Use of fractional laser microablation of skin for improvement of its immersion clearing

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ABSTRACT

We are proposing a new method for enhancement of optical clearing agent delivery into the skin using fractional laser microablation of the skin surface. The Palomar Lux2940 erbium laser with the wavelength 2940 nm and pulse duration of 5 ms was used as a light source. Two regimes of laser action were used in the experiments: the first one realized microablation of skin upper layer and the second one created microchannels in skin. As optical clearing agents mineral oil and PEG-300 were used. *In vivo* studies were carried out with white outbred rats. Both parameters: the permeability coefficient of the agents in the tissue and the optical probing depth were measured using the OCT system at a wavelength of 930 nm. The following values of the permeability coefficient of the skin with microablation were obtained: $(3.41\pm0.46)\times10^{-5}$ cm/s and $(2.35\pm0.30)\times10^{-5}$ cm/s for mineral oil and PEG-300, respectively, at the use of the surface microablation and $(3.32\pm0.09)\times10^{-5}$ cm/s and $(3.61\pm0.34)\times10^{-5}$ cm/s for mineral oil and PEG-300, respectively, at the use of the microporation. The results have shown that the joint application of mineral oil with microablation in the first regime promotes maximal (nearly 2-folds) increasing of optical probing depth in 30 min. Obtained data can be used for development of optical diagnostic methods of skin diseases.

Keywords: erbium laser, skin ablation, optical clearing, optical coherent tomography, permeability.

1. INTRODUCTION

Optical methods are perspective for diagnostics, locating and treatment of diseases of different cutaneous and subcutaneous formations[1]. However, often the complex structure of tissue complicates delivery of visible and near-IR light in a target area. Optical clearing method has a potential to cause light scattering decrease that leads to significant rising of laser beam penetration depth and focusing precision during the photodynamic therapy of subcutaneous tumors[2,3]. The complex organization of skin does not allow for the deep penetration of optical clearing agents (OCAs) especially hydrophilic ones into skin. The natural protective barrier of the skin, i.e., the stratum corneum (SC) with 5 - 20 mm thick consists of plane densely packed cells - corneocytes, merged into the lipid matrix[4]. The hydrophilic pores, transpiercing the epidermis, have the diameter less than 10 nm[5]. The composition and barrier function of the SC limits the number of suitable drugs for transdermal delivery to small, moderately lipophilic, and highly potential molecules. Highly lipophilic molecules do not transfer well from the lipidic SC into the more aqueous viable epidermis and, as a result, are often poorly permeable[6]. Ethyl alcohol, propylene glycol, dimethyl sulfoxide (DMSO), linoleic and oleic acids, azone and thiazone are widely used as enhancers of OCA diffusion through the skin upper layers[1,7-11]. These substances are capable to do temporary and partial dissolving the lipid basis of the SC of the epidermis or to create pores in it. Unlike chemical enhancers, physical methods of increasing of the effectiveness of transdermal penetration of the OCA have two preferences: 1) the enhancer and the OCA are not interacting; 2) these methods reduce the risk of additional irritation[10]. To reduce the protective barrier of the skin epidermis the following methods are usually applied: the removal of the skin upper layers with medical sticky tape strip from the target area[12], microdermabrasion[13], low-and high-intensity laser irradiation of the skin[14,15], iontophoresis[16], the impact by ultrasound[17] and photomechanical (shock) waves[10], needleless injection[18], photothermal and mechanical microperforation[18,19], and epidermis microdamage[20].

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The microporation is the least invasive method used to enhancement of penetration of the immersion agents into the skin at sufficiently large depth. In this case the sufficiently fast (during a few hours) restoration of the barrier integrity occurs after the injection of agents[21]. These specific features also reduce the risk of skin infection caused by the manipulations carried out[5].

In this paper we are presenting the results of the study of delivery of mineral oil and PEG-300 into the rat skin *in vivo* using the fractional laser microablation of the skin.

2. MATERIALS AND METHODS

2.1 Samples

The *in vivo* experiments were carried out with rat skin. Two male and two female animals were used. The age of the animals was 12±6 months. The animals were anesthetized with Zoletil 50 (Virbac, France). The dose was 0.18±0.02 mL. Before the beginning of the experiment the hair was removed from the studied skin area using the depilatory cream Nair (Church & Dwight Co., Inc., USA). The pads of the animals were strongly fixed on the wood support by special holders.

