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ScienceDirect
Biomaterials 27 (2006) 5861–5870
Biomaterials
www.elsevier.com/locate/biomaterials

Graded delamination behavior of human stratum corneum

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Received 25 April 2006; accepted 4 August 2006

Abstract

An in vitro adhesion test method has been adapted to quantify the through-thickness intercellular delamination energy of isolated human stratum corneum (SC). Both untreated and delipidized tissues were tested. Measured delamination energies were found to increase from ~3 J/m^2 near the surface to ~15 J/m^2 for the inner layers of the tissue. For delipidized SC, the location of the initial debond was located closer to the center of the tissue. Delamination energy values were elevated compared to untreated specimens, increasing from ~7 J/m^2 near the surface to ~18 J/m^2 for the inner layers of the SC. Further tests were run to measure delamination energies of SC as a function of hydration (15–100% relative humidity (RH)) at ~25 °C and as a function of temperature (10–90 °C) at several hydrations (15, 45, 100% RH). Delamination energies were observed to decrease with increasing hydration and increasing temperature with the most significant changes occurring for 100% RH conditioned SC. Additional SC was treated with pH-buffered solutions (pH 4.2, 6.7, 9.9) and selected surfactant solutions (1%, 10% wt/wt sodium dodecyl sulfate (SDS)) for comparison to untreated controls. While statistically significant differences were observed, the SC was found to be resistant to large changes in delamination energy with pH and 1% wt/wt SDS treatments with values in the range 4.2–5.1 J/m^2 compared to control values of 4.4 J/m^2. More substantially elevated values were observed for SC treated with a 10% wt/wt SDS solution (6.6 J/m^2) and a chloroform–methanol extraction (11.2 J/m^2).

Keywords: Mechanical properties; Fracture toughness; Epithelial cell; Stratum corneum; Tissue treatment

1. Introduction

Stratum corneum (SC) has a composite structure consisting of heavily keratinized, disk-shaped corneocyte cells bound together by intercellular lipids and degraded desmosomal protein junctions, or corneosomes. To maintain full coverage and effective barrier properties, SC renewal occurs by the continual replacement of mature exterior cells with younger interior cells. Proper SC cell detachment and renewal, or desquamation, has been associated with the progressive degradation of corneosomes toward the outer skin surface [1–6]. Evidence suggests a strong link between this degradation process and the many gradients within SC tissue including water content, pH, natural moisturizing factors, lipids, and desquamatory compounds [7–15]. Normal desquamation has been linked to a variety of factors including appropriate hydration and the presence within the SC of specific molecular compounds such as cholesterol sulfate which inhibits corneosome degradation [8,16–20]. Other factors such as increased hydration have been shown to accelerate corneosome degradation and intercellular lipid disruption which affect mechanical integrity as well [4,8,21].

While the graded character of SC has been long recognized, few attempts have been made to provide

Abbreviations: SC, stratum corneum; RH, relative humidity; FH, fully hydrated; DCB, double-cantilever beam; CMT, chloroform–methanol treated; SDS, sodium dodecyl sulfate; FTIR, Fourier transform infrared; STDEM, standard error of the mean; SEM, scanning electron microscopy; SD, standard deviation

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0142-9612/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2006.08.008

quantitative measurements of mechanical properties as a function of tissue depth. Limited studies have examined the in-plane mechanical and fracture behavior of SC [22–27], while even fewer studies have addressed delamination behavior in the out-of-plane orientation perpendicular to the skin surface [2,28–32]. In one study, the cohesive strength of porcine SC was found to increase with depth into the SC [2]. Additional tape-stripping studies have shown that fewer corneocytes are removed with progressive stripping from outer to inner layers [33]. However, these studies were performed on SC attached to underlying epidermis, and the presence of this soft substrate leads to uncertainties in the mechanics analysis. In addition, similar to peel adhesion testing, the tape stripping technique is difficult to quantify [34]. By isolating SC from underlying skin, its properties can be examined in a more quantitative manner.

Quantifying the through-thickness delamination behavior of SC may also help understanding of why normal desquamation may be impaired by environmental conditions, underlying skin pathology or treatments that may affect normal SC composition and gradients. SC structure and properties can be perturbed by extreme values of humidity or temperature and also by exposure to chemical species such as surfactants in daily cleansers. In the case of existing and emerging technologies that interface with the skin, such as monitoring or transdermal drug delivery devices, knowledge of the graded delamination properties of SC will be important for ensuring adequate adhesion to skin.

