Biomaterials 34 (2013) 3345–3354

Contents lists available at SciVerse ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Surface-mediated functional gene delivery: An effective strategy for enhancing competitiveness of endothelial cells over smooth muscle cells

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ARTICLE INFO

Article history:
Received 26 December 2012
Accepted 15 January 2013
Available online 4 February 2013

Keywords:
Endothelial cells
Gene delivery
Hepatocyte growth factor
Layer-by-layer self-assembly
Smooth muscle cells

ABSTRACT

The non-biorecognition of general biomaterials and inherent biospecificity of biological systems pose key challenges to the optimal functions of medical devices. In this study, we constructed the surface-mediated functional gene delivery through layer-by-layer self-assembly of protamine sulfate (PrS) and plasmid DNA encoding hepatocyte growth factor (HGF), aiming at specific enhancing endothelial cells (EC) compeititiveness over smooth muscle cells (SMC). Characterizations of the (PrS/HGF-pDNA) multilayered films present the linear buildup with homogeneous and flat topographical feature. The amount of DNA can be easily controlled. By using these multilayered films, both human umbilical vein endothelial cells (HUVEC) and human umbilical artery smooth muscle cells (HUASMC) can be directly transfected when they contact with the multilayered films. On transfection, increasing secretion of HGF has been detected in both HUVEC and HUASMC culture, which leads to selective promotion of HUVEC proliferation. In the co-culture experiment, we also exhibit the promoted and hindered growth of HUVEC and HUASMC, respectively, which could be attributed to the inverse influence of HUVEC on HUASMC. These results collectively demonstrate that our system can be served as a powerful tool for enhancing competitiveness of EC over SMC, which opens perspectives for the regulation of intercellular competitiveness in the field of interventional therapy.

1. Introduction

Biomaterials have intensively raised worldwide concern in various applications such as tissue engineering, cardiovascular medical devices and non-invasive diagnosis and treat system [1]. Although the medical devices made of synthetic biomaterials have done lots of contributions to saving lives and improved the quality of life [2], problems still remain in the field of biomaterial implants. The most significant one is that biomaterials are generally fabricated without biorecognition and biospecificity, while the biological systems are biospecific [3]. Currently, how to entitle the biomaterials with property of bioactivities raised a lot of concerns. Many efforts have been made to improve the cellular specificity of material surface. Immobilization or delivery of biological molecules (such as the extracellular matrix (ECM) molecules and cell growth factors) onto the surface of materials has been proven to be an efficient method to improve activity and specificity of cells [4–7]. However, it is difficult to keep long-term efficiency of material surface in retaining cell specificity. Furthermore, these methods have not concerned the intercellular competitiveness which always exists *in vivo*.

Gene therapy has experienced great development in treating human disease over past decades [8]. Gene delivery is a process of introducing foreign DNA into host cells and is one of the crucial steps necessary for gene therapy [9]. It has been widely recognized that successful delivery small quantity of gene to cells can induce long-term expression [10]. Most importantly, many kinds of cells could be simultaneously transfected by a same extraneous gene in the cellular microenvironment. Then, the secretion of proteins (such as growth factors) could specifically influence cellular events because of the special function. Therefore, this process makes gene therapy an ideal candidate for selective regulating of intercellular competitiveness. Hepatocyte growth factor (HGF) is a multifunctional cytokine that enhances many cellular events, including proliferation, migration and differentiation [11]. Recently, it was

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http://dx.doi.org/10.1016/j.biomaterials.2013.01.065

reported that HGF plays very important role in endothelial cells (ECs) regeneration [12]. For instance, HGF has been proven to accelerate the proliferation of ECs and improve endothelial function [13,14] without affecting growth of smooth muscle cells (SMCs) [15,16]. Development of vascular in-stent restenosis is generally attributed to over growth of SMCs after injury of endothelium. Healthy endothelial layer plays a fundamental role in maintaining healthy blood vessel homeostasis because it is involved in prevention of SMC proliferation and coagulation [17]. Rapid regeneration of endothelium is thereby a key point to the success of prevent restenosis. Therefore, local delivery gene encoding HGF could specifically modulate competition between ECs and SMCs.

Immobilization gene (such as DNA and RNA) on the surface of materials is an important issue for successful gene transfection [18–22]. Layer-by-layer (LbL) self-assembly technique, introduced by Decher et al. [23], has been extensively applied to construct gene-embedded thin multilayer on the surface of various substrates [24–28]. LbL technique has many advantages especially in terms of constructing gene delivery system. For example, it helps to precisely control the amount of gene loaded in multilayer and provides temporal and special control over the gene delivery [29,30]. Till now, there is lack of research to construct functional gene delivery system by using LbL self-assembly for the purpose of selective regulation of intercellular competitiveness.