2.2 OCAs

As OCAs mineral oil (Sigma-Aldrich Co., USA) and polyethylene glycol having the molecular weight 300 (PEG-300) (Aldrich, USA) were used. Refractive indices of mineral oil and PEG-300 were measured with refractometer Abbe (Atago DR-M2/1550, Japan) at some wavelengths (450, 589, 680, 1100, and 1550 nm) and interpolated. There were evaluated as 1.457 for the both agents at 930 nm. The OCAs was put on the investigated area once and wasn't added again.

2.3 Experimental setup

To improve the diffusion of OCAs through the skin the technique of fractional laser microablation of the skin surface was used. The Palomar Lux2940 erbium laser (Palomar Medical Products Ltd., USA) was used as a light source. Its parameters were the following: the wavelength 2940 nm, the pulse duration 5 ms. Two regimes of laser action were used in the experiments. In regime I a handpiece that allowed ablation of skin upper layer was used, energy of the pulses was 0.8 J. In the regime, the depth of the damaged area was less than 100 μm. In regime II, 169 vertical microchannels were made in the skin on the area with the dimensions of 6×6 mm. The separation between the centers of the channels was nearly 500 μm. The depth of the channels was about 150 μm. Energy of the pulses was 1 J.

The skin areas affected by the OCAs were visualized with the Spectral Radar OCT System OCP930SR 022 (Thorlabs Inc., USA). The system has central wavelength of 930 nm, spectral bandwidth of 100 nm, output power of 2.0 mW, depth resolution of 6.2 μm, lateral resolution of 9 μm, and optical scanning depth of 1.6 mm in air. As a result we got two-dimensional images consisted of 512 × 2000 pixels. The data acquisition time for the whole image was about 3 s. Besides images of investigated area, the OCT system software allowed to receive data file contained the matrix of intensity values in each image pixel. Each OCT-image was averaged in the lateral direction (~ 1 mm, which is enough for speckle noise suppression). The final single curve of the averaged OCT signal was plotted presenting a one-dimensional distribution of the reflected light intensity over the optical depth. Processing of the experimental data made it possible to calculate such important parameters as optical probing depth in investigated sample Δ and permeability coefficient *P* of the skin for different OCAs and regimes of microablation.

The optical probing depth value was calculated as a level where intensity of OCT signal decreased by a factor of *e* (fig.1). The rat skin permeability coefficient *P* was calculated by division of the thickness of the studied part (with the maximal change in the OCT-signal) by the diffusion time of the agent, $P = z_{reg} / t_{reg}$ [22].

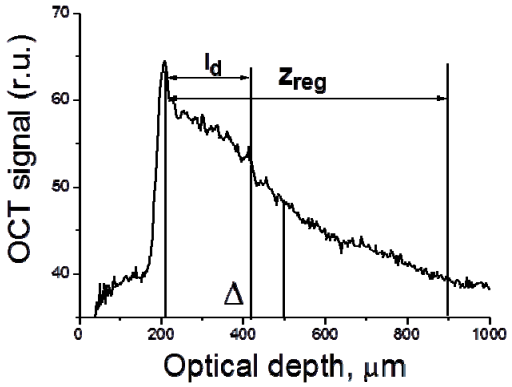

Figure 1. Definition of optical probing depth Δ, the thickness of the studied part (z_{reg}), and the depth of the tissue sample probing (l_d).

Since the immersion agent diffusion manifests itself in the change in the OCT signal Slope (OCTSS) [22], which is relatively constant both before the agent application and after the attainment of saturation, the value of t_{reg} was calculated as the saturation time minus the time of the beginning of the OCTSS variation (i.e., the time of the agent application). OCTSS was calculated as $arctg\left[\frac{(OCT\ signal_{\max} - OCT\ signal_{\min})}{z_{reg}}\right]$.

3. RESULTS AND DISCUSSION

Figures 2 and 3 show the OCT-images of the investigated areas of rat skin *in vivo* and time dependences of OCTSS and optical probing depth for regime I. Figures 4 and 5 demonstrate the same parameters for regime II. The white rectangle shows the area of averaging. The shifting of the area of averaging was caused by factors of animal's breathing and heartbeat. The white lines show size bars. The length of vertical bar is 300 μm and of horizontal bar is 600 μm.

