Optical clearing of blood and tissues using blood components

Olga S. Zhernovaya, Elina A. Genina, Valery V. Tuchin, Alexey N. Bashkatov

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Introduction

Strong light scattering in biological tissues is determined by refractive index mismatch between its components, such as collagen fibers, cellular organelles, interstitial fluid, etc. Scattering prevents the obtaining of good contrast and well-resolved images of microstructure of tissues by optical imaging techniques, such as optical coherence tomography (OCT), visible and near-infrared spectroscopy, fluorescence spectroscopy, microscopy, and others. The optical clearing method is a possible solution for improving imaging quality and increasing imaging depth for optical imaging techniques. Light scattering in blood is mainly caused by the refractive index mismatch between erythrocytes (red blood cells, RBCs) and blood plasma, as erythrocytes have a higher refractive index than blood plasma. Application of biocompatible optical clearing agents (OCAs) decreases light scattering in blood which leads to an increase in optical penetration depth and enhancement of quality of imaging. Determination of optimal type and concentration of optical clearing agents for optical clearing of blood is required for effective and nondestructive usage of the optical clearing method in in vivo applications [10–18]. A number of optical clearing agents, such as dextrans, glycerol, glucose, fructose, hemoglobin, and others demonstrated a potential to be used for optical clearing of blood. The mechanism of optical clearing of blood depends on the properties of a particular OCA. The optical clearing agents can cause aggregation of erythrocytes, refractive index matching between RBCs and blood plasma, alterations of morphology of erythrocytes, and some other effects which change the scattering properties of blood and allow the optical clearing effect in blood to be achieved.

Optical properties of blood

Whole blood consists of plasma and formed elements: erythrocytes (red blood cells, RBCs), leukocytes (white blood cells), and thrombocytes (platelets). Erythrocytes are the predominant group of blood cells. Blood plasma makes up about 55% of whole blood volume and is composed of water (about 90%) and dissolved proteins, glucose, hormones, and other substances. In norm, hematocrit (volume fraction of erythrocytes) in adults is 41%–53% for males and 36%–46% for females [19–21]. An erythrocyte contains about 70% of water, 25% of hemoglobin, and 5% of lipids and glucose. Hemoglobin in erythrocytes is contained in the membrane, which is formed by mostly lipids and proteins. In norm, an erythrocyte is formed as a biconcave disc with the diameter of about 8 μm [20].

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Blood is a highly scattering and absorbing medium in the visible and near infrared regions. Scattering properties of RBCs, the main scatterers in blood, are determined by their size, shape, volume, and mass. Optical properties of RBCs also depend on concentration of hemoglobin in erythrocyte. Absorption properties of blood in the visible range are mainly determined by the absorption bands of hemoglobin. The absorption and scattering spectra of blood and anisotropy factor in visible and near infrared regions are represented in Figure 20.1 [21].

The absorption spectra of blood have characteristic absorption peaks of hemoglobin in the visible range at about 410, 540, and 575 nm (for oxygenated blood), 430 and 555 nm (for deoxygenated blood), and isobestic point at 805 nm. In the near infrared region, the absorption spectra of blood are determined by the absorption bands of water with the maxima at 1450 and 1930 nm. The main source of light scattering in blood is the refractive index mismatch between erythrocytes and blood plasma. The scattering properties of a single RBC depend on its refractive index, which is mainly determined by refractive index of hemoglobin in RBCs. In norm, the concentration of hemoglobin is 120–175 g/L in blood and 310–370 g/L inside the erythrocyte [19]. Refractive index of erythrocytes is much higher than plasma; in the range of 800–1000 nm, the refractive index of hemoglobin with the concentration of 287 g/L is 1.40, while the refractive index of blood plasma is 1.33–1.34 in the same wavelength region [11, 22–24]. Scattering properties of blood mainly depend on hematocrit (the volume fraction of erythrocytes), but there are several factors which can influence the scattering properties of blood, such as aggregation of erythrocytes, sedimentation of blood, hemolysis, and deformation of the RBCs under various conditions. Spectrally, it is clearly seen that in the range from 250 to 630 nm, blood scattering spectrum has dips in the area of hemoglobin absorption bands, and in the range from 630 to 2000 nm, the scattering coefficient monotonically decreases as wavelength increases. The wavelength dependence of scattering anisotropy factor has similar behavior. It is clearly seen that in the range 250–630 nm, the dependence increases but has dips in the area of hemoglobin absorption bands, and in the range from 630 to 2500 nm, the anisotropy factor is slightly decreased in the area of water absorption bands with maxima 1445 and 1930 nm.

