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New Prospects in Optical Imaging
Valery V. Tuchin, Dan Zhu, Elina A. Genina

Light operation on cortex through optical clearing skull window

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Light operation on cortex through optical clearing skull window

Dongyu Li, Chao Zhang, Oxana Semyachkina-Glushkovskaya, Yanjie Zhao, and Dan Zhu

CONTENTS
Introduction ................................................................................................................................................................................. 557
Combination of skull optical clearing and PDT for blood–brain barrier opening ................................................................. 557
Methods .................................................................................................................................................................................. 558
Ex vivo assessment of BBB opening induced by photodynamic effect through optical clearing skull window ................. 558
In vivo observation of BBB opening through optical clearing skull window ....................................................................... 559
BBB opening for GM1-liposomes ............................................................................................................................................... 559
Age differences in photodynamic effect–induced BBB opening through optical clearing skull window ............................... 559
Laser ablation of neuronal dendrites ........................................................................................................................................... 562
Methods .................................................................................................................................................................................. 562
Monitoring dendrites after laser ablation through optical clearing skull window ............................................................. 564
Observation of microglia response after laser ablation through optical clearing skull window ............................................. 564
Vis-NIR-II skull optical clearing window for NIR-II light manipulation ........................................................................... 564
Methods .................................................................................................................................................................................. 565
NIR-II Laser-induced single blood vessel injury in cortex ...................................................................................................... 565
Summary and prospect ............................................................................................................................................................... 566
Acknowledgments ........................................................................................................................................................................ 566
References .................................................................................................................................................................................... 566

Introduction
In addition to cortical imaging, modern optical techniques can be used to manipulate cortical activity and environment. Models such as cerebral arterial thrombosis and brain tissue injury can be established by light irradiation [1–3]. In addition, the optical process can take control of blood–brain barrier (BBB) opening and other neurological functions [4–9]. However, the turbid skull above the cortex strongly attenuates light, causing a limitation of the efficiency and targeting of optical manipulation. To overcome the scattering of skull, targeted optical manipulation is usually performed after craniotomy or by embedding optical fibers in the cortex, which changes the natural environment of the brain and may even further lead to bleeding or inflammation.

The in vivo skull optical clearing technique not only allows optical imaging systems to achieve cortex through the intact skull, but also provides a craniotomy-free optical window for cortical operation [10, 11]. Up to now, an optical clearing skull window–based BBB opening and precisely positioned cortical ablation has been already realized [11–13], suggesting that the in vivo skull optical clearing technique has great potential in noninvasive or minimally invasive cortical modeling.

In this chapter, the applications of optical clearing skull window–based light operation on cortex were introduced, including their methods and progress. In addition, the possibility of using in vivo skull optical clearing technique for a wide variety of optical manipulations were discussed.

Combination of skull optical clearing and PDT for blood–brain barrier opening
The blood–brain barrier (BBB) plays an important role in the central nervous system (CNS), which is formed by endothelial cells that line cerebral microvessels [14, 15]. BBB acts as a selective “physical barrier,” a “transport barrier,” and a “metabolic barrier” [16], controlling penetration of bloodborne agents into the brain and protecting the CNS from toxins and pathogens [17]. However, while BBB blocks harmful substances, it also dramatically reduces the chance that drugs will enter the brain, creating a challenge for effective therapy for the majority of CNS diseases [18], which account for 30% of all diseases [19]. Thus, temporarily opening the BBB for drug delivery has important medical implications, and various methods have been developed in the last decades [20–27]. Among them, photodynamic effect–induced BBB opening has attracted much attention and has been widely used [28, 29].

Photodynamic process is induced by combining light irradiation with photosensitizers. The excited photosensitizer directly oxidizes biomolecules and/or interacts with molecular triplet oxygen (\(1O_2\)), producing singlet oxygen (\(3O_2\)) that causes...
cells apoptosis and/or necrosis through plasma and mitochondria membrane rupture. Therefore, the photodynamic effect will induce edema, which happens at the region surrounding the site of light treatment, suggesting a local degradation of the BBB [29]. It means, theoretically, that targeted BBB opening can be realized using the photodynamic effect, as long as the light reaches a local location precisely. However, the severe scattering of skull tissue causes nonnegligible attenuation when light passes through, and makes it impossible to focus it on a small area of cortex. Thus, targeted BBB opening is hard to perform through the turbid skull. Even for nontargeted BBB opening, it is necessary to increase light dose as well as photosensitizer concentration, which may lead to severe vasogenic edema [29, 30]. To overcome the circumstance, a craniotomy needs to be implemented before applying the photodynamic effect [4, 28]. But this will unavoidably induce changes in intracranial pressure and cortical inflammation, which may cause some misinterpretations of mechanisms underlying photodynamic effect–related BBB opening [31].

