JOURNAL OF THE MECHANICAL BEHAVIOR OF BIOMEDICAL MATERIALS 37 (2014) 251–263

Available online at www.sciencedirect.com

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Research Paper

Rheological behaviour of reconstructed skin

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ARTICLE INFO

Article history:
Received 7 February 2014
Received in revised form
16 May 2014
Accepted 27 May 2014
Available online 5 June 2014

Keywords:
Skin fibres
Relaxation tests
Rheological model
Reconstructed tissue
Viscoelasticity

ABSTRACT

Reconstructed skins have been developed to replace skin when the integrity of tissue has been compromised following severe injury, and to provide alternative methods validating the innocuousness and effectiveness of dermatological and cosmetic products. However the functional properties of tissue substitutes have not been well characterised, mainly since mechanical measurement devices have not been designed to test cell culture materials in vitro. From the mechanical standpoint, reconstructed skin is a heterogeneous multi-layer viscoelastic material. To characterise the time-dependent behaviour of reconstructed skin, spherical indentation load-relaxation tests were performed with a specific original device adapted to measure small soft tissue samples. Load-relaxation indentation tests were performed on a standard reconstructed skin model and on sub-components of the reconstructed skin (3D-scaffold alone and dermal equivalent). Generalised Maxwell and Kelvin–Voigt rheological models are proposed for analysing the mechanical behaviour of each biological tissue. The results indicated a modification of the rheological behaviour of the samples tested as a function of their biological structure. The 3D-scaffold was modelled using the one-branch Maxwell model, while the dermis equivalent and the reconstructed skin were modeled using a one-branch and a two-branch Kelvin–Voigt model, respectively. Finally, we demonstrated that skin cells contribute to global mechanical behaviour through an increase of the instantaneous relaxation function, while the 3D-scaffold alone influences the mechanical response of long relaxation times.

1. Introduction

The histological and the biochemical methods employed to characterise reconstructed tissue models are particularly well-controlled. The main limitation of existing models concerns the determination of their mechanical properties (Monteiro-Riviere et al., 1997; Ponec et al., 2002; Netzlaff et al., 2005). In vitro skin models are based on a three-dimensional (3D) scaffold colonised by dermal cells (fibroblasts) covered by epidermal cells (keratinocytes) to form the 2 outer layers of

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http://dx.doi.org/10.1016/j.jmbbm.2014.05.030
1751-6161/

the skin, the dermis and the epidermis. The hypodermis layer is generally not represented in artificial skins. The 3D scaffold consists of natural or synthetic polymers, or a mixture of both types, assembled to mimic the fibrillar structures of the dermis that support and anchor dermal cells (Drury and Mooney, 2003; Mano et al., 2007; Cheung et al., 2007). Hence the 3D scaffold is used as a template to guide cell growth and tissue development. Several parameters can be modulated to influence the formation of the tissue such as the nature of the polymer, its chemical composition, porosity and pore interconnectivity, 3D organisation and mechanical properties (Shahabeddin et al., 1990; Peppas et al., 2006; Harley et al., 2007; Kanungo and Gibson, 2010).

Although the skin structure is highly complex, the mechanical behaviour of whole skin is mainly linked to the dermis structure (Echinard, 1998). The dermis contains a fibrillar network mainly composed of collagen and elastic fibres that contribute to the tissue's resistance and elasticity, respectively (Silver et al., 2001, 2003). In vivo, the dermis also comprises skin appendages, nerves, and blood and lymph vessels. Under physiological conditions, the dermal fibrillar network is stabilised and consolidated by the enzymatic crosslinking of free lysine residues contained in collagen and elastic fibres. All these fibres are immersed in a liquid medium composed of proteoglycans, ions and water. The surrounding liquid allows the displacement of the proteins that make up the fibres and the fibres themselves, to slide against each other and thus confer to the tissue part of its viscosity.

The upper layer of the skin, the epidermis, has a stratified and cohesive structure resulting from the differentiation of the keratinocytes from the basal to the horny layer. In vivo, for indentation tests, the epidermis layer has less influence on the mechanical properties of whole skin than that of the dermis layer. In contrast, engineered in vitro models have been used to demonstrate that the epidermal layer contributes to the mechanical property of whole skin when tensile tests are performed (Ebersole et al., 2010).

