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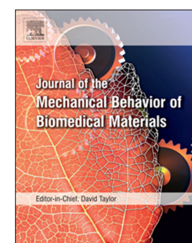

Technical Note

The effects of barrier disruption and moisturization on the dynamic drying mechanics of human *stratum corneum*

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ABSTRACT

We study the dynamic drying mechanics of human *stratum corneum*, the most superficial layer of skin and essential physical and chemical barrier to the external environment. Barrier disruption caused by a depletion of lipids ordinarily found in healthy *stratum corneum* can occur with ageing, aggressive cleansing or with dry skin disorders and diseases such as atopic dermatitis and psoriasis. We establish the effects of severe barrier disruption on the dynamic drying mechanics of human *stratum corneum* by measuring variations in thickness and spatially resolved in-plane displacements in healthy and lipid depleted tissue samples drying in controlled environmental conditions. In-plane displacements recorded at regular intervals during drying are azimuthally averaged and fitted with a profile based on a linear elastic model. The measured thickness of the tissue sample is accounted for in each model fit. Dynamic variations in the drying stress and elastic modulus of the tissue are then established from the model fits. We find that barrier disruption causes dramatic reductions in drying timescales, increases in the elastic modulus of the tissue and larger drying stresses. We expect these changes to increase the propensity for cracking and chapping in skin. The maximum elastic modulus and drying stress of barrier disrupted *stratum corneum* ($E_{SC}=85.4\pm6.8$ MPa, $P_{SC}=10.9\pm0.9$ MPa) is reduced to levels comparable with *stratum corneum* containing lipids ($E_{SC}=26.1\pm3.2$ MPa, $P_{SC}=2.58\pm0.45$ MPa) after treatment with a 5% aqueous solution of glycerol. Neither 2% nor 5% glycerol solutions slow the accelerated drying timescales in barrier disrupted *stratum corneum*.

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Abbreviations: 2% GLY, 2% glycerol aqueous solution; 5% GLY, 5% glycerol aqueous solution; CMT, chloroform/methanol treatment; DIW, deionized water; REF, reference deionized water treatment; R.H., relative humidity; SC, *stratum corneum*

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1. Introduction

The outermost layer of epidermis, or *stratum corneum* (SC) acts as an essential physical and chemical barrier to the external environment (Proksch et al., 2008). This barrier also minimizes, but does not eliminate trans-epidermal water loss (Elias and Friend, 1975; Elias et al., 1977; Froebe et al., 1990; Sahle et al., 2014; Sator et al., 2003; Williams and Elias, 1987). In low humidity environments, SC exhibits reduced hydration levels (Bettley and Grice, 1967; Blank et al., 1984), causing the tissue to dry and shrink. This induces an increase in both the elastic modulus of the SC (German et al., 2012; Levi et al., 2010; Park and Baddiel, 1972; Wildnauer et al., 1971; Yuan and Verma, 2006) and drying stresses; the internal forces within the constrained tissue that build up due to water loss. These mechanical factors govern the damage processes that lead to cracking or chapping (Levi et al., 2010). Healthy skin conditions are regulated by the presence of lipids and hygroscopic natural moisturizing factors (Grubauer et al., 1989a, 1989b; Rawlings and Harding, 2004) that act to minimize water loss and keep the SC hydrated. Ageing and cosmetic cleansing can damage or deplete lipids (Ananthapadmanabhan et al., 2004; Froebe et al., 1990). Lipid content is also reduced with skin diseases such as atopic dermatitis and psoriasis (Sahle et al., 2014; Sator et al., 2003). Barrier disruption from lipid depletion is associated with a reduction in the water-holding capacity of SC (Imokawa et al., 1991), increases in trans-epidermal water loss (Fluhr et al., 2008; Pagnoni et al., 1998) and the onset of xerotic skin (Rawlings et al., 1994) that exhibits flaking and cracking (Boyce et al., 2000; Kedrowski and Warshaw, 2008; Kirkup, 2010; Rawlings and Harding, 2004; Watanabe et al., 1991). While this suggests that lipid depletion is likely to alter the mechanical properties of SC and increase the propensity for tissue damage, this association to date has remained relatively unexplored (German et al., 2013). Common clinical practices to alleviate xerotic skin include regular bathing and application of moisturizers (Bobonich and Nolen, 2014). Treatment of SC with moisturizing products has been shown to reduce the build-up of drying stress in healthy SC (Levi and Dauskardt, 2012) and increase water retention in barrier disrupted SC (Loden et al., 1999). Moisturization is therefore also likely to influence the mechanical properties of barrier disrupted SC. In this article, we employ a simple ex-vivo method to quantify the dynamic drying mechanics of healthy and severely barrier disrupted SC. We further establish the influence of treatment with low concentration aqueous solutions of glycerol on drying behavior. By quantifying and modeling drying induced displacements in isolated human SC samples adhered to a soft deformable elastomer substrate, we establish variations in dynamic drying stress and SC elastic modulus during drying. In comparison with healthy SC that contains lipids, drying is accelerated in barrier disrupted SC and causes significant increases in elastic modulus and drying stress.