**Optical clearing of blood by dextrans**

Dextrans are considered to be promising agents for optical clearing of blood due to their biocompatibility, relatively high refractive index, and ability to induce aggregation of blood. The influence of dextrans with different molecular weights on the optical properties of blood and their applicability for optical clearing of blood were investigated in several studies [10–12]. Addition of dextran to blood in comparison to intravenous contrast agents and their influence on blood transmittance was studied by OCT *in vitro* in circulating blood by Breziniski et al. [10]. Both dextran and intravenous contrast agents were found to significantly increase the light penetration in blood. Application of dextran and intravenous contrast to blood led to the increase of the OCT signal intensity to 69% and 45%, respectively, while the control measurements with saline did not provide any significant effect of reduction in scattering [10]. The increase of the OCT signal intensity after the addition of intravenous contrast agent to blood was suggested to be due to the decrease in the volume of erythrocyte caused by the addition of the agent. The effect of the increased light penetration of blood after application of dextran was mainly attributed to refractive index matching between RBCs and ground matter.

The results of OCT study of optical clearing of blood by several clearing agents, including dextrans with different molecular weights, were presented by Tuchin et al. [11, 12]. It was found that the mechanism and capability of dextrans to improve light transport in blood depends on the molecular weight of the dextrans and their concentration (Figure 20.2).

Application of dextran with high molecular weight (Dx500 – dextran with molecular weight of 473,000) demonstrated better optical clearing efficiency than dextran with low molecular weight [11, 12]. This effect was explained by the assumption that dextran with high molecular weight not only had a greater ability to match the refractive indices of erythrocytes and blood plasma due to the high refractive index of dextrans, but also had a greater ability to induce aggregation of blood, which resulted in enhancement of transmittance of blood. Application of dextran with low molecular weights (Dx10 – dextran with molecular weight of 10,500) improved light transport only due to refractive index matching between RBCs and ground matter. It was also found that high concentration of...
Optical clearing of blood by glucose and fructose

Glucose is widely used as an optical clearing agent for improvement of light penetration in biological tissues for various optical techniques [4, 11, 25–31]. The refractive index of highly concentrated glucose and fructose solutions is higher than that of blood plasma; therefore, glucose and fructose can be used for enhancement of light transport by refractive index matching between erythrocytes and blood plasma.

Theoretical modeling of optical properties of blood upon addition of glucose solutions with different concentrations was performed by Bashkatov et al. [18]. The influence of glucose on the scattering coefficient of blood was estimated for the wavelength range of 400–1000 nm, and it was found that the scattering coefficient of blood decreases at the increase of the concentration of glucose. It was also found that the scattering coefficient of blood nonlinearly depends on the concentration of glucose. The minimum of the scattering coefficient for most wavelengths was observed at the concentration of glucose about 0.6–0.7 g/mL.

The experimental estimation of the optimal concentration of glucose and fructose required for blood optical clearing was performed in vitro using the OCT system working at 930 nm [32]. The decrease of total attenuation coefficient of blood samples with glucose and fructose with the concentrations in the range of 50–400 g/L was observed for all samples, but the most noticeable decrease (about 1.3x) of the total attenuation coefficient was observed for the blood samples with the concentration of glucose and fructose of 400 g/L. A shrinking of erythrocytes and partial hemolysis subsequently took place at high concentrations glucose or fructose solutions added to the blood, of the order of 200–400 g/L, while at their low concentrations of 50 g/L did not lead to the destruction of erythrocytes. The observed shrinking of erythrocytes and hemolysis at high concentrations of glucose and fructose was attributed to the increase in osmolarity of ground matter due to the addition of high concentrations of glucose and fructose. Thereby, the decrease of scattering of blood after the addition of glucose and fructose solutions can be explained not only by the refractive index matching between RBCs and ground matter, but also by the changes in morphology of RBCs at high concentrations of glucose and fructose. Hemolysis also causes the increase of refractive index of ground matter due to release of hemoglobin in blood plasma, which also leads to the decrease of scattering in blood.

