Combination of tissue optical clearing and OCT for tumor diagnosis via permeability coefficient measurements

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Quantifying optical clearing agent (OCAs) permeability and clearing on human tissue with optical coherence tomography (OCT)

Permeability coefficient measurements (PCM) and OCT human tissue imaging in vivo and in vitro

Development of noninvasive imaging methods for functional monitoring and quantification of molecular transport in epithelial tissues as well as controlling of tissues’ optical properties are extremely important for tumor, arteriosclerosis, diabetic retinopathy, and glaucoma research [1]. Many diseases can alter the physiological structure of the tissue and, thus, could affect the permeability rate of the molecules. Knowing this, the change in the permeability of chemicals and analytes could be used to differentiate abnormal from healthy tissues and could potentially be utilized for the development of novel early diagnostic methods [2]. Therefore, exploring the value of permeability coefficients of hyperosmotic agents could be helpful for the diagnosis of disease.

Recently some studies have monitored and quantified the diffusion of an aqueous solution of glucose in normal esophageal epithelium and esophageal squamous cell carcinoma (ESCC) human tissues [3]. Figure 32.1 demonstrated the typical OCTSS graph acquired from normal esophageal epithelium and ESCC tissues of human during glucose diffusion experiments, respectively. From Figure 32.1, we can see that 40% glucose took less time to reach the monitored region of the ESCC tissues than the normal esophageal tissue. In addition, the permeability coefficient of glucose 40% between the normal esophageal tissue and ESCC tissues was (1.74 ± 0.04) × 10⁻⁵ cm/s and (2.45 ± 0.06) × 10⁻⁵ cm/s respectively. The optical backscattering from the tumor tissue appears more heterogeneous compared with that of normal tissue [4]. It means that there is stronger scattering in the ESCC tissues than in normal esophageal tissue.

Figure 32.2 shows the relative 1/e light penetration depth value change graph for human normal esophageal tissues and ESCC tissues after application of glucose at 120 min, respectively. It can be seen that light penetration depth for the normal esophageal treated with 40% glucose was gradually enhanced after 40 min treatment and, after 80 min, penetration depth reached its highest. But for the ESCC tissues, the penetration depth shows a significant increase approximately 20 min after the application of 40% glucose and reached the highest (around 70 min) value with the increasing of time. Such an effect is believed due to the agent migrating into the extracellular and intracellular space; a refractive index matching environment is created by matching the chemical agents with the main scattering components within the tissue, leading to the enhanced light penetration together with a dehydration effect [5, 6]. The light penetration depth for the ESCC tissues is relatively lower than that of normal esophagus tissues in the same time range. It is likely relate to the tumor tissue having stronger scattering due to larger nuclei, the higher nuclear-to-cytoplasmic ratio in tumor cells, and the higher regional tumor cell density of the tumor tissues [7].

At the same time, noninvasive optical methods of early detection of diseases in human organ tissues have been a hot topic for the research of biomedical photonics. In contrast, an optical diagnostic probe could be moved from site to site in succession, with each measurement being recorded in a fraction.
of a second, by simply moving the location of the probe tip. In summary, optical spectrometry offers the potential to improve disease management, with reduced risks for the patient and the potential for earlier diagnosis and immediate treatment. Fluorescence and reflectance spectroscopy are promising techniques for early diagnosis of neoplasia, as numerous diseases are associated with alterations in tissue and cell structure and/or mitochondrial energy metabolic state, reflected in changes in their optical properties [8, 9]. Some studies explore the potential of using the diffuse reflectance (DR) spectral ratio R540/R575 of HbO₂ absorption bands at 540 and 575 nm for in vitro detection of esophageal cancer [10]. Figure 32.3 shows changes in the average of the DR spectra for the normal epithelial tissues and epithelial tissues of ESCC at different heat treatment temperatures of 37, 42, 50, and 60°C in the range of 400–650 nm, and a control group at 20°C was also plotted for comparison respectively. It can be seen from Figure 32.3a that there were three dips in average of DR spectra for the epithelial tissues of normal esophagus around 417, 540, and 575 nm, and for the epithelial tissues of ESCC around 423, 540, and 575 nm. Furthermore, it can be seen that the mean R540/R575 ratios for the epithelial tissues of ESCC were always smaller than that for the normal esophagus at the same heat treatment temperature. In addition, the mean R540/R575 ratios for the epithelial tissues of normal esophagus and ESCC decreased with the increase of heat treatment temperature. This phenomenon may be related to the cancers and precancerous tissues are characterized by increased microvascular volume, and hence increased blood content [11–14].

