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Journal

Lasers in Surgery and Medicine, 42(5)

ISSN

0196-8092

Authors

Yoon, Jinhee
Park, Donghee
Son, Taeyoon
et al.

Publication Date

2010-07-01

DOI

10.1002/lsm.20930

Published in final edited form as:
Lasers Surg Med. 2010 July ; 42(5): 412–417. doi:10.1002/lsm.20930.

A Physical Method to Enhance Transdermal Delivery of a Tissue Optical Clearing Agent: Combination of Microneedling and Sonophoresis

Jinhee Yoon, MS[1], **Donghee Park, MS**[1], **Taeyoon Son, MS**[1], **Jongbum Seo, PhD**[1,2], **J. Stuart Nelson, MD, PhD**[3], and **Byungjo Jung, PhD**[1,2,*]

[1]Department of Biomedical Engineering, Yonsei University, Wonju-si 220-710, Korea

[2]Institute of Medical Engineering, Yonsei University, Wonju-si 220-710, Korea

[3]Beckman Laser Institute, University of California, Irvine, California 92612

Abstract

Background and Objectives—Various physical methods, such as microneedling, laser ablation, sonophoresis, and sandpaper, have been widely studied to enhance the transdermal delivery of tissue optical clearing (TOC) agents. A previous study demonstrated that the microneedling method could effectively enhance the permeability of a TOC agent through the skin barrier.

Study Design/Materials and Methods—In this study, we introduce a new physical combination method which utilizes both microneedling and sonophoresis to further enhance the transdermal delivery of a TOC agent, glycerol. Porcine skin samples were divided into a control group treated only with the microneedle roller and a test group treated with both the microneedle roller and sonophoresis. Glycerol was applied topically after microneedling. The optimal concentration and transdermal delivery efficacy of glycerol were quantitatively evaluated.

Results—A 70% glycerol solution was determined to be the optimal concentration for the combination method. The combination method resulted in approximately a 2.3-fold higher transdermal diffusion rate of glycerol when compared to the microneedling method alone.

Conclusion—The combination method and optimal glycerol concentration effectively enhanced transdermal delivery of glycerol by accelerating the diffusion rate through the skin barrier.

Keywords

glycerol; tissue optical clearing; microneedling; sonophoresis

INTRODUCTION

Tissue turbidity limits the penetration depth of light and, therefore, the efficacy of light diagnosis and therapy. Tissue optical clearing (TOC) agents, such as glycerol, glucose, PPG/PEG, dimethyl sulfoxide (DMSO), and oleic acid, have been studied for their TOC potential and, therefore, enhanced light penetration depth [1]. The mechanisms of TOC were generally described as dehydration of tissue ingredients, partial replacement of interstitial fluid by the TOC agents, and structural modification or dissociation of collagen. The first

*Correspondence to: Byungjo Jung, PhD, Department of Biomedical Engineering, Yonsei University, 234 Maeji, Heungup-myun, Wonju-si, Gangwon-do 220-710, Korea. bjung@yonsei.ac.kr.

and second mechanisms mostly contribute to the refractive index matching of tissue scatterers [2–5].

Vargas et al. [6,7] reported the TOC effect using glycerol and observed the enhancement of Doppler signals from in vivo hamster skin. Glycerol is biocompatible and generally considered as a safe chemical agent by the Food and Drug Administration (FDA) [8]. However, glycerol does not sufficiently diffuse into the dermis because the stratum corneum acts as a skin barrier. Various physical methods have been studied to enhance the permeability of TOC agents into the dermis. McNichols et al. [9] demonstrated that the injection of TOC agents induced rapid dermal clearing but also resulted in necrosis and scarring. Stumpp et al. [10] observed the enhancement of TOC when glycerol was topically applied on skin treated with sandpaper. Also, the delivery of topically applied glycerol could be enhanced by using a 980-nm diode laser [11]. Xu and Zhu [12,13] demonstrated the feasibility of sonophoresis to enhance the delivery of topically applied TOC agents. The sonophoresis resulted in cavitation, thermal, and mechanical effects and, thus, enhanced the permeability of agents into the skin [14–16]. Our previous study reported the efficacy of the microneedling method in TOC [17].

In order to enhance further the transdermal delivery efficacy of glycerol, this study suggests a new physical combination method which utilizes both the microneedle roller and sonophoresis. The microneedle roller created artificial microchannels in ex vivo porcine skin and a 70% glycerol solution was topically applied onto the skin samples. Finally, sonophoresis was applied to the topically applied glycerol. The TOC efficacy was quantitatively evaluated by computing the image contrast of a modulation transfer function (MTF) target underneath the skin samples. In addition, an in vivo mouse study was implemented in order to investigate the dermatological feasibility.

