Porous Nanosheet Wrapping Fabricated by Nanoimprinting Technique for High Quality Bioimaging

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We have proposed freestanding biofriendly ultra-thin films (often called nanosheets) with a thickness of *ca.* 100 nm for biomedical applications [1,2]. These nanosheets represent unique properties such as good adhesiveness, amazingly flexibility, and a high degree of transparency. In the field of biological microscopy technology, it is still a practical challenge to obtain high quality images of suspension cells and tissues, due to the sample desiccation and undesirable cell movement that occurs during observations. In this paper, we propose an innovative technique "polymer nanosheet wrapping" to avoid desiccation and movement of suspension cells and tissues, that is applied to a novel imaging tool for taking high quality images (**Fig. 1**) [3-5].

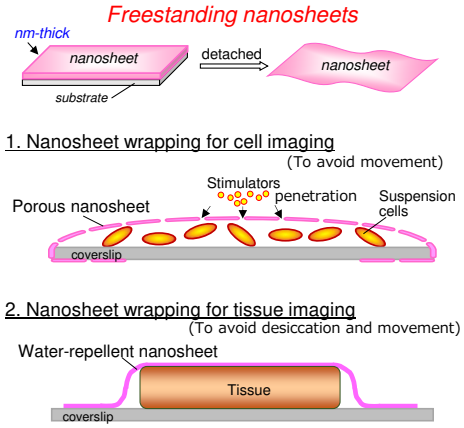

Figure 1. Schematic images of polymer nanosheet wrapping of tissues and suspension cells for high quality bioimaging.

For suspension cell imaging, freestanding porous nanosheets were fabricated by a nanoimprinting technique to enable the addition of external chemicals to cells, ranging from small molecules to macromolecules. In fact, porous nanosheet composed of biocompatible poly(lactic acid) (PLA) with a thickness of *ca.* 60 nm was fabricated by spin-coating process (**Fig. 2A**). Porous structure was then fabricated by a nanoimprinting technique under a nickel pillar mold. The diameter and pitch distance of the pores were designed to be 840 ± 65 nm and 6 μm, smaller than that of ordinary cells (**Fig. 2B**). To obtain a freestanding nanosheet, a water-soluble polymer layer composed of PVA was coated before PLA coating, and the PLA porous nanosheet can then float on the surface of

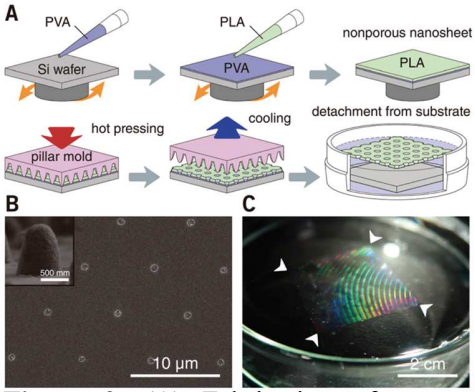

Figure 2. (A) Fabrication of porous nanosheets and nanosheet by spin-coating and thermal nanoimprinting. **(B)** SEM image of a porous nanosheet. **(C)** Photo of a freestanding porous nanosheet floating on the surface of water. (Partially modified from ref. [3]).

water with dissolution of the PVA underling layer (**Fig. 2C**). Through several case studies, such as a live imaging of membrane staining of liposomes and activation of platelets, it is verified that the confined space made by nanosheet could provide a hydrodynamically stable environment for suspension cells, even if an aqueous stimulus is added through the pores in a static or a flowing condition.

For tissue imaging, freestanding water-repellent nanosheets composed of an amorphous fluoropolymer (CYTOP®) were prepared using a spin-coating process. Their hydrophobicity, transparency, water-retentivity and imaging of neurons in brain slices were evaluated. Water-repellent nanosheets for tissue imaging show excellent water retention effect and work well for sample fixation. In fact, by wrapping cleared mouse brain slices with *ca.* 130 nm-thick nanosheet, we achieve high spatial resolution neuron images while scanning over a large area for a long period of time (**Fig. 3**). No visible artifacts arising from sample shrinkage can be detected.

We demonstrated that nanosheet wrapping acts as a powerful tool to obtain high quality images of suspension cells and tissues. The detailed results on other applications of the nanosheets including *in vivo* imaging will be released on the NNT2023.

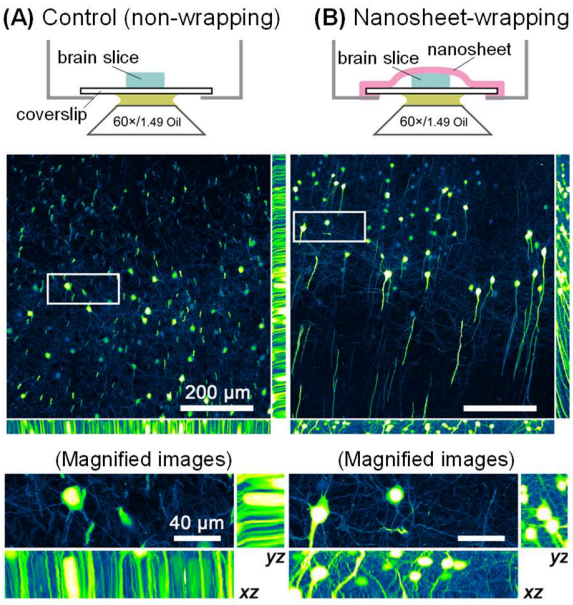

Figure 3. Confocal tissue imaging at tiled scanning of thy-1-EYFP-H mouse brain slices treated with a clearing reagent (Sca*l*eS). **(A)** Control (non-wrapping), **(B)** Nanosheet wrapping. Upper and lower panels show the schematic representation of each mounting process and magnified images, respectively (partially modified from ref. [4]).

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References

[1] Y. Okamura et al. Advanced Materials 2009, 21, 4388. [2] Y. Okamura et al. Advanced Materials 2013, 25, 545. [3] H. Zhang et al. Journal of Materials Chemistry B. 2018, 6, 6622. [4] H. Zhang et al. Advanced Materials 2017, 29, 1703139. [5] H. Zhang et al. PLoS ONE 2020, 15, e0227650.