In addition, the combination of OCT and OCAs can be used for local quantitative measurement of attenuation coefficient, which can provide additional information for the identification of different tissues [3, 15–21]. OCT has been used to discriminate between different structural features of the normal and atherosclerotic vascular tissues [22], apoptosis, and necrosis in human fibroblasts. This shows that OCT is sensitive to the changes of the attenuation coefficient (AC) caused by analyte diffusion, which is induced by morphological changes in biological tissue [23]. Therefore, OCT techniques were used to assess the difference in permeability coefficient and AC between normal and cancerous tissues caused by hyperosmotic agents. This may help distinguish between cancerous and nonmalignant tissues and hold the promise of early diagnosis of colon cancer.

FIGURE 32.1 OCTSS graphs as a function of time from normal and ESCC esophageal tissues during glucose diffusion. Reprinted with permission from Reference [3].

FIGURE 32.2 1/e light penetration depth change as a function of time. Reprinted with permission from Reference [3].
OCT has been also used to conduct real-time monitoring, identification, and quantification of the diffusion of glucose solutions in human normal and adenoma colon tissues [24]. Figure 32.4a–c showed OCT images of normal colon tissue 0, 15, and 30 min after the topical application of 30% glucose, and Figure 32.4 d–f showed images of the cancerous colon tissue. Normal colon tissue had a regular and compact appearance and layers were clearly visible, but the cancerous tissue structure appeared disorganized and nonuniform and had many dark crypts. Figure 32.4 also showed that the visibility, contrast, and imaging depth in both tissues were significantly improved after the application of glucose. This change is due to the diffusion of locally applied analytes into the extracellular and intracellular spaces. By matching chemical reagents with the main scattering components in the tissue, a refractive index matching environment is created, and the light penetration ability is enhanced. Glucose dehydration reduces light scattering and further enhances light penetration [25, 26].

The typical dynamic changes of the 1-D OCT normalized signal intensity curve and the corresponding exponential best-fit curve in the experiment are shown in Figure 32.5. The degree of change in the OCT signal intensity is consistent with
the increase in glucose penetration and glucose concentration in the internal structure. As can be seen from Figure 32.5b, compared with normal colon tissue, the OCT signal produced by cancer tissue in 0–30 min is wider but steeper. These results may be due to the additional effect of glucose diffusion, enhancing the light transmission into the tissue. Therefore, more photons propagate to the deep reflective surface below the tissue, producing a stronger antireflection signal.

As shown in Figure 32.6, a similar trend was observed in the normal and malignant colon tissue. In Figure 32.6a, after the glucose reaches the monitoring area in approximately 20 min, it takes another 47 min to completely finish the diffusion process. In contrast, for the cancer tissue, it only took about 13 min to reach the monitored region, and then another 41 min to completely diffuse the whole region, as seen in Figure 32.6b. The permeability coefficient of 30% glucose for the normal colon tissue in Figure 32.7 was significantly slower at \((3.37 \pm 0.17) \times 10^{-6} \text{ cm/s}\) compared with the permeability coefficient in the adenomatous colon tissue \((5.65 \pm 0.24) \times 10^{-6} \text{ cm/s}\) (\(p < 0.05\)). This may be due to the diffusion of glucose into the tissue, where the tissue contrast is mainly caused by the tissue attenuation coefficient. The tissue attenuation coefficient depends on the volume fraction of interstitial space, cell diameter, and tissue structure.

Figure 32.8 shows that the attenuation coefficients of normal tissue were found to differ significantly from other cancer components with the continuous diffusion of glucose into the tissues. The comparison of Figure 32.8a and Figure 32.8b shows that the decrease in light attenuation was much more prominent in the cancer tissue than that of normal tissue in the same region where we determined the OCTSS. This may be related to some exchange processes in the glucose and differently sized and hydrated (collagen, elastin) structures of tissue because of glucose osmotic impact inducing water flux from these structures (dehydration) and back to them (rehydration) inside tissue [27].

Therefore, these results indicate that OCT technology can assist in the early detection and identification of tumors by

FIGURE 32.5 Normalized OCT intensity profiles with their corresponding exponential best fit curves (a) normal colon tissue and (b) adenomatous colon tissue. Reprinted with permission from Reference [24].

