



Review

From Transparent Cranial Windows to Multifunctional Smart Cranial Platforms

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Abstract: In this paper, we briefly reviewed the development of cranial windows and their functions in brain sciences. We demonstrated that a 3D-printed titanium frame coated with a polydimethylsiloxane (PDMS) film could serve as an excellent transparent cranial window for long-period, in vivo optical experiments in mice and rats, and the devices also allowed multiple injections through the elastic PDMS window, without leaking. Our large-area honeycomb structured Ti-PDMS samples had a relative transparent area ratio of over 90% but a mechanical strength close to that of a human skull, showing a promising potential for applications in large animals as multifunctional cranial windows. We also suggested that more functional modules could be integrated in the large-area Ti-PDMS cranial device, thus turning it into a novel wearable smart platform for wireless data communication, electro-probing and brain stimulation, optical imaging, transcranial injection, and so on, for both fundamental research on neuroscience and clinical practices dealing with brain damage and disease.

Keywords: transparent cranial window; bio-compatibility; 3D printing; titanium alloy; in vivo optical imaging; transcranial injection; wearable electronic device

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1. Role of Cranial Window in Brain Research

The human brain is a very complicated organ. It works in a “black box” skull, where it obtains external information through bundles of neuron axons that connect to body sensors for light, sound, smell, taste, touch, gravity, temperature, etc. The decisions of the brain are also transported out through neuron axons and turn into body actions or inner organ reactions. Most details of the working mechanisms of a brain, such as “How Are Memories Stored and Retrieved?”, remain unknown to date [1–5].

A brain consists of numerous neurons, axons, glial cells, blood vessels, water, and macromolecules, such as protein, a variety of ions with proper concentrations, etc. [6–14]. In the last century, a number of noncontact, whole-brain imaging technologies were developed, including computed tomography (CT) [15,16], magnetoencephalography (MEG) [17,18], positron emission computed tomography (PET) [19,20], functional magnetic resonance imaging (fMRI) [21,22], etc. Among them, PET is applied to monitor the 3D intensity of metabolism status in different regions of a live brain, while fMRI could present the correlation between structure and function at different parts of a live brain down to a spatial resolution of 0.1 mm.

On the other hand, contact probes with various neural electrodes have also been well-developed. By using an external electrode array attached to a head surface, electroencephalogram (EEG) technique measures and records the electrical signals of a whole

brain. Preliminary results showed that by decoding the recorded signals, a computer might roughly understand an idea running inside the brain [23].

Other kinds of electrodes were designed for implantation at the cortex surface, inside a shallow layer of the cortex, or deeply into a brain [24]. These electrodes were used to probe the excitation status of individual neurons or electrical pulses of axons and dendrites to reveal the working mechanisms for body motion, sensing, pain, decision making, etc. [25–32]. Some deep brain stimulation (DBS) electrodes have already been applied in clinical therapy [33–35].

Cranial windows offer a unique approach for brain studies: they open a transparent window on a “black box” skull for direct observation of a live brain and optical imaging of brain tissues and individual neurons [36]. With the help of high-quality cranial windows, two-photon imaging, optogenetics, confocal imaging, optical stimulation technique etc., were applied to perform in vivo optical studies of live animals, and numerous exciting results were obtained [37–47]. Multi-modular studies using combined electrical and optical techniques as well as whole brain imaging techniques led to a better understanding of brain function in complicated animal behaviors [48–62]. In some studies, injection of drugs was applied to regulate brain functions [63–73] for which a cranial window could also play an important role as a transcranial interface.

In this paper we briefly review the development of cranial windows. We describe a novel hybrid cranial device with a 3D-printed titanium alloy (Ti, Ti6Al4V) net-shaped frame combined with a transparent polydimethylsiloxane (PDMS) window, which is suitable for long-period, in vivo optical experiments in mice and rats, and allows transcranial injection without leaking. We will show that large-area honeycomb structured Ti-PDMS samples are good candidates for multifunctional cranial windows in large animals, and they can be further developed into an integrated smart device on the head.

2. Development of Cranial Windows

Basically, a cranial window opens a transparent window on the “dark-box” skull for direct optical observation of brain tissues and related optical experiments [36,45,61,74–76]. The glass cranial window technique was firstly attempted in the 1850s [77,78]. In the next half century, cranial windows have been developed in many different groups [36,79–82]. In the following, we will briefly introduce several cranial windows for optical observation and multifunctional cranial windows that allow optical and electrical measurements or drug delivery.

2.1. Natural Cranial Windows

Natural cranial windows mainly help on-site observation of brain tissues and blood vessels and in vivo optical imaging experiments. They do not require craniotomy operation and thus ensure less risk of surgery bleeding, post-operative hematoma, and inflammation.