Zhang et al. used their newly developed urea-based skull clearing agent (USOCA) to open a switchable optical skull window without craniotomy on mice [10], with which they realized photodynamic effect–induced BBB opening through the optical clearing skull window without causing obvious side effects [12]. In addition, they further investigated age differences in photodynamic effect–induced BBB opening [32]. It is strong evidence that optical clearing skull window will be a promising tool for noninvasive photodynamic effect–related BBB opening.

Methods

In their study [12], USOCA was used to open the optical clearing skull window. Briefly, USOCA consists of two solutions, named solution 1 (S1) and solution 2 (S2), respectively. After the skull was exposed, S1 (a saturated supernatant solution of 75% (vol/vol) ethanol) was topically applied to the skull for 10 min, and then removed. Then S2 (a high-concentration sodium dodecylbenzenesulfonate (SDBS) solution) was topically applied to the skull for 5 min, and the mice could be used for further experiment.

For BBB opening through the established optical clearing skull window, 635-nm laser irradiation was performed with a 30 min intravenous injection of photosensitizer 5-ALA (20 mg/kg). The treated light dose was adjusted by adjusting the irradiation duration (10, 20, 30, 40 J/cm²).

To evaluate the BBB permeability for molecules and liposomes, Evans Blue, rhodamine-dextran (70 kDa), and fluorescently labelled GM1-liposomes were used as tracer agents, respectively. The tracer was intravenously injected 20 min before the injection of 5-ALA. The brain was then irradiated with laser, and followed by in vivo or ex vivo assessment with two-photon/confocal microscopy, respectively. Figure 30.1 shows the schematic diagram of BBB opening through the optical clearing skull window.

Ex vivo assessment of BBB opening induced by photodynamic effect through optical clearing skull window

They firstly analyzed laser dose–dependent photodynamic effects on BBB permeability using spectrofluorimetric assay of Evans Blue dye (EBd) content in the mouse brain [33, 34]. EBD is a 961 Da dye that binds to serum albumin, becoming a high molecular weight complex (68.5 kDa) in the blood, which cannot pass through intact BBB. The laser doses were 10, 20, 30, and 40 J/cm², respectively. As shown in Table 30.1, it was found that there was no EBd leakage with BBB with different doses of irradiation through the intact skull, while strong EBd leakage through the opened BBB was observed with irradiation through the USOCA treated skull.

In addition, histological images suggested that the low laser doses (10 J/cm² and 20 J/cm²) caused mild accumulation of solutes around microvessels, while higher laser doses (30 J/cm² and 40 J/cm²) induced stronger vasogenic edema (Figure 30.2). Considering the fact that the EBd leakage was more pronounced with laser dose of 20 J/cm² versus 10 J/cm², it is reasonable to conclude that the increase in laser dose leads to an increase in BBB permeability.

**FIGURE 30.1 Photodynamic effect induced opening of BBB through optical clearing skull window. Reprinted from Reference [12].**
Light operation on cortex through optical clearing skull window

In vivo observation of BBB opening through optical clearing skull window

For in vivo imaging of BBB permeability, two-photon laser scanning microscopy (2PLSM) was used to image the extravasation of rhodamine-dextran through the USOCA treated skull. Figure 30.3a shows the timeline of the experiment. Firstly, 5-ALA (20 mg/kg) was intravenously injected into the anesthetized mice and circulated for 30 min; during this time, the optical clearing skull window was established by topical application of USOCA. Then the cerebral vessels were imaged through the optical clearing skull window using 2PLSM. After that, a 635 nm laser was applied to irradiate the mouse brain through the window, with the constant power fluence at 165 mW/cm² and 2 min irradiation durations. 1 h after laser irradiation (BBB was opened), the cerebral vessels at the same region were imaged through the optical clearing skull window again. Figure 30.3b–e shows a strong leakage of rhodamine-dextran (70 kDa) from the cerebral vessels into the brain parenchyma via the opened BBB, and the fluorescent signal in the cerebral vessels decreased 2.3-fold due to rhodamine extravasation into the brain tissues, metabolism by the kidney, and even probably some photobleaching (Figure 30.3f).