FIGURE 32.6 The typical OCTSS graphs for human normal (a) and adenocarcinoma (b) colon tissues during a 30% glucose diffusion. Reprinted with permission from Reference [24].
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monitoring and quantifying the diffusion of hyperosmotic agents and the difference in optical communication between normal and cancer tissues by OCT imaging. During the OCT imaging process, due to the complex morphological characteristics of the tissue, it provides a high-scattering medium for the visible and near-infrared bands, reducing the penetration of light into the tissue and limiting the imaging depth [28–32]. It applies OCAs to biological tissues, mainly to reduce random scattering in tissues through better refractive index matching and dehydration [33]. Currently, glycerol [34–36], glucose [37–39], and dimethyl sulfoxide [40, 41] as OCAs can effectively improve the light transmission depth of various biological tissues.

In order to understand how to distinguish normal tissue from diseased tissue by the permeability of 30% glucose solution during OCT imaging, a study was used to monitor and quantify the differences in permeability coefficients of 30% glucose diffusion by the OCTSS method in four kinds of human lung tissue in vitro: normal lung tissue, benign granulomatosis lung tissue, squamous cell carcinoma, and adenocarcinoma tumor [42]. As can be seen from Figure 32.9a, the average permeability coefficient of 30% glucose in normal lung tissue is $(1.35 \pm 0.13) \times 10^{-5} \text{ cm/s}$. In addition, about 85 min into the application on normal human lung tissue, the 30% glucose solution had entirely penetrated through the monitored region.

Compared with Figure 32.9a, Figure 32.9b, Figure 32.9c, and Figure 32.9d showed the OCT signal slope as a function of time recorded from benign granulomatosis, adenocarcinoma, and squamous cell carcinoma lung tissue during the 30% glucose diffusion experiment, respectively. The permeability coefficients of 30% glucose solution in Figures 32.9b, 32.9c, and 32.9d are $(1.78 \pm 0.21) \times 10^{-5} \text{ cm/s}$, $(2.88 \pm 0.19) \times 10^{-5} \text{ cm/s}$, and $(3.53 \pm 0.25) \times 10^{-5} \text{ cm/s}$ respectively. The obvious difference in permeability coefficient of the same hypertonic agent in normal, benign, and malignant lung tissue may be due to several pathological and disease conditions that can change the mechanical properties and microstructure of the tissue. This method illustrated that OCT can distinguish normal tissue from diseased tissue by quantifying the diffusion coefficient of glucose in human lung tissue.

Glucose solutions can also be used as OCA of the skin. Figure 32.10 shows a typical OCTSS graph for skin tissue after application of 40% glucose. 40% glucose took 0.26 h to reach the monitored region, and then another 3.02 h to completely diffuse through the whole region. The permeability coefficient of 40% glucose in skin tissues was found to be $(1.94 \pm 0.05) \times 10^{-5} \text{ cm/s}$. The permeability coefficient of hyperosmotic agent diffusion in human skin might be related to the microstructures, refractive indexes, and molecular sizes of the OCAs.

In summary, applying glucose solutions as hyperosmotic agents to biotissue has been inferred to reduce random scattering within tissue primarily by better refractive index matching and a dehydration action. In addition, by combining the penetration of several therapeutic or diagnostic reagents in normal and abnormal tissues, OCT can become a noninvasive imaging method to distinguish normal tissues from diseased tissues.

![FIGURE 32.7](image_url) Comparison of the mean permeability coefficient of 30% glucose diffusion in normal human and adenocarcinoma colon tissues. Reprinted with permission from Reference [24].

![FIGURE 32.8](image_url) Mean attenuation coefficients of normal (a) and adenomatous colon tissues (b) after topical application of 30% glucose. Reprinted with permission from Reference [24].
Combination of physical and nanoparticles strategy for enhancing optical imaging performance

Evaluation of synergy efficacy with OCAs on tissue using OCT

In the process of optical imaging, accurately acquiring the internal structural characteristics of biological tissues and controlling the optical properties of various biological tissues in vivo and in vivo are of great significance for many medical applications. However, due to the low absorption and high scattering of most biological tissues and biolipids in the visible and near infrared (NIR) wavelength regions, the spatial resolution and light penetration depth of optical diagnostic and therapeutic methods are limited [27, 31, 43, 44]. Therefore, in order to increase the light penetration depth and obtain more imaging information, it is necessary to reduce multiple scattering in biological tissues. A series of recent studies have shown that OCA as an enhancer with different physical and chemical properties can effectively improve the optical clarity of tissues in vivo and in vitro, improve imaging depth, enhance contrast, and increase turbid tissue [45–47]. However, due to the barrier function of OCAs in tissues, many physical methods have been proposed, such as electroporation [48], iontophoresis [49], and microneedling [50]. Recently, it has been reported that the optical cleaning of ultrasound tissue and the synergistic effect of OCAs can be used in the spectral domain optical coherence tomography of human normal tissue and malignant tissue.

