Development of polydimethylsiloxane-based biomimetic scaffolds with cylinder micropillars for retinal pigment epithelial cell cultivation

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Abstract

Background: Age-related macular degeneration (AMD) is one of the leading causes of vision loss. Once the retinal pigment epithelium (RPE) layers are destroyed, the poor visual acuity and recognition are generally irreversible. Cell therapy that possesses enormous potential in regenerative medicine may provide an alternative treatment for several incurable diseases such as AMD. In this study, we developed an innovative polydimethylsiloxane (PDMS)-based biomimetic scaffolds with cylinder micropillars for the cultivation of induced pluripotent stem cell–derived RPEs (iPSC-RPEs). RPEs were cultured on the PDMS-based biomimetic scaffolds and validated the cells gene expression.

Methods: The biomimetic PDMS scaffold was fabricated through spin coating and lithography method. It was further modified on surface with biomolecules to improve cell affinity and stability. The iPSC-RPEs were seeded on the scaffold and analyzed with characteristic gene expression.

Results: PDMS biomimetic scaffold was analyzed with Fourier transform infrared spectroscopy and proved its chemical composition. iPSC-RPEs demonstrated confluent cell monolayer on the scaffold and maintained RPE-specific gene expression, which proved the PDMS-based biomimetic scaffold to be supportive for iPSC-RPEs growth.

Conclusion: The PDMS interface allowed regular growth of iPSC-RPEs and the design of cylinder micropillars further provided the bioscaffold high motion resistance may improve the engraftment stability of iPSC-RPEs after transplantation. Taken together, this innovative PDMS-based biomimetic scaffold may serve as an ideal interface for in vitro iPSC-RPE cultivation and subsequent transplantation in vivo. This novel device exhibits better bioavailability than conventional injection of donor cells and may be an alternative option for the treatment of AMD.

Keywords: Macular degeneration; Pluripotent stem cells; Retinal pigment epithelium

1. INTRODUCTION

Age-related macular degeneration (AMD) contributes to one of the major causes of blindness and is prevailing in persons older than age 55 in the industrialized world. The symptoms of AMD predominantly include profound loss of central vision, losing the ability to read and recognize faces, and poor living qualities. At the advanced stage, AMD is characterized by the gradual loss of retinal pigment epithelium (RPE) cells, outer neural retina, and choroidal capillary vessels. Meanwhile, variable size of extracellular deposits called drusen may accumulate beneath the RPE layer and subsequently disrupt the overlying photoreceptors. Eventually, degeneration of photoreceptors in the macular region often results in functional impairment of the central vision. So far there is no effective treatment that can restore the RPE loss and malfunction in advanced AMD.

RPE cells can be generated in vitro from mesenchymal stem cells, embryonic stem cells (ESCs), and induced pluripotent stem cells (iPSCs).[1–3] The present iPSC platform was developed by Shinya Yamanaka and his colleagues at the beginning[4] and has gradually drawn high attention due to its promising potential in patient-specific therapy. The human iPSCs could be established from somatic cells such as fibroblasts or peripheral blood mononuclear cells through the reprogramming technology. Besides, human iPSCs are able to undergo differentiation into various lineages including cardiomyocyte, neuron, osteoblasts, and RPE cells,[5,6]. In this study, we

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developed a polydimethylsiloxane (PDMS)-based biomimetic cell scaffold that can serve as an ideal interface for the regular attachment and growth of human iPSC-derived RPE cells. In addition, we further introduced cylinder micropillars onto the back of the PDMS bioscaffold using the photolithography techniques, to increase the motion resistance after transplantation into the recipient. By this design, we aimed to provide the tissue-engineered RPE cell sheet grown onto the bioscaffold with high motion resistance to compensate the RPE cell loss in macular degeneration such as AMD.

