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The global mechanical properties and multi-scale failure mechanics of heterogeneous human *stratum corneum*

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ABSTRACT

The outermost layer of skin, or *stratum corneum*, regulates water loss and protects underlying living tissue from environmental pathogens and insults. With cracking, chapping or the formation of exudative lesions, this functionality is lost. While *stratum corneum* exhibits well defined global mechanical properties, macroscopic mechanical testing techniques used to measure them ignore the structural heterogeneity of the tissue and cannot provide any mechanistic insight into tissue fracture. As such, a mechanistic understanding of failure in this soft tissue is lacking. This insight is critical to predicting fracture risk associated with age or disease. In this study, we first quantify previously unreported global mechanical properties of isolated *stratum corneum* including the Poisson's ratio and mechanical toughness. African American breast stratum corneum is used for all assessments. We show these parameters are highly dependent on the ambient humidity to which samples are equilibrated. A multi-scale investigation assessing the influence of structural heterogeneities on the microscale nucleation and propagation of cracks is then performed. At the mesoscale, spatially resolved equivalent strain fields within uniaxially stretched *stratum corneum* samples exhibit a striking heterogeneity, with localized peaks correlating closely with crack nucleation sites. Subsequent crack propagation pathways follow inherent topographical features in the tissue and lengthen with increased tissue hydration. At the microscale, intact corneocytes and polygonal shaped voids at crack interfaces highlight that cracks propagate in superficial cell layers primarily along intercellular junctions. Cellular fracture does occur however, but is uncommon.

Statement of Significance

Human stratum corneum protects the body against harmful environmental pathogens and insults. Upon mechanical failure, this barrier function is lost. Previous studies characterizing the mechanics of stratum corneum have used macroscopic testing equipment designed for homogenous materials. Such measurements ignore the tissue's rich topography and heterogeneous structure, and cannot describe the underlying mechanistic process of tissue failure. For the first time, we establish a mechanistic insight into the failure mechanics of soft heterogeneous tissues by investigating how cracks nucleate and propagate in stratum corneum. We further quantify previously unreported values of the tissue's Poisson's ratio and toughness, and their dramatic variation with ambient humidity. To date, skin models examining drug delivery, wound healing, and ageing continue to estimate these parameters.

1. Introduction

Human skin is a layered composite tissue that acts as an essential physical and chemical barrier to the external environment [1]. The outermost layer of skin, or *stratum corneum* (SC), is comprised of tetradecahedron shaped corneocyte cells [2], approximately 15–30 μm in length [3], surrounded by a lipid rich matrix [4]. The complex network of keratin intermediate filaments within each corneocyte along with corneodesmosome intercellular junctions impart structural integrity to the tissue [5]. Upon rupture, the SC loses its functionality; becoming ineffective at regulating water loss [1] or preventing external pathogens from infecting underlying living tissue [6]. Understanding the mechanical properties and physical limits of SC is therefore critical to predicting fracture risk associated with age or disease. It also has wide ranging applications in the development of artificial mechanical skin models for

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wound healing, drug delivery, cosmetic science, and dermatology [7–14]. Previous investigations of SC mechanical properties are extensive and have explored the effects of ageing [15,16], ultraviolet exposure [17], water content [18–21], lipid composition [10,18] and cosmetic treatments [10,18,22,23]. A diverse range of mechanical properties have been reported to characterize these effects including the elastic modulus, fracture strain, work of fracture, drying stress and cellular delamination energies [12,20,22,23,21,24–26]. To date however, numerous properties such as the toughness and Poisson's ratio of SC that are essential for accurate skin modeling have not been empirically quantified. In-vivo measurements of the Poisson's ratio of full thickness skin have been reported [27], however the influence of skin anisotropy [28–30] or the varying mechanical properties of the different tissue layers [31] on their results are not considered. As a result, skin models continue to estimate these parameters [14,18,32–34]. Moreover, previous studies reporting the mechanics of SC and other biological tissues predominantly use macroscopic testing equipment designed for homogeneous materials [12,19,20,23,31,35–38]. These techniques only provide average mechanical properties and ignore the essential heterogeneity of SC, which has a rich topography and complex cellular architecture across many length scales [39]. Recently, the structural heterogeneity of SC has been shown to notably influence both its drying behavior [26] and the global mechanical properties of full thickness skin [11]. We suspect that this structure will also cause the build-up of heterogeneous stresses and strains within the SC during stretching and impact where in the sample cracks will nucleate [40]. We also anticipate that the inherent structural heterogeneity of SC will play an important role in the way cracks propagate through the tissue. While cracking, chapping and the formation of exudative lesions commonly occurs in SC from exposure to extreme environments, aggressive washing required for sterile environments and with dry skin diseases [10,41,42], a clear understanding of the mechanistic process of failure in SC is lacking. Establishing this insight is not only of importance from a biomechanical and materials science perspective, it is also critical to improving patient health and well-being. Reducing failure in the skin barrier would reduce the risk of skin infections including herpes, impetigo, molluscum, warts, atopic eczema, tuberculosis, and necrotic lesions [43].

