Mechanical Properties of Human Stratum Corneum: Influence of pH-Treatment

Rena Rudavsky, Mechanical Engineering, Columbia University

NNIN REU Site: Stanford Nanofabrication Facility, Stanford University
Principal Investigator: Reinhold Dauskardt, Materials Science, Stanford University
Mentor: Kenneth Wu, Mechanical Engineering, Stanford University
Contact: rmr2003@columbia.edu, Dauskardt@stanford.edu

Abstract:

The stratum corneum (SC), the outermost layer of the skin, is the body's first barrier against the external environment. As such, the underlying microstructure and mechanical properties of the SC are of fundamental and practical interest.

While pH treatments have been shown to influence the lipid lamellar matrix, alterations to the mechanical properties have not been examined thoroughly. Since many topically applied substances like surfactants, creams, and adhesives for transdermal technologies possess varying pH levels, it is important to understand resultant property changes of the SC.

In particular, we looked at pH-induced changes to physical relaxation time and strain-recovery of human SC. We completed time-dependent creep-recovery tests in ambient conditions on pH-treated SC specimens using a dynamic mechanical and thermal analysis (DMTA) device. While various pH levels affected the strain-recovery, they did not appear to significantly alter the physical relaxation time.

Introduction:

The skin has three distinct layers—the epidermis, dermis, and subcutaneous fat. The stratum corneum (SC), the top layer of the epidermis, has a thickness of 10-20 μm. Following a 14-day cycle, cells from lower epidermal layers flatten, rise and fill with the protein keratin to form SC. While a cross-section of the SC reveals inter-digitated cell layers surrounded by a lipid lamellar matrix, similar to bricks and mortar, a top-view reveals complete surface coverage resembling a soccer ball. Keratin, the main component of SC, is the same metabolically inactive protein in hair and fingernails, making it easy to study *in vitro*.

The SC is the body's primary chemical, diffusional, and mechanical barrier. Changes in the biophysical characteristics of this outer layer could greatly influence its mechanical properties and thus its biophysical functions as well. Yet, there is still necessary work needed to fully characterize SC physical properties. Particularly, we focused on the mechanical effects of pH on SC.

Even though healthy SC has a pH of 5.5-7, common skin therapies tend to vary greatly along the pH scale. With anti-wrinkle fighting treatments like pH 3.5 alpha-hydroxy acid and many daily-use soaps of pH 9-10, it is important to identify specific changing mechanisms in this essential barrier. Additionally, with new advances in transdermal technology, it is essential to understand adhesive-induced changes to SC physical properties. Alterations to the barrier could inhibit diffusion or increase tearing during patch removal. Lastly, diseases like eczema and diabetes tend to raise skin pH, thus affecting possible transdermal drug delivery.

Procedure:

We tested human SC separated from full thickness cadaver skin using a trypsin treatment. Specimens were treated for 18 hours with buffered solutions of pH 4.2, 6.7 or 9.9 of constant 0.006 M ionic strength. Other specimens were delipidized with a chloroform-methanol (2:1) soak for 2 hours. All specimens were

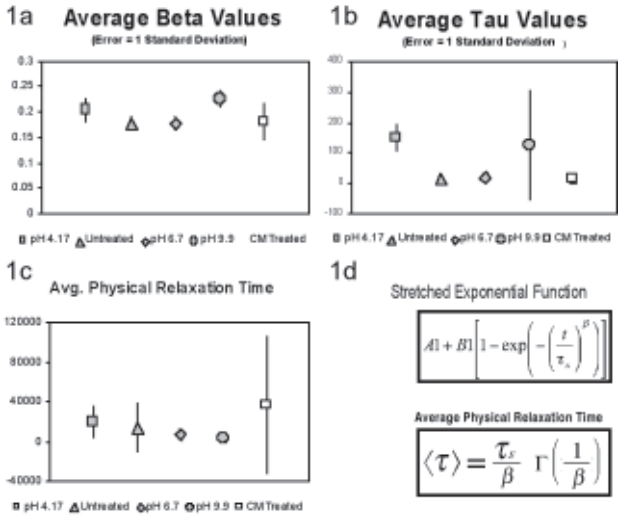

Figure 1: a, b, c) Stretched exponential values/functions; d) Physical relaxation time.

then dried in ambient conditions (~ 25°C; 50% RH).