MATERIALS AND METHODS

Sample Preparation

In order to evaluate the TOC effect by the physical methods, ex vivo porcine skin samples were prepared without the adipose layer and divided into two groups: (1) control group (1.79 mm in average thickness) treated only with the microneedle roller and (2) test group (1.82 mm in average thickness) treated with the microneedle roller and sonophoresis. The skin samples were placed on a MTF target, and 0.1 ml of 70% glycerol was topically applied. In order to prevent glycerol leakage under the skin samples during sonophoresis application, the edges of the skin samples were sealed with transparent tape. Repeatable experiments were performed with three different samples at a room temperature of 24.5°C.

Three additional skin samples were prepared to evaluate the variation of tissue thickness and weight due to the application of glycerol and physical methods. The average thickness and weight of the control sample group were 2.14 mm and 2.96 g, respectively, and of the test sample group were 2.20 mm and 3.03 g, respectively. The changing ratio of the tissue thickness and weight was evaluated after 60 minutes of glycerol application.

Microneedle Roller and Sonophoresis Device

The microneedle roller with 192 needles (8 circular arrays of 24 needles each) was used to create artificial microchannels (Fig. 1a). Each needle had a 70 μm diameter and 500 μm length [18,19]. In general, 240 channels/cm^2 were created after 10–15 repeated applications of the microneedle roller over the same skin site [19,20]. In the experiments, the microneedle roller was gently applied 50 times to minimize the variability in the skin sample thickness due to overpressure during microneedling. A microchannel created by the microneedle roller was imaged with an optical coherence tomography (OCT) system.

Figure 1b shows the sonophoresis device (Ultrasound DM-77; Daeyang, Wonju, Korea) used to accelerate the permeability of the topically applied glycerol. The device was operated in a continuous mode with 1 MHz frequency and average power of 2W to ensure that the sample temperature did not exceed 40°C. The transducer was gently placed on the topically applied glycerol with sufficient contact pressure.

Optical Property Measurement

The optimal concentration of glycerol was investigated with 50%, 60%, 70%, 80%, and 90% glycerol solutions. The skin samples with a thickness of 3.3 ± 0.4 mm were prepared for each glycerol concentration. Microneedling was applied to the skin samples as described above, and then, glycerol was topically applied. The transmittance and reflectance spectra of each sample were measured once with a double-integrating sphere system before and after 60 minutes of glycerol application. The reduced scattering coefficients were computed at a wavelength of 650 nm with an Inverse-Adding Doubling program (Scott Prahl, Oregon Medical Center). Three experiments were repeated with different samples.

Diffuse Reflectance Imaging of Sample

Diffuse reflectance images of ex vivo porcine skin samples were obtained with a polarization variable imaging system (MagVision; Optobiomed, Wonju, Korea) consisting of a linearly polarized LED ring light and rotating linear analyzer. A CMOS camera (Canon 350D, Canon, Tokyo, Japan) was utilized to acquire diffuse reflectance images which were taken every 10 minutes for 60 minutes in cross-polarization mode. Figure 2 illustrates the experimental set-up including the imaging system.

Quantitative Evaluation of Tissue Optical Clearing Efficacy

The region of interest (ROI) of 150 × 150 pixels (about 1.5 × 1.5 cm^2 in actual size) was extracted for analysis. TOC efficacy was quantitatively evaluated by computing the contrast and relative contrast (RC) of the MTF target underneath the skin samples [17]:

$$\text{contrast}=\frac{I_{\max}-I_{\min}}{I_{\max}+I_{\min}} \quad (1)$$

$$\text{RC}=\frac{\text{MC}_t}{\text{MC}_0} \quad (2)$$

where MC_0 and MC_t are the mean contrast of MTF on the skin sample at time 0 and after *t* minutes of glycerol application, respectively. The MC was calculated as follows: (1) the column pixels of ROI were added and mean values at each column were calculated; (2) finally, the MC was computed by averaging the contrasts of MTF at four locations as indicated in Figure 6.

RESULTS

Figure 3 shows the OCT image of a microchannel formed by the microneedle roller. As indicated by the scale bar, the microchannel depth was about 470 μm which is approximately the same length as the microneedle roller. Figure 4 shows the percent reduction of the normalized reduced scattering coefficient as a function of glycerol concentration. After 60 minutes of glycerol application, the normalized reduced scattering coefficients decreased by 26.6%, 32.6%, 54.5%, 40.77%, and 43.13% at the glycerol concentrations of 50%, 60%, 70%, 80%, and 90%, respectively. Figure 5 illustrates the