Application of optical clearing agents which change blood osmolarity, such as glucose and fructose, leads to changes in the morphology of erythrocytes, which can be a possible method to change optical properties of blood and increase the transmittance of blood. In in vivo conditions, the RBCs which were shrunken due to application of an optical clearing agent are expected to return to their normal shape. However, the lysis of some erythrocytes caused by addition of the solutions which change blood osmolarity may prevent the using of these solutions for in vivo applications.

Optical clearing of blood by hemoglobin solutions

Application of hemoglobin, albumin, or multimolecular solutions, such as a whole blood, as optical clearing agents for optical clearing of tissues and blood is promising due to their biocompatibility and high refractive indices. Addition of a highly concentrated hemoglobin solution to blood in order to achieve the optical clearing effect was firstly proposed by Tuchin et al. [13], where the Mie-based theoretical analysis of the scattering coefficient of blood at local hemolysis was presented. It was reported that the scattering coefficient of blood decreased upon increase of the degree of hemolysis (Figure 20.3). Popescu et al. [33] have proven this concept experimentally by studying the dynamics and morphology of erythrocytes by Hilbert phase microscopy.

In the spectral range of 400–500 nm, the scattering coefficient of blood decreases to 30% upon raise of the hemolysis up to 20% (Figure 20.3). In the spectral range of 500–1000 nm, the 40% decrease in the scattering coefficient was observed at the increase of the degree of hemolysis up to 20%. The reduction of the scattering coefficient was supposed to be attributed to the increase of refractive index of blood plasma after release...
of the hemoglobin due to hemolysis, and refractive index matching between erythrocytes and blood plasma, which led to decrease of scattering. Thus, it was theoretically proved that the method of optical clearing of blood by hemoglobin solutions can enhance light transport in blood.

The optical clearing of blood by hemoglobin solutions was experimentally studied in vitro by using of an OCT system working at 930 nm [14]. The total attenuation coefficient and enhancement of transmission of blood after addition of hemoglobin solutions were derived from the OCT measurements for blood samples after addition of hemoglobin solutions with the concentrations of 80 and 160 g/L, which had high refractive indices (1.362 and 1.390, respectively). Addition of hemoglobin solutions to blood led to a noticeable decrease of total attenuation coefficient and to the enhancement of transmittance of blood (Figure 20.4). The total attenuation coefficient of blood mixed with the Hb solutions with the concentrations of 80 and 160 g/L decreased by a factor of 1.3 and 1.5, respectively, compared to the control sample. The enhancement of transmittance for blood mixed with hemoglobin solutions was 30% for the 80 g/L and 51% for 160 g/L concentrations, respectively.

After addition of Hb solutions, the refractive index of ground matter became higher due to high refractive indices of hemoglobin solutions. The high refractive indices of added Hb solutions provided the effect of optical clearing by matching of refractive index of RBCs and blood plasma. The microscopic images of blood samples obtained by smear microscopy did not show any aggregation or considerable deformation of the form of the erythrocytes after addition of Hb solutions compared to the blood samples with saline. Hence, it was assumed that the main cause of the observed optical clearing effect in blood after addition of Hb solutions was the refractive index matching between blood plasma and RBCs.

**Optical clearing of blood by PEG, PPG, and PG**

Among the various substances which can be potentially used for optical clearing of blood, polyethylene glycol (PEG), propylene glycol (PG) and polypropylene glycol (PPG) can be appropriate agents due to their high refractive index (about 1.4, which is comparable to the refractive index of RBCs) and ability to change the aggregation properties of blood. Previously, PEG, PPG, and PG were successfully applied for optical clearing of some biological tissues: the application of PEG and PEG-thiazone mixture improved light penetration in human skin [34]; PPG- and PEG-based polymer mixtures was also found to reduce light scattering in dermis [35]; and PG was used for enhancement of imaging of skin and gastrointestinal tissues [3, 25, 36].

The in vitro OCT experimental study of the influence of PEG, PPG, and PG on scattering properties of blood showed that addition of these agents caused considerable improvement of light transport in blood [14]. For added PEG, PG, and PPG, the enhancement of transmittance of blood samples was found to be 94%, 148%, and 162%, respectively (Figure 20.5). The smear microscopy study showed that the addition of PEG caused shrinking and elongating of the erythrocytes. It was also found that PG possibly induced partial hemolysis of erythrocytes, because after addition of PG, erythrocytes became much thinner. Application of PEG and PPG induced aggregation of blood and led to the formation of big aggregates of erythrocytes, which resulted in changes in the scattering properties of blood and decrease in the total attenuation coefficient of blood after addition of these agents.