One kind of natural cranial window was made with mechanical thinning and polishing techniques on a mouse skull [83,84]. At a target location, a small piece of the skull bone was thinned to 15–30 μm and polished, and it turned into transparent status for studying the blood velocity in vessels [85], optical imaging of cell morphology [83,86,87], and status of neural dendrites [83,88–91]. However, this technique was only applicable to a small area of 0.1–0.3 mm^2 in small animals, such as mice [83,91–93], and bond cells and tissues always regrew a few days after the thinning operation. In some cases, to repeat the same thinning operation, a waiting period of as long as 18 months or longer was needed [36,83,89,94].

Recently, another kind natural cranial window was to turn a whole skull of a mouse into a transparent one by using bio-chemical techniques. Progresses were made to improve the transparency and observable depth of brain tissues [95–103].

2.2. Implanted Optical Cranial Windows

Implanted cranial windows were usually made of thin glass sheets or transparent polymer sheets as the window material. First the target region of an animal was undergone

a craniotomy operation, then a transparent window was implanted to replace a small part of the skull. The edge of the device was then sealed and fixed with the remaining bond by using a bio-compatible glue such as dental cement [104–106]. For general studies, the cranial window was built directly on the dura matter [58,107–118]. However, for high-resolution optical imaging or applications in large animals, the dura matter was usually removed before implantation [75,119–124]. As normal glass sheets were flat and might cause additional pressure against the brain tissues, a curved crystal window was developed, which ensured a continuous optical imaging of millions of neurons for a long period from weeks to a whole year [45,125,126].

Instead of using plain or curved glass, researchers also applied lenses in novel implanted cranial windows [127–133]. By using these novel devices, for instance, two-photon imaging was performed for neurons located as deep as 5 mm under the brain surface [131].

Polymer windows were developed in recent years from PDMS [74,76,134], polyethylene terephthalate [61], PMMA-Plexiglas [135], silicone glue gel [136], and so on. In addition to excellent optical properties, elastic polymer windows could allow penetration of a needle and had a useful self-sealing nature, thus leading to an on-site injection function [74,134].

However, the cranial windows described above were mainly developed for observation with human eyes and for optical imaging experiments. The increasing demands of neuroscience have promoted developments of multifunctional cranial windows, which allowed two or three different functions performed simultaneously.

2.3. Multifunctional Cranial Windows

Over the years, many kinds of multifunctional cranial windows have been developed. Here, we briefly introduce some of them.

Figure 1a schematically illustrates a cross-section view of a chronic cranial window suitable for implantation in a live mouse, where its transparent window is made of a glass sheet [65]. Impressively, this piece of glass window was designed to be removable, adjustable in position, and could be replaced after weeks and months. It allowed in vivo experiments over several months, such as two-photon imaging of targeted region with an attached micropipette. By removal of the window, it allowed micro-surgery such as injections and cutting away overgrown tissues [65].

Figure 1b presents an idea of “lab-on-a-brain” on a mouse, where a micro-optical fluidic device is built in a cranial window [67]. The transparent micro-optical fluidic device enabled long-term in vivo imaging of neurons, as well as delivery of chemicals into brain tissues with low tissue damage. By using this device, the formation and elimination of spines on particular dendrite location was recorded for weeks, and spine shrinkage after two-photon uncaging stimulation was observed in vivo.

Figure 1c shows the structure of another kind of cranial window for mice, where it consists of a glass coverslip, and a small hole with a silicone seal was pre-made on the glass coverslip [64]. Thus, it was capable of self-sealing after multiple injections or retraction of a pipette, an electrode through the silicone seal. Over a period of 7 weeks, repeated injection experiments were performed without causing any infections of the brain.

Figure 1d illustrates a 3D structure of a transparent PDMS cranial window for rats and mice [74]. The PDMS sheet was flexible, penetrable, and elastic. It was glued to the skull with a cyanoacrylate adhesive and sealed with a dental resin. After implantation of the PDMS cranial window, in vivo hemodynamic responses were continuously monitored in cortical vasculatures up to 15 weeks. Insertion of microelectrodes and micropipettes into the cortical tissue through the PDMS window were performed without causing any fluid leakage. Longitudinal two-photon microscopic images of mice were also obtained in similar quality to that using glass cranial window. Interestingly, no obvious adhesion of cell or tissue on the PDMS window was monitored during the long-term observations.

