Subretinal Injection of Micropatterned Nanosheets for Effective Cell Delivery

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Statement of Purpose: There have been ongoing efforts for the development of cell delivery system to overcome the intractable diseases. Age-related macular degeneration (AMD) is the leading cause of visual impairment and blindness in the elderly population, whose main complication is the development of subretinal choroidal neovascularization and degeneration of retinal pigment epithelial (RPE) cells [1]. In this regard, subretinal transplantation of RPE cells to the degenerated site has attracted a great deal of attention as an innovative therapeutics for the treatment of AMD. However, poor viability, distribution and integration of the transplanted cells in suspension to the narrow subretinal space have limited this approach. Therefore, development of cell delivery devices would bring significant benefits for the AMD treatment. Recently, we developed micropatterned polymeric nanosheets which can direct growth and morphogenesis of RPE cells toward local delivery of RPE cells [2]. Here, we examined whether micropatterned nanosheets can be delivered to the subretinal space of rat eyes using a syringe needle.

Methods: Micropatterned nanosheets consisting of biodegradable poly(lactic-co-glycolic acid) (PLGA) and nile red were prepared on a poly(vinyl alcohol) (PVA) coated glass substrate by a combination of spincoating and microcontact printing technique. A typical diameter of circular nanosheets was 300 μm. The freestanding nanosheets were obtained by dissolving the PVA layer with PBS. Sprague-Dawley rats (SLC) weighing 250-300 g were used in this study. All animals were handled in accordance with the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research after receiving approval from the Institutional Animal Care and Use Committee of the Tohoku University Environmental & Safety Committee. After the rats were anesthetized with ketamine hydrochloride and xylazine hydrochloride, micropatterned nanosheets were injected into the subretinal space using a 27G syringe needle (Fig. 1a).

Results: Before injection of the nanosheet, 2 μL of saline was injected to the subretinal space through the sclera in order to the secure the transplanted site. Subsequently, micropatterned nanosheets were injected with 2 μL of saline to the same place by the same route. Figure 1b shows optical coherence tomography (OCT) images of the retina taken one week after the injection of the micropatterned nanosheets. A shadow of the sheet like structure was observed in the subretinal space while there was not such shadow in the OCT image of a control. Also, a shadow of the circular nanosheet was observed at the posterior segment of the isolated eye. These results indicate that the nanosheets were successfully injected and spread into the subretinal space of rat eyes.

Conclusions: We developed micropatterned nanosheets consisting of biodegradable polymers, which can be delivered to the subretinal space using a syringe needle. The injection of micropatterned nanosheets through a syringe needle could be a minimal invasive way to reduce the incision size of the sclera and subsequent inflammatory response. The flexible micropatterned nanosheets injectable by the syringe needle hold great promise to transplant organized RPE cells.

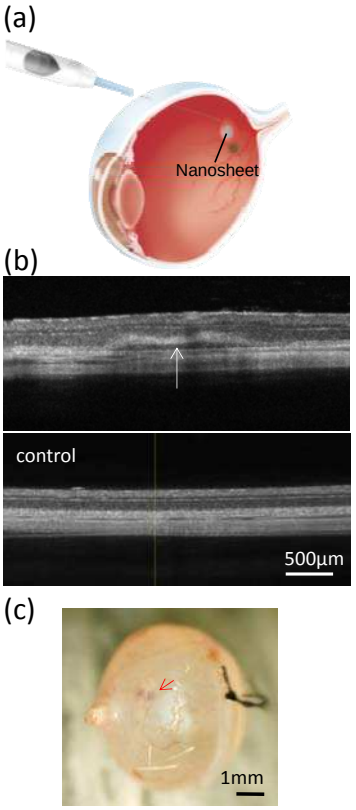

Figure 1. a) Schematic representation of the injection of micropatterned nanosheets into the subretinal space. b) OCT images of (upper) the retina one week after the injection of the maicropatterned nanosheets and (lower) a control. Arrow in the upper image indicates the injected nanosheets. c) Photo of an isolated eyeball. Arrow indicates the injected nanosheets.

References: [1] Hynes SR. Graefes Arch Clin Exp Ophthalmol. 2010;248:763-778. [2] Fujie T. Adv Mater. 2014;26:1699-1705.