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In vitro measurement of the mechanical properties of skin by nano/microindentation methods

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ABSTRACT

The elastic behaviors of stratum corneum, viable epidermis, dermis, and whole skin were investigated by nano/microindentation techniques. Insignificant differences in reduced elastic modulus of skin samples obtained from three different porcine breeds revealed breed type independent measurements. The reduced elastic modulus of stratum corneum is shown to be about three orders of magnitude higher than that of dermis. As a result, for relatively shallow and deep indentations, skin elasticity is controlled by that of stratum corneum and dermis, respectively. Skin deformation is interpreted in the context of a layered structure model consisting of a stiff and hard surface layer on a compliant and soft substrate, supported by microscopy observations and indentation measurements.

1. Introduction

Aside from teeth, cornea, hair, and nails, human organ surfaces consist of epithelial tissue. The outer epithelial layer (epidermis), commonly referred to as the skin, is the largest organ of the human body, providing vital protection to tissue and cells against external intruders, such as bacteria, virus, and fungi, and preventing the loss of water (McGrath and Uitto, 2010; Archer, 2010). Other important functional properties of skin include body temperature regulation, transmission of mechanical stresses, and absorption of light (radiation).

Skin mechanical properties have been traditionally measured with macroscopic instruments. For example, different suction tests have been used to study *in vivo* skin elasticity (Grahame, 1969) and its dependence on ageing (Grahame and Holt, 1969), the role of natural tension on the mechanical behavior of skin (Alexander and Cook, 1977), the dependence of skin elasticity on age, sex, and anatomical region (Cua et al., 1990), the effect of hydration (Auriol et al., 1993) and ageing (Leveque et al., 1980) on skin extensibility, and the influence of fluid volume changes in hemodialysis on the biophysical properties of skin (Brazzelli et al., 1994). An *in vivo* mechanical model of the human skin (Diridollou et al., 2000) and an analysis of the relative contributions of different skin layers to the overall mechanical behavior of human skin *in vivo* (Hendriks et al., 2006) have also been used to study the mechanical response of skin subjected to suction. In addition to elastic stretching, the *in vivo* torsional elastic behavior of human skin has been examined in earlier studies (Finlay, 1971; Sanders, 1973). Several *in vivo* investigations have focused on the effects of ageing, stress, sex, and moisturizing treatment on human skin torsional elasticity (Agache et al., 1980; Kalis et al., 1990; Salter et al., 1993). Most measurements of skin mechanical properties have relied on mechanical instruments (Warren et al., 1991; Gunner et al., 1979; Berardesca et al., 1986; Ohura et al., 1980; Sugihara et al., 1991; Peck and Glick, 1956; Dikstein et al., 1984; Falanga and Bucalo, 1993; Diridollou et al., 1998; Cooper et al., 1985).

Despite important insight into the mechanical properties of human skin provided by the aforementioned studies, very little is known about the mechanical behavior of individual skin layers. Obtaining such knowledge requires the use of microprobe-based methods, such as nano/microindentation, which can permit objectively probing the mechanical response of individual skin layers. Therefore, the objective of this study was to examine the mechanical behavior of individual skin layers using nano/microscale indentation techniques and identify the contribution of each skin layer to the overall mechanical behavior of skin. To accomplish this objective, indentation experiments were performed with porcine skin samples obtained from three different breeds to examine the effect of the breed type on the measurements. Porcine skin is appropriate for *in vitro* studies because its topology, texture, architecture, metabolic rate, and drug permeability are similar to those of human skin (Schmook et al., 2001). In addition, skin properties are not significantly affected by the lack of a physiological environment provided there is significant moisture (Agner and Serup, 1990). The morphology of porcine skin is similar to that of healthy human skin, even after 3 days from harvesting (Fig. 1).

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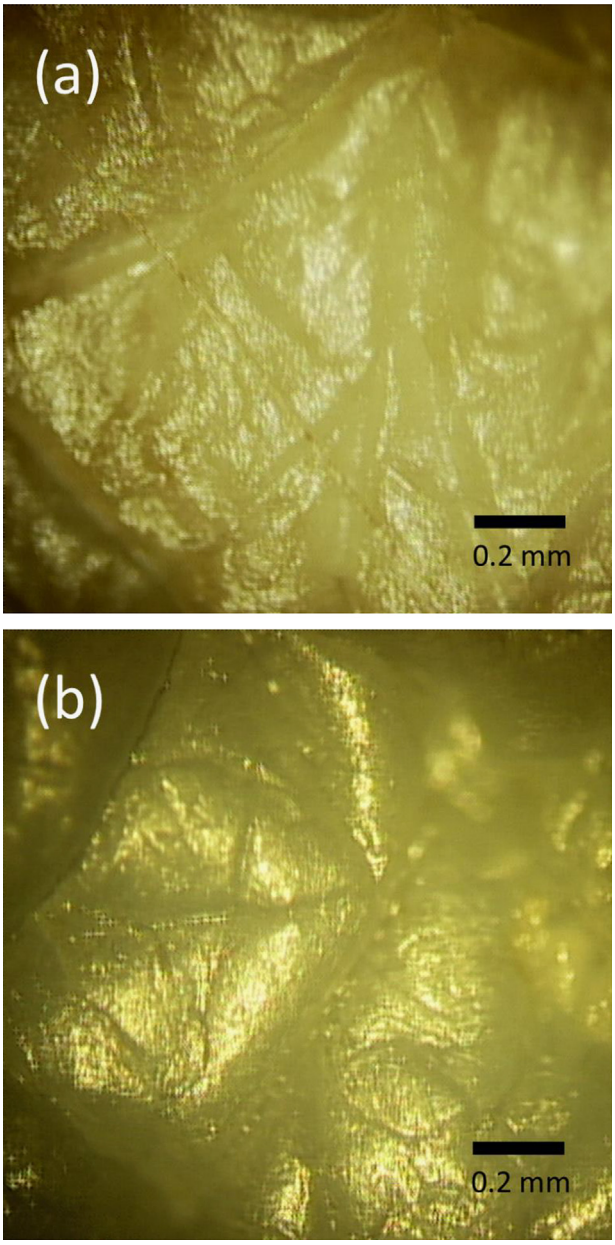