The effect of the optical clearing of blood after addition of PEG and PPG was supposed to be mainly caused by aggregation of erythrocytes. The adhesion (or aggregation) of RBCs possibly led to a decrease of scattering due to the “sieve” effect, when light travels in blood without interaction with scattering elements [37]. High refractive indices of PEG and PPG increase the refractive index of ground matter, which also makes an impact on overall optical clearing effect. The addition of PG causes the lysis of erythrocytes, which can probably provide a decrease in scattering along with refractive index matching between erythrocytes and blood plasma after release of hemoglobin. The addition of saline to the blood samples with PEG or PPG led to recovery of the erythrocytes, which was observed for some cells; therefore, the aggregation of erythrocytes caused by PEG and PPG can be reversible.
Optical clearing of blood in blood vessels

Optical imaging systems such as optical coherence tomography (OCT) and integrated optical and acoustic imaging systems can be applied for imaging of blood vessels and intravascular imaging [15–17, 38]. OCT allows more precise morphological information of atherosclerotic plaques and stent lesions to be obtained than is possible with other conventional intracoronary imaging techniques. The frequency-domain OCT (FD-OCT) imaging systems and combined OCT-ultrasound systems have been used for intravascular imaging using a catheter inserted into the vessel of interest [15, 17]. Application of optical imaging methods for intravascular imaging has a major limitation: strong scattering of blood, which causes signal attenuation. Using optical clearing agents for imaging of blood vessels was proven to be a promising method for improving imaging quality.

For intracoronary imaging, the FD-OCT imaging systems require an injection of contrast media [15]. The increased amount of contrast media used for OCT imaging may lead to impairment of renal function. In order to reduce the risk of that, Ozaki et al. [15] suggested the use of low-molecular-weight dextran L (LMD-L) as a promising alternative flushing agent for intracoronary FD-OCT imaging, which can replace or reduce the use of contrast media. The comparative quantitative and qualitative study of using contrast media or LMD-L for FD-OCT imaging demonstrated that the image quality and lumen measurement of FD-OCT imaging with LMD-L were comparable to those with contrast media. In addition, the volumes of LMD-L and contrast media required for FD-OCT imaging were the same. The obtained results demonstrated that contrast media can be replaced by LMD-L as a flushing agent for intracoronary FD-OCT imaging.

Li et al. [17] experimentally investigated the application of three chemicals (dextran, mannitol, and ioheoxol) as the flushing agents for in vivo imaging of rabbit abdominal aortas by the integrated intravascular ultrasound and optical coherence tomography (IVUS-OCT) system (Figure 20.6). Dextran was found to be the most effective flushing agent for intravascular imaging among these three chemicals, since dextran improved both IVUS and OCT signals, especially when used in high concentrations. The enhancement of OCT imaging quality was attributed not only to displacing of blood by the injected chemicals, but also to refractive index matching in blood between erythrocytes and blood plasma, since dextran, mannitol, and ioheoxol have higher refractive indices than blood plasma.

Recently, experiments on mice demonstrated that injection of saline in a tail vein of a mouse and consequent partial dilution of blood did not lead to a noticeable enhancement of the OCT probing depth when imaging through skin [14]. The injection of fructose intradermally without injection of optical clearing agent in the vein also did not provide a noticeable increase of OCT imaging depth [14]. In order to increase the OCT probing depth, it seems to be necessary to use optical clearing agents, which reduce light scattering in blood by refractive index matching and/or inducing aggregation of erythrocytes.

The enhancement of light transport after intravenous injection of PEG-300 or hemoglobin solution as the optical clearing agents was studied by OCT in in vivo experiments on two living mice [16]. The results of the study demonstrated that the optical clearing effect can be noticed immediately after the injections of PEG-300 or hemoglobin solution in the tail veins of the mice. The injections of the optical clearing agents in the blood vessels provided a rapid enhancement of light transport which allowed the borders of the veins and tissues surrounding the veins to be observed.

