Comparison of skin optical clearing by different drug delivery method

Z. Mao* 1, X. Ge 2, E. Li3
1  Sports Health & Science School, Wuhan Sports University, Wuhan, 430079, China.
2  Department of Fundamental Theories, Shandong Sports University, Jinan, 250102, China.
3  Institute of Physical Education, Huazhong Normal University, Wuhan, 430079, China.
E-mail: mm73@163.com

Abstract. Previous studies have shown that optical clearing techniques have great potential for optical diagnosis and therapy. In order to further discuss the effect by different drug delivery method, diffusion chambers were used to simulate in vivo physiological environment; and the optical clearing agents were acted on the surface of porcine skin, the saline was contacted with the dermal side (epidermal application). Then they were compared with the traditional method-samples immersed in the optical clearing agents (mainly dermal immersion). The transmission intensity of skin was monitored by an integrating sphere system. The changes in thickness were measured before and after experiments. The results showed that the optical clearing effect and the changes in thickness are not coincident under the two different methods. It indicates that the optical clearing effect and mechanism may relate to the drug delivery method.

1. Introduction
The optical clearing technique can increase the penetration depth of light in tissue significantly. Among the investigations of the various tissues, skin attracts much attention [1-5].

At present, the simple drug delivery method for skin optical clearing in vitro is to immerse the samples in the solution [1-4], which means the optical clearing agents act on both sides of the skin, especially the dermis with large permeability. The corresponding way that drug delivery through in vivo skin is subdermal injection [5-7], which is traumatic and difficult to operate. The final goal of the optical clearing technique is to explore the in vivo clinical application. In actual clinical application, the desirable and noninvasive way is to apply the drug on the skin surface directly; and let the drug penetrates through the epidermis to dermis where it will be efficient.

Recently, Yeh finds structural modification or dissociation of collagen is the mechanism of skin optical clearing based on in vitro experiments [8, 9]. Our research [7] indicates the mechanism concerning about dissociation of collagen is inconsistent between in vivo in vitro study; even some of the optical clearing agents can not induce the obvious effect on skin in vivo as that of in vitro. Therefore, different experimental methods may lead different role and effect of agents. However, the previous work mainly focused on in vitro study, such as screening efficient optical clearing agents, using physical-medical methods to achieve ideal optical clearing effect and discussing the mechanism.

* To whom any correspondence should be addressed.
Though research on in vivo skin is difficult, it is important and necessary before the technique carried out for clinical use.

In order to develop a simple experimental setup that can simulate the in vivo physiological environment, we used horizontal diffusion chambers to imitate in vivo physiological environment; and compare the optical clearing effect with the simple drug delivery method - samples immersed in the optical clearing agents.

2. Materials and Methods

2.1. Preparation of optical clearing agents

In previous work, we found that glycerol solution with high concentration achieve good clearing effect. Three typical concentrations of glycerol –100%, 75% and 30% were chosen as optical clearing agents. Saline was set as control. The refractive index was measured with a refractometer (Digital Abbe Refractometer, WAY-2S; Shanghai, China). The concentration and refractive indices of the OCAs used in this study were listed in Table 1.

| Concentration of glycerol | Refractive indices \( \langle n \rangle \) | Data from references \( \langle n \rangle \) |
|---------------------------|------------------------------------------|------------------------------------------|
| 100%                      | 1.470                                    | 1.47                                    |
| 75%                       | 1.435                                    | 1.43                                    |
| 30%                       | 1.375                                    | 1.369                                   |
| saline                    | 1.335                                    | 1.34                                    |

2.2. Horizontal diffusion chambers to imitate in vivo physiological environment

We used horizontal diffusion chambers to imitate in vivo physiological environment in our experiment. The diffusion chambers were made of two colorimetric wares. Each chamber was 50 mm \( \times \) 50 mm \( \times \) 5 mm, with an aperture of 30 mm in diameter on one side, as shown in Figure 1. The samples were cut into 50mm \( \times \) 50 mm sections, and clamped tightly between two apertures. One chamber near the epidermis was filled with OCAs, and the other one was filled with isotonic saline. Both sides of the skin were able to react to the solution through the apertures. The osmotic pressure of isotonic saline was similar to in vivo physiological conditions. By this means, the dynamics of drug delivery is similar to the common clinical manner and the skin can be monitored continuously. On the other hand, the chambers are easy to clean and the dose of solution was limited.

