

Letter to the Editor

Can Topically Applied Optical Clearing Agents Increase the Epidermal Damage Threshold and Enhance Therapeutic Efficacy?

Human skin is a complex and highly scattering medium at visible and near-infrared wavelengths due to variations in refractive indices of different constituents. In light-based therapeutics, epidermal and dermal scattering diminishes the depth of light penetration and attenuates the effective fluence that reaches the targeted chromophores [1]. As a result, higher radiant exposures or fluences may be required to produce the desired therapeutic effect, particularly for more deeply located chromophores. Higher fluences can be beneficial but also increase the risk of epidermal and dermal injury, which can result in dyspigmentation and scarring.

Epidermal injury can be reduced by topically applying certain hyperosmotic solutions prior to laser therapy, such as sucrose, glycerol, and water soluble gels (e.g., surgilube) that have a refractive index matching closely to that of stratum corneum, i.e., ~ 1.4 . Thus, surface scattering from incident light is thereby reduced and the overall intensity of backscattered light is also reduced in the superficial epidermis. However, such solutions are highly hydrophilic and penetrate intact skin very poorly when applied topically [2–4].

Our group has recently reported the use of topically applied hyperosmotic, optical clearing agents composed of a pre-polymer mixture of polypropylene glycol and polyethylene glycol, PPG:PEG that can penetrate intact human skin [5] and temporarily alter local optical properties *in vivo*. Epidermal and papillary dermal scattering events are reduced significantly and more light penetrates deeper into the skin. Moreover, reduced epidermal and dermal back scattering increases the threshold for epidermal injury, which allows safe use of higher fluences. In conclusion, by changing the optical properties of human skin *in vivo* and reducing epidermal and dermal scattering, more photons will reach deeply located chromophores such as hair follicles, tattoo pigments, sebaceous glands, and blood vessels more effectively.

In this letter, we present the use of a topically applied clearing agent (PPG:PEG) prior to Q-switched 532 and 694 nm laser treatment of human skin *ex vivo* and *in vivo*, respectively. Freshly excised skin samples (skin phototype, SPT III) were obtained and randomly divided into three groups. Group I served as control and no topical agents were applied prior to laser exposure. For group II, an index matching lotion (surgilube, refractive index ~ 1.38) was

topically applied on skin samples prior to laser exposure. For group III, a clearing agent (PPG:PEG, refractive index = 1.47) was topically applied prior to laser exposure. Frequency doubled, Q-switched Nd:YAG laser, ($\lambda = 532$ nm) was used at a fluence of 2.8 J/cm^2 with a 2 mm diameter spot size to create a single pass of exposures on skin samples. Punch biopsies were obtained immediately after laser exposure and processed for fresh frozen sectioning and hematoxylin and eosin staining (H&E) (Fig. 1). Control skin samples, without prior topical application, showed significant basal cell vacuolization and separation at the dermo-epidermal junction (DEJ) at various points (Fig. 1A). Group II skin samples, with prior topical application of an index matching lotion, showed less basal cell vacuolization as compared to the control (Fig. 1B). However, cleaving and separation of the DEJ was still seen. Group III skin samples, with prior application of a clearing agent (PPG:PEG) showed no basal cell vacuolization or cleaving or separation of the DEJ (Fig. 1C).

The effects of topically applied clearing agents prior to laser treatment of dermal chromophores (such as tattoos) were also studied *in vivo*. A 40-year-old man (SPT IV) seeking laser tattoo removal for a 15-year-old India ink arm tattoo was recruited in an Institutional Review Board (IRB) approved study. Q-switched ruby laser ($\lambda = 694$ nm) was used at a fluence of 6 J/cm^2 with a 4 mm diameter spot size. The region of interest was divided into three areas; area (1) control, no agent or laser treatment; (2) laser alone; (3) skin pre-treated with optical clearing agent (2 hours under occlusion) followed by laser treatment (Fig. 2A). No post-operative wound care was provided for any areas. One day post-treatment, the patient developed several hemorrhagic blisters on area 2 while area 3 showed only minimal

Contract grant sponsor: Arnold and Mabel Beckman Fellows Program (to BC); Contract grant sponsor: National Institutes of Health (to JSN); Contract grant numbers: 47551, 48458; Contract grant sponsor: National Institutes of Health (to JSN); Contract grant number: 002495; Contract grant sponsor: Dermatology Foundation (to KMK).

