Investigation of skin water loss and glycerol delivery through *stratum corneum*

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ABSTRACT

Investigation of skin water loss and clearing agent delivery through *stratum corneum* is presented. Effectiveness of the method "lattice of islets of damage" for enhancement of *stratum corneum* permeability for water and 71% glycerol solution providing the skin clearing was investigated. Dehydration induced by both stimuli evaporation and osmotic agent action was studied by weight measurements. Measurements of refractive index dynamics of glycerol and water during its interaction with skin samples were carried out. The results have shown that proposed method allows effective transepidermal water loss and delivery of optical clearing agents.

1. INTRODUCTION

Interests in using optical methods in laser diagnostics, therapy and surgery are continuously increasing. However, the main limitation of the methods deals with the transport of the laser radiation through superficial tissues. Scattering properties of tissues define spectral and angular characteristics of light interacting with living objects, as well as its penetration depth.[1,2] One of the methods aimed at the decrease of light penetration depth is the use of hyperosmotic optical clearing agents (OCAs). The response of tissue to OCA is a reduction in light scattering and corresponding increase in optical transparence.[3-6]

Numerous scientific publications discuss advantages and methods of tissue optical clearing using OCAs and understanding of the mechanisms of the clearing.[3-10] There are three mechanisms of light scattering reduction induced by OCA: 1) dehydration of tissue constituents, 2) partial replacement of the interstitial fluid by the immersion substance and, hence, the matching of the refractive indices of the tissue scatterers (collagen and elastin fibers) and the interstitial fluid, and, 3) structural modification or dissociation of collagen.[3-11] In tissues a combination of the mechanisms usually takes place.

It was shown that dehydration induced by osmotic stimuli such as OCAs appears to be a primary mechanism of optical clearing in collagenous and cellular tissue, at that dehydration induces intrinsic matching effect.[9] The fluid space between fibrils and organelles is occupied by water and suspended proteoglycans. Water exiting from tissue with cellular or fibrillar structure is more rapid process than OCA entering into tissue interstitial space due to OCA has greater viscosity then water. As water is removed from the intrafibrillar or intracellular space, proteoglycans become more concentrated and refractive index increases. The resulting intrinsic refractive index matching between fibrils or organelles and their surrounding media may significantly contribute to optical clearing.[9]

Replacement of water in the interstitial space by the immersion substance leads to the additional matching of the refractive indices of the tissue scatterers and the interstitial fluid.

Frequently used agents are glycerol, TMP, 1,3-butanediol, 1,4-butanediol, polyethylene glycol, dimethylsulfoxide, dextrose solution of high concentration as well as linoleic and oleic acids, glucose, mannitol, propylene glycol, polypropylene glycol, X-ray contrasting agents (Verografin, Trazograph, and Hypaque), and their combinations.[3-12]

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Saratov Fall Meeting 2006: Optical Technologies in Biophysics and Medicine VIII, edited by
Valery V. Tuchin, Proc. of SPIE Vol. 6535, 65351G, (2007) · 1605-7422/07/$18 · doi: 10.1117/12.740990

A number of laser surgery, therapy, and noninvasive diagnostic technologies may have a significant benefit at a reversible skin scattering reduction. However, slow diffusion of OCAs through human skin barrier makes practical application of optical immersion effect difficult. To reduce barrier function of skin epidermis a number of different chemical[13-15] and physical methods such as stripping,[16] microdermabrasion,[17] laser ablation of skin surface,[18,19] the use of ultrasound[20] and photomechanical waves,[21] needle-free injection[22] and others were proposed.