Fig. 2 shows that since 1 till 25 min after topical application of mineral oil on the skin with an ablation of skin upper layers (microablation in regime I) OCTSS decreases and optical probing depth increases on average from 160 to 290 μm as mineral oil diffused into deeper layers of epidermis. Thus, in this case the optical probing depth increased in 1.8 folds. Then since 25 till 50 minute we can see the reverse process and the values of these characteristics decreases due to outwashing of OCA because of the metabolism processes. After that the saturation of OCTSS is observed.

The use of microablation in the second regime with mineral oil application (Fig. 4) allows negligible increasing of optical probing depth in 10-15 min and then decreasing below the initial value. However, the respective OCTSS time dependence analysis makes it possible to conclude that OCTSS decreases only during the first 15 min after topical application of mineral oil on the skin with microchannels. It can be by the difficult diffusion of mineral oil, which is a lipophilic agent, into the interstitial space. Interstitial fluid is highly hydrophilic medium filled for the most part by aqueous solution of proteins. Slight increasing optical probing depth and decreasing OCTSS in the initial period of the observation can be connected with the clearing of epidermal tissue. Deep damage of dermis due to the microporation apparently caused water influx in the area. Thus, in this case application of mineral oil does not promote the optical clearing of skin.

The analysis of OCT-images and time dependences of OCTSS and the optical probing depth makes it possible to conclude that the skin treatment of joint application of PEG-300 with skin microablation in regime I promote gradual 1.4-fold growth of optical probing depth in 60 min (Fig.3). Slow growth of the optical probing depth can be explained by diffusion of the hydrophilic agent in the dense epidermal layer. Besides, diffusion of exogenous agent in skin tissues is accompanied by the metabolic washing out of the agent from the area of observation.

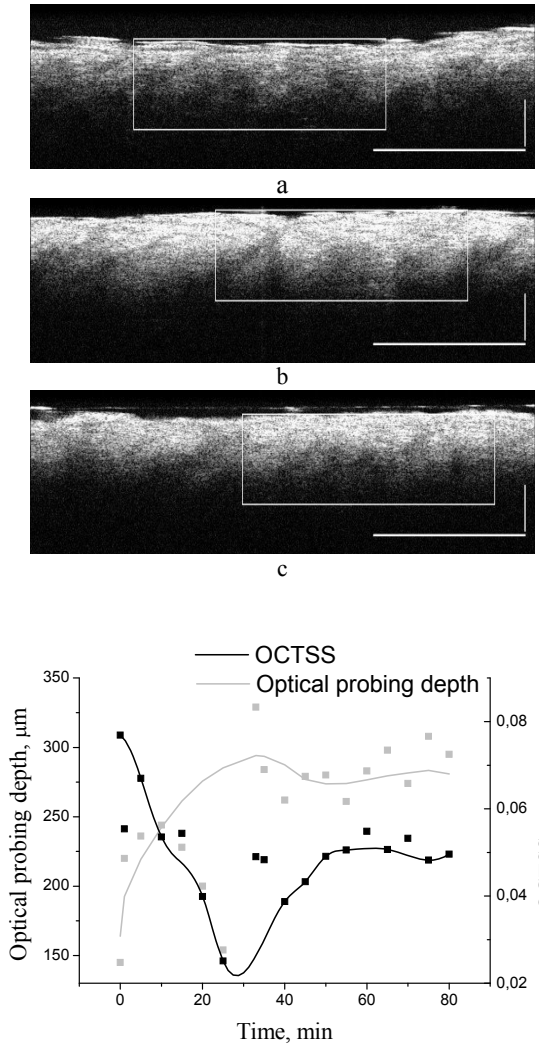

Figure 2. OCT-images of rat skin *in vivo* with microablation in regime I and 1 min (a), 30 min (b) and 70 min (c) after mineral oil application; respective time dependences of OCTSS and optical probing depth (d): dots correspond to experimental data, solid curve – result of interpolation.