TABLE 30.1

Photodynamic effect induced BBB opening for the EBd albumin complex. Reprinted with permission from Reference [12]

<table>
<thead>
<tr>
<th>Groups</th>
<th>Content of Evans Blue in the brain (µg/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated mice</td>
<td>0.685 ± 0.012</td>
</tr>
<tr>
<td>5-ALA</td>
<td>0.650 ± 0.011</td>
</tr>
<tr>
<td>Laser 10, 20, 30, 40 J/cm²</td>
<td>0.708 ± 0.007</td>
</tr>
<tr>
<td>0.673 ± 0.003</td>
<td>0.700 ± 0.006</td>
</tr>
<tr>
<td>0.801 ± 0.011</td>
<td></td>
</tr>
<tr>
<td>PD (5-ALA + laser) without optical clearing skull window</td>
<td></td>
</tr>
<tr>
<td>10, 20, 30, 40 J/cm²</td>
<td>0.741 ± 0.017</td>
</tr>
<tr>
<td>0.687 ± 0.013</td>
<td>0.807 ± 0.015</td>
</tr>
<tr>
<td>0.796 ± 0.010</td>
<td></td>
</tr>
<tr>
<td>PD (5-ALA + laser) with optical clearing skull window</td>
<td></td>
</tr>
<tr>
<td>10, 20, 30, 40 J/cm²</td>
<td>7.618 ± 0.496 ***</td>
</tr>
<tr>
<td>13.863 ± 0.712 ***</td>
<td>18.017 ± 1.171 ***</td>
</tr>
<tr>
<td>18.295 ± 1.466 ***</td>
<td></td>
</tr>
</tbody>
</table>

*** p<0.001 the comparison between untreated mice and mice underwent PD through optical clearing skull window, n = 6 in each group and sub-group.

BBB opening for GM1-liposomes

Liposomes have a phospholipid bilayer structure that makes them more compatible than other nanoparticles with the lipid layer of the BBB, which makes them promising for drug delivery to brain tissue [35–39]. In the study, fluorescently labeled GM1-liposomes were constructed on the basis of a matrix of egg yolk phosphatidylcholine, which contained mol.% BODIPY-phosphatidylcholine in the bilayer (λex = 497 nm, λem = 504 nm). In addition, three different markers were used to reveal the BBB integrity: 1) the endothelial barrier antigen conjugated with antibodies SMI-71 as a marker of cerebrovascular endothelium; 2) the anti-glial fibrillary acidic protein (GFAP) labeling astrocytes; and 3) the laminin, labeling the basal membranes. Figure 30.4 demonstrates effective extravasation of liposomes from the cerebral vessels into the brain parenchyma via opened BBB. The distribution of liposomes was observed among the astrocytes (Figure 30.4a) and outside of the cerebrovascular endothelium and the basal membrane (Figure 30.4b and c). Such results show that the BBB can be open for GM1-liposomes (100 nm) so they could go through all elements of BBB including the vascular endothelium, the basal membrane, and the astrocyte feet.

Age differences in photodynamic effect–induced BBB opening through optical clearing skull window

Zhang et al. [32] further investigated whether there were age differences in photodynamic effects through optical clearing skull window on the BBB, and found more pronounced
photodynamic effect–induced BBB disruption in juvenile mice compared with adult mice. In their work, they introduced photodynamic effect on cortex through the USOCA-based optical clearing window, and studied photodynamic effect–mediated opening of the BBB in a radiant exposure manner (635 nm, 10/20/30/40 J/cm², and 5-ALA, 20 mg/kg) in healthy 4- and 8-week-old mice by using quantitative and qualitative tests for BBB permeability.