effects of the TOC on the control (right images) and test (left images) samples after glycerol application. Over time, visualization of the MTF target on both groups was observed. However, the MTF target on the test group was more clearly observed when compared to the control group after an identical period of time. The TOC was obviously observed after 40 minutes of glycerol application on the test group while only slightly visualized after 60 minutes of glycerol application on the control group. Figure 6 shows the quantitative evaluation of MTF as a function of time. As expected, the test group resulted in better image contrast as compared to the control group. Figure 7 presents the RC of MTF which quantitatively demonstrates the TOC efficacy. The RCs of the test (control) group after 20, 40, and 60 minutes of glycerol application increased by a factor of 3.3 (2.3), 15.2 (6.3), and 33.6 (14.7), respectively. The tissue thickness and weight were reduced 5.50% and 7.26%, respectively, in the control sample group, and 9.30% and 9.88%, respectively, in the test sample group. Figure 8 shows an in vivo optical clearing image of mouse skin following the application of the combination method. Microvascular structures are clearly shown after 20 minutes of glycerol application, confirming the efficacy of the combination method.

DISCUSSION

TOC agents have been used to enhance light penetration depth by minimizing light scattering by tissue. Various physical methods, such as microneedle roller, sand paper, sonophoresis, and laser ablation, have been evaluated to improve the transdermal delivery efficacy of TOC agents [10–12,17,21–23]. However, such methods still have little clinical utility in terms of TOC efficacy due to the long time required for the agent to diffuse across the skin barrier. In order to accelerate the transdermal delivery of glycerol, this study introduced a new physical method which combines the microneedle roller and sonophoresis.

The optimal concentration of glycerol was determined because it may affect the transdermal diffusion time and, therefore, TOC efficacy. In our previous study, the intensity profile of the laser beam demonstrated that a 70% concentration of glycerol was the most effective for TOC in ex vivo porcine skin samples [24]. In addition, a recent study presented a similar result that about 70% glycerol was the most effective concentration for TOC [25]. Figure 4 shows that 70% glycerol might be the optimal concentration for the combination method. The normalized reduced scattering coefficient after 60 minutes of 70% glycerol application decreased by 54.5% as compared to the baseline control value. The diffusion of the TOC agent into the skin may be explained by Fick’s law of diffusion in which skin permeability is inversely proportional to the concentration [26]. As a result, lower glycerol concentration has to have a better diffusion capability. However, 70% glycerol resulted in remarkable decrease of reduced scattering coefficient. It might be that glycerol concentration lower than 70% does not have sufficient osmolarity to induce better TOC effects. For clinical application, a trade-off between the concentration and osmolarity of the TOC agent may be needed to obtain the appropriate TOC effect depending on the purpose.

TOC was visually demonstrated with the MTF target underneath the skin samples (Fig. 5). The TOC was observed after 20 minutes of glycerol application on the test sample (left image of Fig. 5b). However, TOC was not observed on the control sample after identical periods of time. The results might be explained by the fact that the artificial microchannels enhance the diffusion rate of glycerol and sonophoresis accelerates the permeability of glycerol into the skin.

The microneedle roller can easily create multiple artificial microchannels in the skin surface in order to enhance the transdermal delivery of glycerol [17]. The OCT image shows that a channel is around 70–100 μmin diameter and 470 μm in depth. Thus, skin epidermis should be well perforated and the main pathway for glycerol permeation and distribution in the skin

should be dermis. Therefore, it is easy to estimate that when the pathway for glycerol diffusion via dermis is opened, the TOC effect and its kinetics might be defined by diffusion of glycerol and induced opposite water-flux through dermis [27]. Similarly, Bashkatov et al. recently reported that laser-perforated stratum corneum enhanced the diffusion rate of glycerol and therefore, tattoo image contrast [28].

Sonophoresis also enhances the efficacy of transdermal drug delivery by thermal and non-thermal mechanisms [14–16,29–31]. The absorbed vibrating energy increases local temperature, called thermal effects, which affects the viscosity of glycerol. The viscosity of 70% glycerol varies from 22.5 at 20°C to 9.4 at 40°C. Therefore, the reduction of viscosity might result in enhancement of skin permeability [15,16,26,32]. Due to the limited amount of heat that can be safely applied to the skin surface without inducing damage, the temperature was maintained below 40°C in this study. A non-thermal mechanism including cavitational and mechanical phenomena, and acoustic streaming, might also contribute to the sonophoresis-mediated transdermal delivery of glycerol. Cavitation might induce a temporal disorder of the structural lipids located in the intercellular bridges of the stratum corneum and form the temporal channels for transcutaneous molecular diffusion [33].

The TOC efficacy was quantitatively evaluated by computing the contrast of the MTF (Figs. 6 and 7). After 20 minutes of glycerol application, the difference of RC in both groups was observed and gradually increased as a function of time. The TOC in the test group was much greater than in the control sample (Figs. 6 and 7). After 30 minutes, the RC of the test group was a factor of 2.1 higher as compared to the control group.