Fig. 1. Surface morphology of (a) human and (b) porcine (American Yorkshire) skin.

The effects of the mechanical properties of stratum corneum, viable epidermis, and dermis to the mechanical behavior of skin are discussed in the context of nano/microindentation, histology, and microscopy results.

2. Experimental methods

2.1. Sample preparation

Skin samples were harvested from the belly parts of 4–12 months old Berkshire and Duroc–Berkshire Cross porcine breeds from a local abattoir within 3 days from sacrifice. In addition, skin samples of American Yorkshire porcine breed of a similar age range were obtained from the School of Medicine, University of California, San Francisco. To maintain a physiologically similar pH, the skin samples were placed on Petri dishes covered with filter paper, which was previously soaked in 0.9% NaCl or phosphate buffer saline (PBS) solution. Testing was performed within 1–2 days from sample acquisition without any chemical treatment. Before testing, the hairs were carefully removed with surgical blades and the samples were sectioned to the sizes needed for testing. To minimize sample dehydration, testing was performed within ~30 min from sample preparation in a clean-air laboratory environment.

Stratum corneum samples were prepared by removing an outer layer of a few tens of micrometers (consisting of the stratum corneum and a portion of the viable epidermis) from skin samples using a surgical knife. The samples were then epoxy-attached to a steel disk with the viable epidermis facing down or kept on a Petri dish until testing. A chemical method of removing the stratum corneum was not used to prevent any unknown effects on the skin properties. Although the present method does not ensure the full removal of the viable epidermis, this is not a problem because the maximum contact depth in nano/microindentation testing is only a few micrometers. The dermis samples were prepared by removing the hypodermis and a portion of the dermis with a surgical knife and then attaching the stratum corneum surface to a Petri dish covered with filter paper that was soaked in 0.9% NaCl or PBS solution.

2.2. Histology

Skin samples were embedded in an optimal-cutting-temperature compound (TissueTek, Elkhart, IN) on dry ice and kept at a temperature of −62 °C until testing. Before performing histology, 10 μm-thick specimens were cut from the skin samples and stained with hematoxylin and eosin (H&E) following a standard protocol.

2.3. Nano/microindentation experiments

Nanoindentation tests were performed with a custom-made apparatus consisting of an atomic force microscope (AFM) scanner (Nanoscope II, Digital Instruments, Santa Barbara, CA), a three-plate capacitor force–displacement transducer (Triboscope, Hysitron, Minneapolis, MN), and a detector assembly (head) that uses the AFM scanner and the software of a scanning tunneling microscope. Because of the large thickness of the dermis and skin samples, microindentation experiments were performed with a microindentation apparatus (Bruker, Campbell, CA) that has a very large depth range. Details about experimental set-up, associated testing procedures, and mechanics analysis for determining the reduced elastic modulus E_r (Eq. (S5)) and hardness H (Eq. (S6)) as functions of maximum contact depth $h_{c,max}$ can be found in Supporting information.

2.4. Statistical analysis

Significant differences among porcine breeds or samples were determined by a one-way analysis of variance (ANOVA). The F-test was used to validate the assumptions made in ANOVA. Statistically different data were discerned by the corresponding p-value, calculated for a significance level $\alpha = 0.05$. The null hypothesis was used to examine if the mean values were statistically different. The null hypothesis was rejected for $p < 0.05$. Sample sizes were determined for 80% power. Curve fits in figures indicate general trends.

3. Results

The results presented in this section were obtained from three types of layer/substrate samples: (1) stratum corneum/viable epidermis, (2) dermis/epidermis, and (3) whole skin with the hypodermis as the substrate. Samples were attached to a Petri dish via their substrates. Hereafter, these samples will be referred to as the stratum corneum, dermis, and skin samples, correspondingly. Also, h_{max} is measured from the undeformed surface of each sample. To avoid the effect of surface roughness (root-mean-square (rms) $\approx$200–300 nm) on the measurements, data were acquired for depths $>$500 nm. For negligible substrate biasing of the measurements, $h_{c,max}$ must be $\leq$10% of the layer thickness (Bhattacharya and Nix, 1988). Thus, considering the thickness of the stratum corneum (~10 μm) and viable epidermis left after sectioning, $h_{c,\max} < 1500$ nm in all measurements.

3.1. Cross-sectional histology

Optical micrographs of the cross-sectional histology of porcine skin obtained before testing (Fig. 2) showed overall histological features similar to those of human skin (McGrath and Uitto, 2010). As shown in Fig. 2b, the thickness of the darker layer (stratum corneum) is ~10 μm, while that of the underlying tightly packed layer (viable epidermis) varies between 20 and 100 μm. Next is the dermis of several millimeters thickness, followed by the hypodermis.