The BBB permeability to EBd was analyzed every 30 min after 5-ALA-mediated PDT over a 4-hour duration. As shown in Table 30.2, there were no changes in BBB permeability to the EBd albumin complex in mice of both ages in all groups, including laser irradiation itself or 5-ALA injection without laser, as well as in the untreated mice, while significant age differences in photodynamic effect–related opening of the BBB to EBd were observed. For 10 and 20 J/cm² laser irradiation to cause photodynamic effect, the increase of BBB permeability was 1.7 and 1.6 times higher in 4-week-old mice compared with 8-week-old ones. However, application of higher radiant exposures (30 and 40 J/cm²) was accompanied by a significant EBd leakage that was similar for both ages. In addition, the content of EBd in the brain for both 4- and 8-week-old mice with different radiant exposures returned to the normal state 4 hours after photodynamic therapy, indicating that the BBB opening was reversible.

The FITC-dextran (70 kDa) leakages in different conditions were also quantitatively evaluated by confocal imaging brain slices. As shown in Figure 30.5 and Table 30.3, for low and medium radiant exposures (10–20 J/cm²), 4-week-old mice demonstrated more significant changes in BBB permeability to FITC-dextran compared with 8-week-old mice, while high radiant exposures (30–40 J/cm²) caused significant BBB disruption in both age groups. In addition, further experiments demonstrated that FITC-dextran effectively crossed all elements of the BBB, including the cerebral endothelium, the basal membrane, and astrocytes after photodynamic effect performance.

In addition, confocal imaging of photodynamic effect induced BBB opening to FITC-dextran was performed using specific markers of neurovascular unit (NVU) at radiant exposures of 10 and 20 J/cm² for 4- and 8-week-old mice, respectively. The result demonstrated that in both conditions, the selected tracer of FITC-dextran effectively crossed all elements of the BBB, including the cerebral endothelium, the basal membrane, and astrocytes after PDT (Figure 30.6).

To analyze the morphological changes in the brain tissues and cerebral vessels after photodynamic effect performance using different radiant exposures, Zhang et al. performed histological studies in mice of both ages. The results demonstrated that the photodynamic effect–induced BBB opening was accompanied by the formation of vasogenic edema, and it was positively related with the exposure dose. Moreover, changes were more pronounced in juvenile mice compared with adult ones (Figure 30.7).
TABLE 30.2
The photodynamic effect–related changes in the BBB permeability to EBd. Reprinted with permission from Reference [32]

<table>
<thead>
<tr>
<th>Groups</th>
<th>Content of Evans Blue in the brain (µg/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Age 4 weeks</td>
</tr>
<tr>
<td></td>
<td>Untreated mice</td>
</tr>
<tr>
<td></td>
<td>0.60 ± 0.01 (n = 6)</td>
</tr>
<tr>
<td></td>
<td>5-ALA</td>
</tr>
<tr>
<td></td>
<td>0.66 ± 0.01 (n = 6)</td>
</tr>
<tr>
<td>Laser 10 J/cm²</td>
<td>0.59 ± 0.01 (n = 6)</td>
</tr>
<tr>
<td>Laser 20 J/cm²</td>
<td>0.62 ± 0.02 (n = 6)</td>
</tr>
<tr>
<td>Laser 30 J/cm²</td>
<td>0.60 ± 0.01 (n = 6)</td>
</tr>
<tr>
<td>Laser 40 J/cm²</td>
<td>0.64 ± 0.02 (n = 6)</td>
</tr>
</tbody>
</table>