A study has reported that 30% glucose solution (G) alone and 30% G combined with 15 min ultrasound (sonophoretic delivery, SP) were used to observe normal and malignant colon tissues [51]. Figure 32.11a and b show OCT images of 30% G and 30% normal colon tissue alone and G/SP from left to right for 0, 10, 30, and 45 min, respectively. Figure 32.12a and b show the OCT images of adenoma colon specimens with 30% G and 30% G/SP applied from 0–10, 30, and 45 min intervals from left to right, respectively. Figure 32.11 and 32.12 showed the 2D OCT images investigations, after application of 30% G and 30% G/SP; the structural features and optical clearing effect are both more obvious for normal and malignant colon samples as time progresses. Figure 32.11a and b also showed that after 30% G combined with ultrasound treatment, the structure of normal colon tissue and malignant colon tissue are more loose and irregular than that after 30% G treatment alone, especially for malignant colon organization. This phenomenon may be caused by the cavitation effect of the
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ultrasound, which has also been reported in transdermal drug delivery and cancer treatment experiments [52, 53].

Figure 32.13 and Figure 32.14 are the 1D OCT signal normalized to the maximum value of the normal and malignant colon tissue intensity curves, respectively, and the corresponding exponential best-fit curve, showing the dynamics at 30% G and 30% G/SP, respectively. From Figure 32.13a and Figure 32.14a, the intensity of the OCT signal gradually increases with time with the 30% G alone in the upper layers of normal and malignant colon tissues. However, Figures 32.13b and 32.14b indicate that the intensity of the OCT signal and light-penetration depth is greatly enhanced after treatment with 30% G/SP in normal and malignant colon tissues.

Figure 32.15 quantifies the penetration-depth enhancement of light into the tissue with 30% G and ultrasound combination. As can be seen from Figure 32.15a, the corresponding relative 1/e light-penetration depth is limited for the normal colon tissue with 30% G alone, compared with 30% G and the ultrasound. Figure 32.15b shows the same trend in malignant colon tissue. These results also indicate that the depth of light penetration increases when ultrasound is applied. This may be due to the high concentration of chemical enhancer inside the tissue under the action of ultrasonic disturbance [54–56].

Figure 32.16 indicated the dynamic changes of the normalized intensity of the OCT signal as a function of time for the 30% G and 30% G and ultrasound with about 100 min for the normal and malignant colon tissues were summarized. The results showed that in the 30% G and ultrasound groups, the signal intensity of OCT was significantly enhanced compared to 30% G alone in both benign and malignant colon tissues.

Comparing the permeability coefficients of 30% G and 30% G/SP for the normal and cancerous colon tissues presented in Figure 32.17, the permeability coefficients with the 30% G and ultrasound in the normal and cancerous colon tissues are 1.87-fold and 2.18-fold of that without the ultrasound, respectively. These results indicate that the permeability coefficient of hypertonic agent in cancerous colon tissue after ultrasound treatment is greater than that without ultrasound. The reason may be that the intervention of low-intensity ultrasound destroys the characteristics of the tissue structure, resulting in enhanced cell membrane porosity, 30% G permeability, and diffusion in deep tissue.

These findings illustrated that the synergistic effect of ultrasound with glucose can be utilized as an aid to increase glucose permeability and tissue optical clearing and light penetration into deeper biological tissue. Furthermore, more detailed in vivo investigations are required to fully assess the microscopic mechanisms by simultaneous application of ultrasound and OCAs, which can enhance the tissue optical clearing effect and light penetration in tissues. As an important part of the human body, skin can be divided into the epidermal layer, dermis layer, and subcutaneous tissue layer. Its main role is to act as a powerful shield to protect the body from extreme temperatures, sunlight, and chemical hazards in the environment. The stratum corneum (SC) is the outermost layer of the skin, which is an important barrier to prevent skin moisture loss and drug percutaneous absorption [57–59]. Failure in protection of the skin will cause wrinkles, pore bulk, acne spots, yellow calluses, or even more serious diseased states such as seborrheic dermatitis and psoriasis [60, 61]. Thus, the development of chemical peeling (CP) technology has been widely used for facial rejuvenation in the past decade [62–66].

FIGURE 32.10 OCTSS as a function of lime recorded from the human skin in vivo during glucose diffusion. Reprinted with permission from Reference [18].

