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Letter to the Editor

Optical Clearing of In Vivo Human Skin: Implications for Light-Based Diagnostic Imaging and Therapeutics

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The complex morphological nature of human skin provides a highly scattering medium for visible and near-infrared wavelengths due to variations in the indices of refraction of different components therein. Scattering diminishes the depth and clarity of images in light-based diagnostic imaging and attenuates the effective light dose that reaches targeted chromophores in laser therapeutics [1]. Light-based diagnostic techniques and therapeutics would likely be improved if scattering could be reduced, thereby enhancing light penetration into human skin.

One method to enhance light penetration into skin is the use of hyperosmotic and biocompatible agents that induce an optical clearing effect [2]. Hyperosmotic agents result in refractive index matching between cells and ground substance in the dermis, which is believed to cause a reduction in optical scattering. Studies done by Vargas et al. [3] and Wang et al. [4], demonstrated that the injection of hyperosmotic agents into rat dermis could significantly reduce light scattering and thereby enhance the imaging depth of non-invasive techniques such as optical coherence tomography (OCT). If delivered by injection into the dermis, these hyperosmotic agents tend to dehydrate the skin and reduce the index mismatch between inter/intracellular components [5].

Although glycerol and polyethylene glycol (PEG) reduce optical scattering in human skin, their clinical utility has been very limited. Penetration of glycerol and PEG through intact skin is very minimal and extremely slow, because these agents are hydrophilic and penetrate the lipophilic stratum corneum poorly. In order to enhance skin penetration, these agents need to be either injected into the dermis or the stratum corneum has to be removed, mechanically (e.g., tape stripping) or thermally (e.g., erbium: YAG laser ablation). Clinical utility would be improved if the clearing agent could be applied topically onto intact skin and thereafter migrate across the stratum corneum and epidermis into the dermis.

In this letter, we present our evaluation of the use of topically applied optical clearing agents in vitro as well as in vivo. Food and Drug Administration (FDA) approved lipophilic polypropylene glycol-based polymers (PPG) and hydrophilic PEG-based polymers, both with indices of refraction that closely match that of dermal collagen (1.47) were studied alone and in a combined pre-polymer mixture.

To measure the optical properties (diffuse transmission and surface reflectance) of freshly excised in vitro human skin, an integrating sphere was used [6] with an inverse adding doubling algorithm [7] to estimate the reduced scattering coefficient (μ_s') of the samples. The optical clearing potential (OCP) was defined as the ratio of μ_s' immediately before and 24 hours after, agent application. Optical clearing agents including, glycerol and PPG- and PEG-based polymers compounds were applied alone, and in a PPG- and PEG-based combined pre-polymer mixture, topically on the epidermal side of the skin samples which were left undisturbed in an incubator at 37°C for 24 hours. The OCP was found to be significantly higher for the lower molecular weight combined PPG- and PEG-based pre-polymer mixture as compared to glycerol or either pre-polymer alone.

The combined PPG- and PEG-based pre-polymer mixture was also evaluated for its OCP in vivo. The volar forearm of a healthy volunteer was pre-cleaned, shaved and, divided into two areas, i.e., untreated control and where the combined pre-polymer mixture was topically applied onto the skin surface in the form of a thick layer under occlusion. After 2 hours, the mixture was removed and cross-polarized images of the hair shafts were obtained. Visualization of the intradermal portion of the hair shaft (approximately 1 mm deep) was enhanced on the area where the combined pre-polymer mixture was applied (Fig. 1).

The combined PPG- and PEG-based pre-polymer mixture was topically applied to a 2×2 cm area on the leg of a healthy volunteer (that contained vascular telangiectasias). Cross-polarized images of the vessels before, and 2 hours after application under occlusion were obtained. Visibility of the telangiectasias was enhanced (approximately 500 μm deep) and smaller telangiectatic branches