Ischemic stroke is a group of common cerebrovascular diseases with high disability and mortality rate, so it has always been a research hotspot in the field of biomedicine [40–43]. Photothermbsis using photodynamic effects can establish an ischemic stroke model, which has been widely used in the research of repair mechanism and long-term functional recovery after stroke [44, 45]. Photothermbsis involves first injecting a photosensitizer, and then irradiating the brain with light of a specific wavelength to stimulate the photodynamic effect of the photosensitizer to form singlet oxygen, causing endothelial damage, platelet activation, and aggregation, thus forming a vascular embolism [46–48]. By controlling the position and dose of light, a controlled light plug model with a controlled degree can be easily established [49]. Tang et al. [45] applied photothermbsis to rats and performed long-term observation of cortical blood perfusion and tissue damage with optical coherent tomographic angiography (OCTA). In their study, the focus of laser irradiation was adjusted to 500 µm beneath the cortex surface in the photothermbsis experiment, and the ischemic region was observed to spread from the deep irradiation focus up to the surface area. Clark et al. [50] created a single vessel–targeted photothermbsis model by using digital micromirror device (DMD). In their study, targeted vessels were irradiated with the patterned laser. It was found that confining laser illumination to individual arteries on the cortical surface with a digital micromirror device expanded the ischemic penumbra, supporting its usefulness for examining the impact of remodeling events within the penumbra on mechanisms of recovery from ischemia. However, due to the severe light scattering of the skull, it is impossible to perform targeted photothermbsis through the skull in its original state, thus it was only achievable with the skull removed in the previous studies. Fortunately, the in vivo skull optical clearing technique provides a new idea. Since the scattering of skull can be reduced by topically applying optical clearing agents, it is worth trying to combine optical clearing skull window with focused photodynamic effect for targeted photothermbsis modeling.

Besides photothermbsis, the photodynamic effect can also be used for therapy. Photodynamic therapy (PDT) is a method to use photodynamic effect–induced \(^1\)O\(_2\) to perform tumor damage with several accesses: (1) to kill tumor cells by direct injury, leading to tumor cell necrosis or inducing apoptosis;
(2) to damage tumor blood vessels, leading to tumor blood stagnation, collapse, contracture, occlusion, etc., as well as tumor ischemia and hypoxia, thereby indirectly killing tumor cells; (3) to promote the release of cytokines, inflammatory mediators, and immune antigens by target cells, induce inflammatory and immune responses, and damage tumor cells [51–53].

PDT has been widely used for neuroglioma therapy [54–56]. Since the higher the grade of glioma, the greater the damage to the blood–brain barrier and normal brain parenchyma, the grade of glioma is directly related to the level of photosensitizer in the tumor. As a consequence, PDT could damage the tumor with less injury to the normal tissue. It has been reported that PDT can be used in conjunction with other therapies, including radiotherapy, chemotherapy, thermal therapy, immunotherapy, and boron neutron capture therapy [57–60]. In addition, some fluorescent photosensitizers such as 5-ALA could also perform optical-guided therapy. Since the in vivo skull optical clearing technique can reduce the scattering of the skull and the attenuation of light, it holds potential for minimally invasive, precise, and optical guided PDT for neuroglioma.

### Laser ablation of neuronal dendrites

Laser-induced injury is a widely used injury model because the extent and site of the injury are easily controlled [2, 3]. However, the skull is a barrier to avoid cortical laser irradiation. Other than a traditional surgery-based cranial window, the novel optical clearing skull window provides a minimally invasive tool to perform targeted laser ablation in cortex, as well as dynamic optical observation of brain response with synaptic resolution [11].

#### Methods

Zhao et al. [11] used transgenic mice whose dendritic spines and microglia were labeled with yellow fluorescent protein (YFP) and enhanced green fluorescent protein (EGFP), respectively.

---

**FIGURE 30.5** The PDT-related opening of the BBB evaluated by confocal imaging of FITC-dextran (70kDa) extravasation from cerebral vessels into the brain parenchyma. In each group, images in the second row are magnified maps of the area enclosed in the first row, and the profiles of vessels are outlined. Reprinted from Reference [32].

**TABLE 30.3**

<table>
<thead>
<tr>
<th>Light doses</th>
<th>Classification of FITC-dextran extravasation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>4 weeks</td>
</tr>
<tr>
<td>No irradiation (control, n = 6)</td>
<td>/</td>
</tr>
<tr>
<td>10 J/cm² (n = 6)</td>
<td>+</td>
</tr>
<tr>
<td>20 J/cm² (n = 6)</td>
<td>+++</td>
</tr>
<tr>
<td>30 J/cm² (n = 6)</td>
<td>++++</td>
</tr>
<tr>
<td>40 J/cm² (n = 6)</td>
<td>++++</td>
</tr>
</tbody>
</table>
FIGURE 30.6 The confocal imaging of PDT-induced opening of the BBB to FITC-dextran using specific markers of NVU: (a) FITC-dextran leakage outside of the endothelial cells of cerebral vessels labeled by SMI; (b) FITC-dextran leakage outside the basal membrane labeled by the antibodies for the laminin; (c) FITC-dextran extravasation from the cerebral vessels into the brain tissues among astrocytes. The white arrows points to the sites of FITC-dextran leakage. Reprinted from Reference [32].

