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Correlating cell transfectability and motility on materials with different physico-chemical properties

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ABSTRACT

Gene delivery into cells can be facilitated by adding plasmid DNA/transfection reagent complexes in culture medium or pre-adsorbing the complexes on the substrate before cell seeding. Using transfection reagents, however, often causes cytotoxicity. Effective delivery of naked plasmid without any transfection reagent remains a challenge. In this study, we cultured human umbilical cord derived mesenchymal stem cells (hMSCs) on different biomaterial substrates with different physico-chemical properties and examined the transfectability of naked plasmid. Specifically, we synthesized a negatively charged polyurethane (PU) to mimic the hyaluronan-modified chitosan (CS-HA) membranes previously found to promote the transfection of naked plasmid. We observed that the PU membranes were as effective as CS-HA membranes in substrate-mediated delivery of naked plasmid into hMSCs. PU membranes with surface microgrooves further increased the gene delivery efficiency to a similar level as the commercial transfection reagent but without the harmful effect. The gene delivery efficiency was associated with the extent of activation of cellular integrins β1 and α5 on different substrates. Moreover, the delivery efficiency was positively correlated with the cell migration rate on various substrates. The substrate-mediated gene delivery by synthetic polymeric substrates supports that integrin activation and cell behavior (e.g. migration and transfectability) changes can be modulated by synthetic polymer surface with microfeatures. The transfection by PU microgrooves is easy, nontoxic, and as effective as the commercial transfection reagent.

1. Introduction

Gene transfection involves the use of plasmid that transfers the specific gene for intracellular encoding. The exogenous genes can further induce cell transformation or secretion of specific proteins [1]. Transfecting naked plasmid into cells is usually difficult. Therefore, a transfection reagent such as liposome or dendrimer, is used to form a complex with plasmid DNA to increase the transfection efficiency [2]. There are two methods for gene transfection, forward and reverse. In the forward transfection method, cells are plated on materials before addition of the plasmid/transfection reagent complex. Therefore, more experimental time (~84 h) and multiple steps are required [3]. In reverse transfection, the plasmid/transfection reagent complex is tethered on the cell culture substrate before cell seeding [4]. No matter in a forward or reverse transfection, the use of transfection reagent can cause cytotoxicity in transfected cells [5]. The cell survival rate is usually low (<50%). When the targeted cells are difficult to transfect as in the case of stem cells, the choices of transfection reagents are limited and the cell survival can be as low as 30% [6]. Subsequently, using materials with unique surface properties and topographies to reduce the use of possibly cytotoxic transfection reagent and simultaneously promote the gene transfection has become a recent trend [6,7].

Surface topography and features can modulate cell function and thereby gene transfection [8]. The efficiency of gene transfection can be enhanced by pre-adsorbing the plasmid/transfection reagent complexes on rough surfaces [9]. Cells on a rougher surface had a greater migration rate than those on a planar surface [10]. Nanopillars on a flat substrate may pierce through the cell membrane and promote the gene delivery into cells [7]. Microgrooves can influence cell migration, alignment, and the transfection efficiency [11,12]. On the other hand, surface with nanofeatures may regulate the distribution and turnover of integrin receptors on cell

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membrane. Particularly, inhibition of integrin β1 by siRNA was reported to activate the focal adhesion but inhibit the cell migration and endocytosis [13]. The latter suggested that cell migration and endocytosis may be both associated with integrin β1.

In our previous studies, mesenchymal stem cells (MSCs) on chitosan (CS) and hyaluronan-modified chitosan (CS-HA) membranes formed three-dimensional cellular spheroids [14]. These spheroids could be transfected with naked plasmids in serum free medium, which is attributed to the activation of endocytic pathways on CS and CS-HA. In spite of the promising findings, the substrate properties responsible for higher transfection efficiency were not clearly identified. Besides, these natural biomaterials suffered from weak mechanical properties and poor reproducibility among different sources [15]. A synthetic polymer substrate that effectively promotes the delivery of naked plasmid can provide a more stable platform, which allows further examination of the relationship among substrate properties, cell migration, and gene delivery.

Since substrates can modulate integrin receptor and gene delivery while integrin receptor is associated with endocytosis and migration, we assume that substrates that promote cell migration may also mediate gene transfection. In this study, we developed a biomimetic water-based biodegradable polyurethane (PU) that could deliver naked plasmid into human umbilical cord mesenchymal stem cells (hMSCs) effectively without using any vector. We further fabricated PU to have various geometric features including electrospinning fibers and microgrooves. We also modified the transfection method to concurrent transfection (neither forward nor reverse), as displayed in Fig. 1. We evaluated the transfection efficiency, integrin activation, and the cell migration rate to suggest a possible correlation between cell migration and gene delivery in such system. We expected that a general rationale could be generated to design or screen substrate materials for enhancing cell migration and gene delivery into difficult-to-transfect cells.

