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

姓名：常皓（11029036） 导师：计剑教授


**Hao Chang, Ke-feng Ren *, Jin-lei Wang, He Zhang, Bai-liang Wang and Jian Ji***

MOE Key Laboratory of Macromolecular Synthesis and Functionalization, Department of Polymer Science and Engineering, Zhejiang University, Hangzhou 310027, PR China

* Corresponding authors. Department of Polymer Science and Engineering, Zhejiang University, Hangzhou 310027, PR China. Tel./fax: +86 571 87953729.

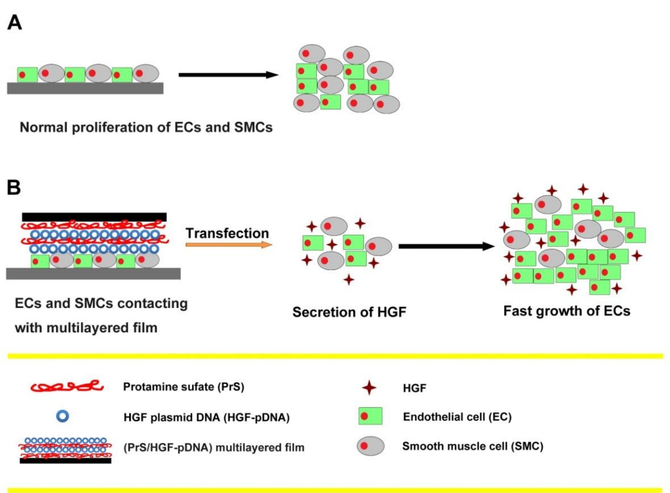
E-mail addresses: renkf@zju.edu.cn (K.-f. Ren), jijian@zju.edu.cn (J. Ji).


## Introduction

Although the medical devices made of synthetic biomaterials have done lots of contributions to saving lives and improved the quality of life, 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 bio-specific [1]. Gene delivery is a process of introducing foreign DNA into host cells and is one of the crucial steps necessary for gene therapy [2]. It has been widely recognized that successful delivery small quantity of gene to cells can induce long-term expression [3]. 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. Recently, it was reported that HGF plays very important role in endothelial cells (ECs) regeneration [4]. Rapid regeneration of endothelium is a key point to the success of prevent restenosis. Therefore, local delivery gene encoding HGF could specifically modulate competition between ECs and SMCs. Layer-by-layer (LbL) self-assembly technique, that introduced by Decher et al. [5] ,has been extensively applied to construct gene-embedded thin multilayer on the surface of various substrates. LbL technique has many advantages especially in terms of constructing gene delivery system [6, 7]. 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.

## Method

In the present study, we aimed 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.** 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.

## Results

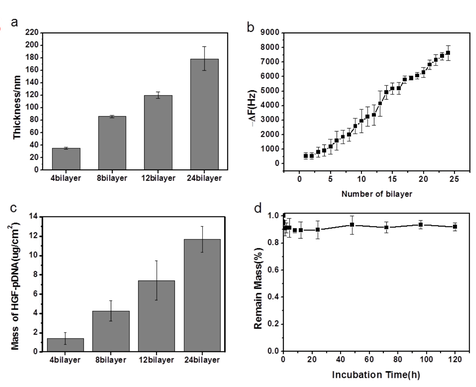



**Fig. 1.** Characterization of (PrS/HGF-pDNA) multilayered films. (a) Ellipsometry measurement of the thickness of (PrS/HGF-pDNA) multilayerd film with 4, 8, 12 and 24 pairs. (b) The change of frequency shift as a function of number of bilayer during buildup of (PrS/HGF-pDNA)n. (c) The mass of HGF-pDNA in 4, 8, 12 and 24 bilayered films. (d) The remaining mass of HGF-pDNA in $(PrS/HGF\text{-}pDNA)_{12}$ as function of time after incubating in PBS at 37 ℃, pH 7.4.

## Results

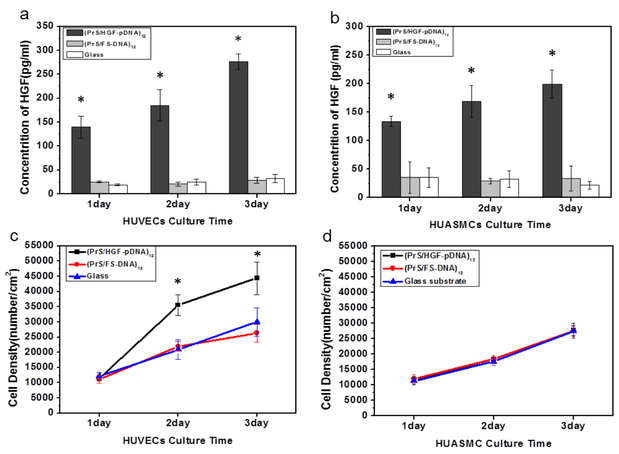



**Fig. 2.** The cell transfection and proliferation assay. ELISA results of HGF secreted by HUVECs (a) and HUASMCs (b) after attachment with $(PrS/HGF\text{-}pDNA)_{12}$, $(PrS/FS\text{-}DNA)_{12}$ and glass substrates over 3 days. The proliferating cell density of HUVECs (c) and HUASMCs (d) in $(PrS/HGF\text{-}pDNA)_{12}$, $(PrS/FS\text{-}DNA)_{12}$ and glass groups for culturing 3 days .The number of cells was calculated from at least 10 images based on the fluorescence images using imageJ software (n=3, mean ± SD, *p<0.05 vs $(PrS/FS\text{-}DNA)_{12}$ and glass groups).

