In vivo optical clearing of human skin under the effect of aqueous solutions of some monosaccharides

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Abstract. The results of in vivo optical immersion clearing of human skin under the effect of aqueous solutions of some immersion agents (monosaccharides of ribose, glucose and fructose, as well as glycerol, a triatomic alcohol) were obtained with the use of the OCT method. Values of average velocity of scattering coefficient change, obtained through an averaged A-scan of the OCT signal in the region of derma with the depth of 350 to 700 µm, were determined to evaluate the optical clearing efficiency. The velocity of scattering coefficient change and the optical clearing potential value appeared to be well correlated. The complex molecular modeling of a number of immersion clearing agents with a mimetic peptide of collagen (GPH) 3, carried out with the use of the methods of classical molecular dynamics and quantum chemistry, allowed to identify correlations between the optical clearing efficiency and the energy of intermolecular interaction of clearing agents with a collagen peptide fragment.

1. Introduction

The application of modern methods of photomedicine and biomedical optics for diagnosis and therapy of diseases entails difficulties arising because of strong scattering in visible and near-infrared regions inherent to skin and many other biological tissues. This scattering happens due to inhomogeneity of refractive indices at borders of different macromolecular structures, basically on collagen fibers that are primarily responsible for light scattering of skin [1]. These difficulties can be overcome with injection of biocompatible molecular agents into the tissue, which to some extent facilitates its optical clearing [2-5]. Today, the mechanisms of optical clearing at molecular level have not been identified clearly yet, and there are very few studies [1, 6, 7] on molecular processes responsible for skin optical clearing. The paper [8] presents the results of the study on clearing agent dehydrating properties and underlines that dehydration is just one of the possible mechanisms that lead to biological tissue clearing. Conducting research in this field provides the understanding of optical clearing processes at molecular level, which, in its turn, will allow to use new efficient clearing agents with tailor-made properties.

This paper is sequel to the authors’ studies on molecular mechanisms of biological tissue optical clearing. The papers [9-10] describe the study on the interaction of a glycerin immersion agent with a collagen mimetic peptide ((GPH) 9) 3 and a fragment of microfibril 5((GPH) 12) 3 with the use of the method of classical molecular dynamics.

The papers [11-13] are dedicated to the studies on interaction of six biotissue-clearing immersion agents (1, 2 and 1, 3-propanediol, ethylene glycol, glycerol, xylitol and sorbitol) with a collagen mimetic peptide (GPH) 1 with the use of the methods of classical molecular dynamics, molecular docking and quantum chemistry (PM6 and DFT/B3LYP). Correlations between the optical clearing potential and such parameters of intermolecular interaction as the time of a hydrogen-bonding state of
agents, relative probability of double hydrogen bond formation and complex formation energy were identified.

In the framework of the present paper, the authors continue studying correlations between the efficiency of biological tissue optical clearing and the energy of forming complexes between clearing immersion agents with a collagen mimetic peptide. A number of monosaccharides – ribose, glucose and fructose – were used as immersion agents.

2. Experiment methods and results
Aqueous solutions (60%) of the following immersion agents were used to study optical clearing of skin: monosaccharides (ribose, glucose and fructose) and, for comparison, glycerol, a triatomic alcohol. The optical coherence tomography (OCT) was used to evaluate the influence of clearing properties of immersion liquids on skin during in vivo experiments. Visualization was carried out with the use of Thorlabs OCP930SR (Thorlabs, USA), an optical coherence tomograph, with the following parameters: radiation wave central length of 930 ± 5 nm, axial and lateral resolution of 6.2 and 9.6 µm respectively (in the air), scan region length of 2 mm.

Permission to conduct a study on in vivo optical clearing of human skin was obtained from the ethics committee of Saratov State Medical University. The measurements were carried out on a skin area of the back of a forearm. The scans were recorded before the exposure with immersion agents, then at 1-minute intervals during 40 minutes of exposure. The measurements involved four volunteers, and a total of five experiments was carried out for each immersion agent.

The attenuation coefficient $\mu_t$ [16] was evaluated by the OCT scans skew on the basis of the single scattering model [14-15]. According to this model, the registered OCT signal power $R(z)$ is proportional to $\exp(-\mu_t z)$ [16]. As the absorption factor $\mu_a$ is far less that the scattering coefficient $\mu_s$ [1] in the spectral range under study, the attenuation coefficient $\mu_t = \mu_s + \mu_a$ may be considered approximately equal to the scattering coefficient, so the value $R(z)$ may be approximated by the expression $R(z) = A\exp(-\mu_s z) + B$, where $A$ is the proportionality factor equal to $P_0 a(z)$, $P_0$ is the optical power of the beam falling on the biological tissue surface, $a(z)$ is determined by the biotissue local ability to disperse light backwards, which depends on a local variation of the refraction index, and $B$ is the background signal. The selection of coefficients in the above expression for approximation of the experimental curve allows to evaluate a depth-averaged (efficient) light scattering coefficient by the tissue sample.

