A Clear, Delicate, Biocompatible Optical Window for Brain Imaging

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***Abstract*— In this work, we present a single-step method to fabricate transparent, flexible optical windows of biocompatible hydrogel material for repeated, long-term optical recordings and stroke monitoring of rodent cortex. The main advantage of the proposed method is that the optical window of any desired shape and size can be conveniently fabricated in a short span of time. The fabrication of the optical window is simple and completed in a single step. Biocompatible hydrogel poly (ethylene glycol) diacrylate (PEGDA) in the liquid form before curing conforms well to the required shape and size after cross linking. Being a light, transparent and stretchable material provide a practical solution to long- term optical imaging in the anesthetized rat.**

I. INTRODUCTION

The ability to record hemodynamic changes from desired cortical sites by using optical windows in awake/controlled animals has an important role in neuroscience research. One of the important requirements is to keep the cortical window completely transparent and maintain the cortex in a good optical condition for long periods by preventing tissue regrowth over the exposed cortex [1]. Selective mapping and manipulation of microcircuit elements over cortical brain regions is possible through optogenetic tools which provides remarkable access to brain [2].

Various strategies are followed to achieve optical window implantation for long-term imaging in anesthetized or awake mice brains. Thinned-skull window method has been used as non-invasive approach in various studies [3], however the limitation of this approach is the control of precise skull thickness. Skull thickness beyond 15 µm may result in the risk of mild cortical trauma due to pushing of skull downwards with the drill bit or microsurgical blade [4]. Biocompatible glues such as agarose [5] and kwik-Sil [6] are used to fix or increase the adhesion between glass window and the cortex. Although this method looks simple and quick, complications exist causing damage to cortical tissue during removal or replacement of the window. In another approach, a PDMS-based dural substitute has been used [7] as an artificial dura. However, PDMS used is considerably thick and not suitable for use in small animal studies such as in rats and mice. Moreover, PDMS is not an FDA- approved material, which cannot be conveniently used for long-term, chronic window applications.

Wichterle and Lim strongly supported hydrogel, as a biomaterial since the publication of their work in 1960. This material has generated huge interest in biomedical material research [8]. Its potential application includes cell delivery, polymer scaffold, wound healing, diagnostic devices and electro conductive biomaterials [9]–[11] . PEGDA is an FDA-approved material, suitable for biomedical applications due to its non-toxic and non-immunogenic nature [12]. The surface properties of PEGDA can be improved by surface modification for specific applications. kinetics of polymerization has been studied, to achieve higher conversion rates, and avoid unreacted monomers and side products.

In this work, we have used biocompatible PEGDA as a material for fabricating optical windows. These networks have a three-dimensional structure, cross-linked together on exposure to a UV source. Thus, the hydrogel biocompatibility and crosslinked structure are suitable for various biomedical applications. Moreover, for such biomedical applications, it is required that the hydrogel matrix maintains physical and mechanical integrity. It should also be non-toxic for it to be suitable for biomedical applications. Different approaches are followed to eliminate contaminants from hydrogels, such as repeated washing and treatment.

II. RAT PREPAPRATION FOR *IN VIVO* TEST

The experiments were performed on an adult female Sprague Dawley rat (250 g) (In Vivos Pte Ltd, Singapore). Prior to the experiments, the rats were ingested for one week with food and water provided *ad libitum* and 12 hours lights on/off. The animal care and use procedures conformed to those specified by the Agri-Food & Veterinary Authority of Singapore (AVA), the Institutional Animal Care and Use Committee (IACUC) and the ethics commission of the National University of Singapore. The rats were anesthetized with a single bolus injection of ketamine/xylazine (150 mg/kg and 10 mg/kg, respectively, intraperitoneal). After an adequate depth of anesthesia was reached, the animal's head was fixed in a stereotaxic apparatus and a scalp incision was

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made to expose the parietal region. A cranial window of 3 mm × 2 mm was made using a dental drill.

III. RESULTS AND DISCUSSION

We have used two methods of fabrication for the optical window, to ascertain the most appropriate method. The schematic representation of fabrication process depicted in Fig. 1. In both the methods (denoted A and B), a thin PEGDA film was coated with parylene C, to form a hydrophobic surface, which is evident from hydrolytic degradation test. Two sets of crosslinked PEGDA samples were prepared for hydrolytic swelling test. One set of samples without parylene coating and other with 200 nm thick parylene coating (Lavida, Femto Science, Korea) which is chemically resistant, biostable, biocompatible and optically transparent [13]. Both the samples were immersed in water and their weight was measured on a weekly basis. Three replicas were measured for each test and standard deviations were shown with error bars in the swelling profile graph. The degree of swelling was calculated as Eq. 1:

$$\text{Degree of swelling} = \left[\frac{(\text{Wet weight} - \text{Dry weight})}{\text{Dry weight}}\right] \times 100\ \% \quad (1)$$

PEGDA samples without parylene coating, start swelling after 2 weeks of immersion time, and disintegrating was observed from the first day and the degree of swelling was almost 90 % at the end of 10th week. On the other hand, Parylene coated PEGDA samples exhibited negligible degree of swelling even after 10 weeks of immersion as represented in Fig. 2. PEGDA film coated with parylene was also subjected to tensile testing, of constant-rate-of elongation type on a Instron mechanical tester to determine the longitudinal elasticity. For tensile testing, the PEGDA film, of specific length and width according to ASTM standard, was glued to a paper holder, which is held between the two testing jaws, with the line of contact perpendicular to direction of applied load. The parylene-coated PEGDA film, indicated reversible recovery and elasticity up to 35% strain, which is good enough for our proposed application as represented in Fig. 3.

