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Directional cell migration through cell–cell interaction on polyelectrolyte multilayers with swelling gradients

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ABSTRACT

The directional cell migration plays a crucial role in a variety of physiological and pathological processes. It can be controlled by the gradient cues immobilized on the substrate. The poly(sodium 4-styrenesulfonate) (PSS)/poly(diallyldimethylammonium) chloride (PDADMAC) multilayers were post-treated in a gradient NaCl solution with a concentration ranging from 3 M to 5 M, yielding the gradient multilayers with a similar chemistry composition (PSS domination) but gradually changed swelling ratio. The gradient nature and physicochemical properties were characterized by X-ray photoelectron spectroscopy and ellipsometry. Compared to the random migration with a lower rate at a smaller cell-seeding density, the vascular smooth muscle cells migrated directionally to the low hydration side at an appropriate cell-seeding density ($1.5 \times 10^4/cm^2$) under the assistance of cell–cell interactions. The cell migration rates on the gradient surface were significantly larger than those on the corresponding uniform surfaces etched by salt solutions of the same concentrations. Relative cell adherent strength and focal adhesion formation were studied to unveil the intrinsic mechanism of the gradient multilayers on the cell migration. It was found that both the gradient cues and cell–cell contact have major influences on the directional cell migration.

1. Introduction

The cell migration plays a crucial role in a variety of physiological and pathological processes ranging from embryonic development, cancer metastasis, blood vessel formation and remolding, tissue regeneration, immune surveillance, and inflammation [1,2]. For example, the leukocytes migrate toward the sites of inflammation and infection, the neurons send projections to specific regions of the brain to find their synaptic partners, and the fibroblasts move into the wound space [3]. Thus, *in vivo* directional cell migration to the special sites is critical rather than random motility. The cell's compass is governed by various directional cues, such as soluble chemoattractants (chemotaxis), surface-attached molecules (haptotaxis), and mechanical cues (durotaxis) [4,5]. It is believed that controlling the cell migration by these various directional cues is one of the paramount problems in regenerative medicine and tissue engineering.

So far the surfaces with chemical and physical gradients have been prepared and their influences on the cell migration have attracted increasing attention [6]. Immobilization of various directional cues on the biomaterials is necessary to guide cell migration, especially for the haptotaxis and durotaxis. For example, cell can directionally migrate on a density gradient of immobilized biomolecules (haptotaxis), such as extracellular matrix proteins (fibronectin (FN) [7], laminin [8], and collagen [9,10]), growth factors (epidermal growth factor (EGF) [11], basic fibroblast growth factor (bFGF) [12] and vascular endothelial growth factor (VEGF) [13]), and small ligands (arginine-glycine-aspartic acid (RGD)) [14–16]. Although many gradients of biological molecules are proved to be effective in inducing directional cell alignment and migration, the complexity of natural macromolecules and their interactions with cells still challenge the design of biomaterials for controlling cell migration because a variety of factors will influence the cell fate. Also, these proteins and growth factors are expensive and easy to reduce bioactivity or denature, limiting their practical applications. On the other hand, the physical signal is also very influential in modulating the cell migration. For instance, the motility of fibroblasts can be governed purely by substrate rigidity (durotaxis), and can directionally move from the soft region towards the rigid side of the substrate [17,18].

Among the various surface engineering methods, the layer-by-layer (LBL) assembly can diversely tailor the substrate properties and is particularly suitable to modify the biomaterials surface [19–21]. The physicochemical properties of the assembled

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polyelectrolyte multilayers (PEMs) can be easily modulated by the deposition or post-treatment parameters, such as ionic type and strength [22–24], pH [25–27] and temperature [28]. Therefore, gradual change of these parameters can fabricate gradient polyelectrolyte multilayers (GPEMs). For example, Nolte *et al.* [29] generated thickness gradients of poly(allylamine hydrochloride) (PAH)/poly(acrylic acid) (PAA) multilayers by a salt etching method. Kirchhof *et al.* established a pH gradient (5–9) by a microfluidic device and generated the heparin/chitosan multilayers with gradient properties [30]. The gradient multilayers pre-adsorbed with fibronectin are able to guide MG-63 osteoblast-like cells' movement from regions deposited under low pH to those under high pH, due to gradual changes in mechanical properties and fibronectin density.

Recently, we found that the structure and physicochemical properties of the poly(sodium 4-styrenesulfonate)/poly(diallyldimethylammonium) chloride (PSS/PDADMAC) multilayers can be changed continuously by the post-treatment in NaCl solutions of increasing concentration (1–5 M) with a transition point at 3 M [31]. For the Multilayers-1M and Multilayers-2M (the multilayers post-treated with 1 M and 2 M NaCl solutions, respectively), the original structure and properties of the multilayers are mostly retained, and their surface is dominated by the positively charged PDADMAC. By contrast, the surface of Multiayers-3M, 4M and 5M is dominated by the negatively charged PSS as a result of larger loss of PDADMAC. The swelling ratio increases along with the salt concentration within a range from 3 M to 5 M. More recently, it was found that smooth muscle cells (SMCs) are preferable to adhere and spread on the negatively charged PSS-dominated surface (the Multilayers-3M, 4M and 5M), compared with the positively charged one (Multilayers-1.2M) which shows cytotoxicity to some extent [32]. Furthermore, the mobility of SMCs is slowest on the dehydrated surface (Multilayers-3M) but can be effectively promoted on the highly hydrated surface such as the Multilayers-5M. Although these uniform multilayers provide a versatile means to control the cell mobility, they cannot govern the directional cell movement due to the lack of gradient cues.

