Optical clearing of human cranial bone by administration of immersion agents

Elina A. Genina, Alexey N. Bashkatov, Valery V. Tuchin
Institute of Optics and Biophotonics, Department of Optics and Biomedical Physics
of Saratov State University, Saratov, Russia

We present experimental results on optical properties of cranial bone controlled by administration of propylene glycol and glycerol. Both transmittance and reflectance spectra of human and porcine cranial bone *in vitro* were measured. Measurements of total transmittance and diffuse reflectance have been performed using a spectrophotometer with an integrating sphere. The reflectance measurements have been carried out with an optical multichannel spectrometer with a fiber-optical probe. For estimation of absorption and reduced scattering coefficients of the bone the inverse adding-doubling method was used. The decrease of reflectance and increase of transmittance of the samples under action of the immersion agents was demonstrated. The experiments have shown that administration of the immersion liquids allows for effective control of tissue optical characteristics, that makes bone more transparent, thereby increasing the ability of light penetration through the tissue. The presented results can be used in developing functional imaging techniques, including OCT and reflectance spectroscopy.

Key words: optical properties, optical clearing, bone, immersion agent

1. INTRODUCTION

Recent technological advancements in the photonics industry have led to a resurgence of interest in optical imaging technologies and real progress toward the development of non-invasive clinical functional imaging systems. Over the last decade, non-invasive or minimally invasive spectroscopy and imaging techniques have witnessed widespread exciting applications in biomedical diagnostics, for example, optical coherence tomography (OCT),[1,2] visible and near-infrared (NIR) elastic-scattering spectroscopy,[3,4] fluorescent[1,3,5] and polarisation spectroscopy.[6,7] Spectroscopic techniques are capable of deep-imaging of tissues that could provide information of blood oxygenation[8] and detect cutaneous, brain and breast tumours,[9] whereas confocal microscopy,[10] OCT,[2,11-13] and multi-photon excitation imaging[10,14] have been used to show cellular and sub-cellular details of superficial living tissues. Besides diagnostic applications optical methods are widely used in modern medicine, for example, for laser surgery of different diseases.[15,16] Interest in using optical methods for physiological-condition monitoring and cancer diagnostics and therapies has been increased due to their simplicity, safety, low cost, contrast and resolution features in contrast to conventional X-ray computed tomography and ultrasound imaging.[9]

The main limitations of the majority of the imaging techniques, including OCT and NIR spectroscopy deal with the strong light scattering in superficial tissues,[9,17-20] which cause decrease of spatial resolution, low contrast, and small penetration depth. Solution of the problem, i.e. reducing of light scattering, and, thus, improving of image quality and precision of spectroscopic information, can be connected with control of tissue optical properties.

It is well-known that the major source of scattering in tissues and cell structures is the refractive index mismatch between cell organelles, like mitochondria, and cytoplasm, extracellular media and tissue structural components such as collagen and elastin fibres.[9,12,21-25] The tissue scattering properties can be significantly changed due to action of immersion liquids.[9,20-22,26-47] Administration of the immersion liquid having a refractive index higher than that of tissue interstitial fluid induces a partial replacement of the interstitial fluids by immersion substance and hence, matching of refractive indices of tissue scatterers and the interstitial fluid. The matching, correspondingly, causes the decrease of scattering. As immersion liquids aqueous solutions of glucose and mannitol, propylene glycol, glycerol and other biocompatible chemicals are used.

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Development of the optical methods in modern medicine in the areas of diagnostics and therapy has stimulated the investigation of optical properties of brain tissues, since the efficacy of optical probing of the tissues depends on the photon propagation and fluence rate distribution within irradiated tissues. Examples of diagnostic use are the monitoring of blood oxygenation and tissue metabolism,[48,49] detection of brain malignancies,[50] and recently suggested various techniques for optical imaging.[51-53]

The possibility of selective translucence of cranial bone is very useful in developing techniques of brain functional imaging. A potential benefit of the optical clearing technique is the improvement of laser therapeutic techniques that rely on sufficient light penetration to a target embedded in tissue. Combining optical clearing with laser radiation could reduce the laser fluences required for a therapeutic effect. Another application of the optical method is the non-invasive visualisation of brain blood vessels, haematomas and small pathologic structures (including cancerous growth) with a high resolution. This is important for diagnosis and treatment of many diseases such as tumours of brain, vascular pathologies, etc. However, despite the numerous studies of optical clearing of such tissues as skin, sclera, dura mater, etc., the bone optical clearing is investigated not enough up to now. Mechanism of the clearing stills unclear due to complex morphological nature of bone.