![Figure 1](image.png) The sample box: horizontal diffusion chambers.

2.3. Part 1: measurement of skins by immersion (mainly dermal immersion)
In this part of our experiment, a double-integrating sphere system was used to measure the optical character parameters; the experimental setup was shown as reference [10]. The samples should be very thin, so rats’ skin was chosen and immersed in agents. The detection system is mainly comprised by the reflective ball and transmitted ball. A 1.6 mW, 1mm beam in diameter He-Ne laser (\(\lambda = 632.8\) nm, 5 mW, Melles Griot, U.S.) was chopped mechanically at 1 k Hz (Model SR 540), and then split two beams by a beam splitter. The reflected beam was a small fraction (20%) of laser beam, which irradiated to a reference sphere of 70 mm in diameter. The other (80%) of laser beam irradiated to the sample mounted in which placed in front of the port (\(\phi = 25\) mm) of a big integrating sphere of 210 mm in diameter. The intensity of the transmitted light from the sample including collimated and diffuse components of transmitted radiation was collected by the integrating sphere. The diffuse transmittance and diffuse transmittance were detected; and then, by transport theory and calculation, we can obtain the optical character parameters of the sample.

Because the structure of rats’ skin is very similar to the human’s, we selected the back skin of rats. The experimental procedures are shown as follows:
1. The hair was removed after the rats were sacrificed. The skin of back region was taken and cut into 5cm \(\times\) 5cm square after the subcutaneous fat layer was peeled carefully.
2. The thickness of sample was measured by using a micrometer.
3. Measuring the initial reduced scattering coefficient of the samples by the double-integrating sphere system;
4. Measuring the thickness, reduced scattering coefficient of the skins after they were immersed in solutions for 10 minutes.
5. We repeated all the procedures above four times. The whole experiment lasted for 60 minutes.

2.4. Part 2: measurement of skins with horizontal diffusion chambers to simulate physiological environment (epidermal application)
In Part 2 of this experiment, we set a drug delivery system, horizontal diffusion chambers to simulate physiological environment, as shown in Figure 1. We chose thick porcine skins to test because the rat skin is too thin to leak when clamped between two chambers. A single integrating sphere system was used to test the transmittance of the samples, as shown in reference [11].

Skin specimens were obtained from the porcine abdomen in a local slaughterhouse within 1 hour postmortem. All samples were measured within 8 h and stored at 4 °C. The stratum corneum and the subcutaneous fat of the whole skin specimens were cleaned before the samples were dissected for measurements. The samples were cut into 50mm \(\times\) 50 mm sections.

After the thickness was measured, each skin sample was clamped between two diffusion chambers, which were held together. 12 ml of OCAs was added to the donor cell in contact with the epidermal side of the skin, while 12 ml of isotonic saline was added to the receptor cell in contact with the dermal side. The chambers were set up with the receptor cell touching to the port (\(\phi = 25\) mm) of the integrating sphere. In order to determine the OCAs induced optical clearing effect of skin; the dynamical transmission intensity of samples was measured at 0, 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 40, 50 and 60 min after the application of OCAs.

3. results
3.1. results of Part 1
3.1.1. The reduced scattering coefficient of samples
To trace the influence of the glycerol on skin optical properties, the reduced scattering coefficient of skins was recorded after immersed for every 10mins by each concentration group. Figures 2 shows the ratio of scattering coefficient measured at every point (\(\mu_s' (t), t=10, 25, 40, 60\)min) and the initial value. The results indicate that the reduced scattering coefficient of skins decreases gradually after treatment with the OCAs. In contrast, there is a little increase in the control group alone after treatment
with the isotonic saline. Among the agents, 100% glycerol caused the greatest changes in reduced scattering coefficient, and 30% glycerol did the poorest.