*Correspondence to: Misbah H. Khan, MD, Beckman Laser Institute and Medical Clinic, University of California, Irvine, California 92612. E-mail: mhkhan@uci.edu

Accepted 13 May 2004

Published online in Wiley InterScience

(www.interscience.wiley.com).

DOI 10.1002/lsm.20078

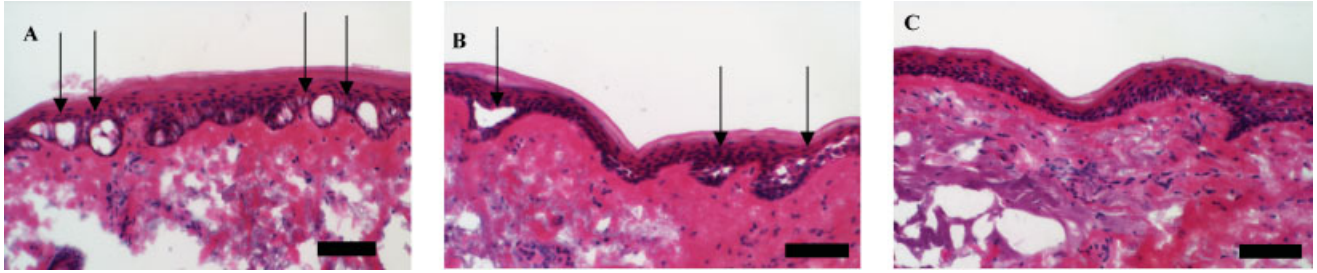


Fig. 1. **A–C**: Histologic vertical sections of human skin immediately after a single exposure to a 532 nm Q-switched laser. **A**: control; **(B)** skin pre-treated with surgilube; and **(C)** skin pre-treated with PPG:PEG. Of note is the extensive basal cell vacuolization and clefting in **(A and B)** (arrows) and the absence thereof in **(C)**. (hematoxylin and eosin (H&E) stain, 200 \times , scale bar 100 μ m).

erythema (Fig. 2B). At 1 month follow-up, significant clearing of tattoo in area 3 was observed as opposed to only minimal clearing in area 2 (Fig. 2C).

Although, it is currently not possible to model precisely the effective light distribution in human skin due to local changes in the scattering coefficient, it is instructive to investigate the effects of gross changes in epidermal scattering. We used the freeware Monte-Carlo multilayered (MCML) code [6] to perform first order calculations

of the heat source term (J/cm^3) in tattooed skin, assuming a slab geometry. Optical properties were derived from data presented in various sources [1,7,8]. Epidermal and superficial papillary dermal (upper 200–300 μ m thickness) scattering coefficients were varied from 0.4 mm^{-1} (assumed to be native skin at 694 nm) to decreased values of 0.2 and 0.1 mm^{-1} after the application of clearing agents. The resulting heat source term profiles (Fig. 3) show $\sim 40\%$ decrease at the DEJ due to reduced epidermal scattering,

