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In vitro indentation to determine the mechanical properties of epidermis

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ABSTRACT

The lack of understanding of the mechanical behavior of the human skin layers makes the development of drug delivery using microneedles or microjets a challenging task. In particular, the key mechanical properties of the epidermis composed of *stratum corneum* and viable epidermis should be better understood.

Micro-indentation experiments were applied, using a spherical tip with a large diameter to the sample thickness ratio. The Young's moduli were derived via an analytical and a numerical method. The tests showed that the analytical method was not appropriate to assess the Young's moduli. That is why a numerical model was used to obtain the correct stiffness. When loaded perpendicularly, the stiffness of both the epidermis and *stratum corneum* vary between 1 and 2 MPa. No significant differences in stiffness between the *stratum corneum* and viable epidermis were observed.

1. Introduction

The outer skin layer possesses characteristics that make it a favorable site for pain-free drug delivery with minimal damage. Indeed, it presents a rich population of immunologically sensitive cells as well as the lack of blood vessels and sensory nerve endings. The development of drug delivery using microneedles or microjets is a challenging task because of deficient understanding of the mechanical behavior of the human skin layers. In particular, the key mechanical properties of the outer skin layer, i.e., the epidermis composed of *stratum corneum* and viable epidermis, should be better understood.

The structure and function of this layer are well-known (Elias and Feingold, 2005). The outer layer, the *stratum corneum*, is an effective physical barrier of dead cells in a "brick-and-mortar" structure: the anucleate corneocytes form "bricks" and the intercellular lipid membranes and corneosomes are considered to represent the "mortar". The viable epidermis mainly consists of keratinocytes migrating towards the *stratum corneum*, continuously changing in composition, shape and function. The junction with the underlying dermis is strengthened by its undulating pattern, such that large cones of epidermal tissue penetrate the dermis (see Fig. 1). Furthermore, epidermal properties are influenced by environmental factors such as temperature, humidity and UV radiation.

In order to deliver drugs transdermally, the microneedle or microjet should penetrate the *stratum corneum* to deliver the drug 100–150 μm below the skin surface, e.g. in the viable epidermis or papillar dermis. A full understanding of the delivery path requires also the understanding of this indentation phase and, therefore, the knowledge of the mechanical behavior of the epidermis.

Recently, Kendall et al. (2007) were the first to report on the mechanical properties of the (viable) epidermis during penetration, using modified standard tips on murine skin. They observed a decrease in storage modulus when the 2 μm probe penetrates through the *stratum corneum*, which is in accordance with studies on *stratum corneum* only (Plewig and Marples, 1970). The authors explained this by an increase in moisture content with depth. In the viable epidermis, the storage modulus remained nearly constant. By contrast, penetration of the 5 μm probe showed a negligible decrease in storage modulus throughout the *stratum corneum* and a gradual increase in the viable epidermis, although the values of the shear moduli were less than that for the corresponding 2 μm probe.

A variety of *in vivo* and *in vitro* indentation techniques were developed to measure the *stratum corneum*. In the eighties, Hendley et al. (1982) developed an indentation device to measure force variations *in vivo* due to age, sex and body site. A needle with a tip radius of 11 μm at the tip was held perpendicular to the surface and moved rapidly into the skin. They claimed that the speed of the indentation ensured that the test was predominantly confined to the properties of the *stratum corneum* (Graves and Edwards, 2002). Measured forces were typically in the order of 3.0 N. Recently, a limited number of nano-indentation studies have been performed on isolated *stratum corneum* (Pailler-Mattei et al., 2007; Pailler-Mattei and Zahouani, 2004; Plewig and Marples, 1970). The tips used varied between 1 and 10 μm, while corneocytes have a diameter ranging from 26 to 45 μm (Holbrook and Odland, 1974). As a consequence,

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very local properties were determined in these experiments. Furthermore, in some of the studies, the three-sided Berkovich tip, that has a sharp three-sided point, is used. This tip easily induces damage on the sample's surface, which interferes with the load-displacements results. Three of the nano-indentation studies were based on continuous stiffness measurements (CSM) protocols (Kendall et al., 2007; Pailler-Mattei et al., 2007). The drawback of CSM is that the results are influenced by the selected amplitude and frequency for viscoelastic materials. Combining the nano-indentation studies on *stratum corneum* reveals measured Young's moduli varying from 10 MPa (Yuan and Verma, 2006) for wet porcine samples up to 1 GPa for dried human samples (Pailler-Mattei et al., 2007). This broad range is likely caused by the differences in testing apparatus and protocols, differences between species and body sites, and the heterogeneity of the material. A reliable method to determine the mechanical properties of the *stratum corneum* on the tissue level only is therefore also required.

The aim of the present study is to present such an indentation method and to use it to determine the Young's modulus of the epidermis, i.e., the *stratum corneum* and viable epidermis. The typical complex geometry, a variable thickness between 30 and 150 μm, and the porosity of the epidermis places high demands on this mechanical characterization. Therefore, isolated epidermis and isolated *stratum corneum* were tested using equipment that is known for its accuracy and reliability. The device is originally designed for solid materials of which well-defined samples can be obtained and therefore the testing protocol needs to be adapted to epidermal samples. To validate that the testing protocol holds for thin materials with a low stiffness, tests have been performed with silicone rubber. Moreover, indentation experiments require a model for the interpretation of the measured results. Next to the analytical model used, which assumes a homogeneous linear elastic material behavior upon unloading, we also adopted a numerical model that allows for taking into account geometrical details and different material properties for different layers.

