Cell Delivery System Using Micropatterned Polymeric Nanosheets

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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. Here, we developed micropatterned nanosheets consisting of biodegradable poly(lactic-co-glycolic acid) (PLGA) [2], which can deliver RPE monolayer to the subretinal space in a minimally invasive way.

Methods: Micropatterned nanosheets were prepared by combination of spincoating and microcontact printing technique. The resulting PLGA/magnetic nanoparticles (MNPs) layer was transferred onto a poly(vinyl alcohol) (PVA) coated glass substrate, on which collagen was spincoated for promoting the cell adhesion. Then, the sample surface was covered with RPE cell suspension, and the freestanding cell/nanosheet construct was obtained by dissolving the PVA layer with PBS. The cell activity on the micropatterned nanosheet was evaluated using LIVE/DEAD staining assay. Moreover, syringe injection of the micropatterned nanosheet was examined using swine ocular globes *ex vivo*.

Results: Despite the mechanical share stress during aspiration and injection by the syringe needle, the RPE monolayer on the nanosheet retained the original shape without any fracture (Fig. 1a), and kept the viability over 80% regardless of the sheet diameter (Fig. 1b). Moreover, we evaluated the thickness effect on the cell viability under the syringe injection. The micropatterned sheet with nanometric thickness (170 nm) retained the viable cells on the surface, while those with micrometric thickness (5.5 μm) hardly retained the cells (less than 30%, data not shown). We assumed that the cells on the micrometric thickness would be scraped from the sheet surface due to the mechanical friction. Therefore, flexible structure of the micropatterned nanosheet is beneficial not only for being folded inside the syringe needle, but also reducing the mechanical stress on the cell monolayer. Finally, we demonstrated the injection of the micropatterned nanosheets to the subretinal space using swine ocular globe *ex vivo*. For example, the freestanding micropatterned nanosheet (1 mm ϕ) was injected by the intravenous catheter (24 G). Then, the nanosheet was successfully released into the subretinal place, and fixed on the macula after removing the prefilled saline without structural distortion (Fig. 1c).

Conclusions: We developed micropatterned nanosheets consisting of biodegradable polymers, on which stable monolayer of the RPE cells were engineered. Owing to the high flexibility of the nanosheets, RPE cells were injected through the syringe needle without significant loss of the cellular viability. Such micropatterned nanosheets were also subretinally injected and placed stably on the macula. The flexible micropatterned nanosheets injectable by the syringe needle hold great promise to transplant organized RPE cells in a minimum invasive way.

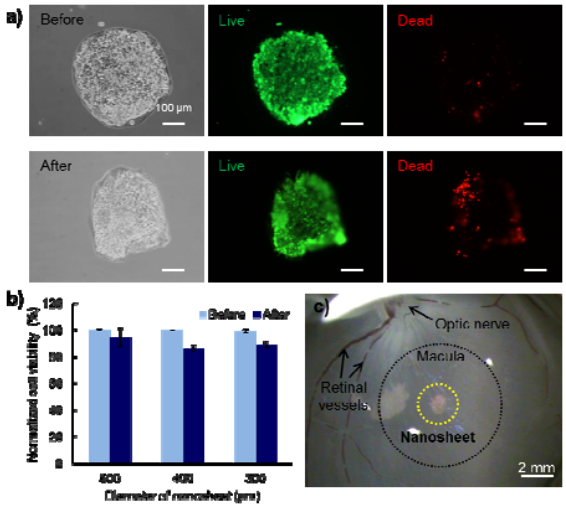

Figure 1. a) Comparison of cell viability before and after injection of the micropatterned nanosheet through 25 G needle, and b) quantification of the cell viability for different diameters. c) Subretinal delivery of the micropatterned nanosheet to the macula.

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