Figure 8 shows the dermatological feasibility of the combination method in terms of diffusion time reduction of glycerol. A previous study showed that it takes 2 hours after TOC agent application to obviously observe the TOC effect in human skin [34]. Unlike human skin, the in vivo mouse skin did not present a TOC effect with only topical application depending on the natural diffusion of glycerol. However, the combination method resulted in clear visualization of microvascular structures after 20 minutes of glycerol application. Based on this result, it is expected to reduce further the diffusion time of glycerol in human skin, and minimizing side effects in dermatological applications.

In conclusion, this study introduced a physical combination method to further improve TOC efficacy by enhancing the transdermal delivery of an optimal glycerol concentration. The combination method demonstrated the reduction of transdermal diffusion time of topically applied glycerol and the feasibility of dermatological application in an in vivo mouse study. In a future study, a new glycerol compound and optimal parameters of sonophoresis need to be studied for in vivo human skin in order to minimize side effects in dermatological applications.

Acknowledgments

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2009-0076112). This project was supported in part by research grants awarded from the National Institutes of Health (AR47551 and EB002495) to J.S.N.

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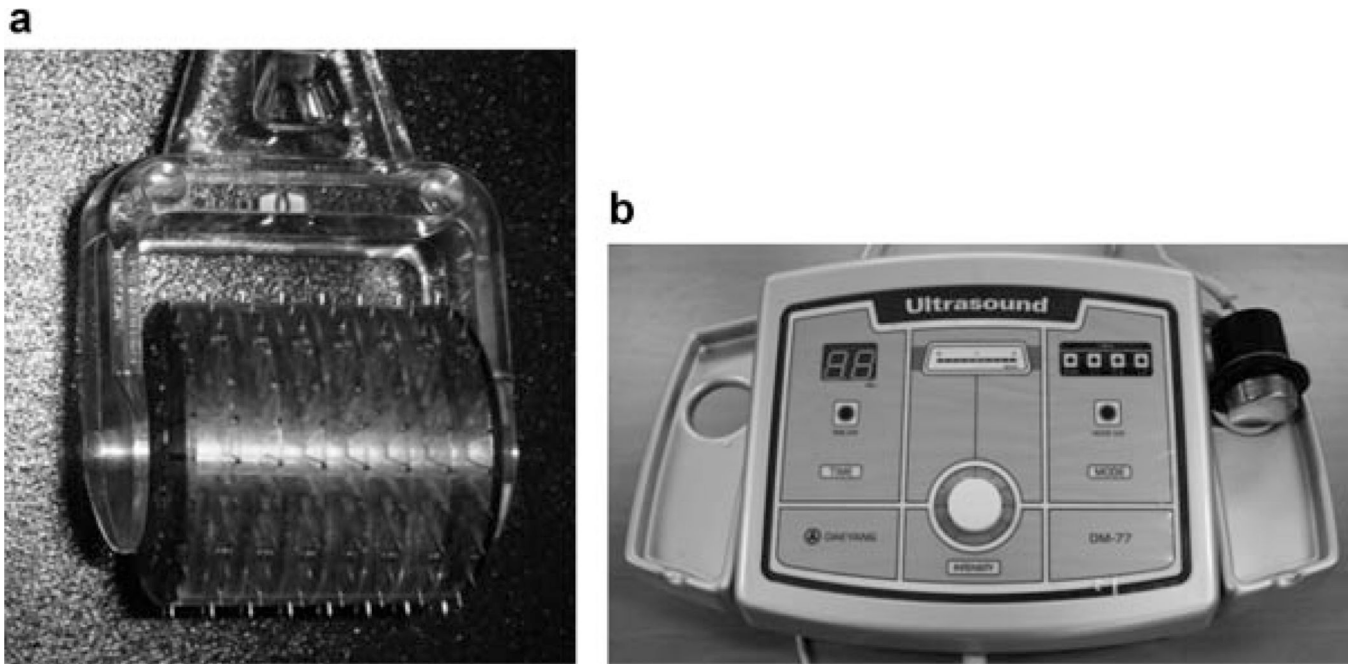

Fig. 1.
Photographs of (**a**) microneedle roller and (**b**) sonophoretic device utilized for the physical combination method.

