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To cite this version:

H Soleimanzad, M Juchaux, D Crepin, H Gurden, F Pain. Skull optical clearing for longitudinal non invasive optical imaging. SPIE West BIOS 2020, Feb 2020, San Francisco, United States. hal-03066940

HAL Id: hal-03066940

https://hal.science/hal-03066940v1

Submitted on 15 Dec 2020

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Skull optical clearing for longitudinal non invasive optical imaging

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We have evaluated in vitro and in vivo the efficiency and practicability of a recently proposed approach for minimally invasive, longitudinal imaging of the rodent brain: reversible optical clearing of the skull[1]. Use of this approach for rodent brain imaging would allow to suppress the need of time consuming and prone to defect chronic optical windows[2,3]. In addition, the method would allow to preserve completely the dura matter and brain tissues thus unaltering the physiology of the tissues. Firstly, in vitro experiments have been conducted on resected mice skulls to determine whether a two steps protocol including calcium chelation followed by topical application of an index matching solution is better than solely the direct optical index matching strategy[4]. The time course of the effect of different chemicals on the optical properties of the skull have been monitored quantitative to provide with an optimal protocol for in vivo skull optical clearing. Secondly, in vivo validation was carried out for mapping of brain blood flow using multiple exposure laser speckle imaging.

Keywords: clearing, skull, longitudinal imaging, laser speckle imaging, cerebral blood flow, mice, brain,

METHODS

In vitro experiments have been conducted on mice skull samples from 20 mice of varying ages, using a two integrating sphere approach depicted on figure 1. Integrating sphere #1 is used to measure total reflectance and integrating sphere#2 is used to measure total transmittance. Both measurements are carried out simultaneously and acquired dynamically. This allows to study the time course of the clarification process while the skull sample is immersed in either calcium chelating or index matching solutions. For further analysis, the thickness of the skull samples was determined using OCT imaging.

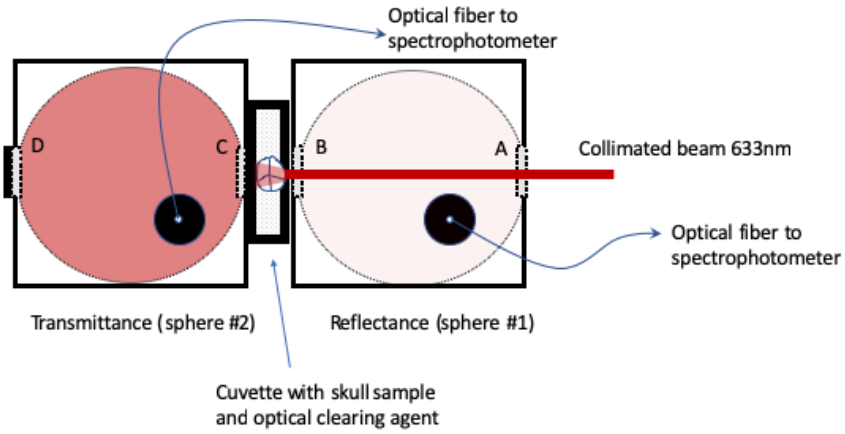

Figure 1. In vitro set-up for the dynamic study of mice skull clarification process.

RESULTS

Representative results of the in vitro experiments are shown on figure 2. The optical clearing agents used were glycerol 80%, EDTA -10% followed by Glycerol 80% or PEG-400. All protocols show a significant increase in transmittance and decrease of reflectance following optical clearing treatment.

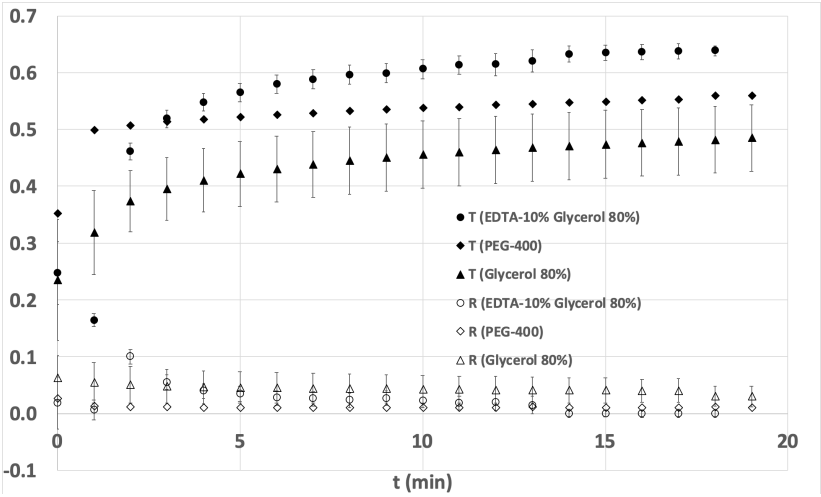

Figure 2 Transmittance (filled symbols) and Reflectance (open symbols) for different skull optical clearing protocols. The optical clearing agents are introduced between 0 and 1 minute.

For all protocols, the data show a relatively rapid time course of optical clearing as about 90% of the maximum transmittance is obtained after 7 minutes. The kinetics is significantly faster for clearing with PEG-400 compared to glycerol-80%. The optimal clearing was obtained for the skull pre-treated with EDTA-10% as a calcium chelator, followed by glycerol 80%. A transmittance of about 65% is obtained combined with a negligible (<1%) reflectance. As it is knowns that the optical properties of skull changes with age in mice[5], further analysis will be presented regarding the influence of mice age on the skull thickness and the clarification process. In vivo validation have been carried out for longitudinal imaging. A representative study is shown on figure 3 where the same mice was imaged twice with a one-month interval between the two sessions. Laser speckle contrast imaging was performed using the multiple exposure approach[6].

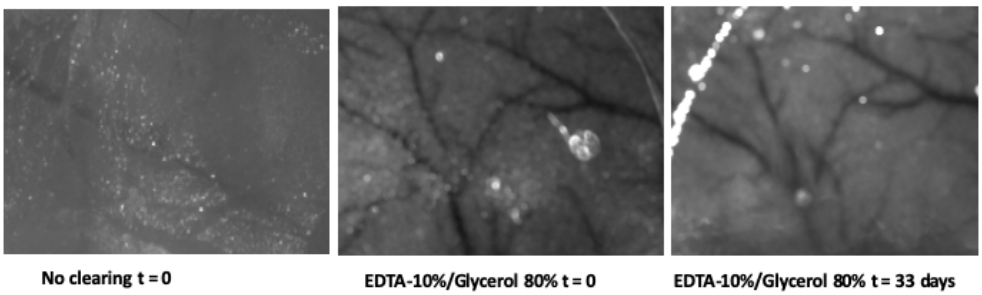

Figure 3 In vivo longitudinal laser speckle contrast imaging after skull optical clearing in mice

Altogether this study proposes a systematic methodology and a quantitative framework for the choice of skull clarification protocols. The in vivo validation demonstrates the practicability of skull optical clearing for longitudinal optical imaging of the mice brain which is of great interest for a vast community of researchers.

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