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Understanding age-induced alterations to the biomechanical barrier function of human stratum corneum

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ABSTRACT

Background: The appearance and function of human skin are dramatically altered with aging, resulting in higher rates of severe xerosis and other skin complaints. The outermost layer of the epidermis, the stratum corneum (SC), is responsible for the biomechanical barrier function of skin and is also adversely transformed with age. With age the keratin filaments within the corneocytes are prone to crosslinking, the amount of intercellular lipids decreases resulting in fewer lipid bilayers, and the rate of corneocyte turnover decreases.
Objectives: The effect of these structural changes on the mechanical properties of the SC has not been determined. Here we determine how several aspects of the SC's mechanical properties are dramatically degraded with age.
Methods: We performed a range of biomechanical experiments, including micro-tension, bulge, double cantilever beam, and substrate curvature testing on abdominal stratum corneum from cadaveric female donors ranging in age from 29 to 93 years old.
Results: We found that the SC stiffens with age, indicating that the keratin fibers stiffen, similarly to collagen fibers in the dermis. The cellular cohesion also increases with age, a result of the altered intercellular lipid structure. The kinetics of water movement through the SC is also decreased.
Conclusions: Our results indicate that the combination of structural and mechanical property changes that occur with age are quite significant and may contribute to the prevalence of skin disorders among the elderly.

1. Introduction

Aging is a universal, inexorable process accompanied by physiological changes, including alterations to the skin. Skin becomes thinner, forms wrinkles, and is more susceptible to xerosis, changes in pigmentation, and bruising [1–5]. Wound healing time is increased [6] and skin cancer is more prevalent [7]. These changes result from intrinsic (genetic) and extrinsic (environmental) factors, including solar UV exposure [8].

The barrier function of the stratum corneum (SC) is not immune from the aging process. Aging is inherently complex, resulting in difficulties quantifying and interpreting experimental results. Hence, the aging literature contains a host of contradictions on barrier function alterations with age. Although transepidermal water loss (TEWL) is either unchanged or reduced in aged skin [9–15], barrier function does degrade which is most apparent when the SC undergoes an insult. Fewer tape strippings are required to perturb the barrier of aged skin and corneocyte cohesion appears to be altered [9,15–17]. However, tape stripping is not a quantitative measure of cellular cohesion, as defined by the energy required to separate intercellular boundaries. It remains unclear how corneocyte cohesion alters with aging. Recovery rates of aged skin after acute barrier disruptions are significantly reduced, with photoaged skin showing an even stronger effect than intrinsically aged skin [18]. Furthermore, the SC's permeability to certain penetrants is altered, often depending on the penetrant lipophilicity [19–26].

Barrier function alteration is accompanied by SC structural changes. A reduction in delivery of secreted lipids to the SC results in a reduction in number of intercellular lipid bilayers [9]. Lipid reduction is global: ceramides, cholesterol, and free fatty acids,

Abbreviation: DCB, double cantilever beam.
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present in approximately equimolar ratios, decline by approximately 30% [27], due to a reduction in activity of rate-limiting enzymes for each lipid. Concurrently, increased pH in the SC decreases the activity of lipid processing enzymes, resulting in abnormal lipid processing [28]. This has important implications for lipid barrier function.

SC protein components also undergo structural changes with age. Corneodesmosomes, critical for corneocyte cohesion, must be degraded by hydrolytic enzymes for proper desquamation. We previously demonstrated the role of corneodesmosomes on corneocyte cohesion [29]. The level of trypsin-like desquamatory enzymes (but not chymotrypsin-like enzymes) is reduced in aged skin [30]. However, the pH at the skin surface is increased [31], which causes an increase in serine protease activity and a reduction in corneodesmosome density [28]. Desquamation itself, however, is slower or unaffected in aged skin while turnover times are longer, resulting in corneocyte buildup on the surface of the SC, which contributes to xerotic skin conditions [17,32]. Corneocytes become larger and flatter with age, consistent with a slower SC turnover rate [33,34]. It is interesting to note that an increased number of layers of larger, flatter corneocytes should result in an increase in SC pathlength and, thus, an increase in passive barrier function and/or resistance to insult [35]. This is not the case, however, which suggests that other deleterious mechanisms are countering this effect [9].

Skin's mechanical properties are also altered with age. Changes in dermal collagen and elastin fiber structure result in skin elasticity and viscoelastic recovery markedly decreasing [12,36,37]. The dermal/epidermal junction flattens, weakening the interface and making the epidermis more prone to separation [38]. Studies to date, however, have focused on alterations to the mechanical properties of full thickness skin and have largely ignored the SC. To our knowledge, few studies have quantitatively determined how the crucial SC biomechanical barrier function is altered with age, and the literature that does exist on age-related structural and biochemical alterations to the SC is filled with contradictions. Since treatment of aged skin generally involves treatment of symptoms and disorders rather than the underlying biochemical process of aging, it is imperative that the symptoms and underlying alterations to the structure and function of the skin be accurately determined.