To study physical relaxation time and strain-recovery of SC, we completed in-plane creep-recovery testing using a Rheometric Scientific Dynamic Mechanical and Thermal Analysis (DMTA V) device. Because SC exhibits viscoelastic and viscous components in addition to elastic properties, response strain to applied stress is time dependent. So, we applied a constant 1 MPa stress for 21020 seconds then lowered the applied stress to 1 kPa for 21020 seconds while measuring the resultant strain (2).

To isolate the elastic modulus of pH-treated specimens, tensile tests were completed at a strain rate of 0.002. Values were used from earlier tests for untreated and chloroform methanol treated specimens.

In preparation for scanning electron microscope imaging, specimens were coated for 45 seconds with gold palladium. SEM images were taken to qualify SC damage.

Results:

We can a see a distinct strain-recovery change in treated specimens in Figure 1c. By comparing the creep strain to the equivalent time interval recovery strain for each treatment, we can see that recovery ranges from 76% for the untreated specimens to as low as 31% for pH 9.9 treated specimens.

Using the stretched exponential equation in Figure 1d, we calculated the viscoelastic response of SC. The viscous component was subtracted from the raw data and the elastic component was isolated using tensile test data- leaving the viscoelastic component to fit with the stretched exponential equation. A β value less than 1 creates a curve stretched from a regular exponential function while τ values characterize the physical relaxation time (τ_s) seen in Figure 1d. Figures 1a,b show little statistical difference between the averaged β and τ_s values for each treatment.

Discussion:

A clear trend indicates that strain-recovery decreases as pH moves farther away from the normal ~ 5.5 pH of SC, increasing plastic deformation and greater tendency of the material to remain deformed after reduction of applied stress. This may be attributed to the isoelectric point (IEP) of ~ pH 5 of keratin and the pKa of ~ pH 7 of lipid fatty acids. As charge in the keratin increases so does swelling and damage [1]. The downward concavity of the strain-recovery data shown in Figure 2b with the lowest point at pH 9.9-, the largest deviation from both the keratin IEP and the lipid pKa- suggests that pH affects both lipids and keratin synergistically. The delipidized specimen, however, suggests that lipid removal does not significantly influence strain-recovery. It appears as though SC alterations are primarily caused by damaged keratin, although more verification is needed.

Harsher treatments, like surfactants, will be analyzed to compare effects on keratin. Interestingly, the physical relaxation times do not vary appreciably in the current data set, but more intensive study is needed for verification.

Acknowledgments:

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References:

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[2] Menard, Kevin P. Dynamic Mechanical Analysis: A Practical Introduction. CRC: Boca Raton, 1999.

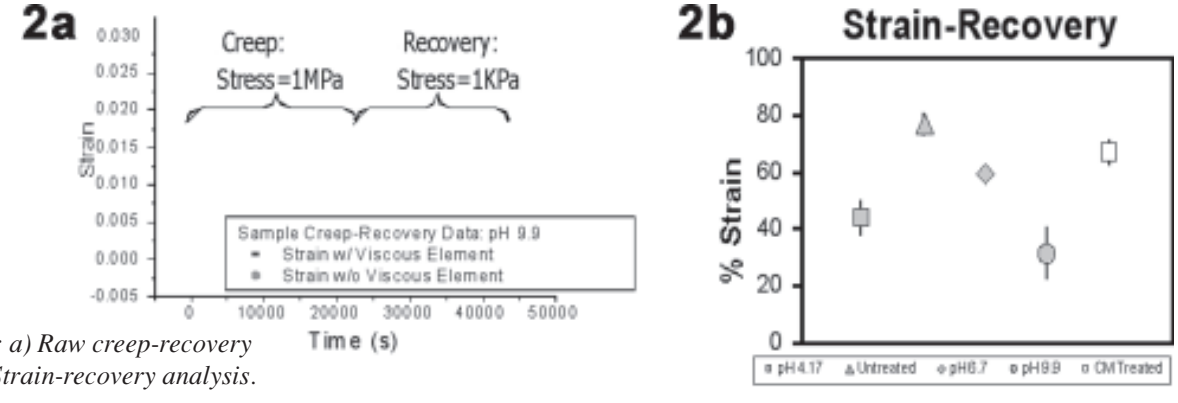

Figure 2: a) Raw creep-recovery data; b) Strain-recovery analysis.