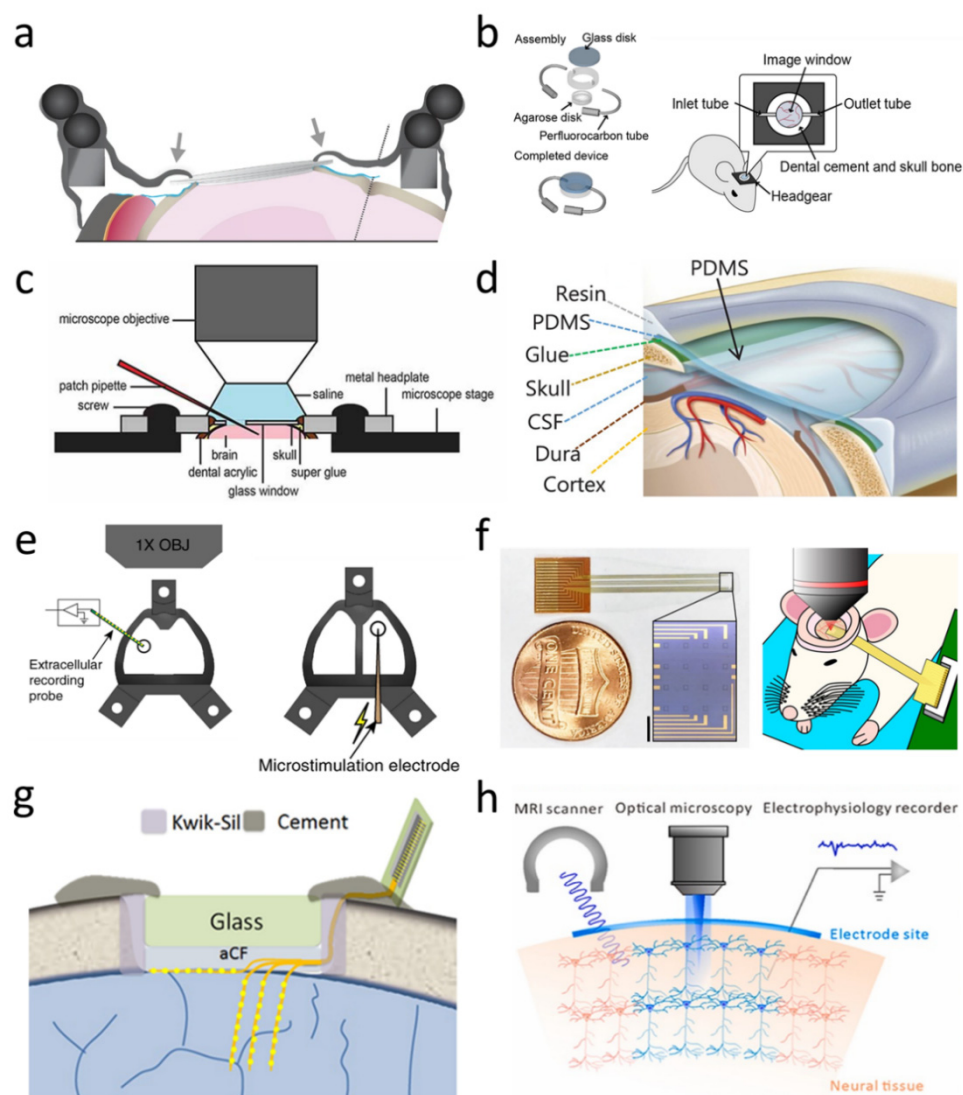


Figure 1. Eight kinds of typical multifunctional cranial windows in brain studies. (a) A cross-section view of a chronic cranial window where the glass sheet could be replaced. Adapted with permission from [65]. 2014, Springer Nature. (b) “Lab-on-a-brain” on a mouse. Adapted with permission from [67]. 2014, Hiroaki Takehara et al. (c) A glass cranial window with a built-in silicone sealed hole for multiple injections. Adapted with permission from [64]. 2014, Christopher J. Roome and Bernd Kuhn. (d) A PDMS cranial window for rats and mice. Adapted with permission from [74]. 2016, Chaejeong Heo et al. (e) A “See-Shells” cranial window for combined electrical and optical experiments. Adapted with permission from [61]. 2019, Leila Ghanbari et al. (f) A transparent graphene sheet was applied as microelectrodes under a glass coverslip. Adapted with permission from [137]. 2018, Martin Thunemann et al. (g) Ultra-flexible nano-electronic threads was applied as microelectrodes under a glass cranial window. Adapted with permission from [50]. 2018, Elsevier. (h) A cranial window with “hydrogel-elastomer neural interface”. Adapted with permission from [62]. 2022, Elsevier.

Figure 1e is a top-view of a so-called “See-Shells” cranial window [61]. It was a large-area, transparent polymer skulls designed for a long-term optical observation of mouse cortex over 10 months. It allowed two-photon imaging of neural structures up to 600 μm deep at a subcellular resolution and calcium imaging from multiple regions. It also had a potential to combine electrical and optical functions, i.e., to perform neural probing for electrical activities and whole cortex imaging at the same time.

