Collagen solubility correlates with skin optical clearing

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Abstract. Biomedical optics and photomedicine applications are challenged by the turbidity of most biological tissue systems. Nonreactive, biocompatible chemical agents can induce a reversible reduction in optical scattering of collagenous tissues such as human skin. Herein we show that a chemical agent’s tissue optical clearing potential is directly related to its collagen solubility, providing a rational design basis for effective, percutaneous formulations.

Keywords: tissues; optical properties; collagen; lasers.

Collagen solubility as a function of chemical agent concentration is shown in Fig. 1a. Solubility is quantified as the amount of collagen remaining in supernatant following fibrillogenesis. In this data set, the initial collagen concentration was 1.25 mg/ml. As would be expected from the data shown in Fig. 1a, ethylene glycol and 1,2-propanediol showed low collagen solubilities. With glycerol, 1,3-propanediol, and sorbitol, their collagen solubilities increased with increasing chemical agent concentration. Sorbitol exhibited twice the collagen solubility of glycerol and 1,3-propanediol, consistent with previously reported results.

Collagen solubility as a function of chemical agent concentration is shown in Fig. 1b. Solubility is quantified as the amount of collagen remaining in supernatant following fibrillogenesis. In this data set, the initial collagen concentration was 1.25 mg/ml. As would be expected from the data shown in Fig. 1a, ethylene glycol and 1,2-propanediol showed low collagen solubilities. With glycerol, 1,3-propanediol, and sorbitol, their collagen solubilities increased with increasing chemical agent concentration. Sorbitol exhibited twice the collagen solubility of glycerol and 1,3-propanediol, consistent with previously reported results.

Overall, our data demonstrated an increase in collagen solubility with increasing sugar-alcohol chain length from ethylene glycol (two carbon chain) to sorbitol (six carbon chain).

Chemical agents that can induce tissue optical clearing increase collagen solubility by suppressing hydrophilic, intermolecular interactions. The propanediols evaluated in this study have been used previously to highlight the role of hy-
Hydrogen bonding in collagen fibrillogenesis. Suppression of these hydrophilic attractive forces can destabilize high-order collagen structures in native and in vitro tissues as observed microscopically and ultrastructurally. Having characterized chemical agent collagen solubilities, we then investigated whether the ability of each agent to suppress hydrogen-bond-mediated attractive forces was correlated with its optical clearing potential (OCP) on in vitro skin.

Using an integrating-sphere-based method, OCP of each chemical agent was evaluated in rodent (from 3- to 6-week-old animals) and human skin. Harvested rodent skin was stored in PBS at 4°C until experiments were performed less than 24 h later. Rodent and cryopreserved (−20°C), dermatomed human skin were cut into 1.5 × 1.5 cm² samples using surgical scissors. Subcutaneous fat was removed using a razorblade and skin thickness was measured using a micrometer (Mitutoyo) after the sample had been placed between two glass slides of known thickness. Transmitted and reflected 635-nm laser light and skin thickness were measured before and after application of specific chemical agents to the dermal side of the skin samples; experiments were performed at room temperature. Chemical agent solutions were volume matched to skin samples; skin samples were exposed for 45 min to chemical agent solutions. The inverse-adding doubling method was used to calculate the reduced scattering coefficient $\mu_s$ before and after chemical agent application. Data are reported as the reduced scattering ratio, $RSR = \frac{\mu_s^{(\text{before})}}{\mu_s^{(\text{after})}}$. Each data point is an average of at least four measurements.

RSR as a function of chemical agent concentration is shown for rodent skin in Fig. 2(a). The slope from linear regression analysis of RSR yields optical clearing potential (OCP) of each chemical agent, shown at right. *Xylitol OCP was not significantly different from that of glycerol, 1,3–propanediol, ethylene glycol, or 1,2–propanediol.
variance test to the RSR data identified three significantly different groups ($p \leq 0.005$): sorbitol; xylitol, glycerol, and 1,3-propanediol; and ethylene glycol and 1,2-propanediol. Of the agents tested, sorbitol had the highest OCP, twice that for xylitol, glycerol, and 1,3-propanediol. In turn, xylitol, glycerol and 1,3-propanediol had twice the OCP of ethylene glycol and 1,2-propanediol.

The RSR of each agent showed similar trends in human skin [Fig. 2(b)]. OCP values were slightly greater in human skin as compared to rodent, indicative of a greater optical clearing effect. Three groups were found to be significantly different ($p \leq 0.05$): sorbitol; glycerol and 1,3-propanediol; and ethylene glycol and 1,2-propanediol. The RSR data of xylitol were not significantly different from ethylene glycol, glycerol, 1,2- or 1,3-propanediol. Sorbitol had the highest OCP in human skin. Glycerol and 1,3-propanediol had twice the OCP of ethylene glycol and 1,2-propanediol.

Our data indicate that a chemical agent’s ability to suppress hydrogen-bond-mediated attractive forces within collagen directly correlates with its OCP. Sorbitol had twice the collagen solubility of glycerol and, correspondingly, exhibited twice the OCP. Traditionally, refractive index matching with collagen ($n=1.45−1.55$) had been used empirically to screen and select potential chemical agents for tissue optical clearing.3,13 The propanediols used in our study had similar potential using in vitro human skin. Some sugars and polyols inhibit fibrillogenesis of type I collagen by disrupting hydrogen-bonded water bridges between the helices, Biochemistry 37, 11,888−11,895 (1998).

Many of the effective optical clearing chemical agents presented herein are already used as sweetening additives in foods and emollients in skin care products. A remaining challenge for their use in conjunction with light-based dermatologic therapeutics (and diagnostics) is percutaneous delivery through the intact stratum corneum of human skin. Some success has been reported when chemical agents were chaperoned with lipophilic compounds.15 We believe that the results reported herein provide a foundation for rational design and development of effective, topically-applied, optical clearing formulations.

Acknowledgments

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Table 1

Chemical agent osmolarity (Osm/kg), refractive index ($n_D$, 20°C), and molecular weight.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Osmolarity (Osm/kg)</th>
<th>Refractive Index</th>
<th>Molecular Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sorbitol</td>
<td>15.2</td>
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<td>182.17</td>
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<tr>
<td>Xylitol</td>
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<td>1.45</td>
<td>152.15</td>
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<tr>
<td>Glycerol</td>
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<tr>
<td>1,3-Propanediol</td>
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</tr>
<tr>
<td>1,2-Propanediol</td>
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<td>1.43</td>
<td>76.10</td>
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<tr>
<td>Ethylene Glycol</td>
<td>9.0</td>
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References