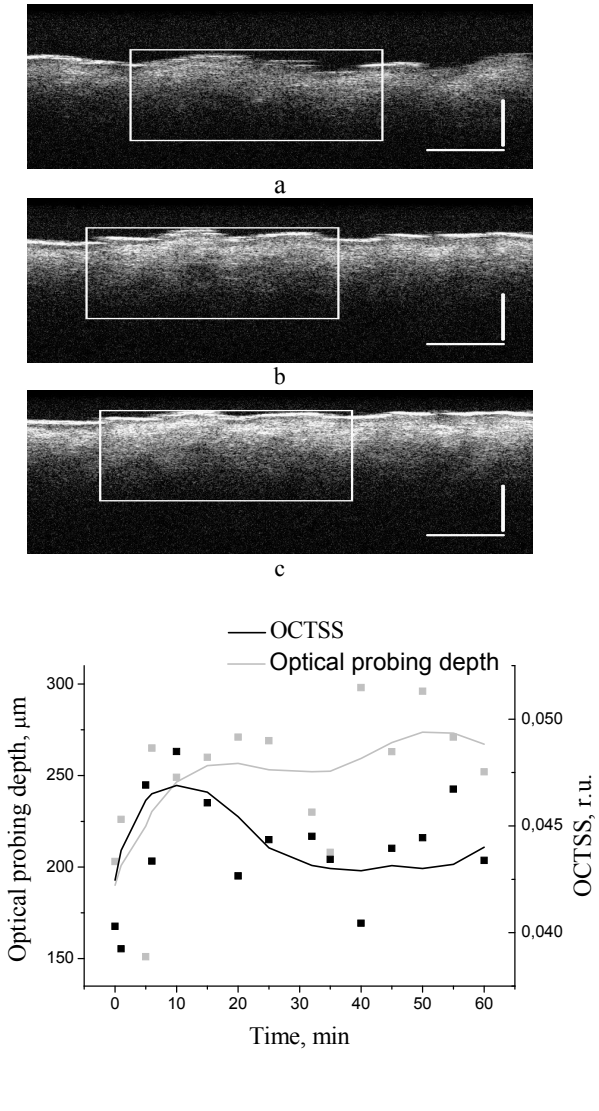

Figure 3. OCT-images of rat skin *in vivo* with microablation in regime I and 1 min (a), 30 min (b) and 60 min (c) after PEG-300 application; respective time dependences of OCTSS and optical probing depth (d): dots correspond to experimental data, solid curve – result of interpolation.

Minor increasing of OCTSS in the initial 10 minutes of the observation can be explained by the brightness increasing of dermal layer that changed the profile of OCT signal. From 10 till 30 min decreasing OCTSS is connected with optical clearing of skin.

In the Fig. 5 it is well seen that the optical probing depth firstly decreased insignificant during 10 min and then increased in 1.2-folds in the case of a combined impact of PEG-300 with skin microperforation. Accordingly OCTSS firstly increased in the same period and then since 10 till 35 min after topical application of PEG-300 on the skin with microchannels (microablation in regime II) we can see the process of diffusion of the immersion agent into the skin, as evidenced by the OCTSS decrease (Fig.5). Since 35 till 50 min we can see the reverse process and the value of OCTSS increases and restore due to washing out of the OCA because of the metabolism processes. After that the saturation of OCTSS and optical probing depth time dependences is observed.