Recently, we proposed a method of accelerating penetration of the OCAs due to enhancing epidermal permeability via creating a lattice of micro-zones (islets) of limited photothermal damage or lattice of islets of damage (LID), in the *stratum corneum* (SC).[23,24] LIDs are created as a result of absorption of a sufficient amount of optical energy by the a lattice of micro-zones. The absorption leads to temperature elevation in the zones of interaction. Since zones of interaction (microdots) contact the skin surface, some of the thermal energy will be conducted to the SC. Such zones have a very small volume but considerable total margin surface. It was shown that the application of regular periodic structures of islets increases the safety margins substantially, depending on the islet separation.[23] As the damage of the SC is not a damage of viable tissue, long-term effect of such damage is only the transient deterioration of skin barrier function. That leads to the local increase of OCAs penetration.[24] The lattice of optical islets can be formed using a variety of energy sources and delivery optics, including application of lenslet arrays, phase masks, and matrices of exogenous chromophores.[23,24]

The goal of our paper is investigation of skin water loss and OSA delivery through SC and effectiveness of the method LID for enhancement of SC permeability for both water and OSA. We present the experimental results on dehydration of *ex vivo* human skin by the following stimuli: evaporation and application of OSA and refractive index dynamics of OCA and water during its interaction with skin sample.

2. MATERIALS AND METHODS

2.1. Tissue samples

Twelve tissue samples included in the study was human skin. Full-thickness samples were obtained post-surgically. The area of the samples was about 20×30 mm. The samples were used after adipose layer removing. Thickness of the samples was measured by micrometer before the dehydration and after that.

2.2. Immersion agent

As immersion liquid 71%-glycerol solution available for sale without prescription was used. Refractive index of the solution measured by Abbe refractometer was 1.4535.

2.2. Flashlamp- appliqué system

For the half of the samples SC was perforated. For the perforation a flashlamp (EsteLux System, Palomar Medical Products Inc. USA) and special designed appliqué were used. The appliqué was a transparent plastic film with a pattern of absorbing centers. Diameter of the dots (absorbing centers) was ~150 μm; distance between the dots was ~500 μm. Regime of the irradiation was 2 pulses with fluence 27 J/cm^2. The dots are made out of inert and biocompatible carbon powder, ensuring high absorption of light energy. When a pulse of light was shone on the appliqué, the dots absorbed the light energy, which resulted in rapid temperature elevation. The appliqué closely contacted with the skin surface to localize light absorption and, thus, the thermal damage, within the thin surface layer.

Parameters of the light/appliqué system were selected in such way that no irreversible damage was caused to the *stratum corneum*, so that integrity of the skin barrier was restored in a short time. Size of the island damage mask (IDM) was 10×20 mm and corresponded to a handpiece window of the flashlamp system. As a result of thermal action LIDs with diameters of craters 200 ± 20 μm and depth 25 ± 5 μm arose on the surface of the skin samples.

To remove products of the thermal action from the craters and enhance diffusion of immersion agent inside skin alcohol compress in 15 minutes was applied on the skin surface. Concentration of ethanol in solution was 40%.

2.3. Weight measurements

Eight samples of skin: perforated and non-perforated were placed on separate glass object-plates and each of them was covered by a polyethylene film with a window 10×20 mm (corresponded to the damaged area) to exclude the drying of

the samples from the site of dermis. The surface of the samples was covered by immersion solution only in the area of the window. Adjacent tissue specimens covered by the same film were allowed to dehydrate in air. All samples were placed in a box with silicone powder to prevent neglected absorption of water from environment.

The samples were weighed on electronic balance every hour in about some weeks. Before weighing glycerol was removed thoroughly and then applied again after the weighting. Experiments were performed at room temperature.

2.4. Refraction measurements

Series of experiments on measurement of refractive index dynamics of glycerol and water during its interaction with both perforated and non-perforated skin sample was carried out. Four samples were put into temperature-controlled cuvette (Fig. 1). For measurements of glycerol refractive index dynamics 2 ml of glycerol solution (3) was poured into the upper reservoir (1). The solution interacted with epidermis (5). Saline (6) was poured into the lower reservoir (2) with the volume 20 ml. It contacted with dermis and maintained the normal state of skin sample. The low reservoir was attached to the thermostat. Temperature of saline was kept constant about 38.5°C. Temperature of the skin surface at this case was about 34°C.