FIGURE 32.11 2D OCT images of human normal colon tissue at different times after topical application of (a) 30% G alone and (b) 30% G/SP, respectively. Reprinted with permission from Reference [51].
Salicylic acid (SA) as a superficial facial CP (SFCP) has been widely used in clinical treatment of acne, freckles, and other facial diseases [67–69]. However, when SA is used as SFCP, its adverse reactions are still under debate in clinic. Therefore, the use of penetration enhancers can optimize the efficacy of SA at the minimum dose, which is of great significance in clinical facial applications. There are currently reports of azone as a chemical permeation enhancer for effective SC peeling-agent delivery into skin. FCPAs can effectively cause morphological structure and optical property variations in skin tissue.

Optical spectrometry combined with SFCP is a useful technology for skin disease diagnosis and monitoring. This technology is mainly based on the differences of various endogenous substances with characteristic optical bands [70].
Salicylic acid (SA) has been frequently used as a facial chemical peeling agent (FCPA) in various cosmetics for facial rejuvenation and dermatological treatments in clinic. However, there is a tradeoff between therapeutic effectiveness and possible adverse effects caused by this agent for cosmetologists. To optimize cosmetic efficacy with minimal concentration, we proposed the use of the chemical permeation enhancer (CPE) azone to synergistically work with SA on human skin in vivo. The optical properties of human skin after being treated with SA alone and SA combined with azone (SA@azone) were successively investigated by diffuse reflectance spectroscopy (DRS) and OCT [71]. To illustrate the dynamics of skin optical properties after the application of chemical permeation enhancer, a set of dynamic spectral changes (from 400 nm to 800 nm) of human skin sample was recorded at regular time intervals over a period of 80 min before and after application of the SA group (S1, S2, S3, and S4 represent 0.5%, 1%, 1.5%, and 2% SA solutions respectively) and SA@azone group (0.5%, 1%, 1.5%, and 2% SA solution combined 1% azone mixture solution marked as S1@A, S2@A, S3@A, and S4@A, respectively) in Figure 32.18 and Figure 32.19 respectively. To quantify the optical characteristic variations of skin resulted by S1-S4 and S1@A-S4@A, the reduction of DR was calculated at three characteristic wavelengths, 420, 540, and 580 nm, at the different time points in Figure 32.20, Figure 32.21, and Figure 32.22, respectively. The results not only revealed that the DR decreased linearly with SA concentration, but also implied that light transmission significantly

**FIGURE 32.15** The dynamic changes of 1/e light-penetration depth (a) normal colon tissue (b) malignant colon tissue after 30% G and 30% G/SP at different times, respectively. Reprinted with permission from Reference [51].

**FIGURE 32.16** Normalized intensity of OCT signal as a function of time during addition of 30% G and 30% G/SP (a) normal (b) malignant colon tissue, respectively. Reprinted with permission from Reference [51].

**FIGURE 32.17** Comparison of the mean permeability coefficients of 30% G alone and 30% G/SP in normal and malignant colon tissues. Reprinted with permission from Reference [51].
increased in skin with SA concentration increment, which sug-
gested SA at higher concentration had greater desmolytic abil-
ity on the SC [72, 73]. In addition, with azone as a penetration
enhancer, the greatest decrease in DR was not in the S4@A
but the S2@A group, since the linear concentration dependent
effect occurred in SA group alone was not applicable for syn-
ergy group. Therefore, the greatest DR decrease in S2@A may
be attributable to the formulation of SA and azone in equal
proportions, which indicated that the two components are
transported through the same microenvironment of the skin.

As shown in Figure 32.23, the epidermal structure of
skin appears compact and hierarchical before treatment.
Structural clarity of tissue and imaging depth of OCT can be
improved by S1–S4 treatment with time. Furthermore, it also
can be seen from Figure 32.23 that there is a remarkable dif-
ference in skin treated with SA alone and SA@azone at differ-
ent concentrations, and the structure of skin becomes looser
and the imaging depth of OCT has significant enhancement in
S1@A–S4@A at the same time intervals, respectively. Figure
32.24 quantified the OCT in-depth reflectance profiles for the
human skin topically applied with S1–S4 alone and S1@A–
S4@A at time intervals of 0, 20, 40, 60, and 80 min. As seen
in Figure 32.24 a–d, the intensity of the OCT signal gradu-
ally increased from the upper layers caused by increase in
SA contents over time. In addition, Figure 32.24 e–h indicate
that the intensity of the OCT signal is greatly enhanced after
being treated with S1@A–S4@A. This phenomenon caused by
azone was through interaction with the lipid domains of the
SC, which increased skin absorption by reversibly damaging
or altering the physicochemical nature of the SC to reduce its
diffusional resistance [74, 75].