2. Materials and methods

2.1. Preparation of hyaluronan-modified chitosan (CS-HA) membranes

Chitosan (CS) was acquired from Sigma (USA). The molecular weight was ~510 kDa and the deacetylation degree was ~77%. Hyaluronan (HA) was acquired from SciVision Biotech Inc. (Taiwan). The molecular weight was ~2500 kDa. CS was dissolved in 1% acetic acid and filtered through a 100 μm mesh. The CS solution was added 300 μL into each well of 24 well tissue culture polystyrene (TCPS) and dried for 24 h. The CS-coated TCPS was immersed with 0.5 N sodium hydroxide for three minutes and washed three times with phosphate-buffered saline (PBS). To prepare CS-HA membranes, the 300 μL of 3 mg/mL HA solution was dropped onto the previous CS-coated TCPS. The coated TCPS was dried for 24 h and further crosslinked by ethyl(dimethylaminopropyl) carbodiimide/*N*-hydroxysuccinimide (EDC/NHS) with a weight ratio of 1.84:0.23 and reacted at 4 °C for 48 h. The CS-HA membranes were washed three times with PBS, sterilized by 75% ethanol, and washed by PBS for three times before cell studies. The amount of HA bound to CS membrane was determined to be ~0.5 mg/cm^2 [14].

2.2. Synthesis of water-based biodegradable polyurethane (PU) and preparation of PU flat films and grooves

PU was based on poly(ε-caprolactone) diol (PCL diol) as the PU soft segment (accounting for ~65% of the PU composition) and was synthesized in a 500 mL round-bottomed four-necked flask. The PCL diol (Mn ~ 2000 g/mol, Aldrich) was placed in the flask with a stirrer operated at 180 rpm and the temperature maintained at 70–80 °C until the PCL diol was dissolved. Isophorone diisocyanate (IPDI, Evonik Degussa GmbH) and the catalyst tin(II)2-ethylhexanoate were added to the flask with continued stirring at 180 rpm for 3 h. Afterwards, 2,2-bis(hydroxymethyl) propionic acid (DMPA, Aldrich) and methyl ethyl ketone (MEK, J.T. Baker) were added to the flask with constant stirring at 180 rpm for 1 h. The temperature was then reduced to 50 °C. Triethylamine (TEA, RDH) was added to the flask and reacted for 30 min. The temperature was reduced to 45 °C and the speed of the stirrer was increased to 1100 rpm. Deionized water was added and the chain extender ethylenediamine (EDA, Tedia) was dropped into the flask to complete the reaction. The residual MEK and TEA were removed by vacuum distillation at 80 °C for 2 h. The synthetic procedures of PU are shown in Fig. 2. The chemical structure was confirmed by ^{1}H-nuclear magnetic resonance spectroscopy (^{1}H-NMR; 400 Hz, Unity Inova FT-NMR, Varian).

To prepare PU flat films, 300 μL of PU dispersion was directly coated on 1.5 cm-diameter coverslip glass. They were placed in petri dish and dried for 2 days. To prepare PU microgrooves, templates were first made of micropatterned silicon wafer. The negative photoresist was coated on silicon wafer at 3000 rpm by a spin-coater and exposed to UV light through a mask at 365 nm for 12 s. The unreacted photoresist was removed to get microgrooves of a 3 μm depth and the aligned ridges and grooves with spacing size of 20 μm on the silicon wafer. Poly(dimethylsiloxane)

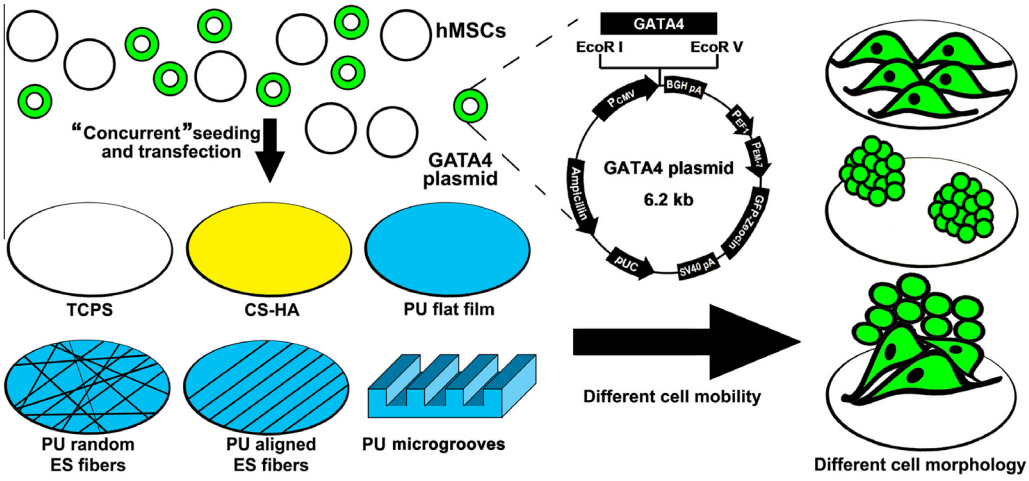

Fig. 1. Concurrent transfection of human mesenchymal stem cells (hMSCs) on biomaterial substrates and those with different microfeatures.