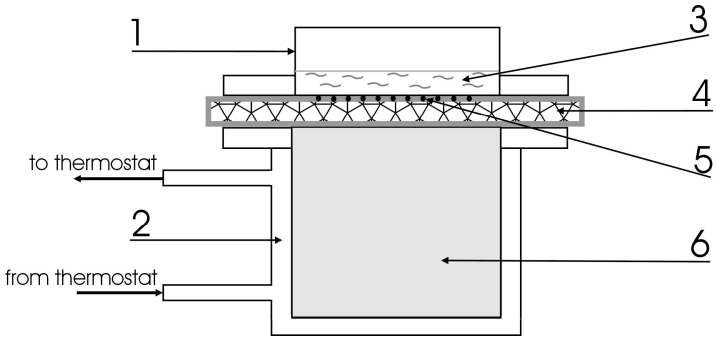

Fig. 1. Scheme of cuvette for the study of the refractive index change: 1 – upper reservoir of the cuvette; 2 – temperature-controlled lower reservoir of the cuvette; 3 – studied agent, in which refractive index is measured (water or glycerol solution), the volume of the liquid is 2 ml; 4 – skin sample; 5 – epidermal layer with/or without LIDs; 6 – glycerol or saline, the volume of the liquid is 20 ml.

For the measurement of the change of water refractive index the liquids were traded places: 2 ml of water was poured into the upper reservoir (1) and 20 ml of the glycerol solution were poured into the lower reservoir (2). Besides, the skin sample was placed epidermis down, to contact with glycerol solution.

0.1 ml of studied agents from upper reservoir was taken for single measurement of the refractive indices with Abbe refractometer.

3. RESULTS AND DISCUSSION

Normalized skin mass dynamics is demonstrated in Fig. 2. The experiment provides the skin clearing by the following stimuli: 1) application of OSA or 2) evaporation (no application of OSA). Symbols present experimental data, solid lines correspond to approximation dependence. Experimental data were approximated by exponential function:

$$y = A \exp(-x/\tau) + y_0, \quad (1)$$

where A - constant; τ - characteristic time, hr; y_0 – weight of the sample at t=∝, g.

The thickness of the first sample, which was dehydrated by glycerol, before and after dehydration was measured as 1.56 ± 0.06 mm and 1.36±0.05 mm, respectively. For the sample, which was dehydrated in air, the thickness was 1.43 ± 0.1 mm and 0.81±0.05 mm, respectively. The epidermis of the both samples was perforated.

It is well seen that tissue mass decreases exponentially with time for all dehydration stimuli. An evaporative process isolates the dehydration mechanism of optical clearing from other mechanisms (i.e. refractive index matching by agents or structural modification of collagen) that allows separating the influence of OSA on the tissue clearing process. The rate of the tissue dehydration by glycerol is more rapid then by air but the degree of the dehydration in the second case is higher. It is connected probably with diffusion of free water from tissue to glycerol solution and retention of attached water. Besides glycerol penetrates into the skin and holds water inside. At the dehydration in air both free and attached water evaporate from tissue.

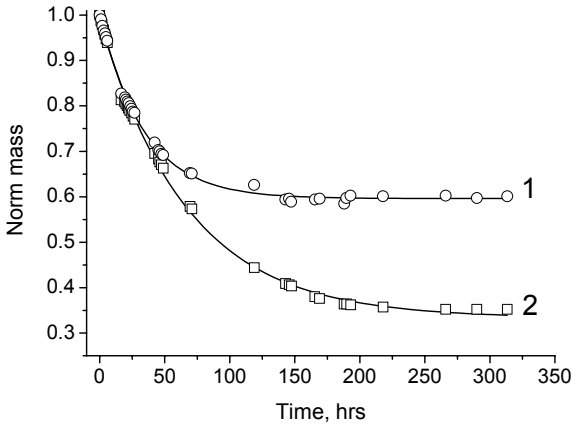

Fig. 2. Normalized mass dynamics of human skin samples with damaged epidermis: 1 – dehydration by glycerol solution, 2 – dehydration in air.