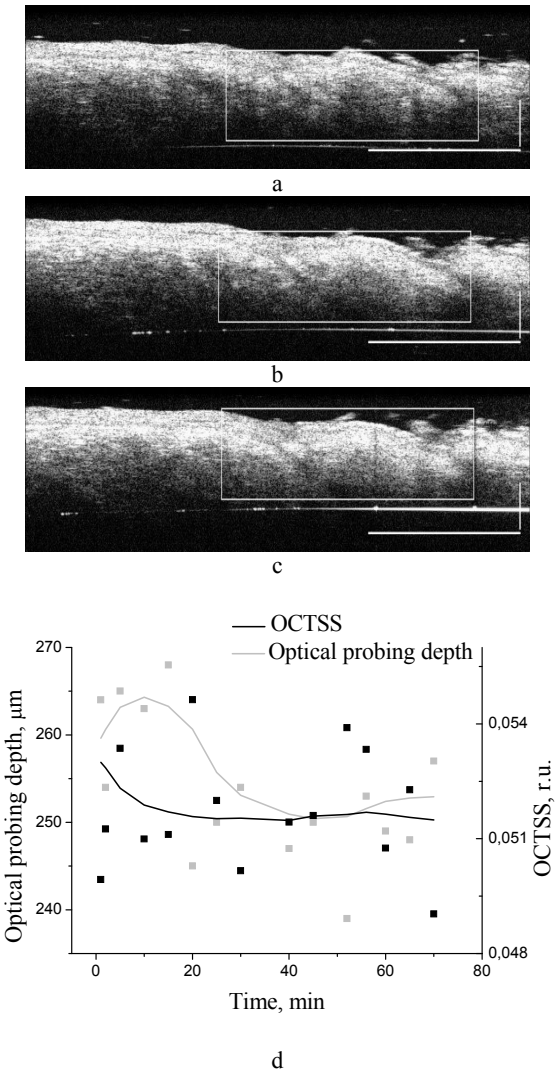

Figure 4. OCT-images of rat skin *in vivo* with microablation in regime II and 1 min (a), 30 min (b) and 70 min (c) after mineral oil application; respective time dependences of OCTSS and optical probing depth (d): dots correspond to experimental data, solid curve – result of interpolation.

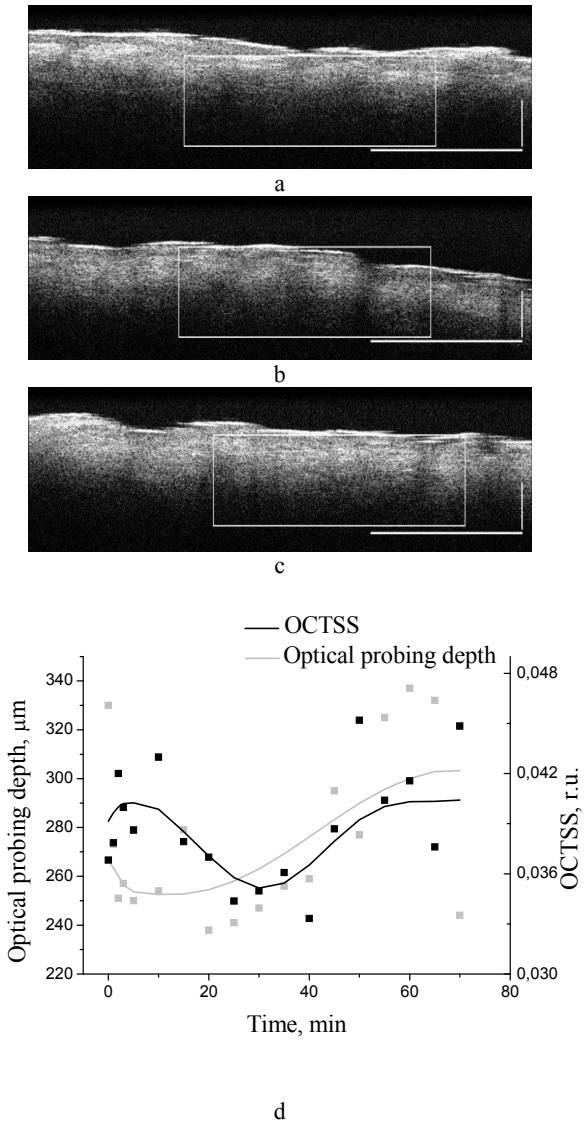

Figure 5. OCT-images of rat skin *in vivo* with microablation in regime II and 1 min (a), 30 min (b) and 70 min (c) after PEG-300 application; respective time dependences of OCTSS and optical probing depth (d): dots correspond to experimental data, solid curve – result of interpolation.

We quantified the values of permeability coefficients of the rat skin *in vivo* and for different regimes of microablation. The permeability coefficients were averaged and found to be $(3.41\pm0.46)\times10^{-5}$ cm/s and $(2.35\pm0.30)\times10^{-5}$ cm/s for mineral oil and PEG-300, respectively, at the use of regime I and $(3.32\pm0.09)\times10^{-5}$ cm/s and $(3.61\pm0.34)\times10^{-5}$ cm/s for mineral oil and PEG-300, respectively, at the use of regime II. The results are presented in figure 6. It could be clearly seen that the permeability coefficient of mineral oil in the case of microblation in regime I is 1.03-fold greater than that for the case of microblation in regime II. For PEG-300 we can see the inverse situation. The permeability coefficient of PEG-300 in the case of microblation in regime II is about 1.5-fold greater than that for the case of microblation in regime I. It means that there is a significant difference in the permeability coefficient of skin for agents with hydrophilic or lipophilic properties and different laser impacts.