Early attempts for fabrication metallic electrodes on transparent cranial windows faced problems in combined optical and electrical measurements [57,138]. A bunch of novel electrode materials, such as graphene [55], flexible nano-electronic threads [56], carbon nanotube films [60], and organic transistor [139,140] were investigated to serve as transparent electrodes on cranial windows. As shown in Figure 1f, a transparent graphene sheet was applied as a microelectrode under a glass coverslip [137]. It allowed integration of in vivo two-photon imaging, optogenetic stimulation, and cortical recordings in one experiment [58,59,137]. In Figure 1g, arrays of ultra-flexible nano-electronic threads were implanted in a mouse for neural recording both at the brain surface and deep-level locations, then a glass sheet cranial window was sealed on the top [50]. This multimodal platform allowed a combination experiment for both laser speckle contrast imaging of cerebral blood flow and electrical recording of neural activity at particular positions [50,56,58].

Figure 1h demonstrates the main concept of a novel cranial window named as “hydrogel-elastomer neural interface”. This unique window allowed multifunctional experiments of electrophysiology, optical microscopy such as two-photon imaging, as well as magnetic resonance imaging [62]. The hydrogel and elastomer showed comparable elastic moduli to neural tissues and dura mater and could fit well to the curved brain surface. Simultaneous electrical recording and optical imaging were performed, and in MRI experiments the soft interface did not cause artifact images.

Nevertheless, the above transparent cranial windows do not seem suitable for large animals. Glass sheets are hard but fragile and easy to break. PDMS sheets are too soft to protect the brain tissue from external strikes. In the following, we present an alternative approach for large-area, transparent cranial window with superior mechanical strength made of Ti frames.

3. A Hybrid Ti-PDMS Structure for Large-Area Transparent Cranial Window

Three dimensional (3D) printing is now a well-developed technology [141–144]. By using laser scanning technique and 3D printing technique, we can fabricate complicated 3D structures with shape, thickness and curvature matching exactly to the designed dimensions from a variety of materials, such as resin, plastic and metal. For example, Figure 2a is a 3D printed resin sample, following the same shape of a rat skull sample [63]. The circular subject fit in the top surface of this resin skull was a Ti cranial window.

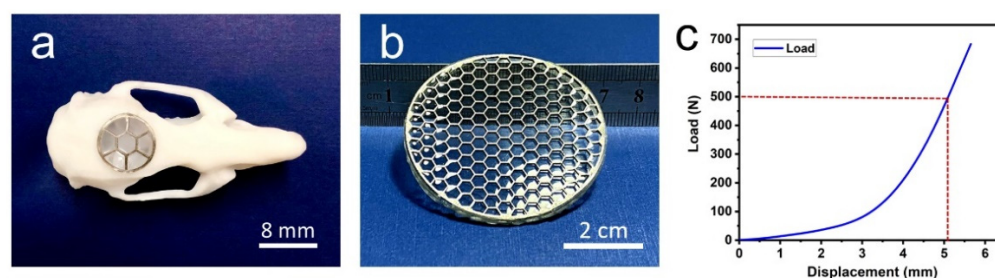


Figure 2. (a) A resin rat skull sample made by 3D printing technique with resin. In the middle of the top surface, a curved, 3D-printed circular Ti cranial window was fitted. Adapted with permission from Ref. [63]. 2022, Nana Yang et al. (b) Optical photograph of a 50 mm diameter, net-like Ti-PDMS cranial window. The circular Ti frame with thin hexagonal grids was firstly 3D-printed, polished, and then covered with a PDMS layer. (c) Raw data of mechanical test for one 50 mm diameter Ti-PDMS cranial window. It needed a static force of 500 N perpendicular to the Ti-frame at the center to let the central region show a 5 mm displacement.

Ti was chosen as the basic material for our cranial windows. Ti is metal with superior properties and has been extensively used in medial and clinical applications [145–151]. Figure 2b is an optical photograph of a honeycomb structured Ti-PDMS cranial window. Made by 3D printing technique, the circular Ti frame had a diameter of 50 mm. Within the circular frame were uniform hexagonal Ti grids, each grid had a thickness of 0.3 mm and

height of 1.0 mm. By using this thin grid structure, we obtained the ratio of the opening area to the total device area as high as $90 \pm 7\%$, depending on the grid size. After 3D-printed, the Ti frame was mechanically polished, then put in a homemade mould to cover a thin layer of PDMS.