In some cases, the injection of an optical clearing agent into the blood vessel does not lead to rapid enhancement of light transport in the area of interest. It was recently reported that for the OCT imaging performed through skin, the combination of intradermal injections and intravenous injections of optical clearing agents can provide fast enhancement of OCT imaging and increase OCT probing depth [16]. The injection of glucose in skin was previously reported to be more effective for the immediate reduction of light scattering compared to the topical application of the clearing agent [26]. A significant decrease in skin reflectance was observed immediately after injection of glucose into the skin [26, 27].

The in vitro experiments with mice, fructose solution with the concentration of 400 g/L was used as an optical clearing agent, and was injected in the skin in order to reduce scattering of the tissues near the tail vein of a mouse, while PEG-300 was injected into the tail veins [16].

The intradermal injection of fructose solution (400 g/L) in combination with the intravenous injection of PEG-300 led to a rapid optical clearing effect, whereas the injection of PEG-300 in the vein did not did not immediately provide any rapid enhancement of optical clearing after the injection. The combination of injections of the optical clearing agents intradermally and intravenously allowed the vein borders and tissues lying below the vein to be observed immediately after the injections. The injection of an optical clearing agent in skin can be used for enhancement of OCT imaging when the injection of the clearing agent into the blood vessels does not provide any noticeable and rapid optical clearing effect.

Overall, the use of optical clearing agents demonstrated the applicability for enhancement of light transport in highly

FIGURE 20.6 The images obtained with alternative flushing agents: (a, b) dextran (c, d) and mannitol. (a, c) are the OCT images of rabbit artery; (b, d) are the corresponding IVUS images. Arrows denote areas where the artery wall is not visible in the image. Reprinted from Ref. [17].
scattering media for in vivo optical imaging techniques. The optical clearing agents which induce aggregation of erythrocytes and/or refractive index matching proved to be effective for application in OCT imaging and combined OCT and ultrasound imaging systems. The aggregation of blood is expected to be reversible in in vivo cases as erythrocytes are able to disaggregate in the flowing blood due to shear stress. The method of optical clearing demonstrated the potential to improve the quality of in vivo OCT imaging and increase the imaging depth in endoscopic mode and also when imaging through skin.

**In vivo optical clearing of skin by blood and hemoglobin**

The efficiency of application of hemoglobin solutions for the enhancement of transmittance of blood was recently theoretically [13] and experimentally proved [14, 16]. Application of blood and hemoglobin solutions as optical clearing agents can also be a potentially effective method for improvement of light transport in other biological tissues, such as skin. Since highly concentrated hemoglobin solutions and whole blood have high refractive indices, they can possibly provide an optical clearing effect in skin due to refractive index matching between collagen fibrils and ground matter (interstitial fluid).

The optical clearing effect upon application of hemoglobin and whole blood as the optical clearing agents was experimentally studied by in vivo measurements of optical reflectance in the near infrared (900–2000 nm) and visible (400–1000 nm) regions using two different fiber optic spectrometers: USB4000-Vis-NIR and NIRQuest-512-2.2 (Ocean Optics, USA). The results of the measurements are presented at Figure 20.7 and Figure 20.8. From the figures, it is clearly seen that the intradermal injection of hemoglobin solution into rat skin (within rat dorsal area) caused the decrease of reflectance both in the visible and in infrared regions. In the near infrared range, the skin reflectance reaches its minimum after 5 min after injection of hemoglobin solution (Figure 20.7a). The reflectance drops more than two-fold after 5 min at the wavelengths of 1000 nm and 1300 nm. For the region of shorter wavelengths (Figure 20.8), a bigger decrease in skin reflectance was observed at 600 nm [almost three-fold in 5 min after the injection of hemoglobin solution (Figure 20.8a)]. The reflectance decreases from 16.1% to 8.4% at the wavelength of 805 nm and from 13.8% to 6.4% at 930 nm.

The main cause of the reflectance decrease after injection of hemoglobin solution was probably the refractive index matching between refractive indices of collagen fibrils and ground matter. This matching occurred rather fast after the injection of hemoglobin solution in skin, since it can be seen that the reflectance drops in the 5 min after injection. The refractive index of collagen fibrils is 1.41–1.47 [39], while the refractive index of interstitial fluid is lower compared to that of collagen fibers and estimated as 1.33–1.35 [24]. The mismatch of refractive indices of collagen fibrils and interstitial fluid is considered to be the main cause of scattering of skin. Since hemoglobin solution with the concentration of 120 g/L has a rather high refractive index (about 1.367–1.384 in the wavelength range used in this study), therefore, the observed optical clearing effect can be attributed to increase of refractive index of the ground matter as a results of the injection of hemoglobin, which caused a decrease in scattering.