2. METHODS

2.1. iPSC culture and in vitro RPE induction

Human iPSCs were generated from human peripheral blood mononuclear cells and cultivated on mouse embryonic fibroblasts (MEFs) derived from 13.5-day-old embryos of C57/B6 mice. The iPSCs were reprogrammed by transduction with retroviral vectors encoding four transcription factors (Oct-4/Sox2/Klf4/c-Myc) or three transcription factors (Oct-4/Sox2/Klf4) as previously described, with some modifications. The mouse iPSCs were transfected with plasmid encoding enhanced green fluorescent protein to constitutively express green fluorescence and were maintained and differentiated in vitro as described previously. Every 3-4 days, colonies were detached with 0.2% collagenase IV (Invitrogen, Carlsbad, CA, USA), dissociated into single cells using 0.025% trypsin (Sigma-Aldrich, St Louis, MO, USA) and 0.1% chicken serum (Invitrogen) in PBS, and replated onto MEFs. For embryoid body (EB) formation, iPSCs were dissociated into a single-cell suspension using 0.25% trypsin-EDTA and were plated onto nonadherent culture dishes in DMEM with 15% FBS, 100 mM MEM nonessential amino acids, 0.55 mM 2-mercaptoethanol, and antibiotics at a density of 2 × 106 cells/100-mm plate. After 4 days in floating culture, EBs were transferred onto Geltrex-coated plates and maintained in the same medium for 24 hours. EBs were then assigned for in vitro RPE differentiation using a protocol as previously described,[7] with some modifications.

2.2. Design and modification of PDMS-based bioscaffolds for RPE cultivation

PDMS-based membranes were generated through a widely employed silicone elastomer kit (Sylgard® 184; Dow Corning, Auburn, MI). The PDMS used in the present work is a liquid bicomponent silicone prepolymer. The liquid silicone mixture containing base and curing agent was first mixed at a ratio of 10:1. The mixture was then poured on a mold produced from lithography technique to endow the PDMS-based scaffold with cylindrical micropillars. After removing air bubbles in the PDMS mixture by vacuum, the PDMS-based membrane was baked in an oven at 70°C for at least 3 hours to thermally cure PDMS-based scaffold.

2.3. Quantitative real-time polymerase chain reaction

The RPE cells were collected, and the total RNA was isolated by TRIzol reagent (Ambion, Austin, TX) according to the manufacturer's protocols. One microgram of RNA was prepared for reverse transcription using Superscript III (Invitrogen) to synthesize complementary DNA strands (cDNA). Quantitative polymerase chain reaction (PCR) was performed using power SYBR green PCR master mix (ABI, Waltham, MA) according to the product's instructions. The real-time PCR reaction was performed and monitored on a step 1 fast real-time PCR System (Applied Biosystems, Foster City, CA). The primer sequences are as follows: ZO-1: CAACATACAGTGACGCTTCACA (forward sequence), GACGTTTCCCCACTCTGAAAA (reverse sequence). PEDF: TTACGAAGGCGAAGTCACCA (forward sequence), TAAGGTGATAGTCCAGCGGG (reverse sequence).

2.4. Statistical analysis

The results are expressed as mean ± SD. Statistical analyses were performed using the *t* test for comparing two groups, and one-way or two-way ANOVA, followed by Bonferroni's test, was used to detect differences among three or more groups. The correlation between expression levels and age were analyzed by the Pearson's correlation coefficient and unpaired Student's *t* test. Results were considered statistically significant at $p < 0.05$. All analyses were performed using SPSS 12.0 (IBM, New York, NY, USA).

3. RESULTS

3.1. Development of the PDMS-based scaffold with cylinder micropillars for stem cell–derived RPE maintenance

In this study, we sought to develop a PDMS-based bioscaffold for the cultivation of iPSC-derived RPEs. At the same time, we further introduced diffusely spread cylindrical micropillars on the back of PDMS-based bioscaffolds to increase their motion resistance. ATR-Fourier transform infrared was applied for analyzing the chemical characteristics of such biocompatible PDMS-based scaffold. As shown in Fig. 1A, the C-H methyl stretch and the Si-CH_3 group presented typical peaks at 2965 and 1260 cm^{-1}, respectively. The broad spectrum of polymer backbone (Si-O-Si) ranged from 1130 to 1000 cm^{-1}. Some unreacted vinyl and hydrosilane (Si-H) groups were detected by tracking the change in intensity of the absorption band at 1410 cm^{-1} (for vinyl) and 2140 cm^{-1} (for Si-H). After this verification, the PDMS bioscaffolds was assigned for the cultivation of human iPSC-derived RPE cells. We generated human iPSCs through reprogramming the peripheral blood mononuclear cells according to the previous protocol.[8] Next, these iPSCs were differentiated into RPE cells. The RPE cells were able to grow to confluence and cover the entire surface of the bioscaffold (Fig. 1B). The cylindrical micropillar structures at the back of the bioscaffolds were designed and arranged at a diameter of 40 μm and the interval length of 10 μm, to provide higher motion resistance and stability after the implantation into the eyes of the recipients (Fig. 1C). Overall, such PDMS-based bioscaffolds exhibited excellent biocompatibility for the cultivation of iPSC-derived RPE cells.