The honeycomb structure has been well studied and applied [152–157]. Our honeycomb structured Ti cranial windows weighed only 6–7 g, yet they had a remarkable mechanical strength. Figure 2c presents a typical raw data taken from mechanical test for a 50 mm diameter Ti-PDMS cranial window. During the test, a stainless-steel rod of 15 mm in diameter was pushed slowly and perpendicularly to the Ti-frame surface at the center. To cause the central region a displacement of 5 mm, it required a static force of 400–500 N [63]. This mechanical strength was close to that of a human skull [158]. If such a Ti frame was implanted as a cranial window on a large animal, it should be able to protect the brain from external strikes.

Note that this Ti grid has a thickness of only 1.0 mm. For application in human skulls, one can increase the thickness to 2–3 mm, thus making the Ti-grid cranial window much stronger. Also, as Ti is a nonmagnetic material, Ti cranial window is compatible to MRI and CT imaging [146].

For the transparent window, PDMS was chosen in our device. PDMS is an elastic transparent polymer and has perfect compatibility to bio-tissues [159–164]. As reviewed in this paper, PDMS has been applied in transparent cranial windows [74,134]. PDMS is a hydrophobic material. Figure 3 presents a typical set of results in contact angle measurements with saline water and PBS solution for our PDMS samples and glass cover slides. It showed that the contact angles for PDMS were approximately 109° for saline water and around 106° for PBS solution. In contrast, for glass cover slides these angles were approximately 37° and 52° , respectively. The hydrophobic nature of PDMS helped a lot in our Ti-PDMS cranial windows, which avoided obvious growth of cells or adhesion of bio-materials on the inner side of these PDMS windows, when they were implanted in live animals. For instance, our experiments showed that after implantation in mice for 140 days, the Ti-PDMS hybrid window retained almost the similar clearness as that of Day 1, and the microstructural details of brain blood vessels were clearly recorded from outside [63].

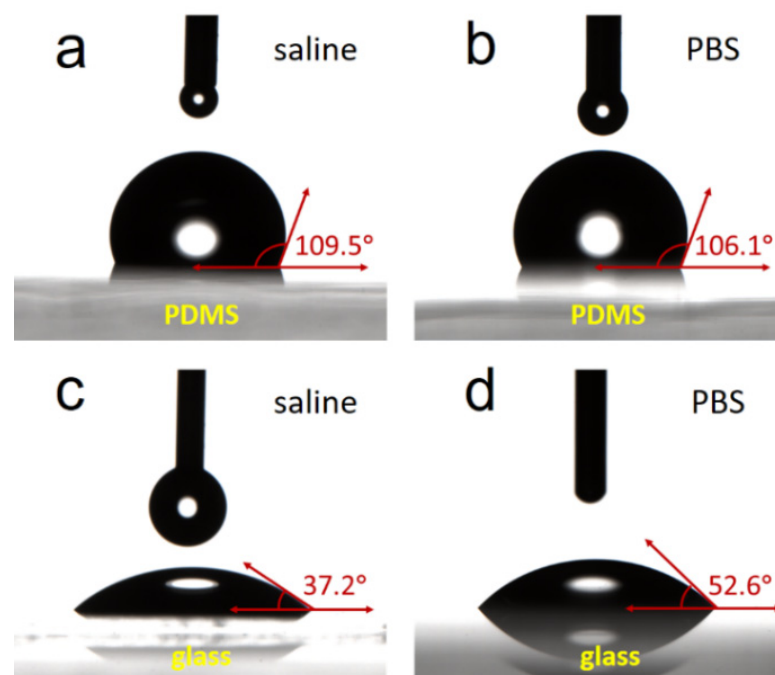


Figure 3. Contact angle measurements for PDMS and glass cover slide. (a) Saline on PDMS. (b) PBS on PDMS. (c) Saline on glass slide. (d) PBS on glass slide.

When coating the polished Ti honeycomb frame with PDMS, some small bubbles were likely to remain in the as-made PDMS windows, as shown in Figure 4a. We have developed a multistage process, where the samples were continuously shaken and degassed with certain equipment, and clear Ti-PDMS samples were obtained without noticeable bubbles, as shown in Figure 4b.