These findings implied that SA mixed with azone may
also be considered by commercial cosmetics to improve the
exfoliation ability of SA, retard skin wrinkling, and maintain
skin vitality. In addition, chemical permeation enhancers of
skin tissue may have some important biomedical applica-
tions connected with the investigation of skin’s structure and
functioning.

TOC technique for improvement
of photoacoustic imaging quality

Photoacoustic microscopy (PAM) technology has played an
irreplaceable role in biomedical imaging. Nevertheless, in
strong scattering tissue such as skin, breast, bioliquids, etc.,
the optical focusing capability degrades due to optical scatter-
ing. In order to overcome these challenges, various physical
and chemical methods have been deployed to enhance opti-
cal imaging depth, such as the utilization of OCAs [76–79].
In recent years, the optical tissue clearing (OTC) technique
**FIGURE 32.19** Dynamic spectral changes of the human skin treated with SA@azone. (a–d) The spectral changes of skin before and after application of the S1@A, S2@A, S3@A, and S4@A at the different time intervals, respectively. Reprinted with permission from Reference [71].

**FIGURE 32.20** Representative diffuse reflectance reduction of human skin treated with (a) S1 and S1@A, (b) S2 and S2@A, (c) S3 and S3@A, and (d) S4 and S4@A at 420 nm, respectively. Reprinted with permission from Reference [71].
FIGURE 32.21  Diffuse reflectance reduction of human skin at 540 nm. Diffuse reflectance reduction at 540 nm for human skin after treatment with (a) S₁ and S₁@A, (b) S₂ and S₂@A, (c) S₃ and S₃@A, and (d) S₄ and S₄@A at 20, 40, 60, and 80 min, respectively. Reprinted with permission from Reference [71].

FIGURE 32.22  Diffuse reflectance reduction of human skin at 580 nm. Reduction in diffuse reflectance at 580 nm for human skin after treatment with (a) S₁ and S₁@A, (b) S₂ and S₂@A, (c) S₃ and S₃@A, and (d) S₄ and S₄@A at 20, 40, 60, and 80 min, respectively. Reprinted with permission from Reference [71].
Combination of TOC and OCT for tumor diagnosis via PCM has shown great potential in inducing optical clearing effects (OCEs) to reduce scattering in tissues using hyperosmotic and biocompatible chemicals agents [25, 41, 80, 81]. In addition, more and more OCAs are being used to investigate whether they can improve the resolution of PAM imaging, such as glucose solution, dimethyl sulfoxide solution, glycerin, and propylene glycol solution, which have refractive index close to that of collagen and are applicable for altering the scattering properties of tissues [41, 80, 82–85]. Among these OCAs, glycerol is one of the most common and efficient OCAs in skin in vitro and in vivo and it has been proven in medical applications such as tooth therapy and cosmetics study [31, 86, 87].

To increase the efficiency of the topical application of OCAs, different concentrations (0%, 20%, 40%, and 60% respectively) of glycerol solution were applied to a piece of fresh pigskin with a thickness of 0.5 mm [88]. The solutions have a mean refractive index of 1.36, 1.39, and 1.41 respectively [45]. Figure 32.25 shows a photo of the registered trademark logo after covering porcine skin tissue with different concentrations of glycerin solution for 15 min. This finding demonstrated that with the increasing of concentration of glycerol, the OCE of skin is dramatically improved. The greatest increase of light transmittance and sign integrality was found with 60% glycerol, indicating that more light may be allowed through the skin tissue and delivered into the sign by applying.
60% glycerol than other concentrations. Visibility differences are mainly due to the hyperosmotic glycerol resulting in refractive index matching between cellular tissue components: cell membrane, cell nucleus, cell organelles, melanin granules, and extracellular fluid.

Glycerol is applied to fresh pigskin to decrease skin reflectance and increase tissue transmittance. Figure 32.26 shows the change of PAM images of the sign covered with a porcine skin tissue at different times (0, 15, 30, 45, and 60 min) immersed in 20%, 40%, and 60% glycerol solution. There were significant differences between different concentration groups. The clarity of PAM imaging is enhanced as the concentration of glycerol solution and the soaking time increase. The photoacoustic (PA) signal amplitude can be caused by light penetration differences or ultrasound transmitting change. From Figure 32.27, it can be seen that PA signal amplitudes of the sign show significant enhancement after applying glycerol solutions with different concentrations. Since the high-concentration glycerin solution has higher sound attenuation, the PA signal amplitude is higher in the group with higher glycerin concentration. This is because the group with higher glycerin concentration improves optical clearing effects, allowing more light to penetrate the skin tissue. The difference in the tendency of PA signal amplitude change possibly reflects the fact that the diffusion of high concentrated glycerol solution is lower than solutions with a lower concentration. This phenomenon is consistent with previous studies on the relationship of the concentration of aqueous sucrose solution and its diffusion through semipermeable membranes [89, 90].