3.2. Comparison of respective RPE characteristics after the cultivation onto control dishes and PDMS biomimetic scaffolds

After reaching the confluence, we compared the morphology, viability, and the expression of RPE-specific gene between iPSC-derived RPE cells grown on control dishes and the modified PDMS bioscaffolds. No significant difference was observed in the morphology (Fig. 2A) and cell viability (data not shown) between iPSC-derived RPE cells grown on control dishes and the modified PDMS bioscaffolds. In addition, iPSC-derived RPE cells grown on the modified PDMS bioscaffold also expressed equivalent levels of RPE-specific markers including ZO-1 and PEDF, compared with that grown on control dishes Fig. 2B). Taken together, these data suggested the PDMS-based biomimetic cell scaffolds exhibited high biocompatibility and supportive characteristics for RPE growth in vitro.

4. DISCUSSION

Human PSCs have been shown to be differentiated into RPE cells with mature functions and thus are an ideal source for RPE transplantation therapy. Nevertheless, PSC is absent in mature organs in adults, and the differentiation capacity of adult stem

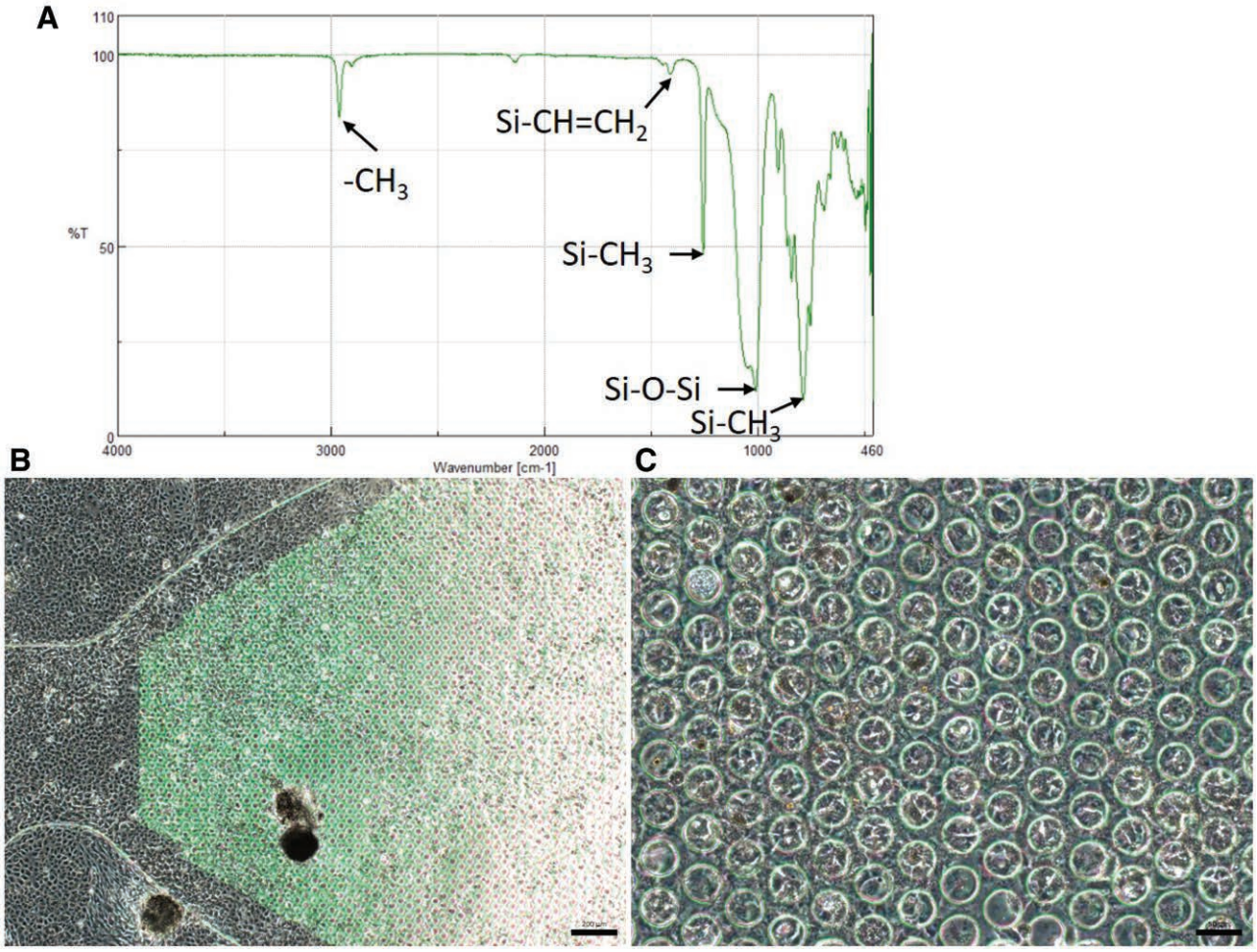

Fig. 1 Fourier transform infrared (FTIR) spectrum and retinal pigment epithelium (RPE) culture on polydimethylsiloxane (PDMS)-based scaffold with cylindrical micropillar for in vitro stem cell–derived RPE maintenance, demonstrating biocompatible and supportive function for RPE transplantation (A) using ATR-FTIR analysis, representative typical FTIR spectrum peaks stretching at 2965 cm^{-1} (C-H methyl), 1260 cm^{-1} (Si-CH_3), 1130 cm^{-1} (Si-O-Si), 1410 cm^{-1} (vinyl), and 2140 cm^{-1} (Si-H), respectively. B and C, Photos of the PDMS-based scaffolds with RPE cells cultivated on it. The RPE cells are cultivated on the flat side, while on the other side a orderly arranged cylindrical micropillars are designed.

cells is largely limited. Overall, cell therapies aiming to replace the lost RPEs or to compensate their functions were generally limited by the graft death after the delivery of large numbers of RPEs. Therefore, strategies to enrich RPE cells and to enhance engraftment of transplanted cells are urgently required. Transplanted ESC-derived RPE cells have been reported to exhibit high safety and tolerability without graft rejection, abnormal proliferation, and teratoma formation, 4 months after the transplantation.