![Figure 2](image)

**Figure 2** The reduced scattering coefficient of skins after treated with different concentration glycerol.

### 3.1.2. The change in thickness of samples

Table 2 lists the thickness of samples before and after the experiment, and the corresponding change. SPSS statistical software was used to perform an F- test among all the groups to compare the differences in the thickness changes after the experiment. Within each group, a paired-sample t- test was performed to compare skin thickness before and after an experiment. It is obvious that the skins shrunk by solutions with high concentration. A paired- sample t- test shows that there are significant differences within groups treated by 100% and 75% glycerol (P< 0.01). But a little swell in control. And an F- test shows that there are significant differences in thickness change between high concentration groups and control (P< 0.01).

**Table 2** Skin thickness before and after experiments and the corresponding changes

| Agents   | \(d_0\) (mm) | \(d\) (mm) | \(\Delta d\) (mm) |
|----------|--------------|------------|------------------|
| 100%     | 1.02±0.05    | 0.85±0.11**| -0.26±0.10**     |
| 75%      | 1.06±0.04    | 0.85±0.08**| -0.20±0.09**     |
| 30%      | 1.10±0.05    | 1.08±0.12  | -0.02±0.12       |
| saline   | 1.08±0.10    | 1.15±0.08  | 0.09±0.07        |

(d0- thickness before experiments; d- thickness after experiments; \(\Delta d\)- thickness changes after experiments. *means P<0.05 ; **means P≤0.01)

### 3.2. results of Part 2

#### 3.2.1. The relative transmittance of skin

The relative transmittance can be deduced as the following formula (1) based on the measurements of transmission intensity:

\[
T = \frac{I_t}{I_0}
\]  \(\text{(1)}\)
\( I_t \) and \( I_0 \) are the transmission intensity measured at time \( t \) and initially \( (t = 0) \), respectively. Figure 3 shows the changes of relative transmittance after treatment with solutions. The relative transmittance of skin was deduced with time. The results indicate that the relative transmittance of skins increases gradually after treatment with the OCAs. In contrast, there is some decrease in the control group alone after treatment with the isotonic saline. Seen from the figure, 100% glycerol achieves the greatest changes in relative transmittance and the best optical clearing effect correspondingly. However, 30% glycerol nearly has no effect on the skin.

![Figure 3 Relative transmittance of porcine skins after treatment with solutions](image)

\[ \text{Figure 3} \] Relative transmittance of porcine skins after treatment with solutions

### 3.2.2. The thickness of skins

The thickness of samples before and after the experiment, and the corresponding change are listed in Table 3. The skins seem a slight shrinkage treated by high concentration glycerol; but a little swell treated by 30% glycerol and saline. However, a paired-sample t-test shows that there is no significant difference within each group, and an F-test shows that there is no difference in thickness change among groups (\( P > 0.05 \)).

#### Table 3 Skin thickness before and after experiments and the corresponding changes (\( \bar{X} \pm SD \))

| Agents   | \( d_0 \) (mm) | \( d \) (mm) | \( \Delta d \) (mm) |
|----------|----------------|--------------|---------------------|
| 100%     | 1.58±0.12      | 1.51±0.11    | -0.07±0.11          |
| 75%      | 1.61±0.11      | 1.59±0.08    | -0.02±0.07          |
| 30%      | 1.60±0.09      | 1.61±0.12    | 0.01±0.05           |
| saline   | 1.66±0.10      | 1.74±0.05    | 0.07±0.01           |

\((d_0\) thickness before experiments; \(d\)- thickness after experiments; \(\Delta d\)- thickness changes after experiments. *means P<0.05 ; **means P≤0.01)