Figure 1 presents analyzed areas of the OCT image, as well as an averaged A-scan of the OCT signal of the human skin dermal layer in vivo (5 minutes after applying 60% ribose solution on the surface) and an approximating curve plotted with the use of the single scattering model. OCT signals were averaged by the A-scan over the whole area of scanning.

Figure 1. Measurements of the scattering coefficient $\mu_s$ in the derma region with the depth of 350 to 700 µm (dotted) on the basis of the analysis of depth distribution of the averaged OCT signal with the use of the single scattering model; (a) is the in vivo fragment of the B-scan of skin that was used to average the OCT signal, (b) is the depth distribution of the averaged OCT signal (thin curve) and the approximation result according to the single scattering model (thick curve). The dashed lines are borders of the regions where the value $\mu_s$ was estimated.
The scattering coefficient values were determined in the region of the averaged A-scan at the depth of 350 to 700 µm.

Within the framework of the present paper, values of the light scattering coefficient, obtained with the use of the averaged A-scan in the derma region with the depth of 350 to 700 µm were determined to evaluate the efficiency of in vivo optical clearing of human skin. Figure 2 presents temporal dependences of the obtained scattering coefficients under exposure to 60% aqueous solutions of three monosaccharides and one trialcohol glycerol.

Figure 2. Dependence of the scattering coefficient value $\mu_s$ in the derma region with the depth of 350 to 700 µm, based on the analysis of the depth distribution of the averaged OCT signal with the use of the single scattering model, on the effect of immersion agents: (a) – glycerol; (b) – ribose; (c) – glucose and (d) – fructose. The linear approximation at the time segment from 7 to 24 minutes is shown with a solid line (the data are marked in white).

As is seen from the figure, the clearing velocity on a long time interval is nonlinear and is described well with an exponential regression model (the determination coefficient $R^2$ is within the range from 60 to 90%). However, at a short time segment, this dependence also can be described well within a simpler linear regression model. The interval from 7 to 24 minutes appeared to be the best time segment for the derma region, chosen within the framework of the present paper. The determination coefficient $R^2$ for the linear regression model is within the range from 50 to 80%, which makes this model acceptable to describe the obtained dependence.

Values of the module of average velocity of the scattering coefficient change at the chosen time segment were used for numerical expression of the skin optical clearing efficiency. These velocities are presented in the graph as a skew value in regression line equations.

3. Molecular modeling methods and results
As in the previous papers [9-13], a mimetic peptide of collagen (GPH)$_3$ [17,18], forming the basis of a great part of regular domains of human collagen, was used as a molecular model of collagen. Such
relatively small synthetic peptides are often used for collagen molecular modeling. A peptide 3D model was built according to the Protein Data Bank (PDB) data with further addition of hydrogen atoms and optimization of the structure by the molecular mechanics method [19]. A number of monosaccharides (ribose, glucose and fructose) were considered as immersion clearing agents. The molecular modeling of interaction of clearing agents with collagen was carried out in two stages.

At the first stage, the method DFT/B3LYP/6-311+G(d,p) [21,22] and the programme Gaussian [22] were used to identify and calculate all the lowest energy conformers of the considered monosaccharides in their isolated state. The calculated geometric parameters and values of the Mulliken atomic charges were further used in modeling of these systems within the framework of classical molecular dynamics. Vibrational transition wavenumbers were also calculated and appeared to be positive, which gives another evidence of molecular systems being at the local minimum. Spatial configurations of the lowest energy conformers of some clearing agents are shown in Figure 3a and 3b.

![Figure 3](image)

**Figure 3.** Spatial configurations: (a, b) – the lowest energy conformers of some clearing agents (ribose and glucose); (c) – a fragment of the mimetic peptide ((GPH)_3)_2 optimized within the semi-empirical method PM6 (figures stand for molecular groups participating in formation of hydrogen bonds with the clearing agents); (d, e) – hydrogen-bonding complexes, formed by the collagen fragment ((GPH)_3)_2 and the above immersion clearing agents. Hydrogen bonds are shown with dotted lines.

At the second stage, a minimum fragment of the mimetic peptide, preserving the regular structure ((GPH)_3)_2, consisting of 231 atoms and having a structure that was preliminary optimized within the semi-empirical method PM6, was used to evaluate the energy of intermolecular interaction of the chosen clearing agents with collagen [23]. The obtained spatial structure is shown in Figure 3c. As is seen from Figure 3c, an entry molecular pocket is a peptide region with an approximate size of 10x12 Å that has four functional groups available for intermolecular bonding: two carbonyl groups (one at the glycine residue (2) and the other at the hydroxyproline residue (3) of the same α-chain) and two alcohol groups (1 and 4) at the hydroxyproline residues of different α-chains. This optimized structure of the collagen model was used to carry out molecular docking with clearing agents within the program AutoDockVina [24].
When the molecular docking was carried out, the first five most suitable configurations were selected for each interacting system, and then they were optimized with the semi-empirical method PM6. Then the total electronic energy of the complexes was calculated with the method DFT/B3LYP/6-31G(d) and through the single SCF procedure. A similar procedure was used to obtain values of the total electronic energy of the clearing agents and the peptide fragment. The intermolecular interaction energy was calculated as a difference between the complex total energies and the sum of energies of its specific components. The largest values of intermolecular interaction energies, corresponding to the most probable complex structures, were chosen to identify correlation with the optical clearing efficiency. Figure 3 (d,e) shows a PM6-obtained spatial structure of hydrogen-bonding complexes, formed by the collagen fragments ((GPH)_3) and some clearing agents.