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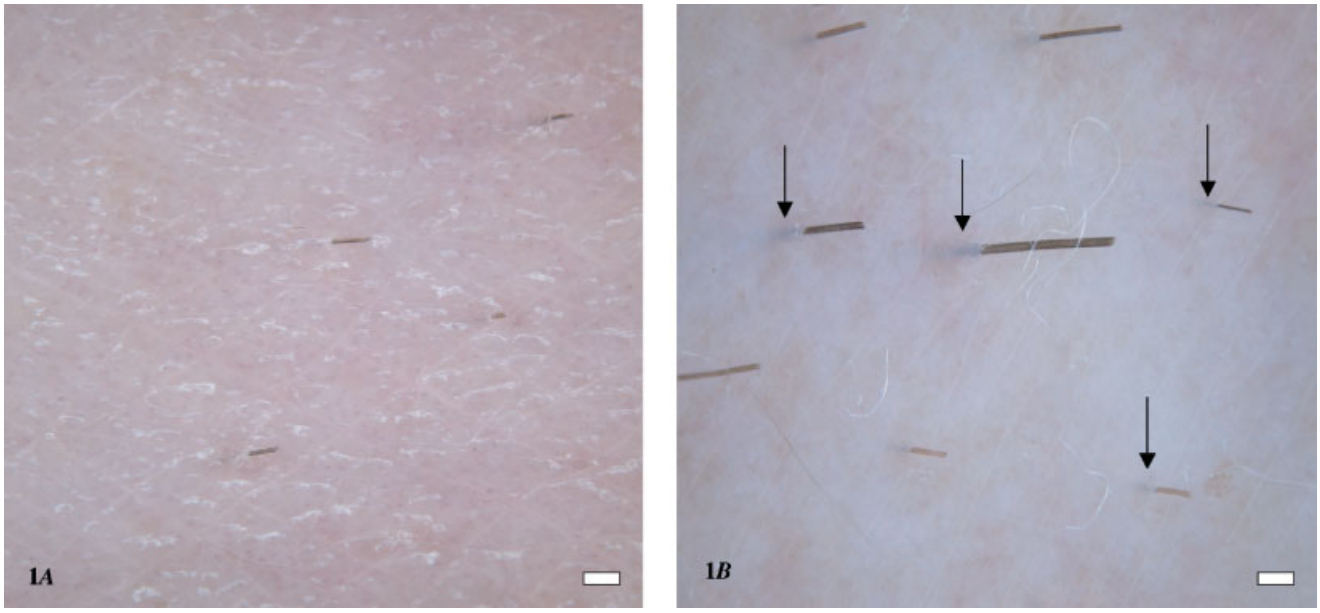

Fig. 1. Cross-polarized images of in vivo human skin before (**A**) and 2 hours after (**B**), topical application of the combined PPG- and PEG-based pre-polymer mixture. Note enhanced visibility of the intradermal portion of the hair shaft (arrows, scale bar = 1 mm).

previously obscured, were seen clearly after the combined pre-polymer mixture was applied (Fig. 2).

The ability of the combined PPG- and PEG-based pre-polymer mixture to enhance the contrast among skin components was also studied using OCT. The lateral field of view was adjusted to 5 mm and the maximum imaging depth in control skin was 1.5 mm. The volar forearm of a healthy volunteer was divided into three, 2×2 cm areas. After baseline images of each area were obtained by OCT, the following were topically applied to the different test areas: combined PPG- and PEG-based polymer mixture, glycerol, or immersion oil. All test areas were covered by a tegaderm dressing and subsequently imaged 5, 10, 30, 90, and 120 minutes after occlusion.

Surface reflection from the stratum corneum was significantly reduced by all three agents after 5 minutes of occlusion. Enhanced contrast among various components in the stratum corneum, epidermis, and dermis was seen 30 minutes after occlusion of the combined PPG- and PEG-based pre-polymer mixture. Images obtained

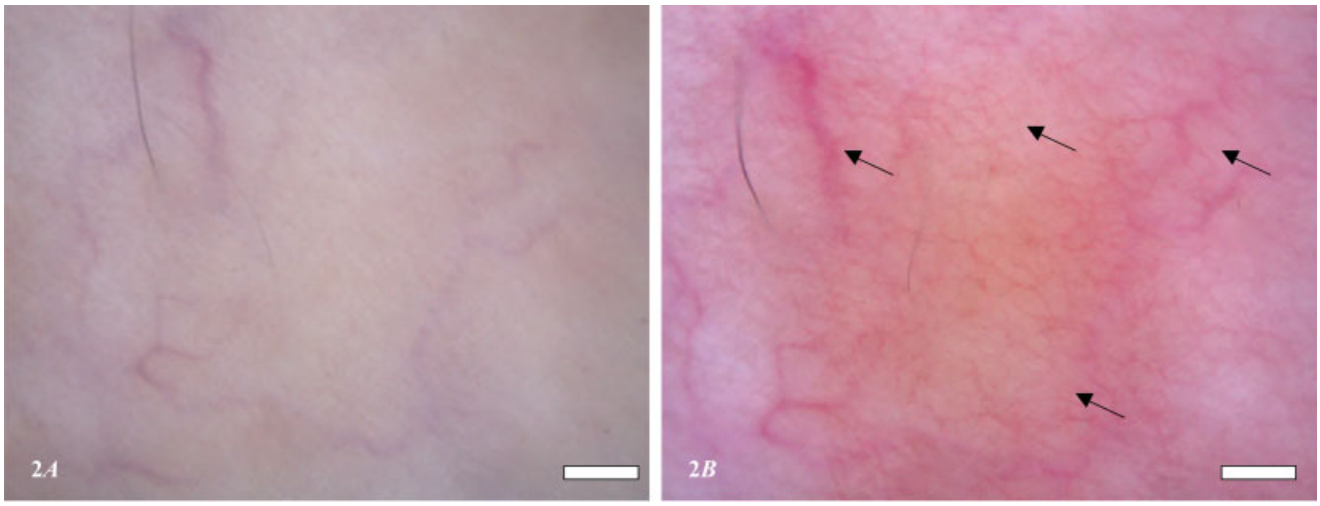

Fig. 2. Cross-polarized images of telangiectasias before (**A**) and 2 hours after (**B**), topical application of the combined PPG- and PEG-based pre-polymer mixture. Noteworthy is the finding that smaller branches of the main vessel, previously invisible, have become more obvious (arrows, scale bar = 3 mm).