In this study, we investigate optical clearing of both human and porcine cranial bone *in vitro* under the action of propylene glycol and glycerol.

2. CRANIAL BONE STRUCTURE

The structural components of the bones consist of an inorganic matrix (largely mineralised) and an organic matrix.[54,55] The inorganic matrix contains calcium hydroxyapatite, which is responsible for the compressive strength of bone, and osteocalcium phosphate. The main components of the organic matrix are collagen, proteins, blood cells, and lipids.[56] Amount of the hydroapatite is 58%, the collagen content is 25%, water content is 12%, and carbohydrate content is 5%.[57] At the same time, Pifferi *et al*[56] reported that the amount of bone mineral matrix is 16%, the lipid content is 54%, the proteins content is 16%, and water contributes 16%. It is the calcium and phosphorus component of the inorganic matrix that makes bone hard and rigid, and the arrangement of the collagen fibres in the organic matrix that makes it strong. Porosity of the bones is 5-10%.[56]

At microstructural length scales, cortical bone is organised into 200-300 μm diameter secondary osteons,[58] which are composed of large vascular channels (50-90 μm diameter) surrounded by circumferential lamellar rings (3-7 μm thick), with so-called "cement lines" at the outer boundary.[59] At the nanostructural level, the lamellae are composed of organic type-I mineralised collagen fibres (up to 15 μm in length, 50-70 nm in diameter, and formed by regular arrangement of subnanostructural collagen molecules) bound and impregnated with inorganic carbonated apatite nanocrystals (about 30 nm in length and width, 2-3 nm in thickness).[60,61]

3. MATERIALS AND METHODS

3.1 Experimental setups

The total transmittance and diffuse reflectance measurements have been performed in the 800-2000 nm wavelength range using the commercially available spectrophotometer CARY-2415 ("Varian", Australia) with an integrating sphere (Fig. 1). Inner diameter of the sphere is 100 mm, size of the entrance port is 20×20 mm and diameter of the exit port is 16 mm. As a light source, a halogen lamp with filtering of the radiation in the studied spectral range has been used in the measurements. The diameter of incident light beam on the tissue sample is 3 mm. Scan rate is 2 nm/sec. The measurements were carried out at room temperature about 20°C.

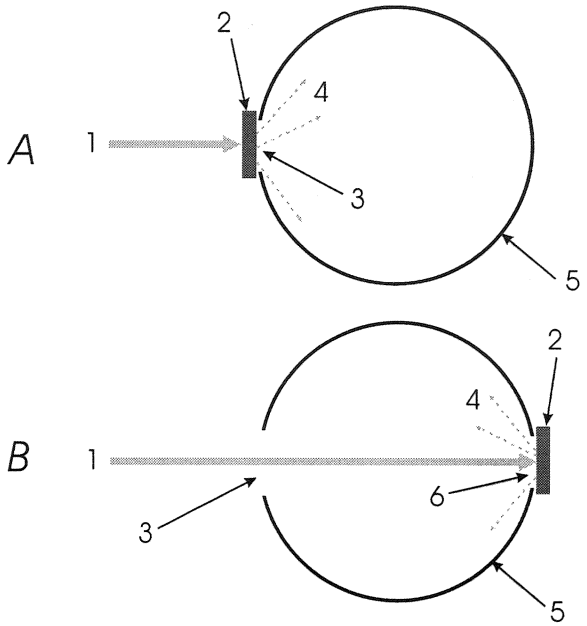


Figure 1: The geometry of the measurements in A) transmittance mode, B) reflectance mode: 1 - the incident beam (diameter 3 mm); 2 - the bone sample; 3 - the entrance port (square 20×20 mm); 4 - the transmitted (or diffuse reflected) radiation; 5 - the integrating sphere (inner diameter is 100 mm); 6 - the exit port (diameter 16 mm).