4. Discussion and findings
To make a discussion on the obtained results more convenient, the qualitative parameters of intermolecular interactions (the values of length of classical hydrogen bonds, formed according to calculations between active groups of the collagen molecular pocket and hydroxyl groups of clearing agents, and calculated values of intermolecular interaction energies), as well as values of the module of average velocity of scattering coefficient change, experimentally obtained with the use of OCT, are presented in Table 1.

Table 1. Lengths of hydrogen bonds (in angstroms), energies of intermolecular interactions (in kJ/mol) between the fragments of collagen (GPH)_3 and different clearing agents, calculated with the use of the method PM6/B3LYP/6-31G(d), as well as experimental values of optical clearing velocity and their standard errors.

| Type of agent | Hydrogen bond lengths | ΔE  | Efficiency of skin optical clearing |
|---------------|-----------------------|-----|-----------------------------------|
| glycerol      | 1.74; 1.91; 1.92; 1.93; 2.44 | -42.8 | 0.69 ± 24.6%                     |
| ribose        | 1.84; 1.90; 1.91; 1.95 | -80.9 | 0.94 ± 16.0%                     |
| glucose       | 1.68; 1.71; 1.84; 1.94 | -94.5 | 1.55 ± 12.9%                     |
| fructose      | 1.82; 1.84; 1.90; 1.96; 2.23 | -89.2 | 1.52 ± 24.3%                     |

For example, as can be seen from Table 1, a molecule of trialcohol glycerol forms four relatively strong hydrogen bonds and one weaker bond with all active groups of the entry pocket; however, the molecule length is insufficient to make all the hydrogen bonds efficient. The transition from trialcohol glycerol to glucose monosaccharide demonstrates a significance increase in the skin optical clearing efficiency. It can be explained by the fact that, according to the calculation, glucose forms stronger hydrogen bonds with collagen than glycerol does. Table 1 shows that, despite fewer hydrogen bonds, their length is notably shorter, which is a determining factor for bonding energy. As is seen from Fig.3e, stronger hydrogen bonds with carbonyl groups are formed both due to a compact ring structure of glucose, which allows it to lower into a collagen molecular pocket, and due to good mutual disposition of interacting groups.

Various parameters are used by different authors to evaluate the degree (efficiency) of optical clearing with immersion agents. For example, in [1] they use the optical clearing potential, introduced as a slope of the dependence of reduced scattering coefficient after 45 minutes of immersion agent effect on its concentration, expressed in moles, i.e. for its determination it is necessary to study the effect of immersion agents with different initial concentrations.

In the present paper, values of the module of average velocity of scattering coefficient change under effect of the solution of a moderate-concentration immersion agent (60%) were used for numerical expression of the skin optical clearing efficiency.
As is seen in Figure 4, these two parameters correlate well with each other. It allows us to use the value of clearing velocity in further research as a way to evaluate the efficiency of optical clearing with immersion agents.

![Figure 4](image)

**Figure 4.** Dependence of the value of the optical clearing potential (left axis) of rat skin (a) and human skin (b) [1], as well as values of the efficiency of optical clearing (right axis) of human skin (c) on the energy of interaction between a collagen peptide molecule and molecules of clearing agents. Immersion agents are indicated with numbers: 1 – ethylene glycol; 2 – 1,2-propanediol; 3 – 1,3-propanediol; 4 – glycerol; 5 – xylitol; 6 – sorbitol; 7 – ribose; 8 – glucose and 9 – fructose. Linear approximations of these dependences are shown with dotted lines.

Figure 4 also shows that the energies of interaction between a collagen peptide molecule and molecules of different clearing agents, calculated with the use of the method PM6/B3LYP/6-31G, correlate well both with the value of the potential of rat and human skin optical clearing [1] and with the experimentally obtained values of velocity of human skin scattering coefficient change. The values of the linear correlation coefficients are 0.94 and 0.88 respectively. It is therefore possible to speak of fundamental importance of a post-diffusion stage, where collagen interacts with clearing agents and influences optical clearing of biological tissues. The study results allow to suggest that during the process of this interaction, a partial substitution of collagen-related water occurs. It leads to disturbance in a binding net of hydrogen bonds and, consequently, to a reversible process of collagen fibril dissolution, which, decreases their value of deflection index and equalizes it to the intercollagen medium. The higher collagen affinity of a clearing agent, the more effective the process is.

The next essential step to increase the interaction efficiency is choosing a molecular agent with specific structural characteristics that would allow it to interact with two or more molecular pockets of collagen at the same time.

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