FIGURE 30.7 Photodynamic effect–induced opening of the BBB evaluated by histological analysis of solute extravasation in 4- and 8-week-old mice. Ctr = the control group, where there was no solute leakage; 10, 20, 30, and 40 J/cm² caused the perivascular edema (black arrows), which appears as empty spaces around cerebral vessels. Reprinted from Reference [32].
They used the optical clearing skull window technique to perform cortical laser ablation. Briefly, the procedure of the establishment of the optical clearing skull window is as follows. After anesthesia, hair removal, and scalp incision, mice are fixed with a custom-built immobilization device that consisted of a custom-built plate and a skull holder. Then, the skull is thinned to around 100 μm (Figure 30.8a), and 10% EDTA disodium is topically applied to the exposed skull for 5–10 min to soften the outermost layer of the skull. Lastly, the EDTA disodium is removed using a clean cotton ball, and 80% glycerol is topically applied to the skull for matching the refractive index. In their study, 2PLSM was used for laser positioning and dynamic observation. Before imaging, a layer of plastic wrap was placed over the cleared skull to separate the water-immersion objective from the glycerol. As shown in Figure 30.8b, the signal intensity and contrast were significantly improved after skull optical clearing.

Once ensuring the position under 2PLSM to perform ablation, the 780-nm femtosecond laser beam was focused on the position of interest for approximately 60 s with power of 60–80 mW to create a tiny injury site.

Monitoring dendrites after laser ablation through optical clearing skull window

With the optical clearing skull window, Zhao et al. [11] kept the laser beam focused on a dendrite to cause ablation. After laser irradiation, continuous two-photon imaging was performed over the course of 1 hour. As shown in Figure 30.9a and b, the dendrites on the laser injury side formed bead-like structures, while the sites that did not suffer damage remained in a normal state.

Observation of microglia response after laser ablation through optical clearing skull window

During a 1-hour observation, microglia soma did not show any significant movement after focused laser irradiation, while the process with bulbous termini immediately moved toward the site of injury (Figure 30.10). The results demonstrate that optical clearing skull windows enable scientists to characterize the effects of laser injury on cortical structures.

In conclusion, the in vivo skull optical clearing technique allows targeted cortical laser ablation to be performed with no need for much thinning or removal of the skull. Despite the fact that it is not comparable with traditional cranial windows in terms of imaging depth, it can still serve as an alternative technique for cortical imaging and manipulation with the brain in its normal state.

Vis-NIR-II skull optical clearing window for NIR-II light manipulation

The NIR-II light (> 900 nm) has higher penetration depth in the cortex due to its lower scattering compared to visible and NIR-I light (700–900 nm). Therefore, NIR-II based optical manipulation holds great potential for deep-tissue cortical operation, which requires an optical clearing agent compatible for NIR-II light.

Actually, water, as the main component of various optical clearing reagents, shows strong absorption in the wavelength range of longer than 1300 nm [61]. Thus, in the NIR-II region, the previous optical clearing window might induce extra attenuation of light due to the strong absorption of water. Li et al. [62] developed a visible-NIR-II compatible skull optical clearing agent (VNSOCA) and used it for 1560-nm
Light operation on cortex through optical clearing skull window

**Methods**

VNSOCA was developed based on USOCA, but the water was replaced by D$_2$O. Briefly, VNSOCA consists of two mixtures, named solution 1 (S1) and solution 2 (S2). S1 is a mixture of urea and ethanol in D$_2$O, and S2 is a high-concentration sodium dodecyl benzenesulfonate (SDBS) in D$_2$O solution.

For skull optical clearing, S1 was dropped onto the exposed skull so that the skull was immersed for 10 min. After that, S1 was removed with medical cotton and S2 was applied to the same area for 5 min, at which point skull optical clearing was established.

