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Controlled surface morphology and hydrophilicity of polycaprolactone toward human retinal pigment epithelium cells

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ABSTRACT

Applying scaffolds as a bed to enhance cell proliferation and even differentiation is one of the treatment of retina diseases such as age-related macular degeneration (AMD) which deteriorating photoreceptors and finally happening blindness. In this study, aligned polycaprolactone (PCL) nanofibers were electrospun and at different conditions and their characteristics were measured by scanning electron microscope (SEM) and contact angle. Response surface methodology (RSM) was used to optimize the diameter of fabricated nanofibers. Two factors as solution concentration and voltage value were considered as independent variables and their effects on nanofibers' diameters were evaluated by central composite design and the optimum conditions were obtained as 0.12 g/mL and 20 kV, respectively. In order to decrease the hydrophobicity of PCL, the surface of the fabricated scaffolds was modified by alkaline hydrolysis method. Contact time of the scaffolds and alkaline solution and concentration of alkaline solution were optimized using Box Behnken design and (120 min and 5 M were the optimal, respectively). Contact angle measurement showed the high hydrophilicity of treated scaffolds (with contact angle 7.48°). Plasma surface treatment was applied to compare the effect of using two kinds of surface modification methods simultaneously on hydrolyzed scaffolds. The RPE cells grown on scaffolds were examined by immunocytochemistry (ICC), MTT and continuous inspection of cellular morphology. Interestingly, Human RPE cells revealed their characteristic morphology on hydrolyzed scaffold well. As a result, we introduced a culture substrate with low diameter (185.8 nm), high porosity (82%) and suitable hydrophilicity (with contact angle 7.48 degree) which can be promising for hRPE cell transplantation.

1. Introduction

The retinal pigment epithelium (RPE) is a light-absorbing and polarized pigmented cellular monolayer that located in the back of the eye. It supports photoreceptor cells and has critical functions in retina. RPE cells perform many crucial functions such as vision creation and processing, interaction with light-sensing photoreceptor outer segments, secretion of neurotrophic factors, phagocytosis of photoreceptor outer segments, providing nutrient, fluid, ion and metabolite transport, response to distinct extra-cellular signals, homeostasis advancement between the photoreceptor and choroid, recycling the visual pigment that is a key in the visual cycle [1–3]. Due to these interactions between RPE and photoreceptors, RPE dysfunction leads to vision loss. Age-related macular degeneration (AMD) and retinitis pigmentosa (RP) are the causes of irreversible visual inabilities like reading, driving and doing daily tasks among people over the age of 50 and characterized by death of the light-sensing photoreceptors. In these diseases, degeneration of photoreceptors and finally blindness happen [4–8]. Unfortunately, there is no fundamental treatment to overcome these illnesses [3,9].

Cell transplantation with sub-retinal injection of suspended RPECs is one of the most promising strategies to replace damaged or lost RPE. However, this therapy results in disorganized or incorrectly localized grafts, even RPE cell stacking, cell death and retinal fibrosis [3,4]. Additionally, allograft cells without identification of host microenvironment such as drusen accumulation, cross-linking and Bruch's membrane (BM) thickening were injected [10]. Since BM plays an essential role in keeping and supporting the physiological function of RPE cells, cell seeding on a biocompatible, biodegradable, porous and synthetic BM would be a promising strategy in treatment of RP.

Among various fabrication methods, electrospinning is a common technique to make a bed with suitable cell adhesion, proliferation and differentiation and useful exchange of nutrients and metabolites

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Table 1
Different groups of scaffolds used in this study.

Groups of scaffold		Description
Un-treated PCL (UPCL)	Chl	PCL dissolved in Chloroform
	Chl:DMF	PCL dissolved in Chloroform:dimethylformamide (7:3)
Hydrolyzed PCL (HPCL)	HChl	Alkaline hydrolysis of Chl scaffold
	HChl:DMF	Alkaline hydrolysis of Chl:DMF scaffold
Oxygen plasma-treated HPCL (PHPCL)	PHChl	Plasma treatment on HChl scaffold
	PHChl:DMF	Plasma treatment on HChl:DMF scaffold

between the nanofibers [3]. Electrospun fibers are a network with high porosity and interconnected pores, have low surface to volume ratio [11].

There are a variety of suitable biomaterials such as synthetic (poly lactic acid (PLA), PCL, poly urethanes and etc.), natural (collagen, gelatin and etc.) or hybrid [3]. A scaffold can potentially improve the organization of cells in to determined layer same as normal retina and high appropriate degree of cell attachment and differentiation. In order to use scaffolds correctly, different features for the scaffolds are needed. Studies showed that an ideal scaffold for retinal tissue engineering should be less than 30 μm thickness to allow the exchange of metabolites and nutrients between the retina and choriocapillaris [4,7].