This evidence indicates that glycerol as an optical transparency agent can significantly improve the quality of PA imaging. The PA signal amplitudes and the visibility of a phantom light absorber covered by skin tissues were found to increase with the concentration of the glycerol solution. Therefore, this method may become an effective tool for enhancing PA imaging of biological tissues.

**Nanoparticles for OCT contrast enhancement imaging**

Gold nanorods (GNRs) as an optical contrast agent and photothermal agent have been widely used in the field of
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biomedical photonics due to their excellent biocompatibility and biocompatibility, which has aroused widespread concern [91]. Figure 32.28 illustrated the highly efficient absorption of GNRs in the near-infrared (NIR) region, a spectral window which permits photons to penetrate biological tissues, allowing deeper imaging depth inside specimens. Therefore, the combination of pure OCT system and GNRs can effectively enhance the structural information of living embryonic tissue. As we all know, embryonic development is a very important stage in life. Early embryonic development will have an important impact on the survival, foraging, movement, and reproduction of individuals. Therefore, the development of a noninvasive, high-resolution, and strong penetration depth imaging technology is essential for the study of embryonic development. A large number of studies have reported that OCT is used as a noninvasive, high-resolution, and convenient imaging technique in the three-dimensional (3D) imaging research on mammalian embryos [92–97]. However, due to the inherent multiple optical scattering characteristics of embryonic tissue, it is still a challenge for pure OCT to obtain satisfactory spatial resolution to display embryonic structure [91, 98]. Contrast agents which enhance the OCT images allow for a wider usage of OCT systems [99–101].

In in vitro experiments of living embryonic tissues, GNRs are added to the embryo culture medium and transported into the embryo as the nutrients are transported through the placenta. In order to quantify the sensitivity of contrast agents in embryonic tissue, the corresponding OCT imaging was applied (see Figure 32.28). Two images of living embryonic tissue sample without and with topical application of GNR solution were captured. It is clear that OCT signal intensity reached the highest when living embryonic tissue was treated with GNRs. By comparison of images of Figures 32.29a and 32.29b, OCT signal intensity reached the highest after the topical application of GNR solution with 20 μg/mL in both 3 and 6 h culture in E 9.5 embryos, whereas without application of GNR solution, the light is almost blocked by the tissue. This is due to the fact that the embryonic tissue highly scatters the incoming light, which degrades the imaging performance.

In addition to enhancing OCT signal intensity, GNRs may have an impact on three-dimensional imaging of the organs of
living embryos. At day E10.5, when the embryo was not treated with GNRs, only a few faint anatomical structures appeared in the three-dimensional reconstruction (see Figure 32.30a). However, after treatment with 10 μg/mL GNRs for 6 h, the 3D reconstruction imaging had more accurate spatial localization with structural features, and better contrasting of the borders among organs can be observed on the GNR-treated embryo (see Figure 32.30b). For GNRs as a contrast agent, these findings confirmed that with an exogenous OCT contrast agent, greater 3D structural detail was obtained as compared to the control embryos. Moreover, the outlines of internal organs are clearer in the GNR-treated embryos.

In summary, GNRs could be delivered to the embryos with a short period of ex vivo culture. The utility of GNRs as contrast agent in OCT system decreased the high scattering of tissues and improved the contrast and penetration depth of images. GNRs also allowed reconstructing clear 3D images of E9.5 and E10.5 embryos. The combination of nanotechnology allows us to improve conventional OCT and assess the morphological changes and structural abnormalities of developing organs in real-time and in situ images.

**Challenges and perspectives**

In summary, optical clearing techniques have enriched our understanding of life sciences and innovated the way we study biological tissues, which are quite crucial for various optical imaging techniques. Evaluating the diffusion and permeability of OCAs in different tissue by OCT is an effective method for differentiating the normal and disease situation. However, further research still has to be done to find the optimal and safest type, concentration, and method of OCAs for the imaging of living biotissue. As mentioned above, optical
.clearing techniques enable us to differentiate normal from pathological tissues based on the molecular permeability rates method, but the specific mechanisms of tissue optical clearing in vivo still remain a challenge. Thus, in practical clinical applications, specific type, concentration, and clearing method of the OCAs should be chosen according to the research requirements.