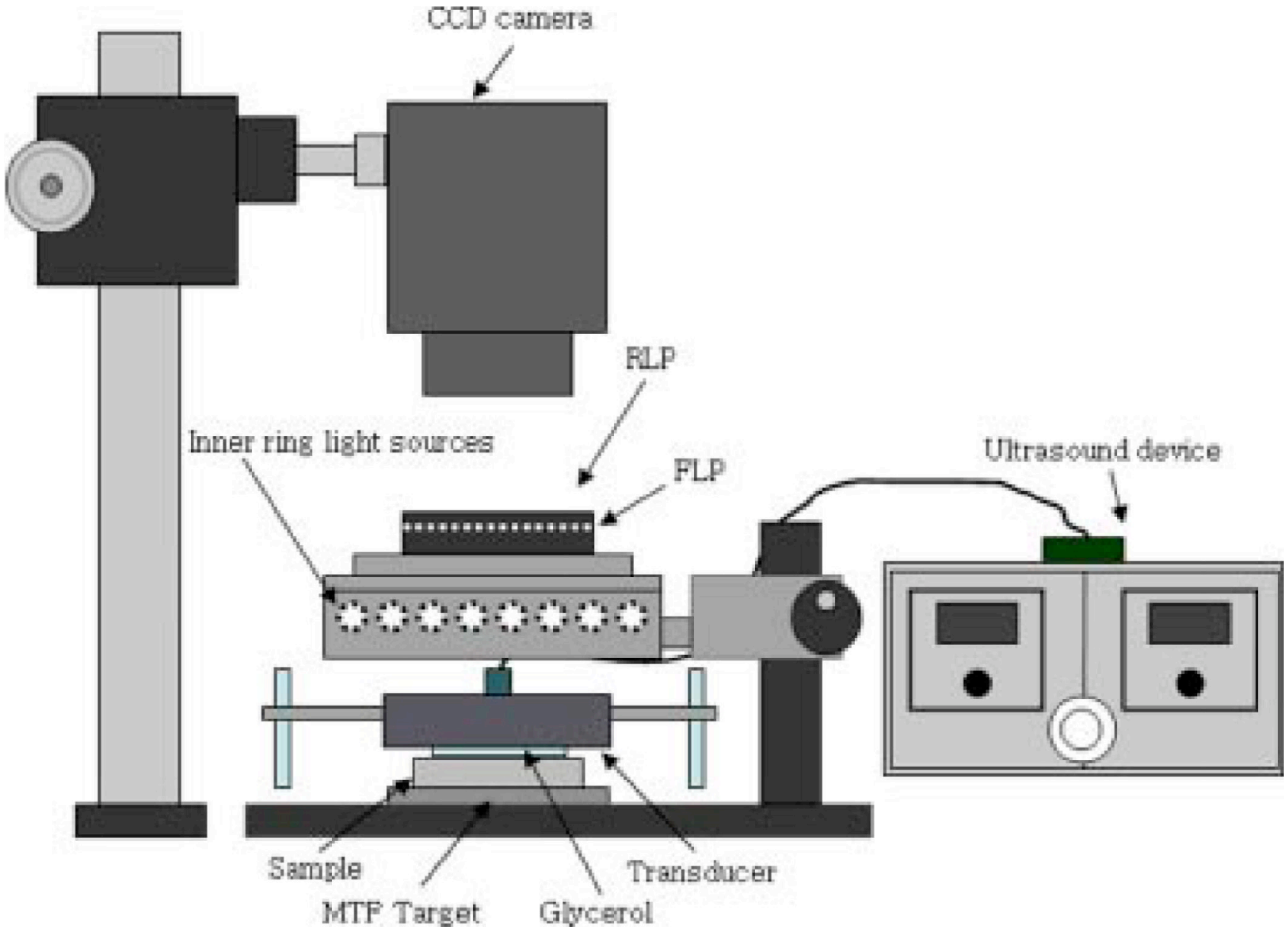

Fig. 2.
Schematic diagram of the experimental set-up utilized to apply sonophoresis and obtain cross-polarization images of skin samples. RLP and FLP indicate a rotating and fixed linear polarizer, respectively.

Fig. 3.
Optical coherence tomography (OCT) image of a microchannel formed in porcine skin by the microneedle roller. The scale bar indicates a length of 470 μm.

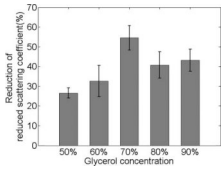

Fig. 4.

The percent reduction of the normalized reduced scattering coefficient in a porcine skin sample as a function of glycerol concentration which ranges from 50% through 90%. It was measured before and after 60 minutes of glycerol application.

