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

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\textbf{A B S T R A C T}

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;
Ni 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 full-thickness 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.

Supplementary material related to this article can be found online at http://dx.doi.org/10.1016/j.jmbbm.2012.05.014.

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 × 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, non-repetitive 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™ 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% CO2. 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 in-plane 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
with a conventional, centred rheometer configuration, thus also enabling the study of the large strain response of soft tissues. In the rheometer experiments, the motor rotations (Fig. 1c) applied a harmonic shear strain to the skin samples, and the resulting torque was measured.

Prior to the experiments, excess and free H-HBSS was gently removed from the samples using medical gauze. The skin sample was glued to the edge of the linear stage and aligned with the end face of the spindle/tool. Then, a thin glue layer (Pattex Ultra gel, Henkel, Düsseldorf, Germany) was brushed onto the stratum corneum. The tool was lowered, and compressive normal loads of 0.032–0.24 N, equivalent to apparent contact pressures of 0.5–3.8 kPa (nominal contact area: 0.64 cm²), were applied to the skin samples. Subsequently, the loaded skin sample was subjected to an oscillating global engineering strain of 10% (angular frequency: 1 Hz, i.e., strain rate 10%/s). The time between sample mounting and testing was 1–3 min. The samples were mounted in a random orientation with respect to the Langer’s lines.

During the rheometer measurements, the through thickness shear deformation of the airbrushed patterned full-thickness skin layers was imaged (Fig. 1a). Films with 8-bit grey value images (640 × 480 pixels) were acquired at a frame rate of 30 frames/s and further processed for DIC. The resolution of the imaged through-plane skin cross-sections were 5.5–6.7 μm/pixel, producing a total field of view between 2.6–3.2 mm in width and 3.5–4.3 mm in height. At each microscope magnification used (× 40–50), the pixel resolution was determined with a microscope calibration reticule. All the measurements were carried out at 37 °C and performed within 3 days after sample preparation.

2.4. Post-processing and data analysis

2.4.1. 2D in-plane digital image correlation

2D in-plane digital image correlation (Chu et al., 1985; Lecompte et al., 2006; Pan et al., 2009; Sutton et al., 1986) was performed to determine skin displacements and deformation in response to the applied shear strain, and used as a basis for local shear strain analysis (see Section 2.4.2).

These image sequences were processed using the ARAMIS digital image correlation software (ARAMIS v5.4.1-5, GOM, Braunschweig, Germany), in order to calculate point displacements of the random spray pattern of the skin sample (Chu et al., 1985; GOM, 2005; Sutton et al., 1986).

In DIC, the locations and signature of areas with a fixed number of pixels (subset or facet) of an image in a deformed configuration are tracked and matched to the locations of the same subset pixels of a reference image (see Fig. 2), using a given correlation function (Lecompte et al., 2006; Pan et al., 2009). A least error squares correlation algorithm correlated the averaged pixel intensities of each subset in the reference and deformed image (Fig. 2), and aligned the facets in the different images to each other (GOM, 2005).

Depending on the microscope magnification and the quality of the speckle pattern, facet sizes of 17–21 pixels (110–141 μm) and step sizes between 7–11 pixels were appropriate for
correlation over the chosen areas of interest, resulting in spatial grid resolutions between 45 and 74 μm. The step size defines the number of pixels over which the subset was shifted in horizontal and vertical direction to calculate the next grid point coordinate (Lecompte et al., 2006), and denotes the distance between two facet centre points in pixels. Following DIC, each successfully matched facet provided a single grid point (new pixel coordinate) from which displacements and strains were calculated, using the first image of the film as a reference.

Displacement precision was 0.08 ± 0.03 μm, the corresponding strain precision was 0.04 ± 0.03%, both being determined from null-deformation measurements of three skin samples, i.e., by comparing in each film two successive images when the skin sample did not undergo shear deformation. The means and standard deviations of repeated null-deformation measurements of different consecutive static images were 0.08 ± 0.02 μm and 0.04 ± 0.02% strain, respectively.

2.4.2. Local strain analysis
To obtain strain information from the experimentally obtained displacement field, a gradient deformation formulation with quadratic interpolation of grid points was used (more details in Geers et al., 1996). The last frame of the second skin deformation cycle (at full 10% skin strain) was analysed (Fig. 2b). Strain calculation was performed using a home-made Matlab code (version 2011b).