### 4. Discussion

The high scattering character of tissue is mainly because of refractive index mismatch among cellular components: cell membrane, cytoplasm, cell nucleus, cell organelles, melanin granules, and the extracellular fluid [1]. It is well known that turbidity of a dispersive physical system can be effectively controlled by providing matching of refractive indices of the scatterers and the ground material. Optical clearing technique proposed by Tuchin attracted intensive attentions. By immersing tissue with biocompatible, hyper-osmotic and refractive index matching agents, tissue scattering reduces due to refractive index matching of scatterers and ground matter. As a result, the turbidity of biological tissues can be effectively controlled, which increases penetration depth of light in tissues [1-6].
In this study, the experiment was divided into two parts based on the medication method: mainly by dermal immersion and by epidermal application. We used diffusion chambers to imitate in vivo physiological environment in Part 2. The optical clearing effect of glycerol with three different concentrations was compared by the different medication above. We found that 100% and 75% glycerol achieved obvious optical clearing effect, but 30% glycerol almost had no influence on skins by epidermal application. In this work, the changes in thickness of skins treated by glycerol solutions were investigated, under two different medications. The results show that skins shrank by immersed in solutions. But their thickness would have no change if just the epidermis contacted with OCAs, and the dermis contacted with saline.

Much previous in vitro research found the dehydration and the shrinkage of skin was marked when the OCAs were directly acted to the dermis [9]. In clinical applications, the OCAs would probably be applied to the epidermis, and then diffuse into the dermis. So we apply the diffusion chambers to simulate transdermal OCAs delivery in vivo. Then the OCAs can not be effective unless they penetrate from epidermis to dermis; on the other hand, part of the OCAs in the dermis was replaced by saline in the receptor cell for the substances exchange. Therefore, in our Part 2 tests, the effective OCAs in the dermis may be less than that in the previous studies [2, 4, 6, 12], and the corresponding dehydration and shrinkage of skins may also be milder. As a result, there is no significant change in skin thickness after treatment of solutions. It indicates that the effect of optical clearing agents may differ between in vitro and in vivo situations in basis of our different medications. Hence, according to our former research, the mechanism may not identical under the corresponding medications [7, 11].

5. Conclusion
The optical clearing effect may relate to the drug delivery method. By using diffusion chambers to simulate in vivo transdermal drug delivery, the skins achieve less optical clearing effect and shrinkage than agents’ immersion. However, we provide a worthy of learning way to imitate in vivo research.

Acknowledgments
This study was supported by the Youth fund of the education department of Hubei province, China (Q20093303). The authors are thankful to Dan Zhu, Xiang Wen in Britton Chance Center for Biomedical Photonics, Wuhan, China, for their help.

References
[1] Tuchin V V 2005 J. Phys. D: Appl. Phys. 38 2497
[2] Jiang J J and Wang R K 2004 Phys Med Biol. 49 5283
[3] Yeh A T, Choi B, Nelson J S, et al. 2003 J. Invest. Dermatol. 121 1332
[4] Xu X and Zhu Q 2007 Opt. Commun. 279 223
[5] Galanzha E I, Tuchin V V, Solovieva A V, Stepanova T V, Luo Q, and Cheng H 2003 J. Phys.-Lond.-D Appl. Phys. 36 1739
[6] Vargas G, Chan E K, Barton J K., et al 1999 Laser. Surg. Med. 24 133
[7] Wen X, Mao Z, Han Z, Zhu D 2010 J. Biophoton. 3 No. 1-2, 44
[8] Hirshburg J, Choi B, Nelson J S, and Yeh A T 2006 J Biomed. Opt. 11 040501
[9] Yeh A T and Hirshburg J, 2006 J. Biomed. Opt. 11 014003
[10] Zhu D, Luo Q, Chen J 2003 Lasers Surg. Med. 33 226
[11] Mao Z, Zhu D, Hu Y, Wen X, Han Z 2008 J. Biomed. Opt. 13 021104
[12] Choi B, Tsu L, Chen E, et al 2005 Lasers Surg. Med. 36 72