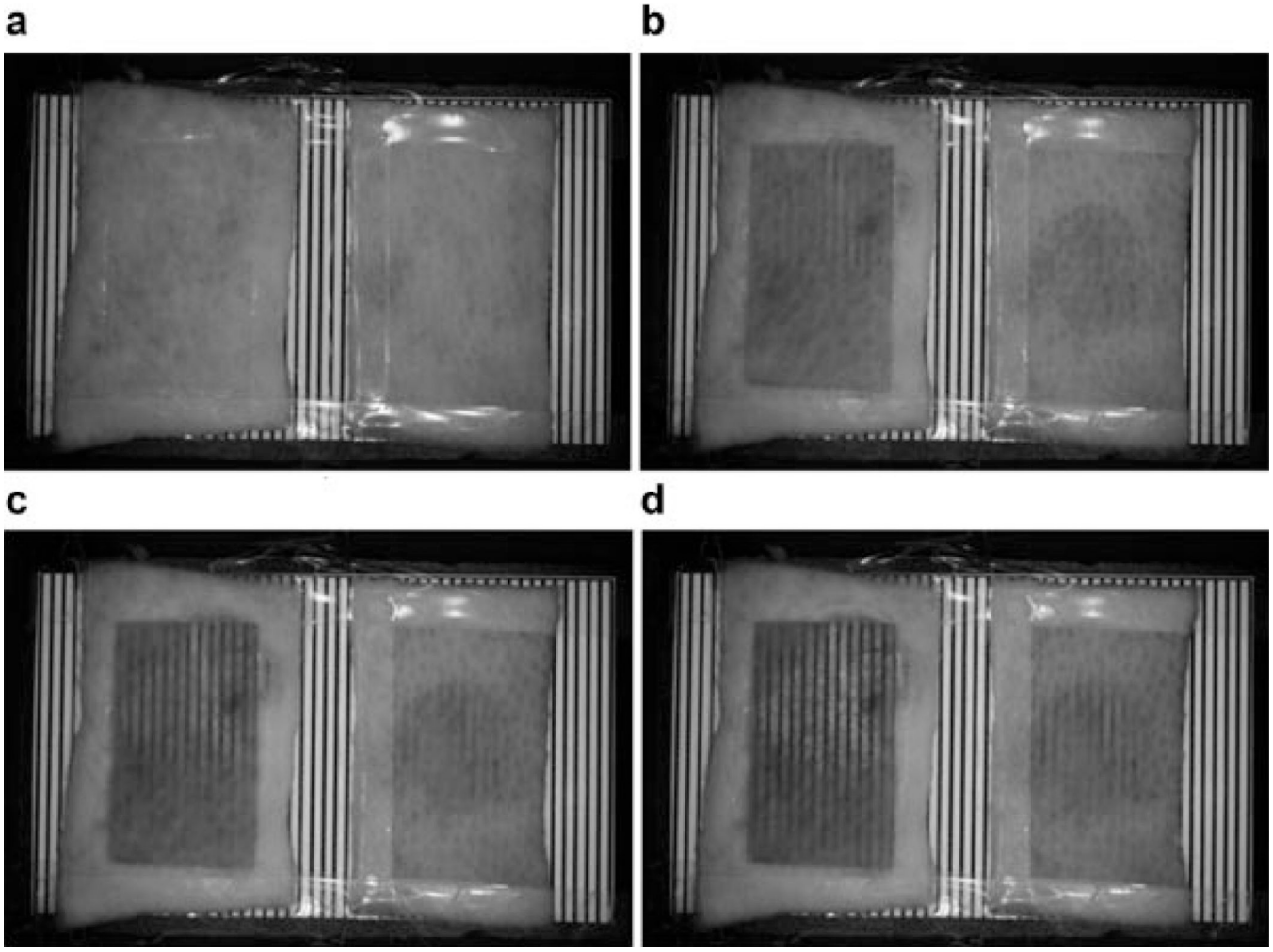

Fig. 5.
Photographs show the dynamic changes of tissue optical clearing on the control (right) and test (left) porcine skin samples after (**a**) 0, (**b**) 20, (**c**) 40, and (**d**) 60 minutes of topical application of 70% glycerol.

