 29, 10126 Torino, Italy
[c] DI, University of Perugia, Via Duranti 67, 06125 Perugia, Italy

ARTICLE INFO

*Article history:*
Received 14 August 2015
Revised 4 February 2016
Accepted 14 February 2016

*Keywords:*
Decellularization treatment
Human dermis
Static mechanical tests
Ultimate stress
Ultimate strain
Young's modulus

ABSTRACT

Human acellular dermal matrices (HADMs) are used in reconstructive surgery as scaffolds promoting autologous tissue regeneration. Critical to the HADM ability to remodel and integrate into the host tissue is the removal of cells while maintaining an intact extracellular architecture.

The objective of this work is to develop a methodology to analyse the mechanical properties of HADMs after decellularization to identify its ideal form of treatment and its duration.

Two different decellularization techniques were used as a benchmark: the first is a well-established technique (incubation in NaOH for 1–7 weeks), and the second is an innovative technique developed by this research group (incubation in DMEM (Dulbecco's modified Eagle medium) for 1–7 weeks). After decellularization, the specimens underwent uniaxial tensile tests, and experimental data were represented with stress strain curves, calculating both engineering and true values.

Mechanical tests have led to the identification of the optimal method (NaOH or DMEM) and duration for the decellularization treatment; differences between engineering and true values can reach 84%, but the engineering values remain useful to make comparisons, providing reliable indications with a simpler experimental set up and data processing.

© 2016 IPEM. Published by Elsevier Ltd. All rights reserved.

## 1. Introduction

Engineered skin substitutes have a significant medical practice for patients with extensive burn wounds [1]. Advances in tissue engineering suggest that skin substitutes will be indistinguishable from the normal skin in the near future [2]. However, current skin substitutes do not restore the full native skin physiology because they lack some components such as hair follicles, sebaceous glands and sweat glands [2]. Additionally, the engineered tissue cannot faithfully replicate the mechanical properties of the native skin [1].

Currently, alloplastic material and skin allografts, taken from multi-organ donors, are the most suitable integumentary replacement for reconstructive surgery [3]. The immune response to allograft skin is directed primarily against epidermal, endothelial and fibroblast cells in the dermis, while the non-cellular component of the dermis (extracellular matrix) has been demonstrated to be relatively non-immunogenic [4]. Glycerolised acellular alloplastic human dermis (HADM) is used as a matrix for various reconstructive plastic purposes, where it retains almost all of the healthy dermal properties: it is compact and elastic, can be taken into the bed wound, and it retains the intact tissue morphology [5].

Different treatments can be used for tissue decellularization [6]. Commonly, a low concentration of NaOH has been used for this aim. The result of this technique is a reliably decellularized matrix. However, surgeons report that this matrix is inferior with reference to handling, ease of use, elasticity and needle penetration resistance. Additionally, decellularization using sodium hydroxide implies the direct contact of the tissue with an aggressive chemical agent, which must necessarily be neutralized by means of incubation in 0.1 N HCl at the end of the decellularization phase. These are the reasons why, in recent times, our research unit has developed an alternative procedure that aims to overcome these limitations. The new methodology consists of keeping the tissue in DMEM (Dulbecco's modified Eagle medium) for a long period of time (several weeks) while being subjected to mechanical action (tilting). From a biological point of view, the efficiency of the different treatments can be verified by means of an immunohistochemistry analysis, but the preservation of the main mechanical properties of the native dermis also needs to be checked [7]. The aim

* Corresponding author. Tel.: +39 075 585 3704.
*E-mail addresses:* mara.terzini@polito.it (M. Terzini), cristina.bignardi@polito.it (C. Bignardi), ccastagnoli@cittadellasalute.to.it (C. Castagnoli), icambieri@cittadellasalute.to.it (I. Cambieri), elisabetta.zanetti@unipg.it (E.M. Zanetti), alberto.audenino@polito.it (A.L. Audenino).

http://dx.doi.org/10.1016/j.medengphy.2016.02.012
1350-4533/© 2016 IPEM. Published by Elsevier Ltd. All rights reserved.

of this work is to evaluate the mechanical properties of tissue subjected to decellularization treatments varying by type and length to establish the best compromise between a reliably complete decellularization and adequate mechanical properties. The mechanical properties here analysed are the loading curve slope and the ultimate load and strain [8], considering that repaired full-thickness burn wounds may be subject to loss due to dermal substitute deficiencies in tensile strength and elasticity [1] and the requirements of soft-tissue augmentation procedures like rotator cuff [9].

The skin is made of three layers, the epidermis, dermis, and hypodermis. It consists of collagen (approximately 75% of the dry weight) and elastin (4% of the dry weight) fibres embedded in a gel-like ground substance consisting of water, small solutes, and macromolecules, predominantly proteoglycans [10]. The dermis provides a major contribution to the overall mechanical characteristics of the skin due to its main constituents, collagen and elastin fibrils, which allow high levels of deformation and flexibility as the fibrils stretch and re-orientate [11]. Collagen fibres are crimped and almost inactive at low strains, while they play a major role at high deformations (where they are stiffer than elastin by approximately three orders of magnitude [8]).

The skin is anisotropic due to the variable orientation of collagen fibres, with prevalence along the orientation of the so-called Langer's lines [8]. The dermis can therefore be described as an anisotropic, viscous, nonlinear [12] and non-homogenous material.

