The Effect of Collagen Vitrigel Nanostructure on Human ESC-Derived Retinal Pigment Epithelial Maturation

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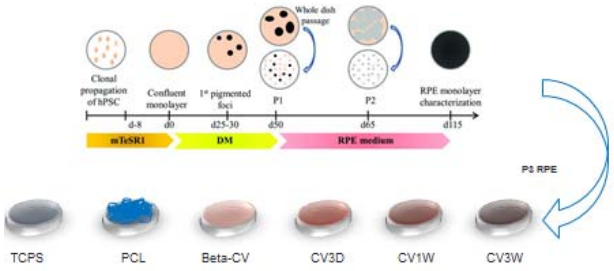
Introduction: Age-related macular degeneration (AMD) is the leading cause of blindness in the US in individuals over the age of 65 years. The current standard for the treatment of wet AMD is monthly intravitreal injections of anti-vascular endothelial growth factor. However it comes with a significant treatment burden on both patients and physicians. Another approach being explored is the transplantation of autologous retinal pigment epithelial (RPE) cells. Although a potentially promising approach, harvesting autologous RPE cells involves complex surgery with possible sight-threatening complications. An alternative approach to obtaining human RPE cells is to generate them from human pluripotent stem cells, either from embryonic stem cells (ESCs) or from induced pluripotent stem cells [1]. These stem cell-derived RPE cells could serve as cell resource for RPE transplantation.

Tissue Engineering provides a promising opportunity to improve cell-based RPE therapies. The fundamental concept is to use a scaffold on which RPE is pre-cultured that supports maturation in to a functional monolayer, followed by transplantation of these RPE cell sheet underneath the retina. An optimal cell scaffold for tissue engineered RPE monolayer should simulate their natural microenvironment, support RPE maturation, along with favorable surgical properties, and biocompatibility. With the aim to develop an optimized tissue-engineered RPE monolayer, we investigated the influence of chemistry and morphology of collagen based materials on stem cell-derived RPE maturation in the current research.

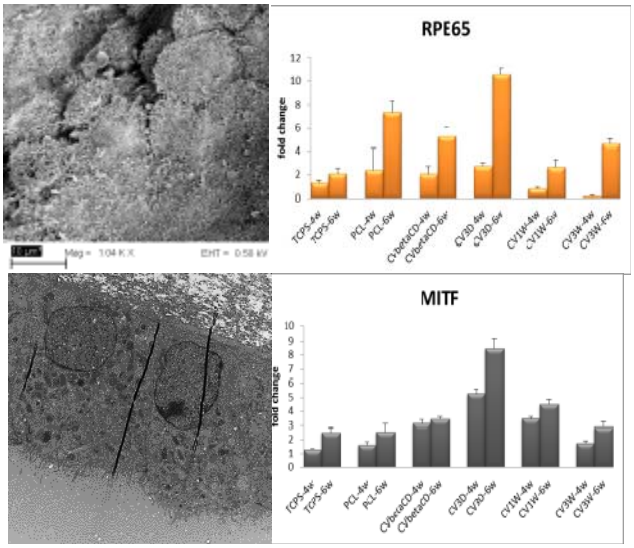
Methods: RPE-like cells were differentiated from embryonic stem cells according to procedures that published previously [2]. In this study frozen cells were thawed and passed for 3 times before seeding to materials, and the schematic is shown below.



To prepare collagen vitrigel (CV), a mixture of 10% FBS and 20 nM N-2-hydroxyethylpiperadine-N-2-ethansulfonic acid buffer in DMEM is mixed with an equal volume of 0.5% acidic collagen solution. Vitrification time varied from 3 days to 3 weeks at 40 °C in order to obtain different nanofibril structure. Beta-cyclodextrin collagen vitrigel (Beta CV) was prepared by mixing 5% β-CD solution with collagen solution then go through vitrification. Polycaprolactone (PCL) electrospun fibers were also prepared for RPE maturation evaluation. Cell morphology, gene expression were tested in all groups.

Results:

Longer vitrification time resulted in thicker collagen fibril (~100 nm) and denser fibril density. CV-3 day has average fibril diameter of ~60-70 nm, while CV-3 weeks has >100 nm fibril diameter. Beta-CV has no distinctive fibril structure and PCL electrospun fibers has diameter around 1 µm. After 4-6 weeks culture, ES-RPE became pigmented and polarized, microvilli can be observed under SEM and TEM among all groups. qPCR results showed significant enhancement of ES-RPE maturation on CV-3D group, which indicates the nanofibril scale of CV3D group is essential for RPE maturation (Figure shown below).



Conclusions: In this study, we evaluated the ES-derived RPE maturation on different substrates, including synthetic polymer and collagen-based materials. RPE cells proliferate well on different substrates and became mostly pigmented and polarized between 4~6 weeks. Quantitative study by qPCR showed RPE characteristic marker expression significantly increased in CV3D group. This study provided an insight of how nanofibril in collagen based material influence RPE maturation.

References:

[1] Al-Shamekh S, Goldberg JL. Transl Res. 2014;163:377-386.
[2] Maruotti J., Wahlin K., Gorrell D., Bhutto I., Lutty G., Zack DJ., Stem Cell Transl Med. 2013;2:341-354.