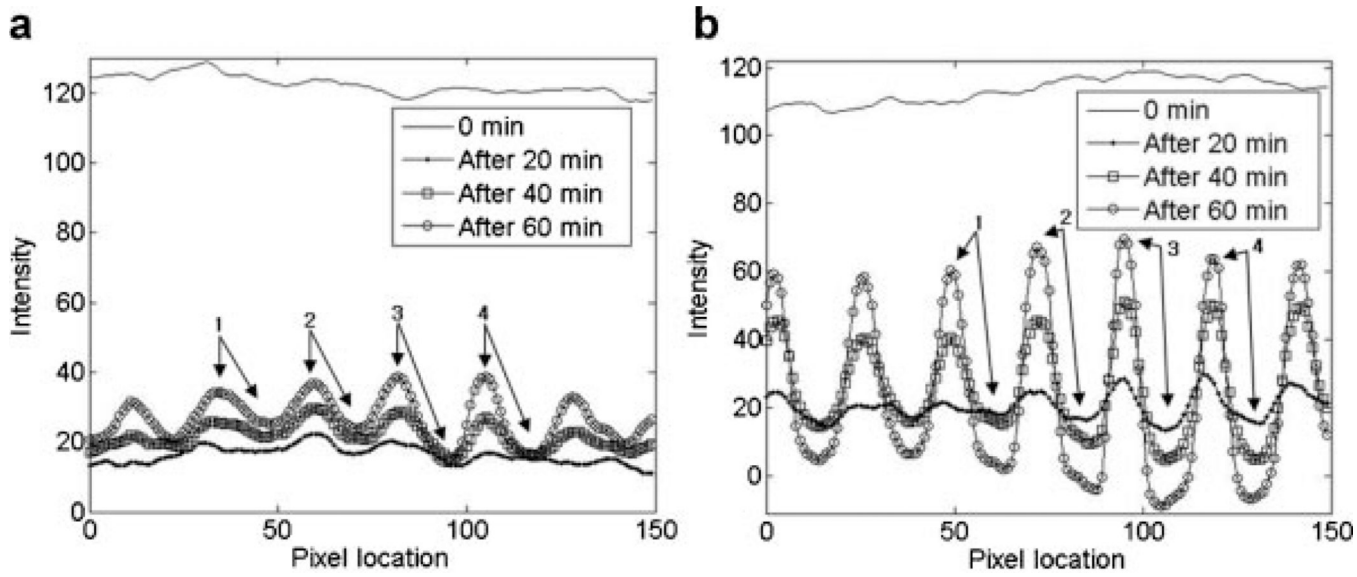

Fig. 6.

Modulation transfer functions on the (**a**) control and (**b**) test porcine skin samples. The contrast of the MTFs was computed at four locations as indicated.

