SUPPLEMENTARY MATERIAL

Article: Development of Keratin-Based Membranes for Potential Use in Skin Repair

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Supplementary Materials and Methods

Rinsing of the leachable components over time

Cylindrical samples were exposed to UV for 6 min (13 mm diameter, 1.5 mm thick) and 96 min (5.3 mm diameter, 1.5 mm thick) as described in the previous section, producing samples groups 6-1.5 and 96-1.5, representatives of low and high energy density (ED) groups respectively. Six samples of each group were collected after casting, lyophilized without rinsing, and then weighed (unrinsed total mass). All other samples were subjected to sequential rinses in phosphate buffered saline (PBS, pH 7.4). Six samples of each group were collected after 1, 3, 5, 7, 10, 12, 15, and 20 rinses. Each rinse consisted of fresh PBS for 15 min, at room temperature. The tenth rinse was a single, extended overnight rinse (14h), part of our usual rinsing protocol. After collection, samples were lyophilized and weighed (rinsed mass) (n=4 to 6).The masses of all lyophilized samples were recorded using a microbalance (Sartorius ME-5, Sartorius, Goettingen, Germany).

Effect of lyophilization on swelling properties

Cylindrical samples (13 mm diameter, 1.5 mm thick) were exposed to UV for 12 and 96 min as described in the previous section, producing samples groups 12-1.5 and 96-1.5, representatives of low and high ED groups respectively. All samples were subjected to sequential rinses in PBS over 24h and then weighed and measured (Original mass, diameter, and thickness). Half of the samples from each group were returned to PBS and stored at 4°C; the other halves were lyophilized. All samples were measured again (Initial mass, diameter, and thickness) and then moved to 2 ml excess minimum essential medium (MEM, Life Technologies, Frederick, MD) at 37°C. The mass and dimensions of the samples were recorded after 30min, 2h, 20h, 46h, 70h, and 116h. At each time point the sample was taken out of solution, gently blotted to remove excess MEM, measured, and then returned to the solution (n=7). The volume of MEM was kept at 2 ml by refilling at the third day.

Supplementary Figures

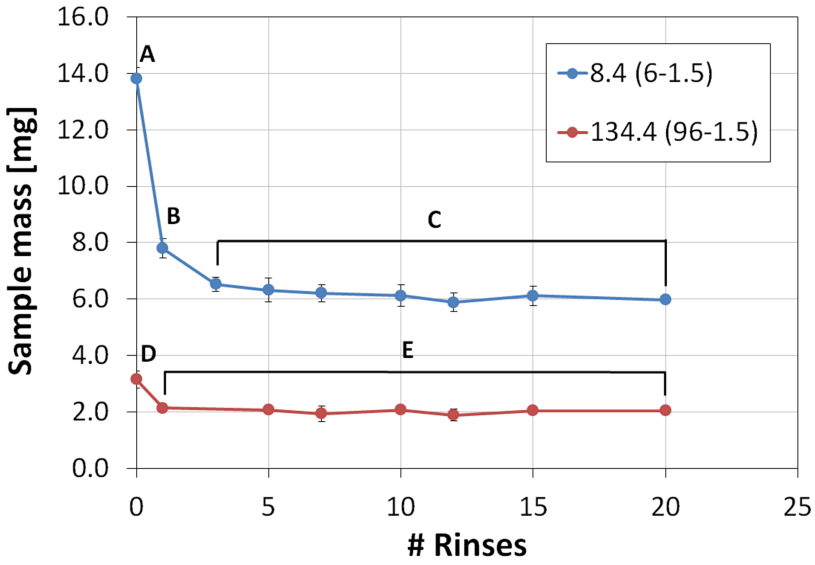

Figure S1. Removal of all soluble mass from the crosslinked membranes. *Low CD samples present a high loss of mass after the initial, but stabilize after the third or fifth rinse; on the other hand, high CD samples, have a lower mass loss and are stable after the first rinse. Measurement of these mass changes on the microbalance, and the low standard deviation errors, allow us to conclude that no mass changes are occurring once the rinsing protocol is complete, indicative of the fact that all leachable products left after the UV crosslinking reaction have been removed. For all plots, samples that do not share the same letter are significantly different (ANOVA with Tukey's comparison, $p<0.05$).*