In the present study, we aim at enhancing ECs competitiveness over SMCs. To achieve this goal, surface-mediated functional gene delivery system was constructed by alternatively depositing protamine sulfate (PrS) and HGF-pDNA on the surface of substrates based on LbL technique (Scheme 1). PrS is a natural protein that facilitates the condensation of DNA in sperm and plays a pivotal role during fertilization [31]. PrS has been approved by FDA as an antidote for the clinical reversal of heparin overdose [32]. In addition, PrS is also extensively studied for constructing drug and gene delivery system [33,34]. In our study, PrS was chosen as a polycation to interact with HGF-pDNA molecules. The buildup process of the (PrS/HGF-pDNA) multilayered films was monitored. The morphology and stability of the multilayered films were then characterized. After that, cellular experiments including transfection and proliferation assays were carried out and analyzed. Furthermore, the co-culture experiments involved ECs and SMCs were established to investigate whether the (PrS/HGF-pDNA) multilayered films could selectively regulate intercellular competitiveness in a multi-type of cells system.

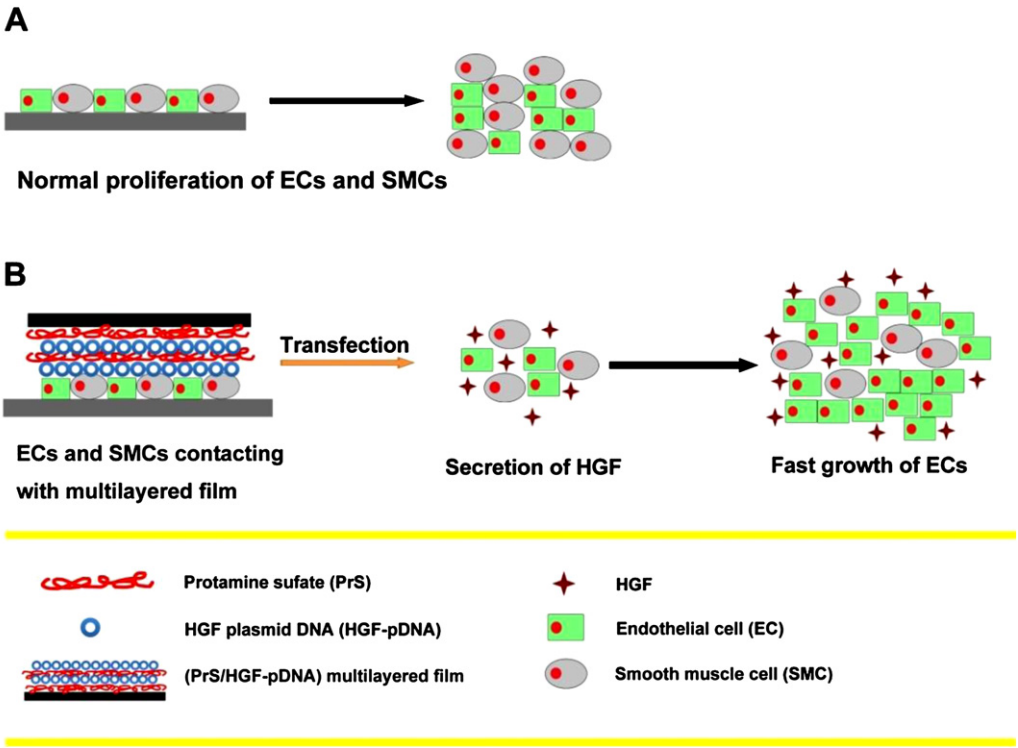
2. Materials and methods

2.1. Materials

Protamine sulfate (PrS), Deoxyribonucleic acid (DNA, fish sperm, sodium salt, FS-DNA), *N*-[2-hydroxyethyl] piperazine-*N*′-[2-ethanesulfonic acid] (HEPEs, free acid, high purity grade) and phosphate buffered saline (PBS) were purchased from Sangon Biotech (Shanghai, China). Plasmid DNA encoding recombinant human hepatocyte growth factor (HGF-pDNA) was provided from Northland Biotech (Beijing, China). Dulbecco's modified Eagle's medium (DMEM), Human Endothelial Serum Free Medium (SFM), MCDB131 and fetal bovine serum (FBS, Gibco), Penicillin and streptomycin (P/S) and 0.25% trypsin–EDTA solution were purchased from Future Biochemical-tech (Shanghai, China). Endothelial Cell Growth Supplement (ECGS) was purchased from BD Biosciences (USA). Recombinant human hepatocyte growth factor ELISA kit and NO assay kit were purchased from Boster Bio-engineering (Wuhan, China). Deionized water (18 MΩ, Milli-Q Ultrapure Water System, Millipore) was used to prepare all buffers. For cell experiments, all the solutions and substrates were sterilized in advance.

2.2. Buildup of (PrS/DNA) multilayered films

Quartz substrates (10 × 20 mm^2), glass coverslips (Φ = 14 mm) and silicon wafers were cleaned by previous method for buildup of multilayered films [25], namely, dipping in a Piranha solution (7:3 (v%) 98% H_2SO_4 : 30% H_2O_2) for 5 min, then 1:1:5 (v%) 30% H_2O_2:25% NH_3:H_2O mixture at 60 °C for 30 min, rinsed thoroughly with deionized water and dried with a stream of nitrogen.



Scheme 1. The schematic diagrams of process that (PrS/HGF-pDNA) multilayered films regulate the intercellular competitiveness between HUVECs and HUASMCs. (A) Normal proliferation state of HUVECs and HUASMCs in co-culture. (B) After covering (PrS/HGF-pDNA) multilayered film on the top of the co-culture cells, the HUVEC growth and competitiveness over HUASMCs were enhanced.