Figure 3 shows normalized mass dynamics of the skin samples with non-damaged (1) and damaged (2) epidermis dehydrated in air. Symbols present experimental data, solid lines correspond to approximation dependence. Initial thickness of the samples with both non-damaged and damaged epidermis was 1.98±0.12 mm and 1.43 ± 0.1 mm, respectively. After the dehydration the thickness of the sample was 0.93±0.05 mm and 0.81±0.05 mm, respectively. The figure demonstrates that the degree of the dehydration of the samples was almost equal because the both ones dried in air in equal terms. However, it is well seen that dehydration of the second sample went on in about 4.5 folds more rapidly then of the first one. Thus, formed LIDs facilitate significant water loss from epidermal surface of skin.

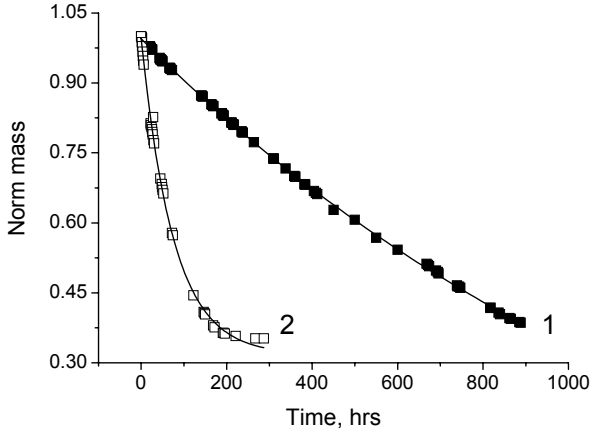

Fig. 3. Normalized mass dynamics of the human skin samples with: 1 - non-damaged epidermis and 2 - damaged epidermis dehydrated in air.

Normalized weight dynamics of the human skin samples with non-damaged (1) and damaged (2) epidermis under action of glycerol solution is demonstrated in Fig. 4. Symbols present experimental data, solid lines correspond to approximation dependence. The thickness of the first sample before and after dehydration was measured as 1.44 ± 0.06 mm and 1.21±0.05 mm, respectively. For the sample, epidermis of which was previous damaged, the thickness was 1.55 ± 0.05 mm and 1.36±0.04 mm, respectively. The figure shows that dehydration of the second sample went on in about 3 folds more rapidly then of the first one. However, it takes note that the degree of dehydration of the sample with previously damaged epidermis is lower then that of the non-damaged sample. It is connected with formed LIDs served as centers of enhanced permeability of epidermis not only to water leaving skin but to hydrophilic agent, as glycerol, which diffuse from environment solution into skin. In this case two flows take place: the first one is water diffusion from low layer of epidermis and from dermis to the glycerol solution and the second one is glycerol diffusion from environment solution to the tissue. For the non-damaged sample, apparently, the second flow is less then for the perforated one, therefore as a result the degree of dehydration of the sample #1 is more then that of the sample #2. Thus, free admission of glycerol into skin prevented formation of high concentration gradient between interstitial water and environment glycerol solution that decreased dehydration of tissue.

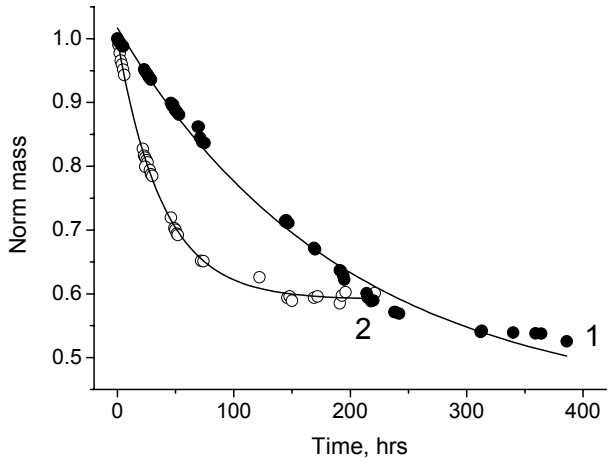

Fig. 4. Normalized mass dynamics of the human skin samples with: 1 - non-damaged epidermis and 2 – damaged epidermis dehydrated by glycerol solution.

