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#### **Technical Note**

# A novel method for visualising and quantifying through-plane skin layer deformations

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#### ABSTRACT

Skin is a multilayer composite and exhibits highly non-linear, viscoelastic, anisotropic material properties. In many consumer product and medical applications (e.g. during shaving, needle insertion, patient re-positioning), large tissue displacements and deformations are involved; consequently large local strains in the skin tissue can occur. Here, we present a novel imaging-based method to study skin deformations and the mechanics of interacting skin layers of full-thickness skin. Shear experiments and real-time video recording were combined with digital image correlation and strain field analysis to visualise and quantify skin layer deformations during dynamic mechanical testing. A global shear strain of 10% was applied to airbrush-patterned porcine skin (thickness: 1.2-1.6 mm) using a rotational rheometer. The recordings were analysed with ARAMIS image correlation software, and local skin displacement, strain and stiffness profiles through the skin layers determined. The results of this pilot study revealed inhomogeneous skin deformation, characterised by a gradual transition from a low (2.0-5.0%; epidermis) to high (10-22%; dermis) shear strain regime. Shear moduli ranged from 20 to 130 kPa. The herein presented method will be used for more extended studies on viable human skin, and is considered a valuable foundation for further development of constitutive models which can be used in advanced finite element analyses of skin.

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#### 1. Introduction

Knowledge on the mechanical properties and deformation behaviour of skin is a prerequisite to optimise surfaces and materials which come in contact with skin, and essential for the understanding of fundamental skin-friction mechanisms (Derler and Gerhardt, 2012).

Skin is a multi-layered composite material composed of an upper avascular cellular layer (epidermis), intimately connected to the collagen and ground substance-rich dermis, and the underlying subcutaneous fat tissue. From a mechanical point of view, skin is characterised by (non-)linear viscoelastic, anisotropic material properties similar to those of soft elastomers (Kang and Wu, 2011; Meyers et al., 2008;

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Ní Annaidh et al., 2012; Oomens et al., 1987). The mechanical properties of skin depend on many factors such as age, anatomical region, strain (rate), and skin hydration level (Diridollou et al., 2001; Gefen, 2011; Gerhardt et al., 2009; Hendriks et al., 2004; Sanders, 1971; Sopher and Gefen, 2011; Wildnauer et al., 1971). Depending on the experimental length scale and contact conditions, contributions of the varying mechanical properties of the individual skin layers (Geerligs et al., 2011a; Hendriks et al., 2006; Holt et al., 2008; Wildnauer et al., 1971) need to be taken into account in the global mechanical response.

In a series of studies on the mechanical properties of human skin, Geerligs et al. (2011a, 2011b, 2010, 2008) investigated the small strain behaviour of the stratum corneum (SC), viable epidermis, dermis, and subcutaneous fat tissue, using various in vitro setups. The studies demonstrated linear viscoelasticity for shear strains <0.5%, and similar stiffness of the SC and viable epidermis; results from indentation and shear experiments revealed highly anisotropic material behaviour with shear moduli being about 100 times lower than compression moduli (see Section 3.2).

In several investigations (e.g. Groves et al., 2012; Hendriks et al., 2006; Hendriks et al., 2004), attempts were made to describe skin layer deformations based on the analysis of external skin-surface deformation measurements. However, until now, there has been no reported experimental method allowing one to directly assess through-skin thickness deformations, and to study interactions of the individual skin layers and their contributions to the global mechanical response of skin (e.g. flattening, stretching or folding/bulging of the skin and epidermal-dermal junction; Sopher and Gefen, 2011, delamination of stratum corneum from viable epidermis; Wu et al., 2006). In order to gain insights in the deformation behaviour of skin and the mechanics of interacting skin layers of full-thickness skin, we developed an experimental-analytical method, as presented in this article.

Here, we describe experiments with a novel imaging-guided method to visualise and quantify skin layer deformations of full-thickness skin. Oscillating shear experiments and real-time video recording were combined with digital image correlation (DIC) and strain field analysis to determine local displacement, strain and stiffness profiles throughout the skin layers of fullthickness skin. Whereas in recent studies, DIC was used to determine skin surface displacements and skin surface strains (Evans and Holt, 2009; Groves et al., 2012; Guan et al., 2004; Hollenstein et al., 2011; Krehbiel et al., 2010; Marcellier et al., 2001; Staloff et al., 2008; Staloff and Rafailovitch, 2008; Tanaka et al., 2008), this paper presents a novel methodology for the assessment of individual skin layer deformations and strain distributions in deeper tissue layers during cyclic shear loading.

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#### 2. Materials and methods

#### 2.1. Preparation and preservation of skin samples

Porcine abdominal skin flaps were obtained from a local abattoir (Ballering, Son, The Netherlands). All pigs were

Landrace Hybrids, having a dressed carcass weight of approximately 83 kg, and were 4–6 months old. The skin samples were prepared according to previously developed methods in detail reported elsewhere (Geerligs et al., 2008, 2011a). Briefly, skin was immediately processed within 4–6 hours after slaughtering. An electric dermatome (D42, Humeca, Enschede, The Netherlands) was used to prepare full-thickness skin slices (thickness: 1.2–1.6 mm) from which square samples ( $8 \times 8$  mm) were punched. The skin samples had an aspect ratio (thickness/width) of 1:6–1:5, which is close to the ratio of 1:4 recommended to reduce/minimise effects of strain concentrations at boundaries (Abraham et al., 2011; Horgan and Murphy, 2011).

Pilot tests showed that the surface of the through thickness plane of the as-received skin did not present a unique, nonrepetitive high contrast pattern that is required for image correlation (Lecompte et al., 2006). To create a random grey value spray pattern with high contrast on the surface, the sample cross-sections were sprayed with black paint (Molotow<sup>TM</sup> One4All signal black, Molotow, Lahr, Germany) using an airbrush system (Micron-CM-SB, Iwata-Medea, Portland, USA). Sample spraying with paint has been reported to cause no significant changes in the mechanical properties of soft tissue (brain tissue) (Libertiaux et al., 2011). The airbrushed tissue samples were then preserved in HEPES-buffered Hanks Balanced Salt Solution (H-HBSS) supplemented with 2% penicillin/streptomycin and incubated at 37 °C and 5% CO<sub>2</sub>. Under these storing conditions, it has been demonstrated that the viability and integrity of the skin tissue can be maintained for 3 days (Geerligs et al., 2011a). In the pilot experiments presented here, three different skin samples were investigated.

#### 2.2. Experimental setup

A microscope-video camera based test setup was constructed to visualise skin deformations during rheometer measurements (Fig. 1). The setup consists of a stereo-microscope (Olympus SZ11, Zoeterwoude, The Netherlands) equipped with a monochrome CCD-camera (DMK 21AU04, Imagingsource, Bremen, Germany), and a flexible light source for sample illumination. A vertical positioner (lab jack model 271, Newport, Utrecht, The Netherlands) and micrometre actuators allow camera adjustments and fine tuning in the inplane and vertical direction (Fig. 1a and b).

A high precision linear stage that was fixed to the motor of the rheometer by means of a steel flange, enabling accurate positioning of the sample relative to the in-plane end face of the rheometer tool (Fig. 1a–d).

#### 2.3. Mechanical testing

All measurements were performed using an ARES LS-LC rotational rheometer (Scientific Instruments, USA), in combination with a Peltier environmental control unit and a fluid bath. Each sample was tested in an eccentric loading configuration using parallel plate geometry (Fig. 1c), as reported in detail by van Turnhout et al. (2005). With the eccentric configuration, skin deformation can be assumed to be much more homogeneous over the tested skin sample compared

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