The analysis of a skin sample subjected to 2D simple shear deformation parallel to the x-axis direction (Figs. 1e and f, 2) was conducted neglecting dynamic and time-dependent (i.e., viscoelastic) effects, which are small at low frequencies (Geerligs et al., 2011a; Holt et al., 2008). According to the reference frame shown in Fig. 1e, the following shear deformation ϕ is applied to the sample:

\[
\phi(XY) = \begin{bmatrix} x(XY) \\ y(XY) \end{bmatrix} = \begin{bmatrix} X + s(Y) \\ Y \end{bmatrix}
\]

being \( s(Y = h) = 0 \), with \( h \) the thickness of the skin sample (see Fig. 3b). Note that shear displacement \( s(Y) \) should be linear for a homogeneous and isotropic material under through-thickness shear deformation (see Fig. 3c).

The deformation gradient tensor \( F \) can be then computed as follows:

\[
F : = \partial \phi / \partial X
\]

\[
[F] = \begin{bmatrix} 1 & s' \\ 0 & 1 \end{bmatrix} \quad s' = \partial x / \partial Y = \tan(\theta)
\]

with \( \theta \) being the shear angle (see Fig. 1f).

The deformation gradient tensor \( F \) was estimated in each data point from the 2D-displacement field (central particle) with a least squares fit through 8 neighbouring points of the displacement field (details on the procedure in Geers et al., 1996).

Using the right Cauchy-Green deformation tensor \( C = F^T \cdot F \), the Green-Lagrangian strain tensor \( E = 1/2(C - I) \) was then calculated, with \( I \) being the identity tensor \( \begin{bmatrix} 1 & 0 \\ 0 & 1 \end{bmatrix} \).

The matrix components of tensor \( E \) are given by

\[
[E] = \begin{bmatrix} E_{xx} & E_{xy} \\ E_{yx} & E_{yy} \end{bmatrix} = 1/2 \cdot \begin{bmatrix} 0 & s' \\ s' & s'^2 \end{bmatrix}
\]

In addition to the assumption of simple shear, as a first simplified approach, isotropic, incompressible and linear...
3. Results and discussion

3.1. Shear deformation behaviour of skin

Fig. 2 shows the shear deformation response of full-thickness porcine skin (see film in the supplementary information). From the film, it can be qualitatively seen that the upper skin layers deform less than deeper tissue structures.

A critical issue in the experimental test setup was the sample fixation to the linear stage and spindle in order to keep the skin in position during shear testing. Several options were studied: the use of different sandpapers, micro-textured steel surfaces and a non-penetrating/non-diffusing viscous adhesive glue (see Section 2.3). The latter provided the most efficient and reproducible fixation to avoid slip at the skin-to-steel interfaces (Fig. 1c and d).

Quantitative displacement and strain analyses confirmed the qualitative observations from the films. Fig. 3 shows the horizontal displacement and shear strain profiles through the skin layers of full-thickness porcine skin.

Following the application of an oscillating global shear strain (10%), inhomogeneous skin deformation (Fig. 3b) was found, characterised by different shear gradients (Fig. 3b) and gradual transition from a low $(2E_{xy}=3.0-5.0\%)$ to high $(2E_{xy}=5-15\%)$ shear strain regime (Fig. 3c). If the skin was ideally homogeneous, a constant shear deformation gradient (i.e., straight line/diagonal), and consequently a constant Green-Lagrangian shear strain of theoretically 5% would be the expected when the sample is strained/stretched to an engineering strain of 10% (indicated by red dotted lines in Fig. 3b and c). This is true if a simple shear stress state holds in the specimen. Therefore, if we neglect inelastic effects such as damage or geometrical non-linearity, the results shown in Fig. 3b–d suggest that non-linear through-thickness shear deformation is attributed to skin inhomogeneities and anisotropy.

Zero shear strain was found for all other regions that can be related to rigid body motions of the rigid steel parts of the measurement setup. However, here it should be mentioned that small shear strain was measured at the interface between spindle and upper skin layers (Fig. 3c). This strain is a direct consequence of averaging pixel intensities over subsets, and the chosen DIC parameters (subset size, step size).
Taken the length scale of the different strain regimes into consideration (Figs. 3–5), the low strain region (250–300 μm) can be ascribed to the epidermis and the region with larger strains to the dermis (Avon and Wood, 2005; Meyer and Zschemisch, 2002).