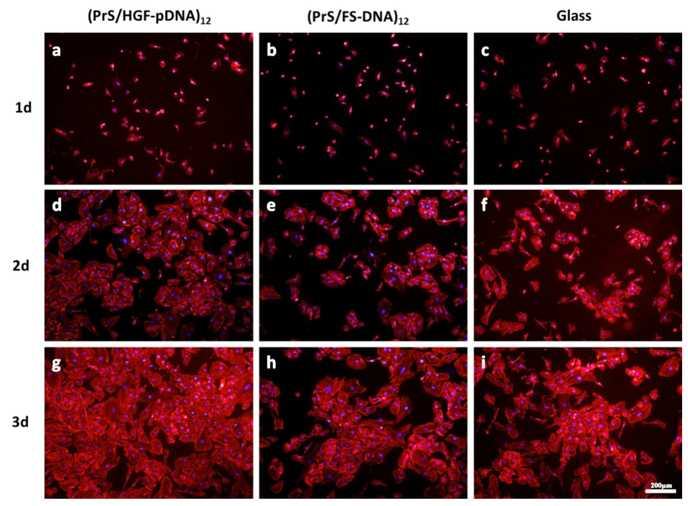



**Fig. 3.** Immunofluorescence images of cytoskeletal actin (red) of HUVECs in $(PrS/HGF\text{-}pDNA)_{12}$ (a, d, g), $(PrS/FS\text{-}DNA)_{12}$ (b, e, h) and glass (c, f, i) groups after 1, 2 and 3 days of culture. The nuclei of HUVECs were stained by DAPI (blue). Scale bar is 200 μm.

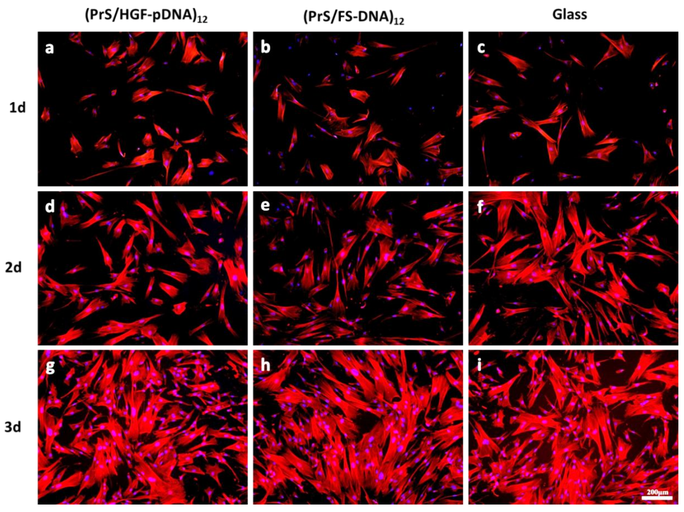



**Fig. 4.** Immunofluorescence images of cytoskeletal actin (red) of HUASMCs in $(PrS/HGF\text{-}pDNA)_{12}$ (a, d, g), $(PrS/FS\text{-}DNA)_{12}$ (b, e, h) and glass (c, f, i) groups after 1, 2 and 3 days of culture. The nuclei of HUASMCs were stained by DAPI (blue). Scale bar is 200 μm.

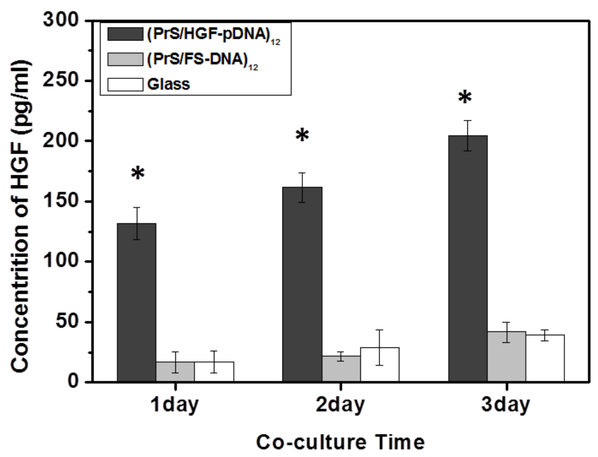



**Fig. 5.** The transfection assay in co-culture system. ELISA results of HGF in the cell culture media after 1, 2 and 3 days culture. ( n=3, mean ± SD *p<0.05 vs $(PrS/FS\text{-}DNA)_{12}$ and glass groups).