For future optical imaging applications, if we want to make them more effective, it is very important to understand the optical properties of the research object and screening optimal OCAs. Understanding the optical properties of the sample is not only the basis for screening or designing OCAs, but is also helpful for exploring the mechanisms of TOC. In addition, we believe that increasing numbers of new and safe enhancement permeability methods and other delivery techniques for OCAs diffusion will be developed and applied in biomedicine imaging.

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REFERENCES


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Combination ... vitro study of control of rat skin optical properties by 


51.  Q.L. Zhao, H.J. Wei, Y.H. He, et al., "Evaluation of ultra - 

52.  G.A. Husseini, W.G. Pitt, "Ultrasonic-activated micellar 

53.  N. Kim, A.F. El-Kattan, C.S. Asbill, et al., "Evaluation of 

54.  N. Dujardin, P. V. Der, V. Preat. "Topical 

55.  P.M. Elias, "Epidermal lipids, barrier function and desqua -


57.  A.S. Michaels, S.K. Chandrasekaran, J.E. Shaw, "Drug 
permation into rat skin using electroporation," Pharm. 

permation through human skin: Theory and in vitro 


60.  M.E. Hartstein, G.G. Massry, J.B. Holds, et al., Pearls and 

effects of physical dermabrasion combined with chemical 
peeling in porcine skin," J. Cosmet. Laser Ther. 17(1), 24– 

62.  N. Zakopoulou, G. Kontochristopoulou, "Superficial chemical 

peeling and microdermabrasion of the skin: 
Comparative immunohistological and ultrastructural stud - 

peeling and microdermabrasion of the skin: 
Comparative immunohistological and ultrastructural stud - 

65.  B. Marczyk, P. Mucha, E. Budzisz, H. Rotsztejn, 
"Comparative study of the effect of 50% pyruvic and 30% 
salicicyc peels on the skin lipid film in patients with acne 

66.  J.A. Bouwstra, M. Ponec, "The skin barrier in healthy and 
diseased state," Biochim. Biophys. Acta Biomembr. 1758(12),

67.  H.S. Lee, I.H. Kim, "Salicylic acid peels for the treatment 
of acne vulgaris in Asian patients," Dermatol. Surg. 29(12),

68.  C.M. Burgess, Cosmetic. Dermatology Books, Springer, 
Berlin Heidelberg (2005).


70.  W.P. Rowe, A.R. Shally, "Effective over-the-counter acne 
treatments. Seminars in cutaneous medicine and surgery," 

of salicylic acid with a penetration enhancer on human skin 
monitored by OCT and diffuse reflectance spectroscopy," 

72.  C. Thomas, "Cosmeceutical agents: A comprehensive 
review of the literature clinical medicine," Dermatology 1, 

73.  C. Huber, E. Christophers, ""Keratolytic" effect of salicylic 

74.  A. Hassain, P.H. Andrzej, et al., "Potential enhancers for 
Pharm. 4(181), 19–22 (2014).

75.  K. Swain, S. Pattnaik, S.C. Sahu, et al., "Drug in adverse 
type transdermal matrix systems of ondansetron hydrochlo- 
ride: Optimization of permeation pattern using response sur-

76.  G. Ku, L.V. Wang, "Optical clearing-aided photo-
acoustic microscopy with enhanced resolution and imaging 

77.  G. Ku, L.V. Wang, "Deeply penetrating photoacoustic 
tomography in biological tissues enhanced with an optical 

78.  X.D. Wang, G. Ku, M.A. Wiegel, et al., "Noninvasive 
photoacoustic angiography of animal brains in vivo with 

79.  R.K. Wang, "Signal degradation by coherence tomogra-
phy multiple scattering in optical of dense tissue: A Monte 
Carlo study towards optical clearing of biotissues," Phys. 

80.  V.V. Tuchin, "Tissue optics: Light scattering methods 
and instruments for medical diagnosis," in SPIE Tutorial 
Texts in Optical Engineering, SPIE Press, Bellingham, 

81.  V.V. Tuchin, D.A. Zimnyakov, L.L. Maksimova, et al., "The 
coherent, low-coherent and polarized light interaction with 
tissues undergo the refractive indices matching control," 

82.  A.N. Bashkatov, E.A. Genina, V.I. Kochubey, et al., "In vivo 
and in vitro study of control of rat skin optical properties 

83.  A.N. Bashkatov, E.A. Genina, V.I. Kochubey, et al., "In vivo 
and in vitro study of control of rat skin optical properties by 
87. N.A. Trunina, V.V. Lychagov, V.V. Tuchin, “OCT monitoring of diffusion of water and glycerol through tooth dentine in different geometry of wetting,” *Proc. SPIE* 7563, 75630U (2010).