2. Materials and methods

2.1. Preparation of the stratum corneum

Full thickness skin is received from elective surgery. An exempt approval (3002-13) was obtained to perform research using de-identified tissue samples pursuant to the Department of Health and Human Services (DHHS) regulations, 45 CFR 46.101(b)(4). The tissue source; 36 year old African American female breast, is used for all studies. The choice of breast tissue is based on attainability from surgery. The full thickness skin tissue specimen is stored in phosphate buffered saline solution, transported in a chilled container and received within 24 h of surgery. Isolation of SC is performed within 48 h of receiving the tissue specimen using a standard water bath and trypsin solution based technique [10,23,44]. Blunt nosed specimen forceps are used for separation to minimize the risk of piercing or tearing the SC. After isolation, the SC sheet is allowed to dry to ambient conditions (25 °C, 40% relative humidity (R.H.)) on plastic mesh for 24 h. To ensure a consistent sample geometry, the SC is separated from the mesh and cut to size (7 mm width × 20 mm length) using a scalpel and cutting board with grid lines. All samples are inspected for cracks and holes that can form during the isolation process. Samples exhibiting defects are not used for mechanical characterization as they can induce premature fracture during mechanical testing by acting as sites of crack nucleation.

2.2. BODIPY staining

Surface and intercellular lipids in both intact and fractured SC samples are stained with a fatty acid fluorescent analog (BODIPY FL C_{12} lipid dye, Invitrogen, Carlsbad, CA). A quantity of 100 μL of 0.6 mg/mL solution of BODIPY FL C_{12} in ethyl alcohol is dissolved in 1 mL of carbonate buffer with $pH = 10$. SC samples are agitated in the solution for 60 s, then rinsed with citric buffer with $pH = 5.5$. Samples are then deposited on a glass coverslip with their topside facing outward.

2.3. Transmitted light and fluorescent imaging of SC

Images are acquired using a Nikon Eclipse Ti-U inverted microscope (Nikon, Melville, NY) with 20X objective lens (Nikon Plan Fluor). BODIPY stained SC samples are excited using a SOLA 6-LCR-SB (Lummencor light engine, USA) with FITC filter (510–560 nm emission bandpass). Fluorescent and transmitted light images are recorded using a digital CCD camera (Andor Clara, Belfast, Northern Ireland) at a resolution of 1392 × 1040 pixels. The field of view (FOV) of each image is 0.42 × 0.33 mm. Larger FOVs of the crack interface at the same magnification are acquired using an automated x-y stage (Nikon, TI-SH-U) and subsequent image stitching.

2.4. Scanning electron microscopy

Hydrated SC samples are laminated to a glass coverslip, and then dried in an oven at 60 °C for 1 h. This strongly adheres the samples to the glass. The coverslip is then carbon sputtered in a carbon coater (208C High Vacuum Turbo Carbon Coater, Cressington) and imaged in a Scanning Electron Microscope (ZEISS FEG-SEM Supra 55 VP) with a voltage of 0.7–1.5 kV.

2.5. Confocal imaging of SC

Confocal image stacks are acquired using a spinning disk laser confocal microscope (Leica SP5, Wetzlar, Germany) with a 40X oil immersion lens (Leica HCX PL APO 40X, Wetzlar, Germany) with a numerical aperture of 1.25. BODIPY stained SC samples are illuminated with an argon laser with excitation wavelength 476 nm. Fluorescent and brightfield images are recorded using an integrated imaging system (Leica DMI6000, Wetzlar, Germany) with a spatial resolution of 0.38 μm/pixel (1024 × 1024 pixels). The fluorescent emission spectrum of the BODIPY lipid stain is captured between 520 and 525 nm. The composite image shown in Fig. 3 (c) is created by recording an image stack of 109 images separated by $\Delta z = 170$ nm and imaged using the LAS-AF software (Leica).

2.6. Mechanical characterization

Uniaxial tensile testing is performed using a uniaxial tensometer (CellScale UStretch, Waterloo, ON, Canada) equipped with a 4.4 N load cell, all housed in an environmental chamber. In order to prevent slippage of the samples in the tensometer grips, the ends of each SC sample are taped; leaving an exposed area of 7 mm × 15 mm. Fine graphite particles are sprayed on the face of the sample to increase the number of trackable features. Individual SC samples are mounted into the opposing tensometer grips separated by 15 mm. Each sample is equilibrated for 24 h to specified relative humidity conditions ranging between 7 and 100% R.H. prior to mechanical testing. This humidity affects tissue water