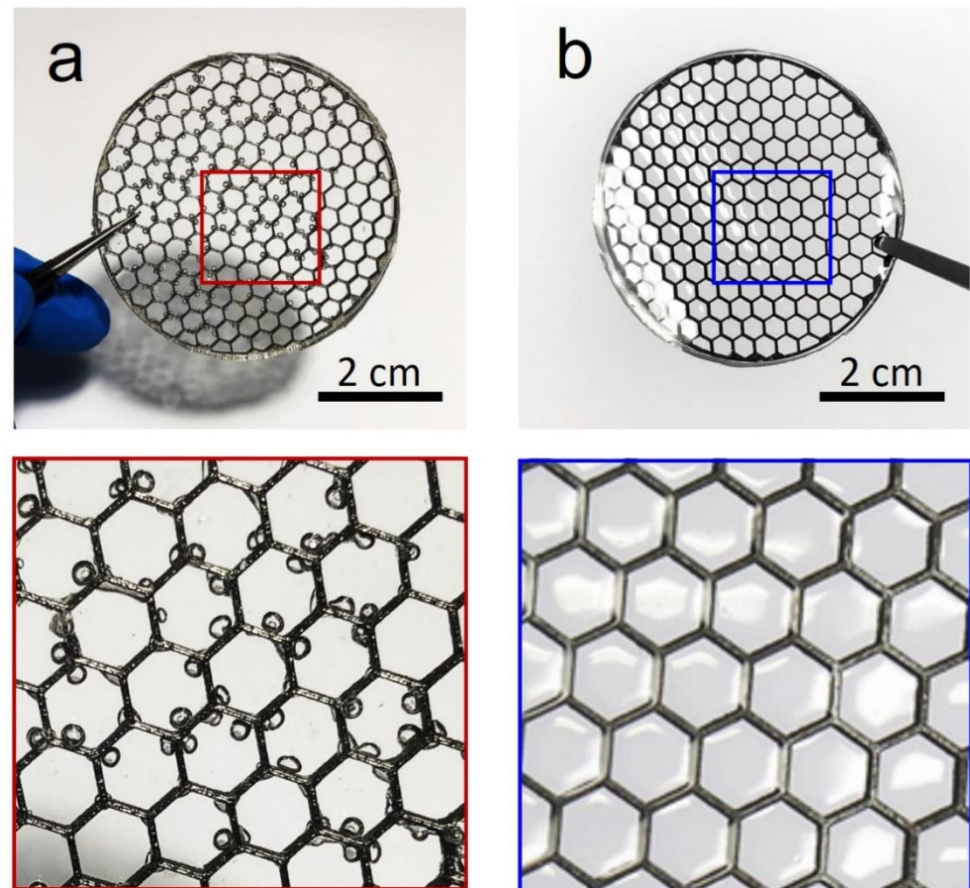


Figure 4. Photographs for Ti-PDMS cranial window samples. (a) Simple dipping process of the Ti frame in PDMS caused micro-bubbles. (b) By using a multistage shaking and degassing processes, the gas bubbles were totally removed from the sample.

In the wavelength range of 380–1100 nm, PDMS layer (0.3–0.5 mm in thickness) showed a higher transmittance than that of #1 glass coverslip. Therefore, our small-size Ti-PDMS transparent cranial windows implanted in mice allowed excellent two-photon imaging results [63]. The excellent plastic nature of PDMS was utilized to perform multiple injection of drugs using commercial 1 mL syringe through the same unit window of PDMS on a honeycomb Ti frame. For continuous injections by 15 times, no leaking of the injected liquid was observed. Figure 5 showed a typical result after injection of DiI (0.1% methanol in water, 0.2 μL , 0.1 $\mu\text{L min}^{-1}$) and fluorescence dye CTB488 (1 mg mL^{-1} in PBS, 0.2 μL , 0.1 $\mu\text{L min}^{-1}$) at three different spots of a mouse cortex through an implanted Ti-PDMS cranial window. No leaking was observed after the injections. Two days later, confocal imaging experiments were performed at the injection region. As shown in Figure 5d, the three injection spots were clearly observable, and fluorescence dye was recorded in deeper tissues around the injection spot [63].