The measurements of bone reflectance have been performed in the spectral range 450-1000 nm using a commercially available optical multichannel spectrometer LESA-5 (BioSpec, Russia) with fiber-optical probe at room temperature about 20°C. The scheme of the experimental setup is shown in Fig. 2.

The fiber-optical probe consists of the seven optical fibers. All fibers had 200 μm core diameter and a numerical aperture of 0.22. The central fiber (S) delivers incident light to the tissue surface and the six fibers (D), placed around the central fiber, collect reflected light. Distance between the delivering and receiving fibers is 290 μm. As a reference a white slab $BaSO_4$ with a smooth surface has been used. For the spectrophotometric measurements each sample was fixed in the special cuvette with immersion liquid inside.

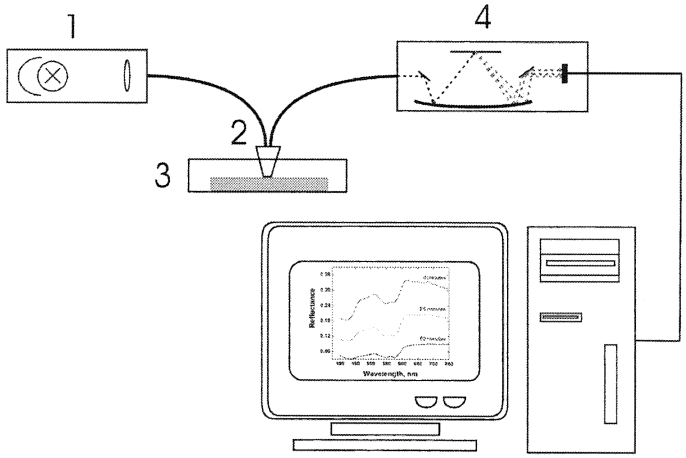


Figure 2:The geometry of the measurements in reflectance mode with the spectrophotometer LESA-5: 1 - galogen lamp; 2 - fiber-optical probe; 3 - cuvette with a bone sample; 4 - grating and photodiode array.

3.2 Experimental samples

For this study five porcine and ten human cranial bone samples were used. All bone samples were cortical (or compact) bones. The samples of human cranial bone were obtained from *post-mortem* examinations. All samples were kept in saline at temperature about 5°C until spectroscopic measurements. The bone samples were measured during 4-6 h after autopsy. The area of the samples was about 25×25 mm^2. The thickness of each bone sample was measured with a micrometer in several points over the sample surface and averaged. Precision of the single measurement was ±50 μm. Thickness of the samples varied from 1.6±0.1 to 5.0±0.5 mm.

3.3 Immersion liquids

In this study, the commercially available pure propylene glycol (Reactive, Russia) and pure glycerol (Ph. Eur., Germany) have been used as clearing agents. The refractive index of propylene glycol and glycerol has been measured by Abbe refractometer at wavelength 589 nm as 1.435 and 1.471, respectively.

3.4 Processing of experimental data

For processing the experimental data and determination the change of the optical properties of tissue, the inverse adding-doubling (IAD) method developed by Prahl *et al*[62] has been used. The method is widely used in tissue optics for processing the experimental data of spectrophotometry with integrating spheres.[63-68] This method allows one to determine the absorption (μ_a) and the reduced scattering coefficients ($\mu'_s=\mu_s(1-g)$) of a tissue from the measured values of the total transmittance and the diffuse reflectance. Here μ_s is the scattering coefficient, and g is the anisotropy factor of scattering. In these calculations the anisotropy factor has been fixed as 0.9, since this value is typical for the tissue in the visible and NIR spectral ranges.[12,69]