The equation (1) allows estimating characteristic time τ showing typical time of water loss process. The values of the diffusion constants for all investigated samples are presented in the Table 1. It is well seen that the use of osmotic agent as glycerol promotes significant accelerate of the water loss of skin. LIDs provide enhancement of dehydration process that leads to the increase of water loss rate even greater. However, as it is seen from the figures, at the use of the LIDs the degree of the dehydration increases in the case of evaporation and decreases in the case of osmotic stimuli action.

Table 1. Characteristic time of the skin dehydration process at different stimuli

Dehydration stimuli	**Epidermal perforation**	**τ, hrs**
osmotic agent	+	36.4 ± 0.7
osmotic agent	-	193.4 ± 12.3
evaporation	+	71.2 ± 1.9
evaporation	-	1408.9 ± 36.3

To make sure that two processes: water loss and osmotic agent diffusion into skin took place simultaneously the study of dynamics of refractive indices of both glycerol solution and water at the interaction of skin with osmotic agent was carried out.

Fig. 5 shows dynamics of glycerol refractive index during skin dehydration through epidermis for samples with both non-damaged (1) and damaged (2) epidermis. During four hours the refractive index of glycerol solution decreased insignificantly in the first case unlike the second case. It means that diffusion of water from skin through epidermis to environment solution went more rapidly at the use of the LIDs.

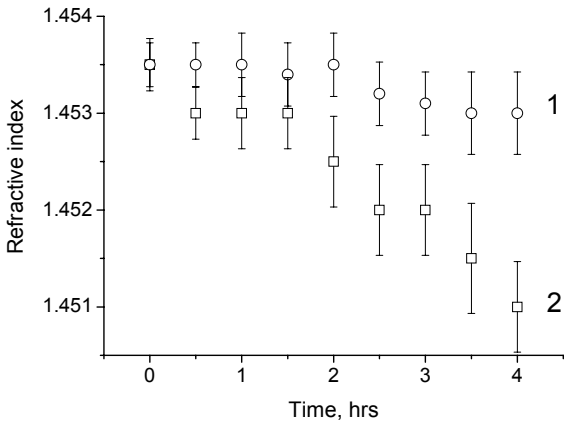

Fig. 5. The dynamics of glycerol refractive index during skin dehydration through epidermis. 1 – the sample with non-damaged epidermis and 2 – the sample with damaged epidermis.

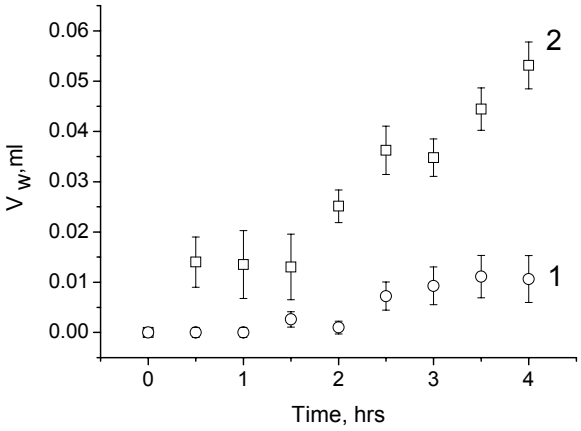

Fig. 6. The dynamics of water volume in glycerol solution due to diffusion from skin. 1 – the sample with non-damaged epidermis and 2 – the sample with damaged epidermis.

Basing on Gladstone-Dale law it is possible to obtain volume fraction of water incoming from the skin to the glycerol solution through epidermis:

$$n_{\text{solution}}=n_{\text{water}}C_{\text{water}}+n_{\text{gl}}C_{\text{gl}}, \tag{2}$$

where n_{solution}, n_{water} and n_{gl} are refractive indices of environmental solution, water and glycerol; C_{water} and C_{gl} are volume fractions of water and glycerol in environmental solution.