[9,10] Medium-term to long-term safety have also been reported in the follow-up of two open-label phase 1/2 studies.[10,11] To compare and elucidate the suitable formula of RPEs for subretinal transplantation, ESC-derived RPE cells have been subretinally transplanted into immunocompromised rats as either a cell suspension or a polarized monolayer.[11] Although there was no teratoma formation in recipients with transplantation of either a cell suspension or a polarized monolayer, RPE cells apparently exhibited better viability as the polarized monolayer.[11] Further evidence indeed supported that polarization of RPE cells protected them from oxidative insult.[12] These findings indicated that polarization and monolayer morphology might be critical for the maintenance of regular viability and RPE physiology after transplantation into the subretinal space. Based upon these findings and backgrounds, we designed a PDMS-based biomimetic scaffold to allow the regular growth and proliferation of iPSC-derived RPE cells as a monolayer. The monolayer characteristic of iPSC-derived RPE cells is supposed to be beneficial after the implantation into the subretinal microenvironment in vivo.

PDMS is widely applied in plastic surgery and is facile for modification. Besides, it is a bio-inert and highly biocompatible materials, preventing the release of acidic by-products into surrounding microenvironment. In this study, we designed a PDMS-based membrane on which RPE cells resided and exhibited cell growth and RPE-specific gene expression equivalent to that grown on control dishes. The cylinder micropillars regularly arranged on the back of bioscaffolds may provide higher motion resistance and prevent graft detachment, the loss of transplanted cells, and decompensation of regular RPE functions. Taken together, our findings have provided an innovative PDMS-based biomimetic device for iPSC-derived RPE regular growth as a monolayer. Such device may be beneficial for transplanted RPE cells to maintain regular RPE physiology and may further improve regenerative medicine and the strategies of cell therapy in macular degeneration (Fig. 3).