For vasculature injury, firstly, Li et al. performed cortical vascular THG microscopy through the VNSOCA-induced optical clearing window with the assistance of a THG probe, and then chose a small capillary in the imaging vision. Secondly, the 1560-nm fs laser (80 mW after objective) was focused onto the vessel for 10 seconds to make ablation, followed by a 3-second dynamic THG observation of the cortical hemorrhage. In addition, another larger blood vessel was chosen and laser was then focused onto the vessel for 15 s, followed by observation for 10 min.

**NIR-II Laser-induced single blood vessel injury in cortex**

As shown in Figure 30.11a, a small vessel was chosen to be irradiated by the 1560-nm fs laser for 10 s, after which the THG probe quickly diffused into the surrounding tissue. As shown in Figure 30.11b, a larger blood vessel was also partially
scanned by the laser, but for 15 s. The fracture of the vessel wall was clearly observed. 10 min later, the broken blood vessel was blocked, and the THG probes still remained in the surrounding tissue. The results indicated that precise NIR-II light manipulation could be performed through the established skull optical clearing window.

In conclusion, the VNSOCA, due to its high transparency, allows NIR-II light (1560 nm) to realize targeted optical manipulation in cortex. The previous skull optical clearing windows were demonstrated to be suitable for visible/NIR-I optical manipulating, such as photodynamic opening of the blood–brain barrier (635 nm) [12, 32], and laser ablation of neurons (720 nm) [11].

Apart from photodynamic effect–related applications and laser-induced targeted injury, the skull optical clearing technique can also provide a tool for optogenetic research. Optogenetics is a new type of cell control technology, where light-sensitive ion channel proteins are expressed on excitable target cells or organs, and light of a corresponding wavelength activates the light-sensitive channels to achieve fine regulation of physiological functions of cells, tissues, organs, and animals [6, 63–66]. Although the traditional means to control the behavior of cells or biological organisms can change the activity of cells, they cannot be precisely located in a certain cell, so it has a wide range of effects and a large number of toxic and side effects. Optogenetics technology can achieve higher spatial control accuracy by using advanced light-feeding technology. For example, by changing the power intensity of the light, the light stimulation can reach a certain depth on the tissue, so as to achieve precise spatial positioning [7]. Another advantage of optogenetics technology is that it can make the occurrence of light stimulation and specific behavioral effects almost synchronous, without waiting for the blood or other media to transport the stimulus to a specific site, so that the stimulation occurs when light occurs, and the stimulation immediately stops when light ceases; thus the time-control accuracy is very high. However, due to obstruction of the skull, in the previous study, scientists usually remove the skull before optical irradiation, or introduce optical fibers into brain tissue [67–70]. Serving as a noninvasive light-transparent skull window, the optical clearing skull window holds great potential to be combined with optogenetics to perform optical manipulations.

Summary and prospect

Recent reports have clearly shown that some light operations can be performed well through optical clearing skull windows, including BBB opening and laser-induced targeted cortical injury.

Photodynamic effect–related BBB opening with low doses of photosensitizer and laser irradiation can be realized through the intact skull with topical treatment of USOCA [12], which permits minimizing of brain tissue injuries after modeling. Using an optimal laser dose, the BBB can be opened not only for high weight molecules, but also for 100 nm GM1-liposomes that passed through the vascular endothelium and the basal membrane and were distributed among astrocytes. In addition, the in vivo optical clearing skull window was used to investigate age differences in photodynamic effect–mediated BBB opening, and presented a novel understanding that young mice demonstrated a more pronounced photodynamic effect induced increase in BBB permeability to high weight molecules as well as increase in vasogenic edema [32].

The in vivo skull optical clearing technique can also be used for laser ablation of dendrites and vasculature, as well as dynamic visualization of dendrites, microglia, and micro-blood vessels after injury [11, 71]. Furthermore, compared to an open skull window, the optical clearing skull window technique was safe, with less risk of producing inadvertent damage or inflammation, providing a window for cortical optical operation extremely similar to environments within the normal state of the brain, which would be more suitable for the study of immune cells (such as microglia), which are highly sensitive to the microenvironment.

In conclusion, the in vivo skull optical clearing technique has been used as an alternative to craniotomy but with lower invasiveness, providing a skull window for various optical cortical operations, including BBB opening and laser ablation. In the future, it might attract more attention in the fields of photothermosis, PDT, and optogenetics.

Acknowledgments

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