2. Methods

2.1. Sample preparation

Indentation tests have been carried out on *ex vivo* abdominal skin of Caucasian women from a similar age 43 ± 4 years old undergoing abdominoplasty surgery. All patients gave informed consent for use of their skin for research purposes under a protocol approved by the ethics committee of the Catharina Hospital, Eindhoven, The Netherlands. Abdominal skin with striae markers, cellulite, damage due to UV exposure or excessively hairy skin is excluded from the study.

Immediately after excision, the skin was brought into the laboratory and processed within 4 h. Epidermal sheets were obtained using a dermatome (D42, Humeca) in which the prescribed thickness was refined for this purpose by the supplier. The dermatomed slices of 100 μm thickness were cut in pieces of approximately 1 cm^2. Depending on various factors such as skin surface roughness, tissue hydration and the amount of cones and ridges (see Fig. 2), samples may consist of epidermis and/or some papillar dermis (see Fig. 1b and c).

To obtain *stratum corneum* samples, dermatomed skin slices of 200 μm were immersed in a solution of 0.1% trypsin (Hyclone, SV30037.01) in an incubator at 37 °C for 2–3 h. Thereafter, the sheets were rinsed in PBS and also cut into pieces of approximately 1 cm^2. All samples were stored at −80 °C until further use.

In order to validate the experimental procedure is valid for thin samples, a highly elastic silicone rubber (Köraform 42A, Alpina Siliconee, Germany) was measured using various sample thicknesses. The silicone rubber was poured under vacuum into various thicknesses: 0.05, 0.12 and 2.0 mm. Thereafter, samples of about 1 cm^2 were cut out.

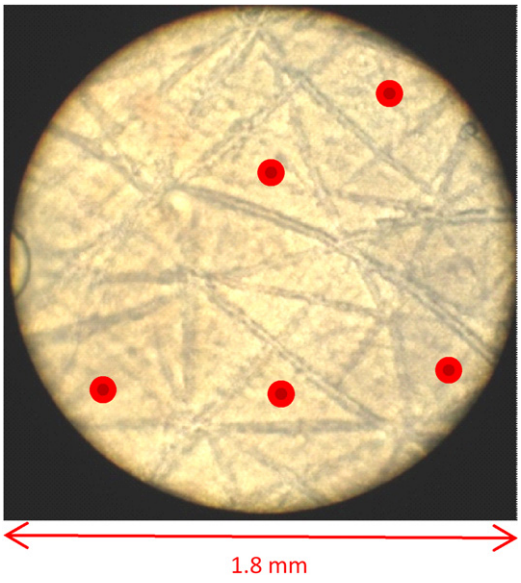

Fig. 2. The top center of the triangles, highlighted by the large red points, formed by the glyphics was chosen as indentation location on the skin samples. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

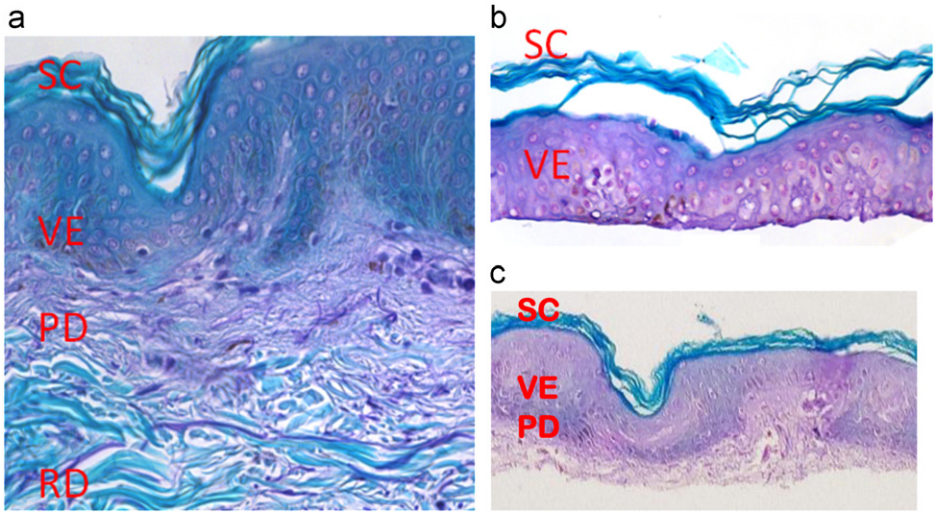

Fig. 1. An aldehyde–fuchsin staining is used to visualize the morphology of the various skin layers: (a) full-thickness skin including the *stratum corneum* (SC), viable epidermis (VE), papillar dermis (PD) and reticular dermis (RD), (b) dermatomed skin with a set thickness of 100 μm consisting of the epidermal layer only and (c) dermatomed skin of 100 μm consisting of epidermis and some fragments of papillar dermis.