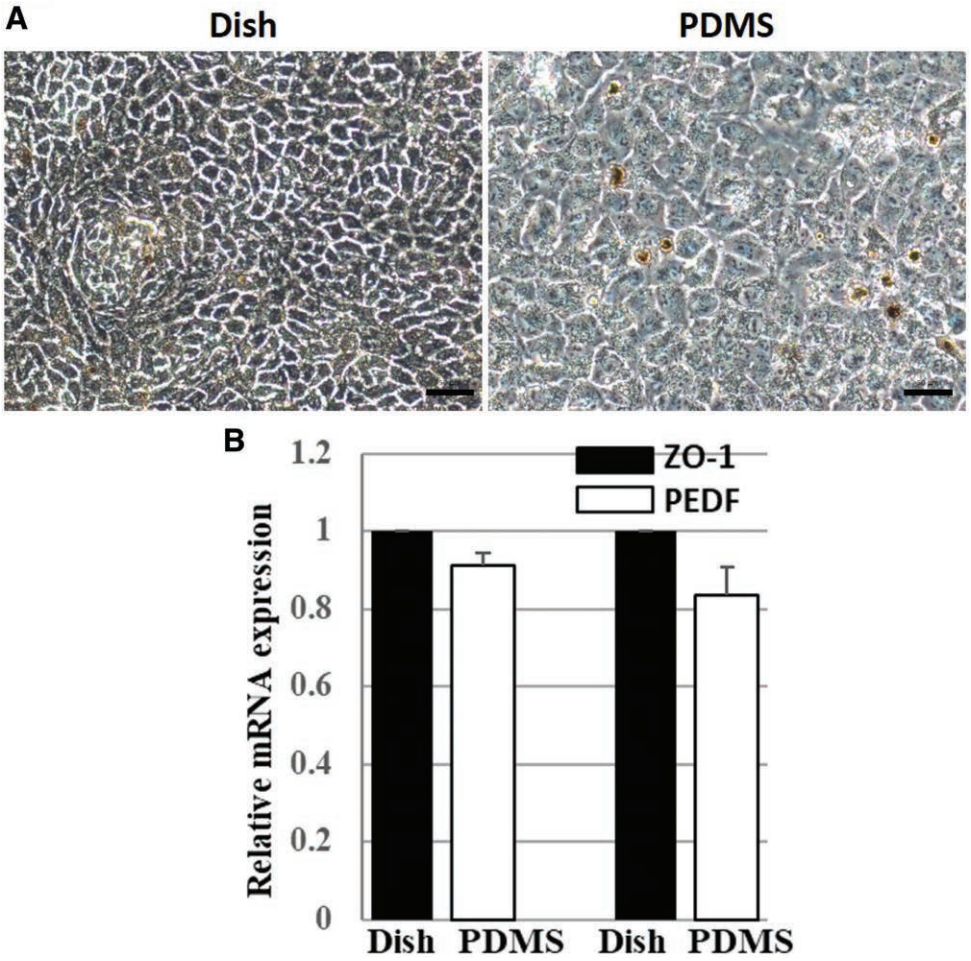

Fig. 2 Respective characteristics of retinal pigment epithelium (RPE) cells cultured on control dish or on polydimethylsiloxane (PDMS)-based scaffold. A, RPE cells are cultivated on control dish (left) or on PDMS-based scaffold (right), and it is observed that the RPE cells on PDMS scaffold exhibit similar morphology to those on the dish. B, Representative RPE gene expression analysis through quantitative polymerase chain reaction method show that the RPE cells cultivated on PDMS-based scaffold exhibit similar gene expression profile to those on the dish.

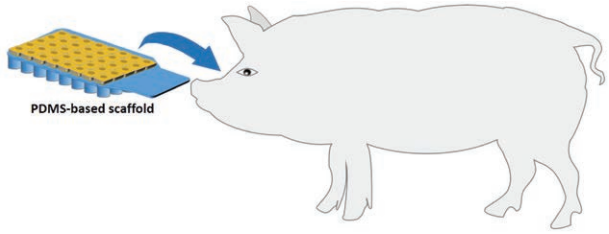

Fig. 3 Polydimethylsiloxane (PDMS)-based scaffold with cylindrical micropillar and retinal pigment epithelium (RPE) cells culture is of high potential in cell therapy. The schematic representation show that the innovative device can be further employed for in vivo study on animals including porcine, which is characterized with eyes structures similar to humans. The device is expected to efficiently deliver RPE cell sheet to targeted retinal site and support RPE cells adaptation and growth, improving cellular survival and patients' prognostics.

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