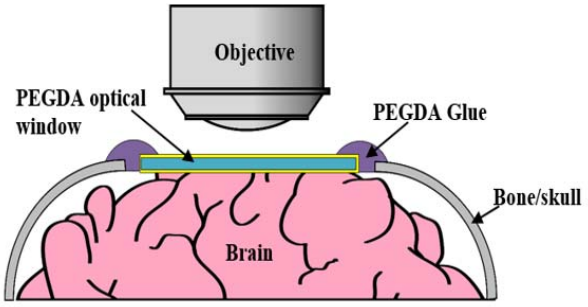

Fig. 1. (a) schematic illustration of hydrogel optical window fabrication and assembly

In method A, PEGDA mixed with photoinitiator at low concentration (0.1 %, v/v) was cured with portable UV exposure equipment for 1 minute, to facilitate formation of a thin film of thickness 100 µm and of dimension slightly smaller than the cranial window. PEGDA film fabricated externally was placed on the brain window. Liquid PEGDA is filled in the gap between cranial window and edges of the film and cured immediately for 30 seconds to secure the film in place as illustrated in Fig.4 (a).

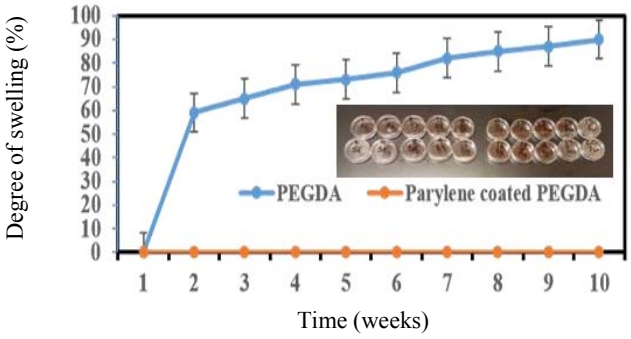

Fig. 2. Hydrolytic degradation test of PEGDA sample coated with parylene.

In method B, PEGDA mixed with photoinitiator at low concentration (0.1 %, v/v) was cured with portable UV exposure equipment for 1 minute to form a thin film of thickness 100 µm of dimension 5 mm × 3 mm, larger than the cranial window. This is to facilitate easy removal and replacement of optical window. PEGDA film fabricated externally was placed on the brain window. Liquid PEGDA is poured around the edges of the film and cured immediately to secure the film in place as illustrated in Fig.4 (b). The cured film is bendable and stretchable in both methods as shown in Fig.4 (c).

Thin layer of PEGDA film of dimension slightly smaller than the cranial window fabricated with method A, smeared well on the brain. There was no gap between the brain and film, cured PEGDA was found to adhere to the cortex with good degree of conformability, demonstrating method A as an appropriate procedure for window fabrication. On the other hand, thin layer of PEGDA film of dimension larger than the cranial window fabricated with method B, adherence of the film to the cortex was not firm, resulting in the regrowth of tissue and opaqueness of the optical window rather quickly at the end of the day. The cause of failure was the gap between the brain and the PEGDA film placed over the window.