One of the main health challenges of the next twenty years will be meeting the needs of the aging population. Understanding the SC aging process will enable development of treatments designed to maintain skin's vital barrier function, youthful feel and elasticity, along with improved strategies for UV protection. According to the UK Office For National Statistics [39], by 2037 the population aged 65 and over will account for 24% of the total population, while the number of people aged 85 and over is projected to be 2.5 times larger than current figures, reaching 3.6 million and accounting for 5% of the total population. A very similar situation exists in the US [40].

The average life expectancy in the US and UK has increased by approximately 10 years since 1950, which results in significant increases in exposure to extrinsic causes of aging. For example, a ten year increase in life expectancy results in a lifetime increase of 250,000 J/m^2 of UV radiation exposure [41]. As life span and healthy active years are expanded, controlling the skin aging process becomes increasingly important.

2. Materials and methods

2.1. Tissue preparation

Experiments were performed on abdominal SC from female cadaveric donors ranging in age from 29 to 93 years old (29 y.o. specimen was African American, all others were Caucasian). Gender and body site was chosen to minimize variation between specimens and reduce the presence of hair, which can introduce small holes in the tissue.

The tissue processing protocol has been described previously [8,42–46] and is used extensively in the skin science community. Briefly, full thickness skin was harvested immediately after death and stored until use at −80 °C. To isolate the SC, the skin was thawed and epidermal tissue was mechanically separated from full thickness skin after immersion in a 60 °C water bath. The SC was isolated from the underlying epidermis by soaking in a trypsin enzymatic digest solution (0.1% wt/wt in 0.05 M, pH 7.9 Tris buffer) at 35 °C for 120 min. After processing, the SC was dried and stored in a desiccating cabinet (~22°C, ~10% RH). Due to the SC's lack of vascularization, unlike most other soft tissues in the human body, the SC does not undergo rapid apoptosis and structural damage after harvesting. The trypsin digest only causes removal of uncornified cells of the full thickness epidermis. The structure of the cornified SC layer is unaltered, and SC isolated in this manner can be stored with minimal change for extended periods [47].

2.2. Biomechanical testing

We performed micro-tension, bulge, double cantilever beam (DCB), and substrate curvature experiments as previously described [42,45,48] and briefly reviewed here.

Micro-tension experiments were performed using a tensile testing apparatus (Bionix 200, MTS Systems Corporation, Eden Prairie, MN, U.S.A.) equipped with a 44.48N load cell to study the uniaxial biomechanical behavior of the tissue. The specimens (25 × 6 mm, 10 mm gauge length) were tested to failure in all cases (initial strain rate = 0.01 s^{-1}).

Bulge testing determined the biaxial stress-strain behavior of the SC. SC specimens (5 mm × 5 mm) were clamped onto the orifice of a cavity drilled in a Plexiglas unit with two channels, and one side of the specimen was exposed to DIW for 1 h prior to testing. The SC was bulged outwards with distilled water (flow rate = 0.0445 μL s^{-1}), and the pressure inside the system was measured by a pressure transducer. To determine the biaxial stress and strain in the SC, the pressurized tissue was modeled as a section of a thin-walled spherical pressure vessel.

DCB testing measured the cohesion of the corneocytes, quantified by the critical value of the strain energy release rate, G_c. G_c is the amount of energy per unit area required to propagate a (intercellular) crack through the SC. SC was adhered between two elastic substrates of polycarbonate with cyanoacrylate adhesive. Cyanoacrylate adhesive polymerization is readily initiated by the presence of small amounts of water on the bonding surfaces, limiting the adhesive to the SC exterior [42]. Substrate dimensions (40 × 10 × 3 mm) were chosen to ensure purely elastic deformation. The specimens were mounted at the SC-free end via loading tabs in an adhesion test system to propagate a debond through the SC layer (displacement rate = 2 μm/s).

Substrate curvature experiments measured the stress that develops when the SC is exposed to a drying environment. SC was adhered to 22 mm × 22 mm × 177 μm borosilicate glass coverslips (Fisher Scientific, 12-541-B) with reflective Cr/Au (35 Å/465 Å) films deposited on one surface. To adhere the SC to the substrate, the SC was submersed in water for 25 min. Water was removed with pipettes, allowing the SC to settle on to a glass cover slip. Excess SC was removed from the edges. The SC was placed in a substrate curvature instrument (23 °C, 7% RH), where a scanning laser measured the angle of deflection versus position on the substrate and calculated an average curvature. The SC stress was calculated from the curvature using Stoney's equation.

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