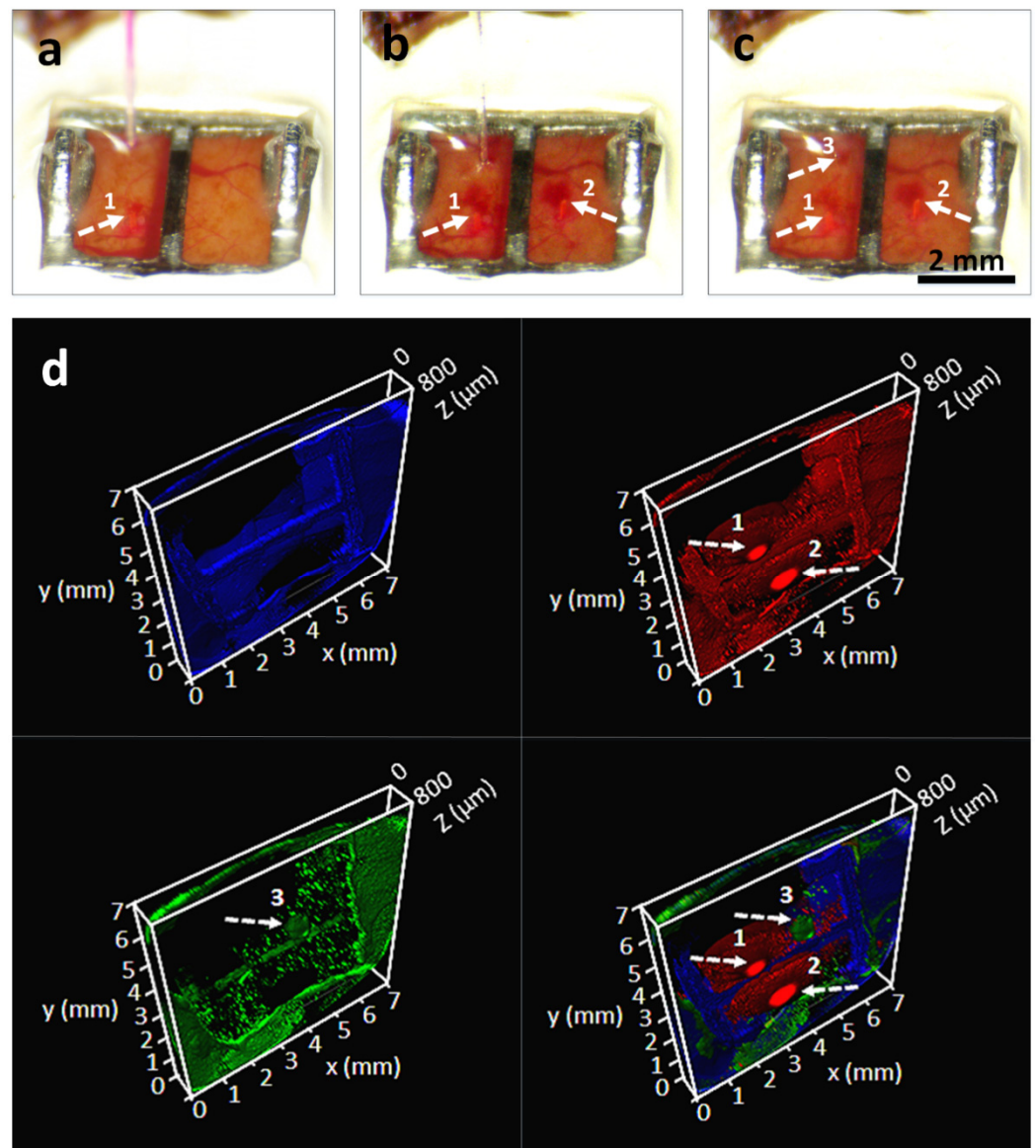


Figure 5. (a–c) Photographs for injections of DiI at spots 1 and 2 (0.1% in methanol, $0.2\ \mu\text{L}$, $0.1\ \mu\text{L min}^{-1}$) and fluorescence dye CTB488 ($1\ \text{mg mL}^{-1}$ in PBS, $0.2\ \mu\text{L}$, $0.1\ \mu\text{L min}^{-1}$) at spot 3 through a Ti-PDMS cranial window implanted on a mouse head. (d) Confocal imaging micrographs for the injected spots after two days. Adapted with permission from [63]. 2022, Nana Yang et al.

4. Discussion

Our small size Ti-PDMS cranial windows worked well in rats and mice for optical experiments and drug injections. Our preliminary results have shown that Ti-PDMS cranial windows could be implanted in live animals for 5 months without causing any observable inflammation of brain tissues or other side effects [63].

Aiming at potential applications as large-area transparent cranial windows, our 3D-printed honeycomb-structured Ti frames showed excellent mechanical strength against external strikes. Weighing only approximately 7 g, the 50 mm diameter, 1 mm thick honeycomb-structured Ti frame presented a displacement of 5 mm at the center under an external static force of 400–500 N. On the other hand, the 3D printing technique used in this work allowed personal designs for special size, curvature, thickness, and transparent ratio in various applications on different animal skulls [63].

The excellent transparent properties of PDMS in a hybrid Ti-PDMS cranial window offered an ideal optical window for long-period, real-time observation of brain status or

to perform a variety of in vivo optical experiments such as two-photon imaging, confocal imaging, optogenetics, etc. [63,74].

The good elastic nature of the PDMS window also allowed multiple impaling of injectors for injection of drugs into the brain or extraction of hydrocephalus from the brain, without leaking. This merit was quite valuable in both brain studies and clinic applications [63,74]. For example, it could be applied to perform drug delivery bypassing the blood–brain barrier.

Finally, the non-magnetic nature of both Ti and PDMS also made the Ti-PDMS devices compatible to CT and MRI measurements.

The above merits made the hybrid Ti-PDMS device a promising candidate for implantation in big animals as a large-area, transparent, high-strength, multifunctional cranial window.