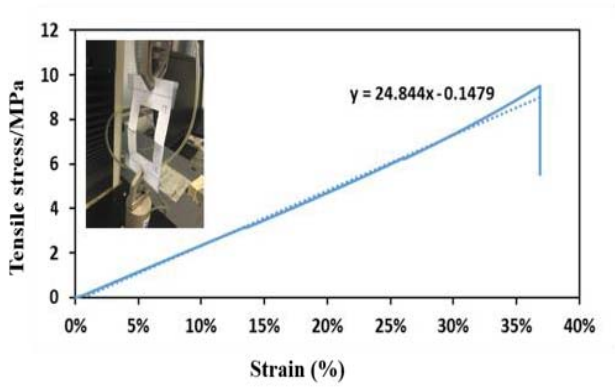

Fig. 3. Tensile strength test of PEGDA sample coated with parylene

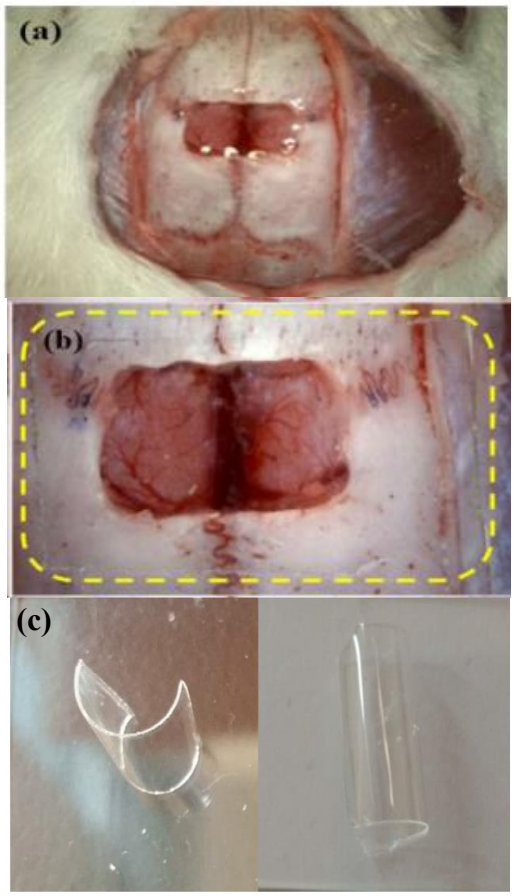

Fig. 4. PEGDA film over window (a) PEGDA film of size slightly smaller than cranial window placed, Method A (b) PEGDA film of size larger than cranial window placed, Method B (b) Flexibility of fabricated PEGDA film.

Henceforth, we have used method A to fabricate an optical window. The scalp was sutured back and opened every 4 hours to check clarity and transparency of the optical window during different time intervals. The window was very clear at the end of 4, 8 and 12 hours as represented in Fig. 5(a, b, c) respectively. The dura was not removed during entire duration to keep the animal's brain in an intact condition for long periods. The process of dura removal could damage the brain resulting in undesirable consequences. If the dura is retained, it will keep the brain intact, thereby facilitating long-term chronic studies. The results were encouraging during this acute test, and this method will solve a major difficulty in chronic cortical microelectrode stimulation or recording experiments.

The removal of the optical window at required intervals prevents skull regrowth and dura, as it will conform and adhere well to the cortical surface, providing strengthened clarity, while extending the lifetime of imaging through this window. The removal and replacement approach have not been used by other groups in rat or mice, similar technique is commonly and widely used in chronic cortical imaging in primates. This method will also facilitate insertion of microelectrodes, through the window for stimulation/recording in the rat brain as the PEGDA film is thin, robust and elastic. Direct observation of pial microcirculation through a transparent window attached to the cortical surface is possible while stimulating with microelectrodes as it is transparent.

IV. CONCLUSIONS

The single-step hydrogel optical window that we describe here has many advantages compared with the state-of-the art. The window thickness can be precisely controlled with a good degree of flexibility and confirmability. The material used, photocurable PEGDA, is of low molecular weight, which is flexible, biocompatible, FDA- approved and tissue-friendly material with very low young modulus suitable for proposed application. We compared two differing fabrication and experimental procedures for implementing the PEDGA window for brain imaging. The method A we describe here enables removal and replacement without causing any damage to rat brain. Therefore, we have demonstrated this to be an enabling technology for a wide range of applications involving observation of the brain activity. In future work, this will be a useful tool for photoacoustic imaging and brain electrical stimulation.

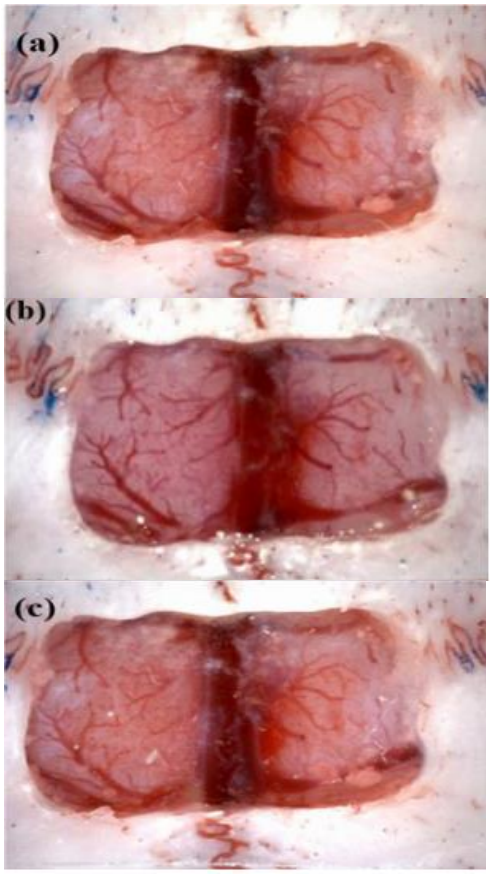

Fig. 5. Clarity of PEGDA window (a) after 4 hours (b) 8 hours and (c) 12 hours of window placed securely following fabrication method A.

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