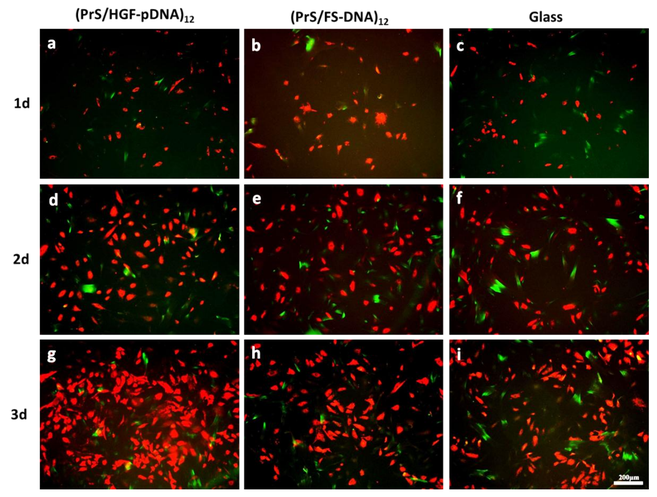



**Fig. 6.** Immunofluorescence images of co-culture of HUVECs and HUASMCs in $(PrS/HGF\text{-}pDNA)_{12}$ (a, d, g), $(PrS/FS\text{-}DNA)_{12}$ (b, e, h) and glass (c, f, i) groups after 1, 2 and 3 days of culture. HUVECs and HUASMCs were stained by anti-vWF (red) and anti-calponin (green), respectively. These Images from different microscope channels were combined together. Scale bar is 200 μm.

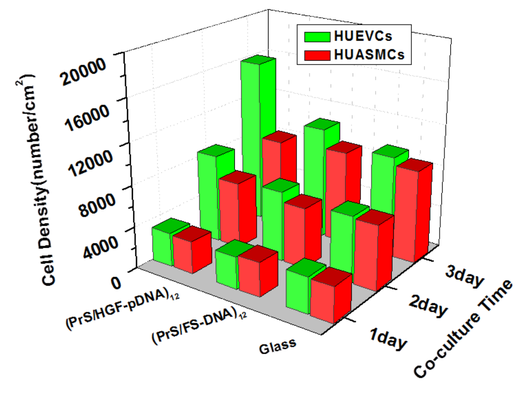



**Fig. 7.** The three-dimensional column chart of cell density in $(PrS/HGF\text{-}pDNA)_{12}$, $(PrS/FS\text{-}DNA)_{12}$ and glass groups over 3 days in co-culture system. The number of HUVECs (green column) and HUASMCs (red column) was calculated separately according to immunofluorescence images of co-culture.

## Conclusion

In summary, we demonstrated the surface-mediated functional gene delivery system is an effective strategy to regulate the intercellular competitiveness. LbL technique was applied to successfully incorporate HGF-pDNA and PrS into multilayered film. The approach that combination of LbL technique and functional gene offers a promising strategy to selectively regulate intercellular competitiveness in a multi-type of cells system, highlighting possibilities for various implant applications in biological system where the cell-specific regulation is desired.

## References


[1] Ratner BD. The engineering of biomaterials exhibiting recognition and specificity. J Mol Recognit. 1996;9:617-25.

[2] Felgner PL, Rhodes G. Gene Therapeutics. Nature. 1991;349:351-2.

[3] Niidome T, Huang L. Gene therapy progress and prospects: Nonviral vectors. Gene Ther. 2002;9:1647-52.

[4] Hayashi K, Nakamura S, Morishita R, Moriguchi A, Aoki M, Matsumoto K, et al. In vivo transfer of human hepatocyte growth factor gene accelerates re-endothelialization and inhibits neointimal formation after balloon injury in rat model. Gene Ther. 2000;7:1664-71.

[5] Decher G. Fuzzy nanoassemblies: Toward layered polymeric multicomposites. Science. 1997;277:1232-7.

[6] Jewell CM, Lynn DM. Multilayered polyelectrolyte assemblies as platforms for the delivery of DNA and other nucleic acid-based therapeutics. Adv Drug Deliver Rev. 2008;60:979-99.

[7] Bechler SL, Lynn DM. Characterization of Degradable Polyelectrolyte Multilayers Fabricated Using DNA and a Fluorescently-Labeled Poly(beta-amino ester): Shedding Light on the Role of the Cationic Polymer in Promoting Surface-Mediated Gene Delivery. Biomacromolecules. 2012;13:542-52.


## Acknowledgements


Financial support from the National Natural Science Foundation of China (50830106, 21174126, and 51103126), China National Funds for Distinguished Young Scientists (51025312), the National Basic Research Program of China (2011CB606203) and Research Fund for the Doctoral Program of Higher Education of China (20110101110037 and 20110101120049) are gratefully acknowledged.