The injection of blood solution into the skin also led to a decrease in reflectance, but after a longer time (30–40 min) (Figures 20.7b and Figure 20.8b). Moreover, the relative drop in skin reflectance after blood injection was less than that after the injection of hemoglobin. This fact can be explained by the assumption that since the refractive index of blood diluted in heparin is 1.343 (at 589 nm), which is lesser than the refractive index of the hemoglobin solution (1.384), the hemoglobin solution therefore provides better matching of refractive indices between tissue components than the blood solution.

It is also can be noted that in the first 10–15 min after the injection of blood solution, the reflectance of skin is higher than it was initially (Figure 20.7b and Figure 20.8b). This can be attributed to the contribution of the injected erythrocytes, which increase the overall scattering of skin. The reflectance of the skin started to decrease after about 30 min after the injection of blood solution. This time can be associated with the lysis of erythrocytes, which result in the decrease of the amount of the ground matter before the injection; “0” corresponds to the reflectance of skin before the injection; “00” corresponds to the reflectance of skin immediately after the injection.

![FIGURE 20.7 Temporal optical reflectance of rat skin after injection of hemoglobin (120 g/L) (a) and blood (2:1, in heparin) (b) solutions measured at 1000 and 1300 nm by the fiber optic spectrometer #1. “00” corresponds to the reflectance of skin before the injection; “0” corresponds to the reflectance of skin immediately after the injection.](image-url)
Optical clearing of blood

Optical clearing of blood

The contactless laser coagulation and ablation of pathologic formations in different organs is widely used in modern clinics [5, 6, 24, 40]. The minor invasiveness of the procedure reduces the risk of postoperative complications. The sources used for this purpose include, particularly, the ytterbium (1075 nm), CO2 (9.4–10.6 µm), Nd:YAG (1064 nm), diode (980, 808, 810 nm), and other lasers. The main advantage of lasers generating at the wavelengths that coincide with the absorption bands of water is the small depth of light penetration into tissues, which prevents damage to the adjacent healthy tissue underlying the affected area. However, cheaper diode lasers have recently become more and more widely used. The radiation from infrared diode lasers in the range 800–1100 nm penetrates deep enough, since in this range, the absorption of such tissue components as hemoglobin, melanin, proteins, and water is relatively small [24]. Thus, the danger of tissue damage or perforation arises. To prevent these complications, the authors of some papers [41–43] propose increasing the thickness of the irradiated object. For example, in order to protect the mucosa of stomach and intestinal wall in the course of laser endoscopic resection, the layers of mucosa and submucosa were separated by certain spacing, filled with glycerol, sodium hyaluronate, or hydrogel. An alternative possible approach is to optimize the laser’s impact by varying the optical parameters of the tissue.

The control of optical parameters can be implemented both by enhancing the absorption properties of the object itself and by reducing the scattering in the tissues adjacent to the lesion focus. In the first case, on the one hand, the laser beam penetration depth is reduced and, on the other hand, the fraction of energy absorbed in the lesion focus is essentially increased, thus improving the laser coagulation efficiency. In the second case, the precision of laser radiation focusing is facilitated.

The absorption and scattering properties of the stomach wall mucosa are well-studied [44]. It has been shown that the use of biocompatible immersion agents, such as solutions of glycerol, propylene glycol, etc., results in efficient optical clearing (i.e., light scattering reduction) of the gastric tissues in the near IR spectral region [45–49]. Earlier, we proposed using hemoglobin in order to control the scattering properties of blood by creating a local hemolysis site in a blood vessel [13]. In this section, we propose using blood hemoglobin as an absorbing agent, and demonstrated the effect of aqueous hemoglobin solution on the optical properties of gastric wall mucosa in order to improve the conditions for laser coagulation in the visible and near IR spectral ranges.