It seems that natural polymers have the better cell survival and function, however, synthetic polymers may be more attractive because of easier modification and reproducible. PCL is an aliphatic polyester and a synthetic polymer with high compatibility in a subretinal space, biodegradable with degradation products less acidic compare with polylactic acid (PLA) and poly(lactic-co-glycolic acid) (PLGA) [10]. Hydrophobic nature of PCL was the reason to attempt for improve the overall performance of scaffolds [12]. Surface modification methods were applied to create or/and enhance topographical and functional changes of the surface to accelerate cell adhesion and cell-material interactions. Plasma treatment is one of the frequent techniques to make reactive radicals to the surfaces which is easily converted to functional groups [13]. Alkaline hydrolysis is a simple and inexpensive technique that is used for this purpose. In this method, the hydrophilic terminal groups (hydroxyl and carboxyl) on the surface of material are created [14].

The aim of this study was to improve the surface hydrophobicity of PCL scaffolds as well as finding the effect of two types of surface modification methods on RPE cell adhesion and proliferation. So, an electrospun PCL scaffold was fabricated and the surface of the hydrolyzed PCL scaffolds were modified by alkaline hydrolysis and plasma treatment. Fabrication and modification conditions were optimized with response surface methodology (RSM) based on contact angle measurement and scanning electron microscope (SEM) results. Human RPE cells were seeded on different groups of scaffolds. Various analyses such as biocompatibility and immunocytochemistry (ICC) were done to obtain the best scaffold to act as a suitable synthetic BM for RPE transplantation.

2. Methods and materials

2.1. Scaffold fabrication and optimization

PCL solution was made by dissolving PCL (M_w = 80.000, Sigma Aldrich Co.) in two kinds of solvent: chloroform (CHL, Merck Co.) and chloroform:dimethylformamide 7:3 v/v (CHL:DMF, Merck Co.) and mixed for 6 h at room temperature to obtain a clear solution [15,16]. Then polymer solution was electrospun from a 3 mL plastic syringe having flat-end metallic needle at a feed flow rate of 0.3 mL h^{-1}. Tip to collector distance was 15 cm and for better cell position and proliferation on scaffold and to uniform thickness of scaffold, used a rotating drum with 600 rpm which was covered with aluminum foil.

In order to use optimum scaffold in cell culture based on minimum diameter, different concentrations of solutions were prepared. So a set of experiments based on response surface method (RSM) was designed to study the influence of composition, solvent and voltage on diameters of scaffolds by changing variable parameters like amount of PCL using for making solution, adding DMF to Chloroform with 3:7 v/v and considering a range of voltage for making scaffold [17,18].

According to previous study [9], the range of solution concentration and voltage were considered 0.08–0.12 g PCL mL^{-1} solvent and 15–25 kV, respectively. By analyzing SEM of each experiment and measuring diameter of fibers by Image J, the optimum condition for each solution was obtained. For CHL scaffolds, voltage 18.4 kV and 0.08 g/mL concentration of solution and diameter of fibers 526.3 nm were obtained. Whereas, the optimized diameter was gained 185.8 nm with 20 kV voltage and 0.12 g/mL solution concentration for CHL:DMF scaffolds.

2.2. Surface modification and optimization

Since PCL has a hydrophobic nature, for increment of cell adhesion and better cell culture, its surface was modified by alkaline hydrolysis using NaOH (Merck Co.). A set of experiments based on RSM was designed in order to investigate the effect of exposure time of scaffolds in alkaline solution and concentration of NaOH solution. So different concentrations of NaOH solution (1–5 M) were prepared then scaffolds soaked in it during various time (60–120 min). Finally, they were rinsed 3 times with distilled water to return pH 7.

Optimum condition of alkaline hydrolysis was obtained by using contact angle measurement. In this method, a droplet of deionized water (4 μL volume) was dropped on treated scaffolds and the contact angle of water was measured with an optical bench-type contact angle goniometer (Dataphysics, CA 15 plus).

In order to investigation the effect of applying two kinds of treatment on cell culture simultaneously, some of treated scaffolds were carried out using a low pressure radio frequency (RF) oxygen discharge. They were placed in the plasma chamber and treated at 150 W for 10 min according to previous data [19,20].

Various groups of scaffolds used in this study were detailed in Table 1.

2.3. Water-uptake

Dry scaffolds (3 pieces from each group) were cut into ($1 \times 1\ cm^2$) and weighed (W_{dry}) and immersed in distilled water at 37 °C for 24 h. Then the scaffolds were removed, dried on filter paper to remove excess water, and weighed (W_{wet}) to determine water uptake [21,22].

$$\text{Water uptake } (\%) = \frac{W_{wet} - W_{dry}}{W_{dry}} \times 100 \quad (1)$$

Table 2
The coded and actual values of the variables.

Independent variables	Symbol	Coded levels				
Weight volume concentration (gr/mL)	a	−1.41	−1	0	+1	+1.41
	A	0.071	0.080	0.100	0.12	0.129
Voltage (kV)	B	13.97	15	20	25	21.03
Concentration of alkaline solution (M)	C	0.18	1	3	5	5.82
Time of hydrolysis (min)	D	48	60	90	120	132