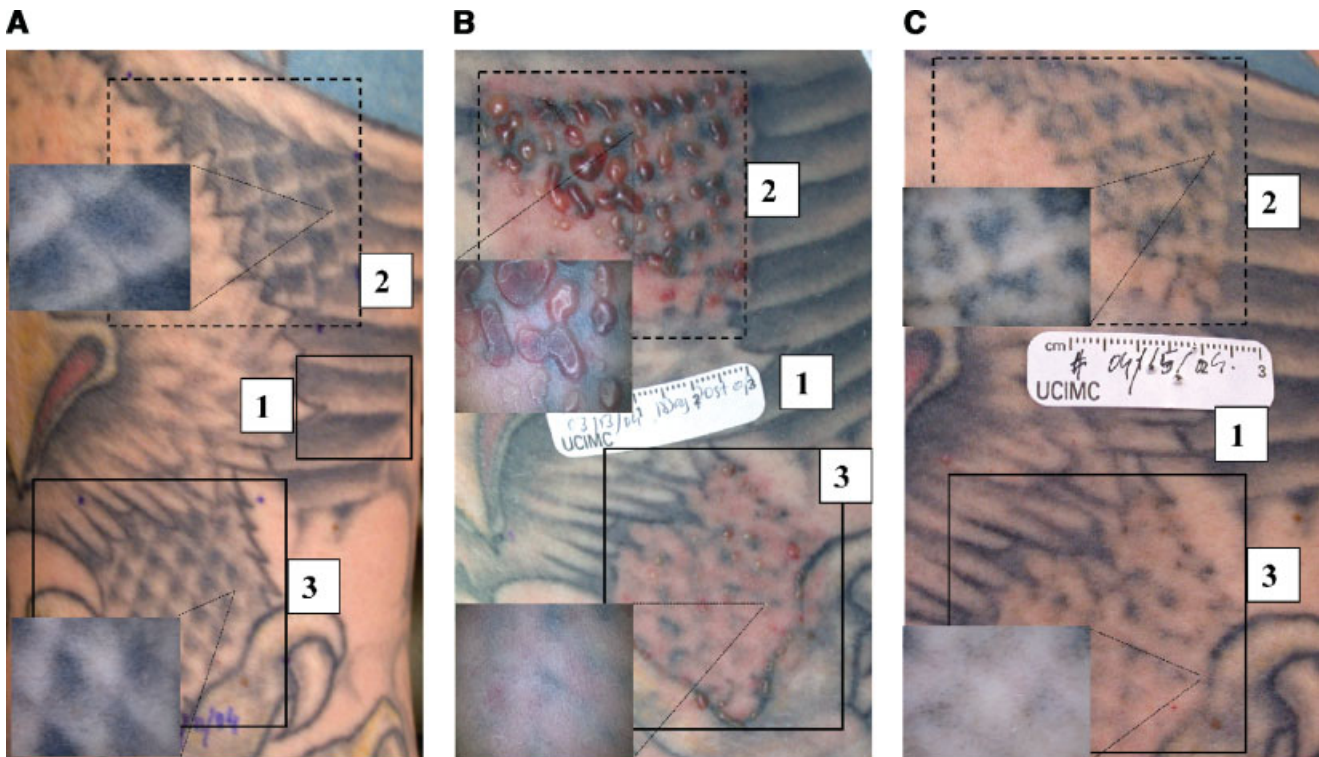


Fig. 2. **A–C**: Gross and close-up digital images of a 15-year-old tattoo before and after laser treatment. **A**: before treatment, **(lane 1)** control, no agent or laser treatment; **(lane 2)** laser alone; **(lane 3)** topical application of clearing agent followed by laser treatment. Insets represent cross-polarized close-up views (4 \times) of areas 2 and 3. **B**: One day post-treatment. Insets

represent linear-polarized close-up views (4 \times) of the surface topography of areas 2 and 3. Of note is the extensive blistering in area 2. **C**: One month after single laser treatment. Insets represent cross-polarized close-up views (4 \times) of areas 2 and 3. Note the significant clearing of the tattoo ink in the area pre-treated with clearing agent.

