Proceedings of the British Pharmacological Society at http://www.pA2online.org/abstracts/Vol13Issue3abst084P.pdf

An Improved Protocol for Electrophoretic Clearing of Solid Organs

CLARITY (Clear Lipid-exchanged Acrylamide-hybridized Rigid Imaging/Immunostaining/in situ-hybridization-compatible Tissue hYdrogel)is a technique to immobilize proteins in whole organs while increasing transparency and making the tissue suitable for 3D imaging using visible light. It preserves the ultrastructure and allows fluorescent staining of proteins and nucleic acids in situ (1). This isachieved by infusing a cold cocktail of acrylamide/bisacrylamide, formaldehyde and a thermally triggered initiator into the tissue, followed by polymerization of the hydrogel at 37 °C. After hydrogel polymerization is complete, lipids can be removed using a strong ionic detergent-based clearing solution (borate-buffered 4% SDS), either passively by diffusion or by accelerating clearing by moving lipid/detergent micelles through the tissue in an electric field electrophoretic forcing.

CLARITY has been developed and mostly used on mouse brains, adult zebrafish and post-mortem human brain tissue (2), and has already revolutionized neuroscience. This technique has also been adapted to other tissues, such as pancreas, lung, intestine, liver and kidney but the time reported in the literature (2-4 weeks, (2,3)) for clearing solid organs remains a major disadvantage of the technique The aim of the project has been to evaluate whether the CLARITY technique could be acceleratedfor solid intact organs, such as kidney from adult and P5mice.

For this purpose, adult and P5mice were anesthetized and perfusedtranscardially with ice cold 0.01% heparin solution, followed by ice cold hydrogel solutionwith a mixture of 4% PFA, 4% acrylamide, 0.05% bisacrylamide,0.05% saponin, 0.25% VA044 in PBS; kidney and brain were extracted and incubated for a few hours in the same solution. Acrylamide was then cured for 3-5 h at 37 °C.

After overnight incubation in 8% SDS/clearing solution, hydrogel-embedded organs were placed in a small custom-made electrophoretic tissue clearing (ETC) chamber. The small size allowed effective cooling and increasing field strength from the reported ~10 Vcm^{-1} to 30 Vcm^{-1} which cut down clearing time to1 or 2 days for P5 or adult mouse kidney, respectively.

We immunostained clarified kidneys by incubation with anti-D28K calbindin, with custom-generated anti-NCC and anti-barttin antibodies for 3 days, followed by incubation with the respective secondary antibodies. Imaging was carried out on a Femtonics live/*in vitro* 2-photon microscope and Alexa488 and 546-coupled secondary antibodies were visualized simultaneously using 970 nm excitation.
Although we achieved a significant reduction in the clearing time, immunostainingremained time-consuming and tissue penetration remained superficial. We achieved a penetration depth of 150-200 µm, and several freeze-thawing cycles prior to staining did not result in deeper penetration.

In order to make CLARITY fast enough to test several conditions for more problematic primary antibodies, antibody penetration must be improved, possibly by electrophoresis (4). Whether applying pulsed fields, such as used for electrophoresis of large DNA molecules (5), remains to be investigated.

(1) Tomer R *et al*. (2014). *Nature Protocols* **9**: 1682–1697

(2) Lee H *et al*. (2014). *BMC Developmental Biology* **21**: 14-48

(3) Kim SY *et al*. (2013). *Trends CognSci* **17**: 596-599

(4) Liu H and Kao WW (2009). *Mol Vis* **15**: 505–517

(5) Tenover FC *et al*. (1995). *J ClinMicrobiol **33**: 2233-2239*