Indeed, such a multifunctional cranial window may lead to a wearable, smart electronic device for both medical and health care applications. Recent developments of microchips, 5G wireless communication technology, and neural probing techniques make this proposal practical.

For instance, Neuralink Co. has demonstrated that a brain–computer interface mounted in the skull of a pig or a monkey, with the size of a quarter coin, and consisting of built-in processing chips, circuits and battery, could collect the real-time neural signals of the animal through thousands of microelectrodes implanted in the brain cortex [165]. This could be considered as a simple prototype of a smart cranial electronic device.

As illustrated in Figure 6, a complicated smart cranial electronic device, once developed, may have built-in modules of multichannel electrodes for cortex probing, deep brain stimulators, data processing chips, and wireless communication devices. For brain studies, it may also consist of an injection window and microfluidic sensors, as well as optical windows at multiple spots for long-time observation and optical experiments. The skull of a human adult has a large surface area; thus, it offers a large room for implantation of such a smart device.

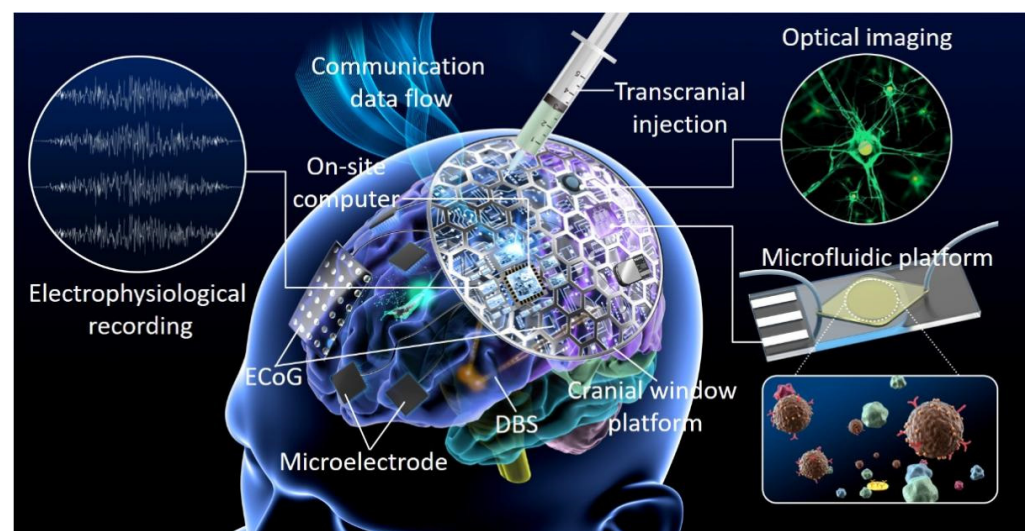


Figure 6. A proposal for a large-area multifunctional cranial window as a wearable smart electronic device for both medical and health care applications.

Such a smart cranial device may be applied in clinical surgery, such as treatment for cerebral apoplexy, repairing damage in brain tissue or skull bone from car accidents or shootings, removing cerebral hemorrhage, etc. It may also be used in treatment for chronic diseases, such as injecting drugs for inflammation and monitoring development for Alzheimer’s diseases or tumors.

5. Conclusions

In summary, in this paper we briefly reviewed developments of cranial windows, which offered a unique and direct approach for long-time direct observation of brain tissues and in vivo optical experiments with cortex neurons.

We presented preliminary results for our novel hybrid Ti-PDMS cranial windows. The Ti frames were made with 3D printing technique, and the PDMS layer was fixed on the frame with a multiple-stage process to ensure no remaining bubbles were in it. Experiments on mice and rats had shown excellent bio-compatibility of the device and excellent optical property for in vivo two-photon imaging and confocal imaging, as well as good self-sealing capability in multiple injections with commercial syringes. Tests showed that the 50 mm diameter, 1 mm thick honeycomb structured Ti frames had mechanical strength comparable to that of a human skull bone, meanwhile it had a large transparent area ratio of approximately 90%. Therefore, the Ti-PDMS hybrid devices have a promising potential for applications in brain studies in pigs, dogs, monkeys, and even humans as long-period, large-area, transparent and multifunctional cranial windows.

We also suggested that such a large-area multi-functional cranial window is indeed a good platform for a smart wearable electronic device implanted on the head that plays a key role in hybrid optical-electrical brain-computer interface in brain studies and serves as a drug delivery window for clinical applications. A smart cranial electronic device may have integrated modules for electrical probing, deep brain stimulation, data processing, and wireless communication, as well as a variety of on-site sensors, optical windows, and injection spots.

The results of this paper may shed light on the development of novel brain-computer interfaces and wearable electronic devices.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

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