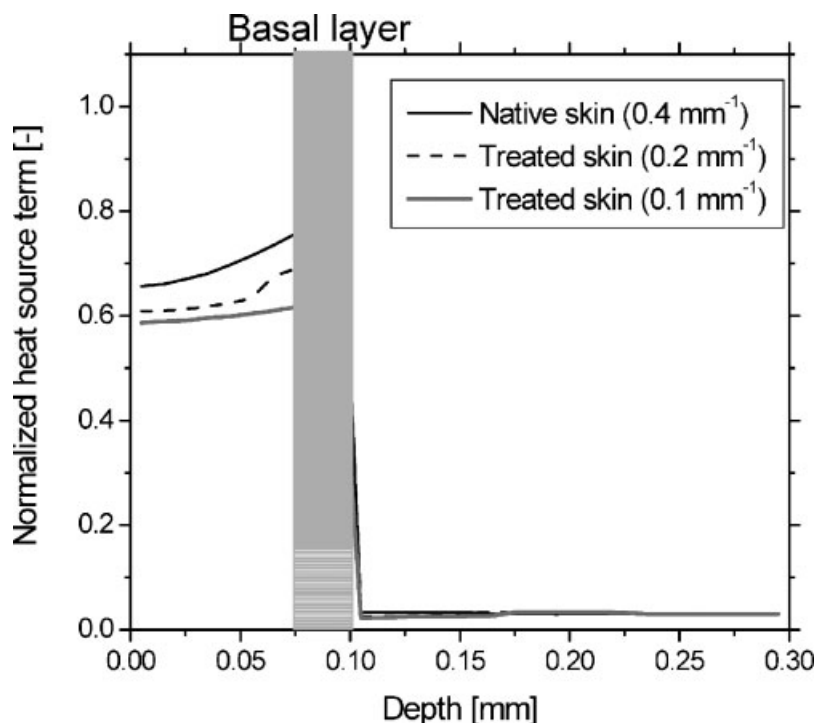


Fig. 3. Normalized heat source term in the upper 0.3 mm of human skin, calculated with a Monte-Carlo simulation of laser tattoo removal. Note that the tattoo is modeled at a depth of 1 mm and thus not included here. The numbers in parentheses in the figure legend represent assumed epidermal

scattering coefficients before (black solid line) and at two stages after clearing agent application. The heat source term in the epidermis, and in particular at the DEJ, decreases as epidermal scattering coefficient is decreased.

thus serving as preliminary support for our current working hypothesis.

Our *in vivo* results (Fig. 2) suggest that a reduction in optical scattering in human skin improves the outcome of light-based therapeutic procedures. Topically applied clearing agents (PPG:PEG) allow more photons to reach the target as well as reduce the back scattered light. Higher fluences for deeper dermal targets can be used much more safely and effectively without damaging the epidermis, especially when treating patients with darker skin types.

PPG:PEG is safe, effective, and well tolerated for optical clearing of human skin. Prospective, comparative, and controlled clinical studies are being performed at our Institute so that the role of topically applied optical clearing agents in light-based therapeutics can be fully understood.

ACKNOWLEDGMENTS

We thank Dr. Brain Wong for his support.

Misbah H. Khan, MD^{1*}
 Samuel Chess, BS²
 Bernard Choi, PhD¹
 Kristen M. Kelly, MD^{1,2}
 J. Stuart Nelson, MD, PhD^{1,2}

¹Beckman Laser Institute and Medical Clinic

²Department of Dermatology
 University of California, Irvine
 California 92612

REFERENCES

- Jacques SL. Skin optics. <http://omlc.ogi.edu/news/jan98/skinoptics.html>
- Vargas G, Chan EK, Barton JK, Grady HG III, Welch AJ. Use of an agent to reduce scattering in the skin. *Lasers Surg Med* 1999;24:133–141.
- Wang RK, Tuchin VV, Xu X, Elder JB. Concurrent enhancement of imaging depth and contrast for optical coherence tomography by hyperosmotic agents. *J Opt Soc Am B* 2001; 18: 948–953.
- Tuchin VV, Maksimova IL, Zimnyakov DA, Kon IL, Mavlutov AK, Mishin AA. Light propagation in tissues with controlled optical properties. *J Biomed Opt* 1997;2:401–417.
- Khan MH, Choi B, Chess S, Kelly K, McCullough J, Nelson JS. Optical clearing of *in vivo* human skin; implications for light-based diagnostic imaging and therapeutics. *Lasers Surg Med* 2004;34:83–85.
- Wang L, Jacques SL, Zheng L. MCML-Monte-Carlo modeling of light transport in multi-layered tissues. *Comput Meth Prog Bio* 1995;47:131–146.
- Ho DDM, London R, Zimmerman GB, Young DA. Laser-tattoo removal—a study of the mechanism and the optimal treatment strategy via computer simulations. *Lasers Surg Med* 2002;30: 389–397.
- Taylor CR, Anderson RR, Gange RW, Michaud NA, Flotte TJ. Light and electron-microscopic analysis of tattoos treated by Q-switched ruby laser. *J Invest Dermatol* 1991;97:131–136.