3.2. Local strain and stiffness profiles through skin

For the strain profiles illustrated in Fig. 3c, the corresponding shear moduli were qualitatively estimated according to Eqs. (5) and (6) and assumptions therein. The results showed that the epidermis (70–110 kPa) exhibits about 2–4 times higher stiffness than the dermis (20–50 kPa), when a global engineering strain of 10% was applied (Fig. 3d).

The observed two to four times higher stiffness (see also Fig. 4) of the epidermis is consistent with previous results obtained from shear experiments on separate/individual skin layers (epidermis: 10 kPa, dermis: 2.5–3 kPa), measured at engineering strains <0.5% (Geerligs et al., 2011a). It was hypothesised that loading in shear causes cell deformation in the epidermis without affecting the desmosomes. In the dermis, the shear response is assumed to be determined by the ground substance, because collagen and elastin fibres are mainly oriented transversally (reticular dermis) (Geerligs et al., 2011a). Thus, it is likely that this substance has a lower shear resistance than the highly organised epidermis.

The absolute shear moduli values (20–110 kPa) were found a factor of 10–20 higher than those reported in (Geerligs et al.,

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**Fig. 4** – Mean shear strain and corresponding shear moduli profiles through full-thickness porcine skin. For clarity, error bars for strains are not included in (a) as they were in the same order of magnitude (mean SD = 0.7 ± 0.2%) than the ones shown in Fig. 3c. The red dashed vertical line denotes the computed average strain across the entire skin thickness.

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**Fig. 5** – Influence of the field of view (FOV = width x height) on through thickness strain and stiffness profiles. Field widths of less than 1 mm demonstrated low variations in strain and stiffness. The variation coefficients of the three different regions of interests were similar to those of an individual FOV (see Section 3.3), with variation coefficients of 10 ± 8% (range: 1–28%) for Green-Lagrangian strains and 9 ± 7% (range: 1–24%) for shear moduli. For clarity and better illustration, error bars for strains (1.1 ± 0.1%) are not included.
2011a; Holt et al., 2008), which either indicates strain stiffening, or differences which can be attributed to different tissue species studied (animal skin vs. human skin). Here, we need to point out that the common slaughtering procedure includes treatment of the skin to remove the hair from the skin. The skin of the pigs was scalded (with 65 °C water) and singed, both causing heat damage to the tissue and the underlying collagen resulting in less skin flexibility and pliability due to the coagulated and denatured collagen fibres. Therefore, it cannot be completely ruled out that stiffness changes due to collagen denaturation have extended the low strain regime ($E_{xy} < 2.5\%$) towards the papillary dermis. Furthermore, the assumed simple shear stress state (i.e., linear elastic, non-viscoelastic material behaviour) may have affected the estimations of the shear stiffness across the thickness of the skin specimens. However, since our main goal was to demonstrate the performance of the new method, we do not consider both issues a significant drawback at this stage of the work.

Similar qualitative through-thickness skin strain and stiffness profiles were found for all samples tested (Fig. 4). With increasing tissue depth, the shear moduli decreased from 112 ± 29 kPa (epidermis: $E_{xy} = 1.4 ± 0.6\%$; tissue depth: 60 ± 14 μm) to 16 ± 5 kPa (dermis: $E_{xy} = 9.4 ± 1.7\%$; tissue depth: 1075 ± 60 μm). The considerable variations in the strain and stiffness profiles/values (Fig. 4; variation coefficients: 18–40%, mean: 28 ± 9%) can be attributed to differences in the morphology of the epidermal–dermal junction or collagen network, as well as to different epidermal hydration levels which were not monitored during the present pilot experiments.

Sample positioning in a random orientation with respect to Langer's lines, as well as differences in the tissue pre-compaction level, the latter possibly inducing stiffening effects in the tissue, may have also contributed to the variations.

The repeatability standard deviations of local strains and shear moduli were 2.1 ± 1.2% (median: 1.9%), and 25 ± 16 kPa (median: 27 kPa), respectively; both reflecting variations between the three samples, all being measured under the same experimental condition within a short period of time.