Herein, the gradient PSS/PDADMAC PEMs with a gradually increasing swelling ratio are prepared, which are expected to guide the directional cell migration by providing the cell adhesion force in a gradient manner. For this purpose, the as-prepared multilayers are etched in a gradient salt solution with a linearly increasing concentration from 3 M to 5 M (Fig. 1). For the first time we find that the directional migration of SMCs are guided by both the gradient physical cue and the cell-seeding density.

2. Experimental section

2.1. Material

Polyethyleneimine (PEI, M_w = 25 kDa), poly(diallyldimethylammonium chloride) (PDADMAC, M_w = 200–350 kDa) and poly(sodium 4-styrenesulfonate) (PSS, M_w = 70 kDa) were obtained from Sigma–Aldrich. Water used in this experiment was purified by a Milli-Q water system (Millipore, U.S.A.). All the polyelectrolytes were prepared to a final concentration of 1 mg/L aqueous solutions. PEI was dissolved in water, and PSS and PDADMAC were supplemented with 1 M NaCl. Quartz, glass, and silicon wafers were cleaned in piranha solution (7:3 v/v% H_2SO_4/H_2O_2). After rinsed thoroughly with water, they were dried under a smooth stream of N_2.

2.2. Assembly of polyelectrolyte multilayers

To ensure the successful adsorption, a precursory layer of PEI was deposited on the substrates. PSS and PDADMAC were then alternately assembled by auto dipping at 20 °C. Between alternate exposures to the two kinds of polymer solutions for 20 min, there were 3 rinses with 0.1 M NaCl solutions for 3 min. In the last step, the films were immersed in water for at least 5 min to eliminate the adsorbed salt. Taking into account our previous results on the physicochemical properties after salt etching [31] and the cell responses on the uniform salt-etched multilayers [32], herein a total of 7 bilayers were assembled and the multilayers are expressed as $(PSS/PDADMAC)_7$.

2.3. Preparation of gradient solution

A linear density gradient of NaCl solution was obtained by using a home-made device which is consisted of two reservoirs, two channels and a gradient column (Fig. 1a). A and B reservoirs are loaded with the same amount of NaCl solutions of high and low concentrations (high and low densities), respectively. The solution in the reservoir A was pumped into the reservoir B and mixed by a magnetic stirrer, and consequently the concentration (density) of the solution in reservoir B was gradually increased. The flow velocity(s) was controlled by the peristaltic pump, and the rate ratio of s_A to s_B was 1:2. When the solution in reservoir B was pumped into the gradient column with the prolongation of time, the gradient column would be filled first with the salt solution of a lower concentration and gradually higher concentration. Due to a higher gravity of the higher concentration, the new concentrated flow could locate stably in the bottom of the gradient column and then the solutions of lower densities were pushed upwards gradually (the outlet of the tube was mounted at the bottom of the gradient column). After the solutions in A and B reservoirs were exhausted eventually (and simultaneously), the pump B was turned off to avoid the generation of air bubbles in the gradient salt column. Finally, the solution of gradually increasing concentration (density) was collected in the gradient column. By this fabrication strategy, the concentration (density) of the gradient salt solution is continuously increased from the top to the bottom in the range from the initial concentration of the solution in the reservoir B to that in the reservoir A. The concentration range and gradient length in the gradient column could be adjusted by the initial concentration and volume of the solutions in the reservoirs A and B. In this study, the concentration range of the gradient solutions was tuned from 3 M to 5 M and the length was adjusted to 2.5, 5 and 20 mm through the various initial volumes of the solutions in the reservoirs A and B, respectively.

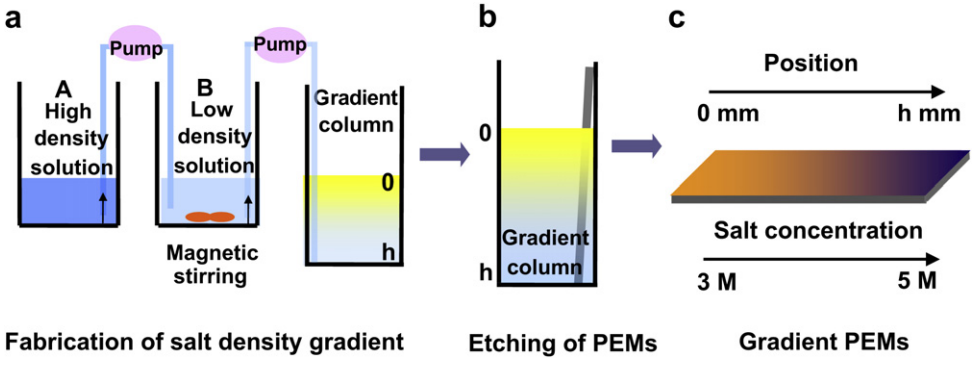

Fig. 1. Schematic illustration showing the fabrication of gradient salt solution and gradient polyelectrolyte multilayers thereof. (a) Generation of a continuous density gradient of NaCl solution. The details are presented in the Experimental section. (b) Vertical incubation of the as-prepared $(PSS/PDADMAC)_7$ multilayers into the gradient NaCl solution for 2 h. (c) The gradient multilayer film is obtained after rinsing with water. The upper level of the salt gradient solution (b) and the multilayers treated at this place is defined as the 0 position.