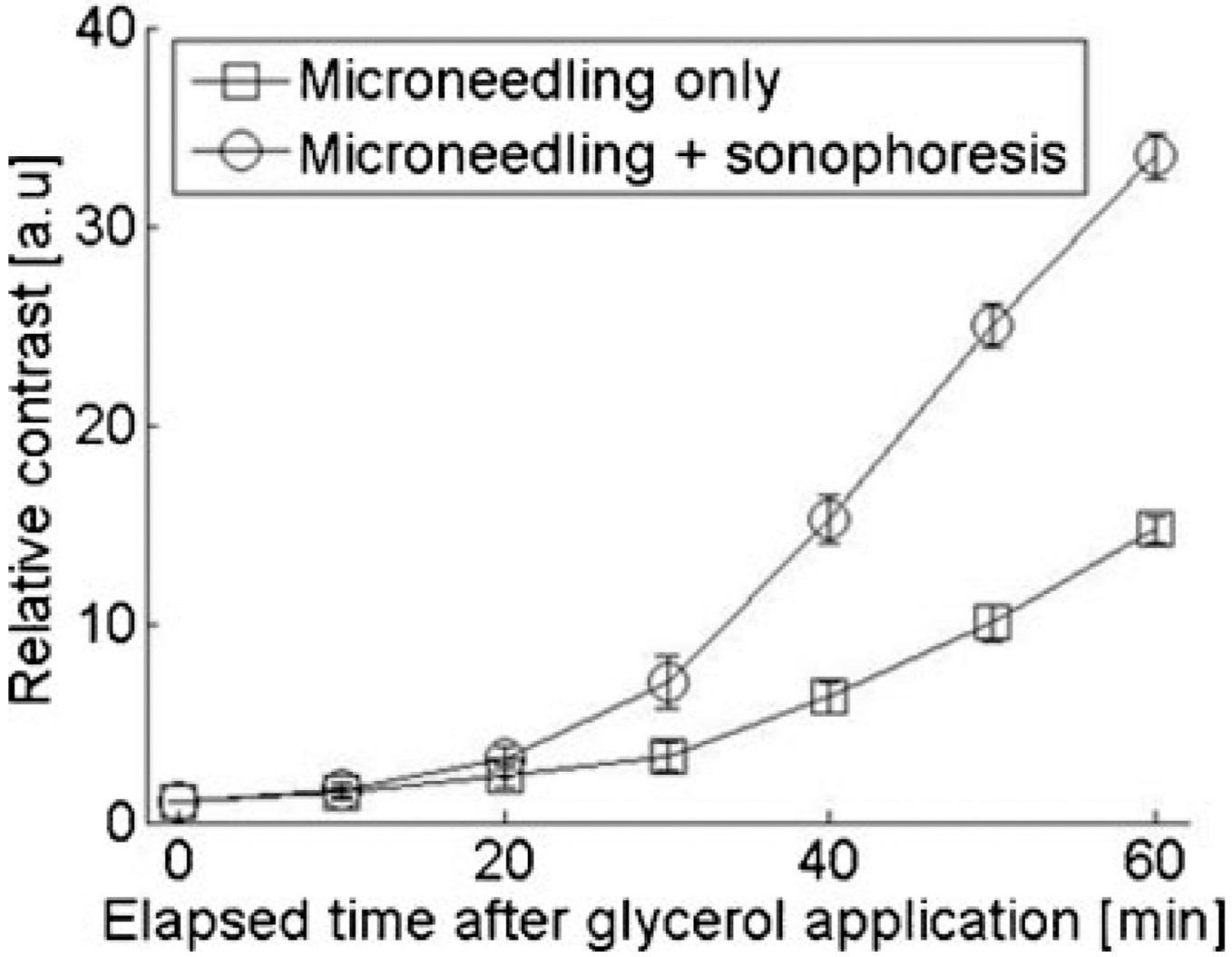

Fig. 7.
The relative contrast of MTFs on the control (□) and test (○) porcine skin sample groups as a function of time.

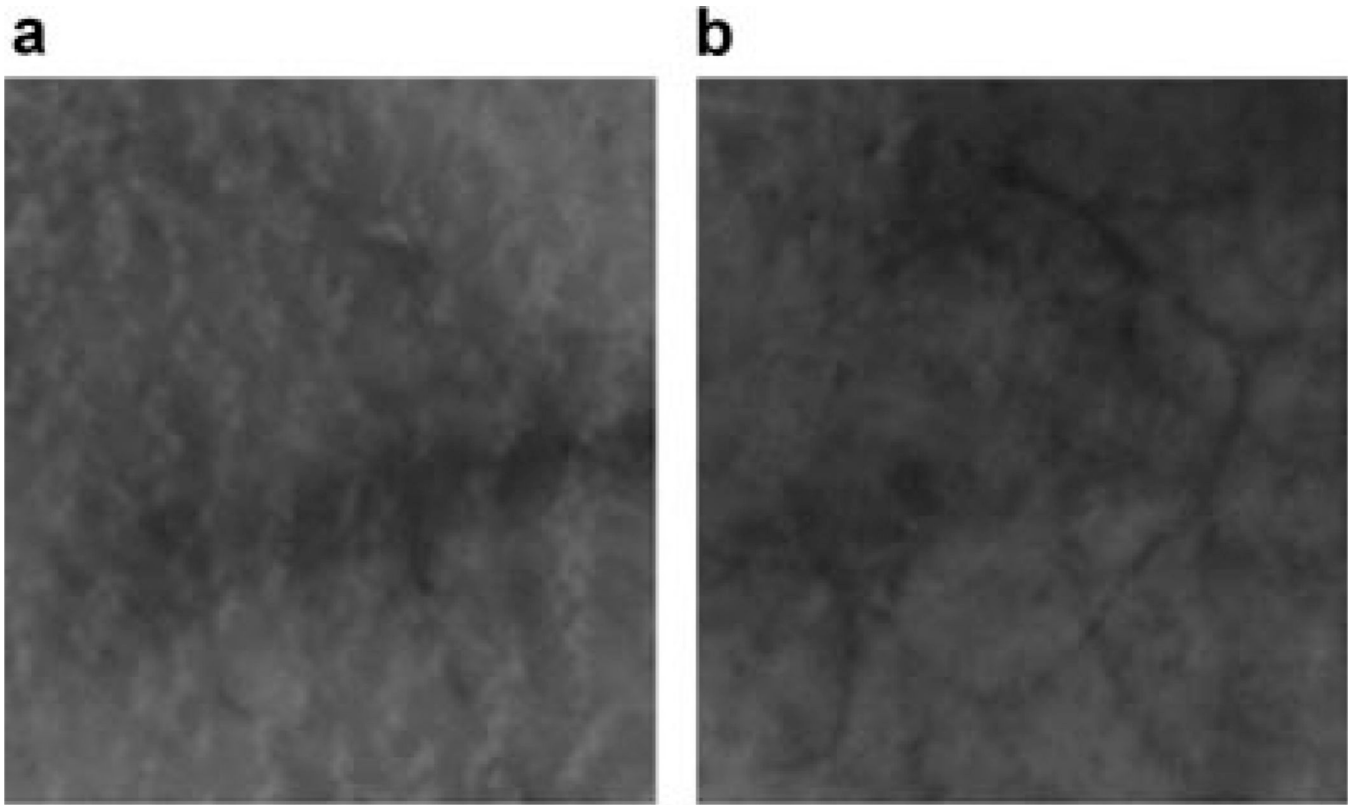

Fig. 8.
Optical clearing images of in vivo mouse skin (**a**) before and (**b**) after glycerol application using the combination method.