Dual Gene Coating for Promoting HGF and Inhibiting TGF-β1 Expression

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Introduction

Interventional therapy, which is the main method to treat coronary heart disease now, faces restenosis after stenting. There are still many drawbacks of the clinical stents, and they indicate that either merely promoting endothelialization or only suppressing hyperplasia is insufficient to solve the problem of restenosis effectively.

Here, in order to weaken extracellular matrix (ECM) secretion while enhance endothelialization at the same time, we developed a kind of polyelectrolyte multilayers consisting of protamine sulfate (PrS) and plasmid DNA (pDNA) of tow functional genes using layer-by-layer (LBL) technique. The pDNA encoding hepatocyte growth factor (HGF-pDNA) and short hairpin RNA vector gene of TGF-β1 (TGF-β1-shRNA gene) were incorporated into the films. This dual gene multilayer film can transfect NIH-3T3 cells, enhancing HGF secretion while suppressing TGF-β1 secretion at the same time, thus it is able to achieve the goal of promoting proliferation of endothelium cells and suppressing ECM secretion synchronously, and may become a new stent coating to solve restenosis.

Method

- LBL was applied to embed the two kinds of plasmid DNA (HGF-pDNA and TGF-β1-shRNA-pDNA) into multilayers with PrS to form surface-mediated gene delivery system.
- The fabrication of the polyelectrolyte multilayers (PEMs) was characterized by UV-vis absorption spectroscopy and ellipsometry. The morphology of $(PrS/pDNA)_{12}$ multilayered films was studied by a field-emission scanning electron microscope (SEM). We also tested the stability of the films by incubating them in phosphate buffered saline solution (PBS) at 37 ºC.
- NIH-3T3 cells were transfected being covered by the gene multilayer films, and the secretion of HGF and TGF-β1 were studied by ELISA *in vitro*.
- Secretion of FN and Collagen I was studied by immunofluorescence.

Results

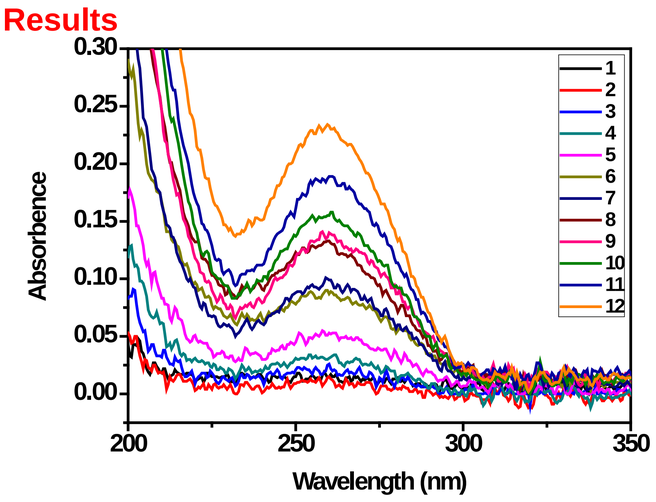


Fig.1. UV-vis absorption spectra of PrS/pDNA films as a function of layer number

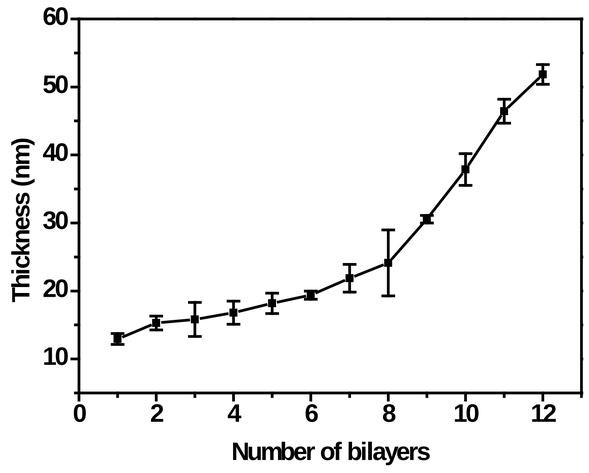


Fig.2. Thickness of multilayers as a function of PrS/pDNA films' bilayer number.

(The PrS/pDNA multilayered films could be built up successfully.)