4. RESULTS AND DISCUSSION

Figs. 3 and 4 demonstrate dynamics of optical properties of human cranial bone after administration of pure glycerol calculated by IAD method on the basis of measured values of the total transmittance and the diffuse reflectance. Fig. 3 presents the wavelength dependence of the bone absorption coefficient before glycerol administration and after hour of glycerol action. It is well seen that in the wavelength range from 800 to 1400 nm absorption coefficient of the bone sample does not change. In the range from 1400 to 2000 the increase of the absorption coefficient is observed. In the spectrum maxima at 978, 1192, 1464 and 1930 nm are connected with the absorption bands of water.[70,71] Maximum at 1745 nm corresponds to absorption band of lipids.[72] The increase of absorption of water and lipids in the longer wavelength range can be explained by the decrease of the scattering in bone tissue causing the increase of photon's free path length and, thus, more photons are absorbed.

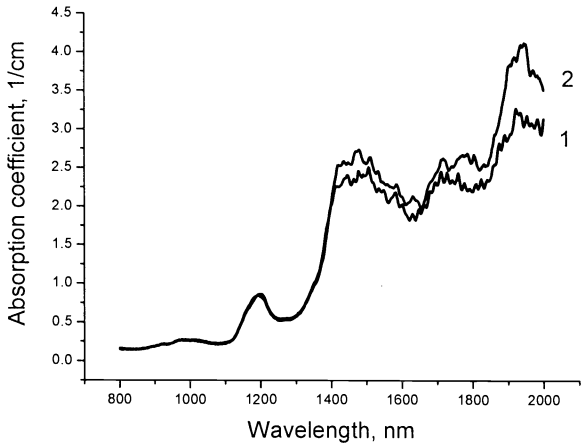


Figure 3: The scattering spectra of bone sample measured before (1) and after administration of glycerol (2) during an hour

Fig. 4 shows the wavelength dependence of the bone reduced scattering coefficient before glycerol administration and after hour of glycerol action. The decrease of tissue scattering is observed in whole studied wavelength range. With increasing of wavelength the effect becomes more significant. Analysis of the experimental results has shown that the average decreasing of the reduced scattering coefficient was about 8% in the range 900-1300 nm and about 30% in the range 1400-2000 nm.

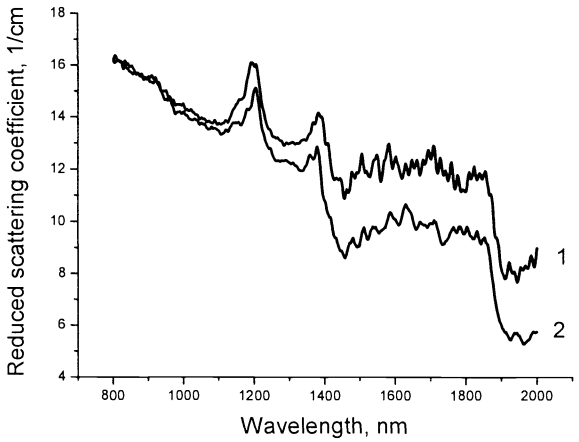


Figure 4: The reduced scattering spectra of bone sample measured before (1) and after administration of glycerol (2) during an hour.

In Fig. 5 some spectra of reflectance of cranial porcine bone during the action of propylene glycol are presented. In the spectra it is well seen three spectral bands corresponding to blood absorption in the visible. There is the Soret band with maximum at 415 nm, the α-band with maximum at 537 nm and the β-band with maximum at 568 nm of oxyhemoglobin absorption.[73] The action of immersion agent produces the decrease of the reflectance of the bone tissue in all studied spectral range that signifies that bone becomes more transparent. During 20 minutes the decrease of the reflectance of bone was about 70%. Such dynamics of the reflectance is apparently connected with the change of regime of photon scattering from multiple to low.