Soft tissues like skin present anisotropic and viscoelastic mechanical properties (linear or non-linear depending on the level of deformation). Many techniques, such as stress relaxation tests (Jachowicz et al., 2007), static indentation (Pailler-Mattei et al., 2013), dynamic indentation (Boyer et al., 2009) and oscillating shear tests (Gerhardt et al., 2012; Lamers et al., 2013) have been developed to characterise the viscoelastic behaviour of skin. When the experimental conditions exceed the small strain regime, the quasi-linear viscoelasticity (QLV) method is applied to characterise the viscoelastic behaviour of soft tissues, and corresponds to an adaptation of linear viscoelasticity appropriate for non-linear materials (Fung, 1993). On the basis of classical tensile tests, biaxial traction, applied multi-axial loading and rich set of deformations tests, soft biological tissues have been reported to present anisotropic behaviour (Flynn et al., 2011; Groves et al., 2013; Jor et al., 2011; Limbert, 2011). Generally, the experimental conditions required for these kind of tests are very severe and the mechanical behaviour of skin is non-linear (Flynn et al., 2013; Ni Annaidh et al., 2012). The aim of this paper is to characterise the viscoelastic mechanical properties of reconstructed skin using an indentation load-relaxation test. Indentation load-relaxation tests are often encountered in the literature for characterising the time-dependent behaviour of biological materials (Ledoux and Blevins, 2007; Hidaka et al., 2011) and cutaneous tissues (Jachowicz et al., 2007). In this study, the generalised Maxwell and Kelvin–Voigt rheological models are first proposed to model the time-dependent behaviour of reconstructed skin. Furthermore, the analytical solutions of the two previous models used to obtain the time-dependent load for a spherical indentation test are also suggested. Relaxation tests are then performed on each structure composing the reconstructed skin: 3D-scaffold, 3D-scaffold+dermis (dermal equivalent), 3D-scaffold+dermis+epidermis (reconstructed skin). The experimental relaxation curves are analysed using the generalised Maxwell and Kelvin–Voigt models. The results permit understanding and modelling the effect of each biological structure on the global mechanical response of the reconstructed skin. Finally, whenever possible, the viscoelastic parameters of each structure composing the reconstructed skin are compared with the values obtained in the literature.

2. Experimental details

This study was performed with reconstructed skin samples licensed by BASF® Beauty Care Solutions France. All the human tissues were obtained after informed consent was received. The principle underlying the preparation of reconstructed skin is summarised in Fig. 1a.

2.1. *Isolation of human dermal fibroblasts and keratinocytes*

Normal dermal fibroblasts and keratinocytes were isolated from human adult skin biopsies. The fibroblasts were amplified in medium consisting of Dulbecco's modified Eagle medium (DMEM) (Invitrogen, Carlsbad, CA, USA) supplemented with 10% foetal bovine serum (FBS) (HyClone, Logan, UT, USA), 25 mg/l gentamycin (Sigma-Aldrich, Saint-Quentin Fallavier, France), 100,000 UI/l penicillin (Sigma) and 1 mg/l amphotericin B (Sigma-Aldrich). The keratinocytes were amplified in K-FSM (Invitrogen) medium with 25 mg/l gentamycin, 100,000 UI/l penicillin and 1 mg/l amphotericin B. Cells were used at early passages (below 5) to construct the skin equivalent.

2.2. *Scaffold preparation*

The 3D-scaffold used in this study is a biomaterial with a base of collagen, chitosan and glycosaminoglycan. Eighteen 3D-scaffold substrates were prepared. Briefly, 1.5% bovine collagens (95% type I, 5% type III) were solubilised in an acetic acid solution at a concentration of 0.025 M. Chitosan from crab-deacetylated chitin was dissolved at 0.7% in an acetic acid solution at a concentration of 0.084 M. Chondroitin-4-sulfate from bovine cartilage was prepared at 2.5% in deionised water. These compounds were mixed to obtain the following final composition in dry matter: 72% collagen, 20% chitosan and 8% chrondroitin-4-sulfate. The final preparation was poured into Snapwell inserts (Corning Life Sciences, New