Taking into account the immersion agent volume change during the measurements allows us obtaining water volume in the glycerol solution. Fig. 6 shows the change of water volume in glycerol solution due to water diffusion from skin with non-damaged and damaged epidermis. It is well seen that for the sample with damaged epidermis in four hours water volume in glycerol solution decreased from zero to 0.05 ml that is more then 4%. For the sample with non-damaged epidermis the decrease of water content is nonsignificant (about 1%).

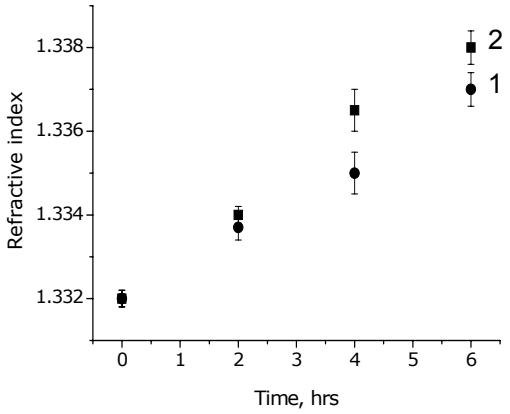

Fig. 7. The dynamics of water refractive index during skin interaction with glycerol solution. 1 – the sample with non-damaged epidermis and 2 – the sample with damaged epidermis.

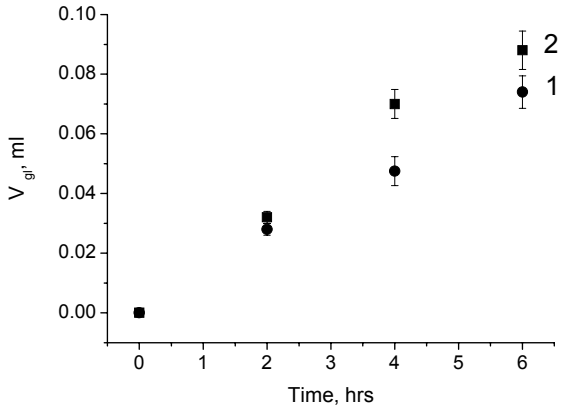

Fig. 8. The dynamics of glycerol volume in water due to diffusion from skin. 1 – the sample with non-damaged epidermis and 2 – the sample with damaged epidermis.

Analogical measurements of water refractive index were made. In this case skin dermis was contacted with water; epidermis with/without LIDs was washed by glycerol solution. Results of the measurements are presented in Figs. 7. We can see that the refractive index of water decreases not only for the sample with LIDs in epidermis but for the sample without ones. It can be explain by penetration of glycerol through tissue in water. At that penetration of the glycerol solution into the skin is accelerated at creating the corresponding pattern of enhanced permeability channels.

Thus, OCAs induce optical clearing by combination of dehydration and refractive index matching due to water exiting and OCA entering skin. LIDs provide enhancement of dehydration process and OCA delivery through *stratum corneum*.

4. CONCLUSION

The results of the experiments have shown that the method LID is effective for transepidermal delivery of optical clearing agents. Microdamages of the skin surface served to reduce *sratum corneum* barrier function and promoted penetration of hyperosmotic agent into skin. Free admission of glycerol into skin prevented formation of high concentration gradient between interstitial water and environment glycerol solution that decreased dehydration of tissue.

Island damage method could be used successfully in *in vivo* conditions for the enhancement of optical clearing of treated skin area and as enabling faster effect of the medication.

ACKNOWLEDGEMENTS

The research described in this publication has been made possible, in part, by grants PG05-006-2 and REC-006 of U.S. Civilian Research and Development Foundation for the Independent States of the Former Soviet Union (CRDF) and the Russian Ministry of Science and Education, grants RUB1-570-SA-04 of CRDF, grant of the Russian Federal Agency of Education Russian Federation 1.4.06, and grant of RFBR No. 06-02-16740.

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