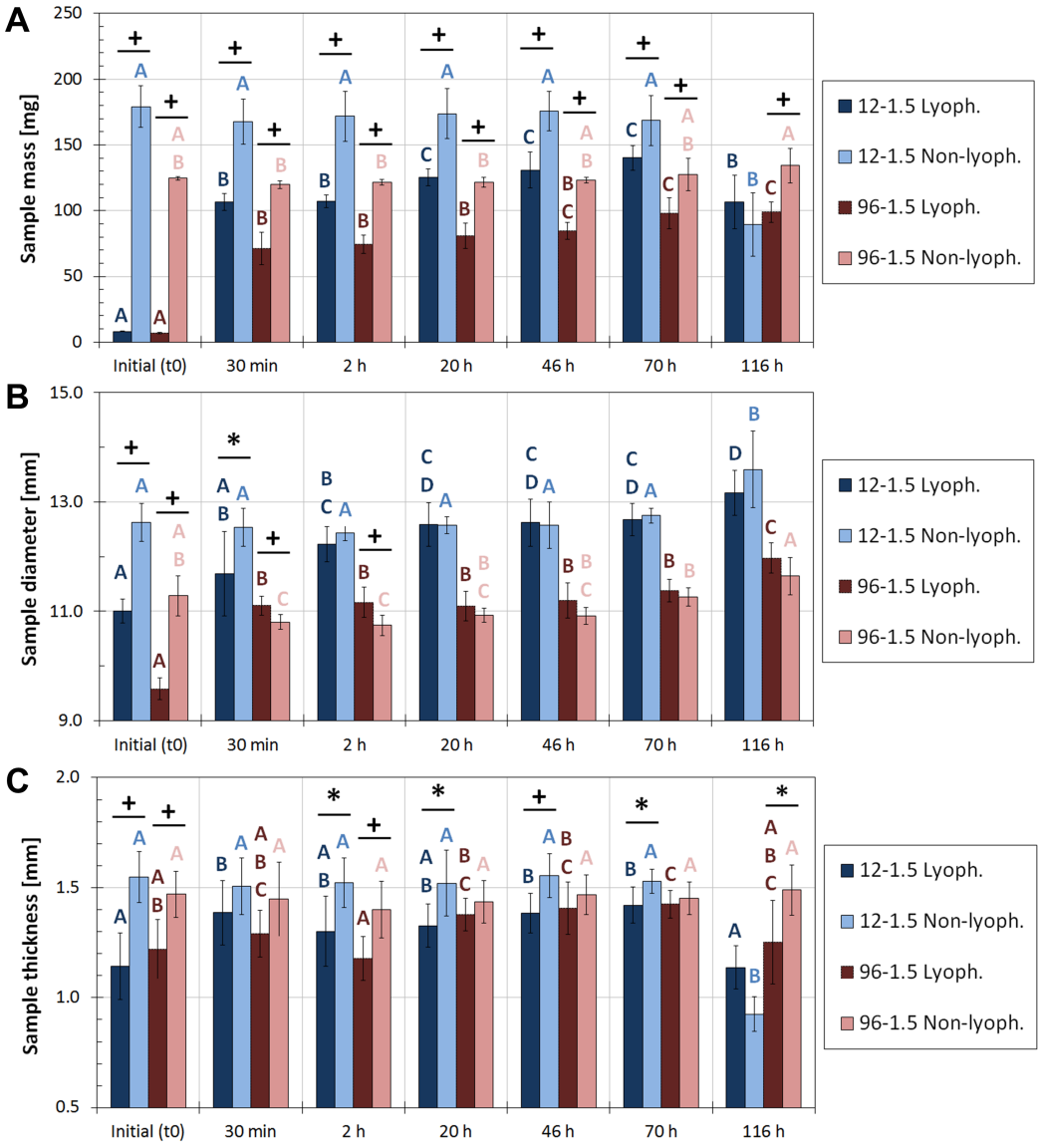

Figure S2. The effects of lyophilization on swelling of the crosslinked membranes. *Lyophilized and non-lyophilized keratin membranes were rehydrated to assess any additional entanglement caused in the freezing and drying processes. A) During rehydration, there are significant differences due to lyophilization at all time points for both low (12-1.5, ED 16.8 mJ/mm²) and high (96-1.5, ED 134.4 mJ/mm²) CD membranes. On the other hand, diameter (B) and thickness (C) are recovered over time and are comparable to non-lyophilized samples. Overall, dimensions can be broadly restored with rehydration, but the mass uptake can be irreversibly decreased; this is indicative that swelling assessments, which require a lyophilization step, can be offset due to additional entanglement. ANOVA with Tukey's comparison was performed on each group independently, samples that do not share the same letter are significantly different within that group as denoted by color ($p<0.05$). Comparison between lyophilized and non-lyophilized samples was assessed at each time point using two-sample t-test for the mean ($p<0.01$ (+) or $p<0.05$ (*)).*