2. Methods

2.1. Preparation of elastomer coated coverslips

A silicone elastomer is first prepared by mixing a base (Sylgard 184; Dow Corning, Midland, MI) with the curing agent in a weight ratio of 55:1. After mixing and degassing, it is spin-coated onto a glass coverslip at 2000 rpm for 1 min. The sample is cured at 60 °C for 24 h, resulting in a 36 μm thick elastomer film with a Young's modulus of $E = 16 \pm 1$ kPa and a Poisson's ratio of $\nu = 0.5$ (Cesa et al., 2007). The Young's modulus of the substrate is purposefully chosen to be less than the reported stiffness of epidermal tissue (Kuwazuru et al., 2008) in order to better resolve differences between different glycerol treatments. Red fluorescent microspheres (535/575 nm, 500 nm diameter) are chemically bonded to the elastomer substrate for SC thickness measurement experiments using a technique previously described in German et al. (2012). The resulting density of beads is ~300 per 100×100 mm^2. No fluorescent bead layer is required for independently performed experiments quantifying in-plane drying displacements.

2.2. Preparation of the stratum corneum

Full thickness skin is received from elective surgery. The tissue source (44 yrs, African–American female thigh) is used for all drying studies. The choice of thigh tissue is based on attainability from surgery. Isolation of SC is achieved using a standard water bath and trypsin solution based technique (German et al., 2012). After separation, the SC is allowed to dry to ambient conditions (25 °C, 40% R.H.) on plastic mesh for 24 h. The SC sheet is then cut into half. One half is agitated in deionized water (REF) for 60 min. The other is agitated in chloroform/methanol (2:1 by volume, Fisher-Scientific, Pittsburgh, PA) for 60 min, then washed for a further 60 min in deionized water. The chloroform/methanol treatment (CMT) induces severe barrier disruption by fully depleting intercellular and surface ceramides, cholesterol and free fatty acids ordinarily found in healthy human SC (Bligh and Dyer, 1959; Fulmer and Kramer, 1986; Lampe et al., 1983; Rogers et al., 1996; Weerheim and Ponec, 2001). The SC sheet treated only with water retains high levels of lipids (Lampe et al., 1983). Both SC sheets are then allowed to dry again to ambient conditions on plastic mesh for 24 h. To ensure a consistent sample geometry, the SC is separated from the mesh and cut into circular samples of radius, $R = 3.1 \pm 0.25$ mm, using a hole punch (Harris Uni-Core, Redding, CA). An indelible marker is used to place a spiral mark at the center of each sample to highlight the topside of the SC. The center of the SC sample is chosen as this corresponds to where drying displacements are smallest.

2.3. Sample treatment and deposition

SC samples are agitated for 30 min in 15 ml deionized water containing 90 μl fluorescent marker beads (505/515 nm, 1 μm diameter, carboxylate-modified, Molecular Probes, Invitrogen, Grand Island, NY) for in-plane drying experiments. An identical protocol is used for SC thickness measurement experiments without the addition of the fluorescent beads. Samples are then adhered to the silicone elastomer substrates. To minimize residual stresses and avoid entrapment of bubbles between the SC and the substrate, substrates are first partially immersed in the deionized water at a shallow angle. An edge of the SC sample is then pinned at the contact line between the substrate and water interface. Raising the substrate vertically out of the water enables complete lamination of SC samples to the substrate without the subsequent need to physically press the SC down.

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