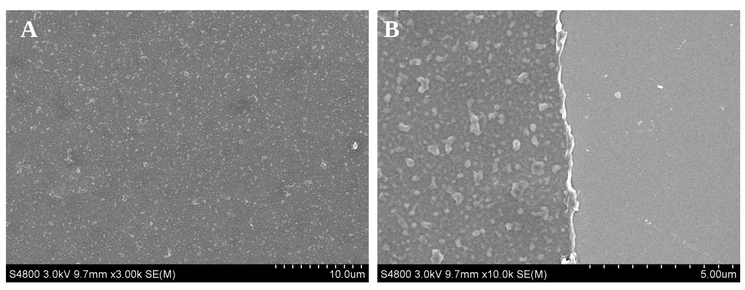


Fig.3. Topographical SEM images of $(PrS/pDNA)_{12}$ films under magnification factors of 3k (A) and 10k (B). The left part of (B) is multilayer and the right part is silicon substrate.

(The multilayered films clearly existed against the silicon substrate and had homogeneous surface morphology with small protuberances which might be polyelectrolyte complexes.)

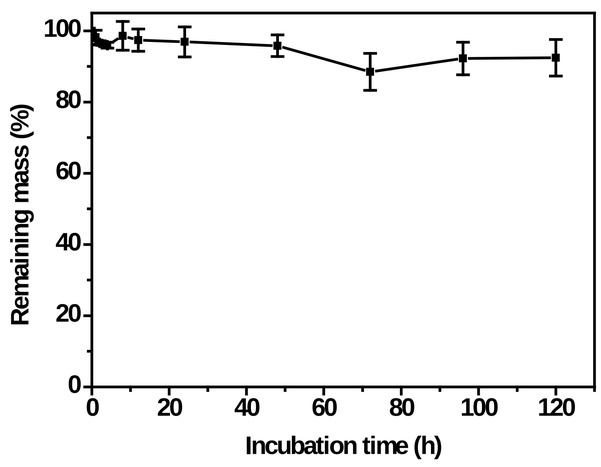


Fig.4. Stability of $(PrS/pDNA)_{12}$ films in PBS solution (pH = 7.4 37°C).

(The multilayered films exhibited good stability under physiological condition without disintegration.)

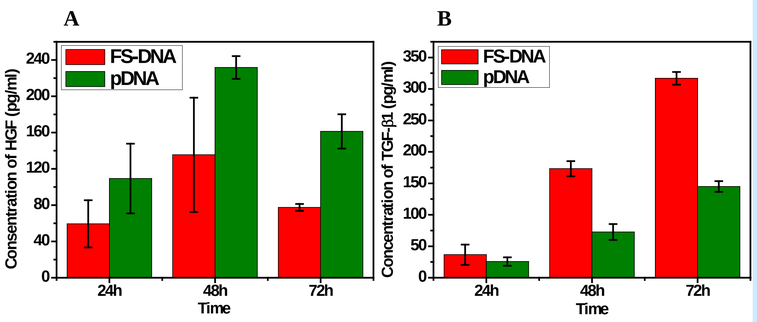
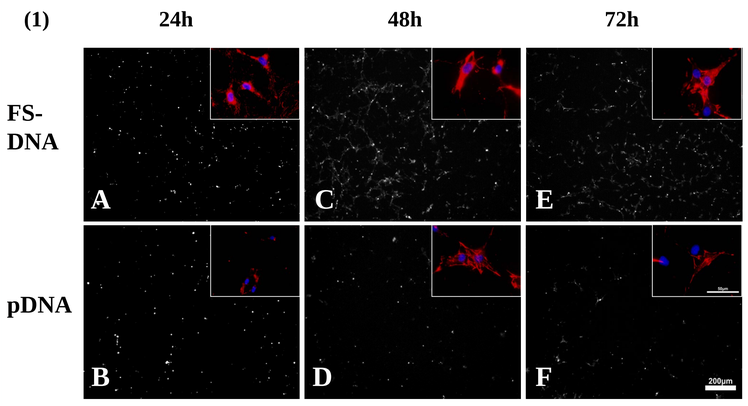


Fig.4. Concentration of HGF (A) and TGF-β1 (B) secreted by NIH-3T3 cells after multilayer transfection characterized by ELISA. Films of $(PrS/FS\text{-}DNA)_{12}$ were chosen as control groups.

(NIH-3T3 cells could be transfected by the gene multilayers and up-regulation of HGF and down-regulation of TGF-β1 were achieved at the same time .)