The tensile test is the most widely used mechanical test performed on *ex vivo* skin specimens. Using this method, the anisotropic, non-linear and viscous behaviours of skin have been explored, as well as its failure properties [13], creep [14], fatigue [15] and preconditioning behaviour [16]. This test is here being used to assess changes in the biomechanical behaviour produced by alterations of the skin's structure, similarly to the approach followed by those authors who studied variations in the collagen content [14] or elastin and proteoglycans contents [10].

Due to section narrowing taking place during the specimen loading, different formulations of stress in mechanical tests can produce different results: these are the so called 'nominal' or 'engineering values'; their respective 'true' values or 'true' values obtained from engineering values under specific assumptions such as volume constancy [17,18]. Experimentally measured true values provide the most faithful representation of the material properties, but their estimation requires a complex and demanding experimental set up; therefore, this work is also an attempt to quantify differences among all these expressions and their limits, establishing if they can or cannot be used for tissue characterization and/or to make comparisons among decellularization treatments.

## 2. Materials and methods

### 2.1. Specimens

Strips of skin tissue, collected from the backs of human donors, were dissected along the cranio-caudal direction. They were decellularized using two different methods based on incubation in 0.06 N NaOH or DMEM for 1–7 weeks. Immunohistochemistry has been performed for all treatments to verify the decellularization, according to the following procedure. Biopsy samples were washed in physiological solution, fixed in 4% neutral-buffered formalin and embedded in formalin by routine processing (FFPE). FFPE samples were sectioned at a thickness of 2–3 $\mu$m for immunohistochemistry reactions, and immunohistochemistry was performed using an automated slide-processing platform (Ventana BenchMarckXT Autostainer, Ventana Medical Systems, Tucson, AZ, USA)." HADMs, preserved at 85% glycerol in a 4 °C refrigerator at the Turin Skin Bank (Italy) and unfit for transplantation, were used for these experiments after the approval of the Institutional Ethical Board of *Azienda Ospedaliera Universitaria Città della Salute e della Scienza* of Turin, Italy, (approved on January 23rd, 2012 with protocol number 0006730), and written informed consent was obtained from all study participants. Before use, the dermis grafts were washed to remove all of the glycerol, dipping them sequentially in three different beakers filled with abundant saline solution 0.9% at +37 °C for more than three minutes each, as prescribed by the Euro Skin Bank [19]. The specimens were obtained by cutting out approximately 2 × 4 mm strips along the cranio-caudal (CC) and medio-lateral (ML) directions using a custom made die cutter; this cutting method avoids generating notches and defects that could bias tests. The resulting specimen sizes were measured by means of photogrammetry before mechanical testing: $4.33 \pm 0.57$-mm width, $2.21 \pm 0.32$-mm thickness, $10.10 \pm 0.38$-mm length (average ± std).

On the whole, there were 3–4 specimens (depending on the original strip size and shape) for each combination of decellularization method (NaOH or DMEM), duration (called 'Tx' in the following, where *x* represents the number of weeks of incubation) and cut orientation (CC or ML), for a total 96 specimens. Intact human skin was used as a control (called 'T0' in the following, as it did not undergo any decellularization treatment).

### 2.2. Photogrammetry set-up

Two different photographic set-ups have been developed to measure the specimens. The first was finalized to measure the specimens' size at rest and was made of a full-frame digital camera (Canon EOS 5D Mark II) with an autofocus lens for macro photography (Canon EF 100 mm *f*/2.8 Macro USM), a camera stand with two light stands, and a tripod. A second set-up was developed to follow tensile tests; it included the previously described digital camera as well as a second digital single-lens reflex camera (Canon EOS 400D). When the two cameras were triggered, they acquired the frontal and lateral views of the specimen through a remote capture software (DSLR Remote Pro). The width and the thickness of the specimens were measured using the image analysis software ImageJ (NIH, USA) (as an average of five different measurements), reaching a 0.01 mm/pixel measurement resolution given a 21.0 MP image (5616 × 3744 pixels).

### 2.3. Mechanical tests

Samples were subjected to uniaxial tensile tests along both the cranio-caudal and medio-lateral directions to quantify the influence of the chemical treatment on the skin tissue's biomechanical behaviour. Testing parameters have been set according to the physiological loads, the expected tissue behaviour, and the Bose Electroforce features. For example, the strain rate could reach very high values in reality due to impact forces, but the characteristics of the material are strain rate dependent [20], and the test speed had to be limited to 3.2%/s so as not to exceed the load cell range and risking rupture. The specimen length also had to be chosen considering the physiologic peak strain (over 100%) and the machine stroke (±6 mm), together with the limited sample extension; these considerations led to the selection of a 10 mm specimen length. The specimens were clamped by titanium machine grips that were specifically developed for biomaterials and have knurled-flat faces to prevent slipping. The analysis of the video recordings demonstrates that there were neither anomalous behaviours nor failures near the clamps. Sliding through the testing grips was excluded, too, as no abrupt increase or decrease was detected in the experimental curves. No marks were observed on the specimen ends, and the extension of the grasped ends was found to be unchanged.

Up to the instant preceding the tensile test, all specimens were kept hydrated in physiological solution; no additional hydration

Download English Version:

https://daneshyari.com/en/article/875587

Download Persian Version:

https://daneshyari.com/article/875587

Daneshyari.com