Figure 20.9 presents the spectra of the absorption coefficient of the gastric wall mucosa before and after the injection of aqueous hemoglobin solution with concentration 70 g/L. The averaging was executed over five samples of the tissue. From Figure 20.9(a), it follows that in the spectral range from 350 to 1250 nm, one can observe an essential increase in the absorption coefficient of the mucosa (by 2–4.5× depending on the wavelength). In the spectral range 1250–2500 nm, the increase is expressed essentially weaker. Such behavior of the absorption coefficient is related to the characteristic absorption of hemoglobin in the visible wavelength range. From Figure 20.9(b), it follows that the injection of the aqueous solution of hemoglobin has practically no effect on the scattering characteristics of the mucosa.

The result of estimating the depth of light penetration into the tissue \( d = 1 / \sqrt{3\mu_a(\mu_s + \mu'_s)} \) is shown in Figure 20.10. The expression used is applicable to the case when the tissue surface is uniformly illuminated by the radiation from a point source, located at some distance from the surface. This corresponds to the conditions of real laser surgery of gastric wall, since in this case the illuminating probe is introduced directly into the
stomach cavity without contact with the mucosa surface. The depth of light penetration into the mucosa was calculated using the values of the absorption coefficient (see Figure 20.9 a) and the reduced scattering coefficient (see Figure 20.9 b). Another important parameter is the fraction of the incident radiation energy absorbed in the mucosa. This parameter was calculated in the course of Monte Carlo modeling with algorithm presented in [50], and the result is presented in Figure 20.10.

In this figure, one can clearly see that the depth of laser radiation penetration essentially decreases in the visible range of wavelengths (at the wavelength of Nd:YAG laser radiation (1064 nm) the penetration depth decreases by nearly 60%, whereas the fraction of absorbed energy at this wavelength increases by nearly 90%). The computer simulation allows the evaluation of the change of the absorbed energy fraction at the wavelengths of diode-based laser systems used for the photodestruction of tissue neoplasms. Thus, for the wavelength 810 nm, the injection of hemoglobin aqueous solution leads to the increase of the absorbed energy fraction by nearly 80%, with an almost 50% reduction in the laser radiation penetration depth. For the wavelength 970 nm, the increase of the absorbed energy fraction amounts to nearly 65%, with a decrease of about 50% in the laser radiation penetration depth.

Conclusions

Light scattering in biological tissues and blood can be effectively reduced by application of biocompatible optical clearing agents. The method of immersion optical clearing showed applicability for decreasing light scattering and enhancing light transport in tissues and blood. Various biocompatible optical clearing agents can be effectively applied for enhancement of light transport in blood and, therefore, for improving image quality and increasing imaging depth for optical imaging techniques.

The effect of optical clearing of blood can be caused by several factors, such as refractive index matching between erythrocytes and blood plasma after addition of optical clearing agents, alterations in size and form of erythrocytes, hemolysis, changes in aggregation properties of blood, and other factors. Refractive index matching between blood plasma and erythrocytes is considered to be one of the main causes of the improvement of light transport in blood. Along with refractive index matching, several other effects can influence the transmittance of blood after addition of clearing agents. For example, the optical clearing agents which induce aggregation of blood were experimentally proved to induce a noticeable optical clearing effect. Deformation of RBCs and variations in their form and size also change the scattering properties of blood. The partial lysis of RBCs can be a possible reason for the enhancement of transmittance of blood due to reduction of scattering between blood plasma and RBCs, followed by destruction of the RBCs. The release of hemoglobin from
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The experiments carried out on animals demonstrate the possibility of using the optical clearing method for in vivo applications. The optical clearing method has the potential to increase the probing depth of optical imaging techniques not only in endoscopic mode, but also when imaging through skin. For more effective in vivo optical clearing in blood vessels through skin, the injection of optical clearing agent into the vein can be made along with injection of optical clearing agent in skin above the vein in order to reduce light scattering in skin and achieve the optical clearing effect more rapidly and effectively.

Due to the high refractive index of highly concentrated hemoglobin solutions and blood, these substances can also be potentially applied as optical clearing agents for enhancement of light transport in biological tissues. The in vivo experiments carried out in animals demonstrate that the injection of highly concentrated hemoglobin solution into skin caused an immediate and considerable drop in optical reflectance of skin; therefore, the application of hemoglobin solutions can be a promising method to achieve a fast and efficient optical clearing effect in skin and other biological tissues for in vivo applications of optical imaging techniques.

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