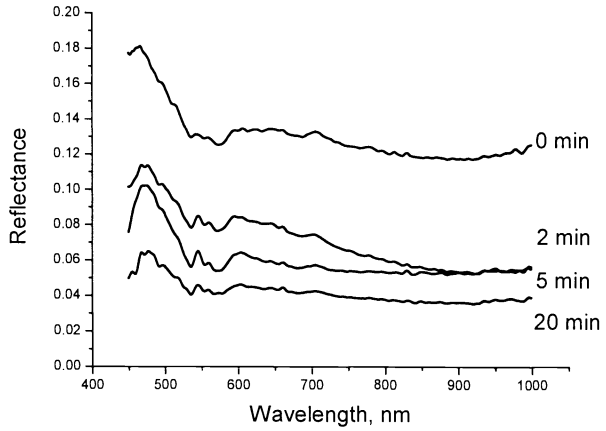
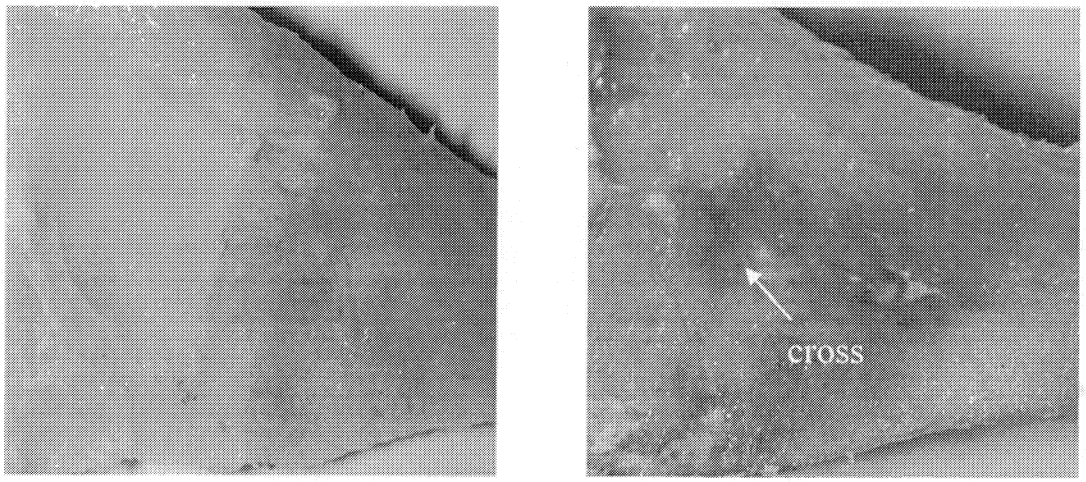


Figure 5: The reflectance spectra of the bone sample measured concurrently with administration of propylene glycol at different time intervals.

The complete clearing of the bone took place in about 24 hours. Fig. 6 shows images of cranial porcine bone before (a) and after (b) optical clearing by pure propylene glycol during 24 hours. The cross pictured on a paper was covered by the

bone. It is well seen that before immersion agent action the tissue was turbid, and the cross under the sample was not seen. After 24 hours the bone became more transparent and the cross was clearly seen. Besides blood vessels being under surface of bone tissue were also clearly seen.



a b

Figure 6: The bone sample before (a) and after (b) optical clearing by pure propylene glycol during 24 hours. The cross pictured on a paper under the bone is clearly seen.

Thus, administration of immersion liquids into fibrous tissue such a bone allows for effectively control of its optical characteristics. The scattering properties of the bone are effectively reduced by the refractive indices matching of the collagen fibrils and interstitial substance.

5. CONCLUSION

The dynamics of the change of reduced scattering and the absorption coefficients of the human cranial bone *in vitro* under action of pure glycerol have been determined over the wavelength range 800-2000 nm using the integrating sphere technique and the inverse adding-doubling method. The dynamics of the reflectance change of the porcine cranial bone *in vitro* under action of propylene glycol have been determined over the wavelength range 450-1000 nm with optical multichannel spectrometer. The decrease of reduced scattering coefficient of the samples up to 30% under action of glycerol has been demonstrated. The dynamics of bone reflectance under action of propylene glycol has demonstrated the decrease up to 70%.

The experiments have shown that administration of the immersion liquids allows for effective control of tissue optical characteristics, that makes bone more transparent, thereby increasing the ability of light penetration through the tissue. The presented results can be used in developing functional imaging techniques, including OCT and reflectance spectroscopy.

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