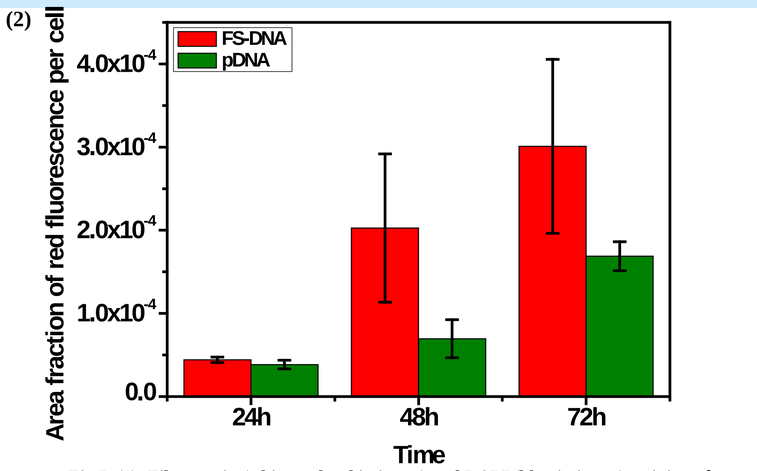


Fig.5. (1): Fibronectin (white and red in insets) and DAPI (blue in insets) staining of the studied NIH-3T3 cells cultured for distinct times under the studied different medias. (2): Area fraction of fibronectin fluorescence per cell calculated by Image J.

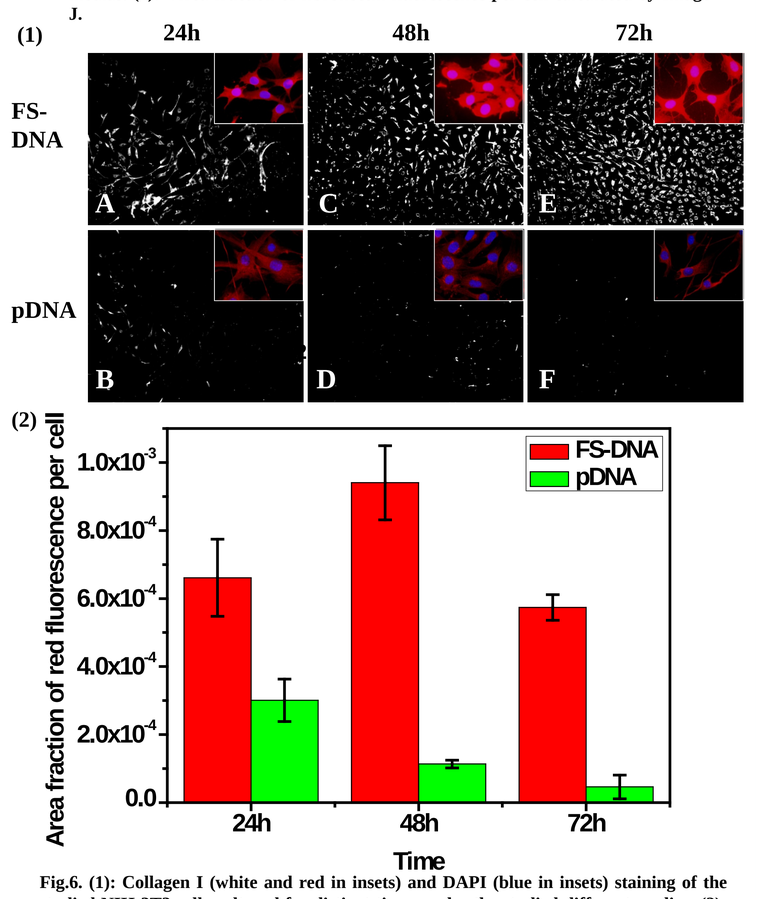


Fig.6. (1): Collagen I (white and red in insets) and DAPI (blue in insets) staining of the studied NIH-3T3 cells cultured for distinct times under the studied different medias. (2): Area fraction of Collagen I fluorescence per cell calculated by Image J.

(Secretion of FN and Collagen I was efficiently suppressed by gene transfection.)

Conclusion

The polyelectrolyte multilayer could be built up successfully, exhibited good stability under physiological conditions, was able to transfect NIH-3T3 cells and made cells express the desired genes. This film is considered to have the gene therapy function of regulating the two growth factors at the same time and suppressing secretion of FN and Collagen I, and may become a new stent coating to inhibit restenosis.

References

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Acknowledgements

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