We recently presented a technique to quantify the resistance of isolated SC tissue to delamination in terms of delamination energy measured in units of J/m^2 [31,32]. Out-of-plane delamination energies were shown to change significantly with hydration, temperature, and chloroform–methanol lipid extraction. Results were consistent with increased cohesive strength values measured by cohesometry tests after similar solvent and chloroform–methanol lipid extraction treatments [2,31,35]. In the present study, this delamination method has been further developed and employed to quantify human SC intercellular delamination energy as a function of depth into the SC. The delamination energy was found to increase significantly from $\sim 3\,J/m^2$ near the surface to $\sim 15\,J/m^2$ for inner layers of the tissue. Delamination energies also were observed to decrease with increasing hydration and temperature with the most significant changes occurring for 100% relative humidity (RH) conditioned SC. Additional SC was treated with pH-buffered (pH 4.2, 6.7, 9.9) and selected sodium dodecyl sulfate (SDS) surfactant solutions. These treatments have been shown previously to affect lipid fluidity and SC swelling [36]. While statistically significant differences were observed, SC delamination energy was found to be relatively insensitive to pH and 1% wt/wt SDS treatments. More substantially elevated values were observed for SC treated with a 10% wt/wt SDS solution and after chloroform–methanol extraction. For delipidized SC, the initial debond was located closer to the center of the SC layer and delamination energy values were elevated compared to untreated specimens. SC specimen failure surfaces were examined with scanning electron microscopy (SEM) to visualize surface morphologies and to determine failure paths. The measured delamination energies are interpreted in terms of SC microstructure and constituents.

2. Materials and methods

2.1. Tissue preparation

Human cadaver SC used in these experiments was obtained from abdominal or thigh skin of three female Caucasian donors, 57–101 years old. Comparative tests were performed on single donor tissue specimens to reduce variability within test sequences. Epidermal tissue was separated from dermis by immersion of donor tissue cleared of adipose tissue in a 35 °C water bath for 10 min followed by a 1 min soak at 60 °C then mechanical separation from the dermis using a flat-tipped spatula. Subsequently, SC was isolated from 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. During separation, the orientation of the outer SC surface was recorded. The SC was then rinsed with room temperature water and allowed to dry on filter paper (Grade 595 General-Purpose Filter Paper, Schleicher and Schuell MicroScience GmbH, Dassel, Germany) then removed and stored in ambient conditions of ~18–23 °C and ~35–55% RH.

For measurement of delamination energy as a function of temperature and hydration, specimens were equilibrated in an environmental chamber (Model LH-6, Associated Environmental Systems, Ayer, MA) or in an enclosure over a water-filled container for 11–43 h before specimen fabrication. Specimens were equilibrated at 15%, 30%, 45%, 60%, 75%, 100% RH at ~25 °C prior to testing. Additional SC was treated in several pH-buffered and selected surfactant solutions to examine their effects on SC mechanical integrity. The solutions used have been shown to affect lipid fluidity and SC swelling indicative of possible internal corneocyte damage [36]. They were prepared as previously described [36]. Potassium hydrogen phthalate (Lancaster Synthesis Inc., Pelham, New Hampshire)—HCL was used to buffer pH 4.2 solutions. NaOH–potassium dihydrogen phosphate (Lancaster Synthesis Inc., Pelham, New Hampshire) was used to create a pH 6.7 buffer. Borax (sodium tetraborate decahydrate—Sigma-Alrich, Inc., St. Louis, Montana) + NaOH buffer was used to obtain pH 9.9. Buffer concentration was adjusted to 0.006 M in each case. Surfactant solutions consisted of two unbuffered SDS (Sigma-Alrich Inc., St. Louis, Montana) solutions with concentrations of 1% and 10% wt/wt and initial pH values of pH 6.6 and pH 7.3.

For both buffered and unbuffered solutions, SC was submersed in a given solution for 18 h at ~20 °C. The SC used was ~40 mm wide by ~80–90 mm in length and soaked in 100 mL of solution. For the 10% wt/wt SDS solution a smaller $\sim 40 \times 50\,mm^2$ specimen of SC was treated in 45 mL of solution. Subsequently, the treated SC was transferred to water-dampened filter paper and lightly rinsed then allowed to dry in ambient conditions. Additional tissue for graded delamination energy measurements was not further processed after separation from underlying epidermis except for test specimen preparation as described in the following section. For comparison, SC, $\sim 60 \times 60\,mm^2$, was delipidized with a 120 min 30 mL chloroform:methanol (2:1 by volume) soak with two subsequent 30 min 30 mL water rinses as described previously [31].

2.2. Delamination energy measurements

The fracture mechanics technique developed to measure the delamination energy of SC tissue as a function of hydration, temperature, and