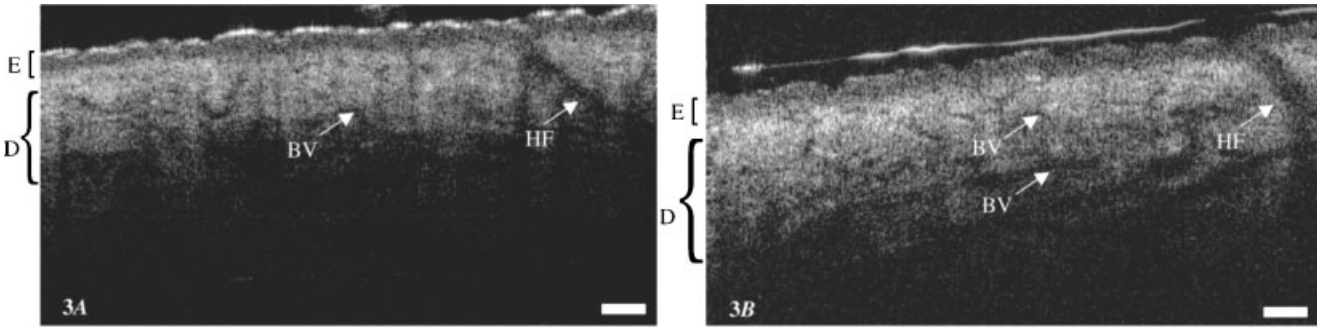

Fig. 3. OCT images of in vivo human skin before (**A**) and 90–120 minutes after (**B**), the topical application of the combined PPG- and PEG-based pre-polymer mixture. Noteworthy is the absence of surface scattering and enhancement of dermal vasculature and hair follicles (arrows, scale bar = 200 μm). E = epidermis, D = dermis, HF = hair follicle, BV = blood vessels.

90–120 minutes after occlusion showed significant improvement in contrast enhancement (Fig. 3) and an increase in imaging depth (from 1.5 mm to approximately 1.75 mm). In particular, imaging of dermal vasculature and hair follicles was substantially improved. OCT imaging of the areas where glycerol and immersion oil were applied, did not show any enhancement in contrast or imaging depth over 120 minutes.

These intriguing findings merit further discussion. Although glycerol is an effective optical clearing agent when injected into the dermis, it does not penetrate intact human skin. Our preliminary experiments show that the combined PPG- and PEG-based polymer mixture can penetrate intact skin and reduce dermal scattering and significantly enhance OCP. The mechanism of optical clearing by these topical agents remains incompletely understood. It is, however, speculated that the PPG-based polymer acts as a permeation enhancer because of its highly lipophilic nature, which allows better penetration of the hyperosmotic PEG-based polymers, which are hydrophilic.

The concept of reduced tissue optical scattering using topically applied clearing agents can benefit a number of light-based diagnostic imaging techniques and therapeutic applications. Although the combined PPG- and PEG-based pre-polymer mixture is hyperosmotic with a high refractive index (1.47), it was demonstrated by OCT to enhance contrast among dermal components and to reduce scattering in the stratum corneum, epidermis and dermis, thereby increasing imaging depth [8]. Optical clearing of skin, in vivo, might improve non-invasive diagnostic imaging techniques by providing higher resolution of cellular microstructure over a greater imaging depth.

Reduction in skin surface and dermal scattering might also improve light-based (e.g., laser) therapeutics. The highly turbid nature of human skin reduces the incident light that reaches targeted chromophores. A significant reduction in dermal scattering induced by use of topically applied clearing agents will allow more photons to reach the target. This, in turn, should reduce the amount of energy, from lasers and other light sources, required to achieve the desired clinical effect such as the removal of port wine stains and other hypervascular skin lesions, hair, benign pigmented skin lesions, and tattoos.

Our results clearly demonstrate that a combined PPG- and PEG-based polymer mixture causes optical clearing in vitro and in vivo when applied topically onto intact human skin. This pre-polymer mixture is FDA-approved, safe, effective and well tolerated for optical clearing of skin in vivo. Prospective, comparative, and controlled clinical studies on a multi-center basis against accepted treatment regimens are required so that the role of topically applied clearing agents to assist the treatment of various dermatoses amenable to laser therapy can be fully defined.

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