The strain and stiffness variations between different skin samples (Fig. 4) were larger than variations within a particular sample (Fig. 5). As shown in Fig. 5, averaging over different fields of view did not significantly change the overall shape of strain and stiffness profiles.

The largest local shear strains ($2E_{xy} = 19.0 ± 4.0\%$) reached almost twice the globally applied engineering strain of 10% (Figs. 3–5). For instance, in Fig. 3b, the maximum local engineering strain value $2E_{xy}$ was ≈ 15%. This difference is a direct consequence of the tissue heterogeneity, clearly illustrated by the heterogeneous distribution of the local strain field, as suggested by Jacquemoud et al. (2007), who reported that local Green-Lagrangian shear strain of the three different skin strain profiles shown in Fig. 4 was 4.4 ± 0.8%. This value is in good agreement with the theoretically expected $E_{xy}$ of 5%, when a global engineering strain of 10% is applied and also the overall measurement accuracy of 0.6% is taken into account.

3.4. Advantages of new method

We believe that the herein described method to visualise and quantify skin layer deformations has several advantages over conventional dynamic mechanical testing using a rheometer. First of all, the novel imaging based method allows to perform shear tests and to study layer dependent skin properties using full thickness skin; there is no need to separate skin layers which is a time consuming procedure (Geerligs et al., 2011a), and possibly disrupts skin layers. In addition, the visualisation of the shear experiment provides real-time optical feedback improving quality assurance and reliability of the results. For example, a direct control on the anticipated boundary conditions (no slip) is available from the imaging.

Moreover, our method can be used to directly measure large strains, i.e., skin mechanics in the non-linear viscoelastic strain regime ($> 0.5\%$; Geerligs et al., 2011a; Holt et al.,
2008), in which moduli are strain dependent and the analysis and interpretation of conventional rheometer measurements is complicated (Hyun et al., 2011). In this pilot study, a global engineering strain of 10% was defined a priori to investigate the mechanical response of full-thickness skin at “moderately large” strain conditions, in which skin layers cannot be studied separately without disregarding interactions between the tissue layers and losing information on the global mechanical skin behaviour.

Last but not least, our novel method can be upgraded to record time and strain dependent torque signals (harmonic mechanical skin behaviour. The herein presented method is currently being used as a foundation for more extended studies (e.g. strain, strain rate, implementation of constitutive models) on viable human skin and is considered a valuable tool to effectively determine previously unavailable information on the mechanical properties of the different skin layers.

3.5. Limitations and future work

The present results are based on a small number of measurements and should therefore be verified with more studies, including experiments on human skin. Future experiments should also be carried out under controlled laboratory conditions, including sample conditioning (skin hydration, temperature). In this study, we analysed the second skin deformation cycle only (when the skin was strained horizontally from the left to the right direction; see Fig. 2). In future, it would also be interesting to study possible directional/differential shear strain or skin deformation effects, as well as skin fatigue or ratchetting (i.e., progressively increasing strain with increasing number of strain cycles), as recently reported for skin under uniaxial cyclic loading (Kang and Wu, 2011).

Moreover, detailed histological investigations are essential to fully validate and verify future experiments. There is especially need to compare layer dependent strain regimes derived from DIC with the corresponding morphological tissue structures of epidermis and dermis to study whether skin layer length scales match with histological features.

The herein presented method is currently being used as a foundation for more extended studies (e.g. strain, strain rate, implementation of constitutive models) on viable human skin and is considered a valuable tool to effectively determine previously unavailable information on the mechanical properties of the different skin layers.

Future research will also strive to capture and implement the experimental data in nonlinear, finite strain models, taking into account material phenomena such as strain stiffening, collagen/elastin fibre orientation, viscoplasticity or anisotropic damage.

4. Conclusions

To our knowledge, this is the first study which reports on the combined visualisation and quantification of through-plane skin layer deformations. The results revealed two to four times higher stiffness of the epidermis compared with the dermis. Although of preliminary nature/character, our results showed gradual local stiffness transitions from the skin surface into deeper tissue structures. The through-skin deformation method presented here can be directly applied to the evaluation of constitutive models for shaver development, surgical simulation, or wound prevention, as well as for computational modelling, e.g. by implementing strain and stiffness profiles in multi-layered numerical models.

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Appendix A. Supplementary materials

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