Microablation in regime I promotes mineral oil diffusion into the dermis and displacement of water by OCA. Microchannels in regime II cause, apparently, skin swelling and water influx, which contribute to the rapid outwashing of the OCA from the dermis. In the case of usage of microablation in regime I we damage epidermal skin layer which contains a small amount of interstitial liquid so the swelling is not observed. In the case of usage of microablation in regime II, the damage of the dermal layer causes more active response of the organism, which is expressed in the influx of interstitial liquid to the place of injury, resulting in a swelling.

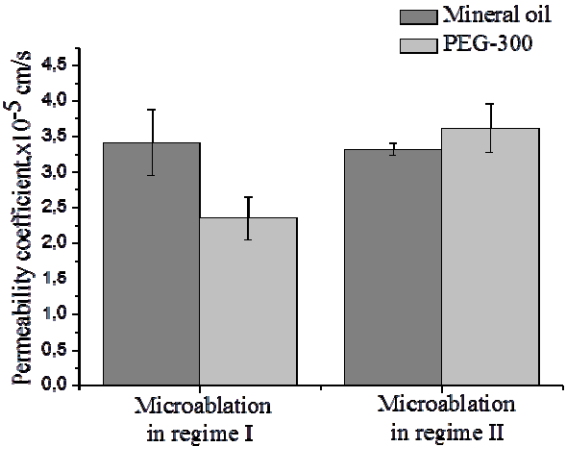

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Figure 6. The permeability coefficient of mineral oil and PEG-300 at the skin microablation in regime I and in regime II.

Xiao Guo et al. reported the permeability coefficients of the human skin *in vivo* for such aqueous solutions of OCAs as 40% propylene glycol, 40% glucose and 40% glycerol quantified by the method of OCT signal slope analysis[23]. The permeability coefficient values were averaged and found to be $(2.52 \pm 0.02)\times10^{-6}$ cm/s, $(1.94 \pm 0.05)\times10^{-6}$ cm/s and $(1.82 \pm 0.04)\times10^{-6}$ cm/s for 40% propylene glycol, 40% glucose and 40% glycerol, respectively. The comparison of these results and results presented in at our work allows concluding that permeability coefficients in the case of skin microblation are order of magnitude greater than that for the intact skin. It proves the fact that application of microablation method promotes more quick and deep penetration of OCAs into the skin and its distribution in dermis and, as a consequence, more sufficient optical clearing effect.

4. CONCLUSIONS

A new method for enhancement of OCA delivery into the skin *in vivo* using fractional laser microablation of the skin surface was studied for both lipophilic and hydrophilic agents. The performed results have shown that the use of fractional laser microablation allows efficient insertion of OCAs into skin dermis. The OCT probing depth of the rat skin has been increased after its treatment with mineral oil and PEG-300 excluding the joint using of skin microporation and mineral oil. In this case hydrophilic interstitial fluid of dermis hinders in diffusion of lipophilic agent. So, the joint application of mineral oil with an ablation of skin upper layers promotes nearly 2-fold increasing of optical probing depth in 30 min. Joint application of PEG-300 in the same regime promotes less sufficient growth of optical probing depth. It's just 1.4-fold difference in 60 min. Optical probing depth increased by 1.2 times at the combined impact of PEG-300 with skin microporation.

The following values of the permeability coefficient of the skin with microablation were obtained: $(3.41\pm0.46)\times10^{-5}$ cm/s and $(2.35\pm0.30)\times10^{-5}$ cm/s for mineral oil and PEG-300, respectively, at the use of the surface microablation and $(3.32\pm0.09)\times10^{-5}$ cm/s and $(3.61\pm0.34)\times10^{-5}$ cm/s for mineral oil and PEG-300, respectively, at the use of the microporation. Obtained data can be used for optical diagnostics and treatment of skin diseases.

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