In this study, we prepared fusion proteins containing diverse combinations of PTDs such as Tat and 9R, and protein to find an optimum combination for efficient transduction effect. Various combinations of PTDs were fused with green fluorescent protein (GFP) in a recombinant way, which allows intracellular protein trafficking and measurement of transduction efficiency. We focused on not only transduction efficiency but also retention ability of PTD-conjugated GFP. The tat-conjugated GFP showed high transduction efficiency, while 9R-conjugated GFPs had good retention abilities. GFPs conjugated with both Tat and 9R demonstrated synergistic effects showing high transfection efficiency and long retention ability.

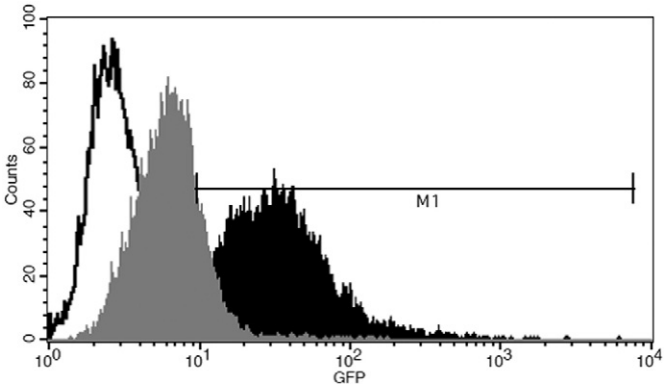

Fig. 1. Flow cytometric analysis of 3T3-L1 cells incubated with GFP (white), Tat-GFP (solid gray), Tat-GFP-9R (solid black) fusion proteins.

Keywords: protein delivery, protein transduction domain, intracellular uptake, transduction efficacy

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PEI modified biodegradable complex micelles as gene transfer vector for proliferation of ECs

Juan Lv[a], Xuefang Hao[a], Jing Yang[a], Yakai Feng[a,b,c,*], Marc Behl[b,c,d], Andreas Lendlein[b,c,d]
[a]*School of Chemical Engineering and Technology, Collaborative Innovation Center of Chemical Science and Chemical Engineering (Tianjin), Tianjin University, Weijin Road 92, Tianjin 300072, China*
[b]*Tianjin University, Weijin Road 92, 300072 Tianjin, China*
[c]*Helmholtz-Zentrum Geesthacht, Joint Laboratory for Biomaterials and Regenerative Medicine, Kantstr. 55, 14513 Teltow, Germany*
[d]*Institute of Biomaterial Science and Berlin-Brandenburg Center for Regenerative Therapies (BCRT), Helmholtz-Zentrum Geesthacht, Kantstr. 55, 14513 Teltow, Germany*
*Corresponding author.
E-mail address: yakaifeng@tju.edu.cn (Y. Feng).

Polyethylenimine (PEI) is one of the most successful cationic polymers for gene delivery. PEI of high molecular weight provides high transfection efficiency but at the same time a high cytotoxicity, while the toxicity of low molecular weight PEI is significantly lower, however its transfection efficiency as well. It is reported that the copolymerization of PEI with other polymers could enhance the transfection efficiency. A strategy of co-assembling several block polymers into the complex polymeric micelles offers a convenient preparation process of nanosized vectors. Particularly, micelles co-assembled from multiple block polymers with mixed shell provide an elegant way to tune various physical and biological functions, such as targeting, reduction-sensitivity and "stealth" property for immune evasion, easily via changing the ratios of different shell-forming blocks.

Herein, 1800 Da PEI was used, and a complex micelle with a biodegradable PLGA core and a mixed MPEG/PEI shell was prepared by self-assembly of methoxy-poly(ethylene glycol)-*b*-poly(lactide-*co*-glycolide) and polyethylenimine-*b*-poly(lactide-*co*-glycolide)-*b*-polyethylenimine in aqueous solution. Then, the pEGFP-ZNF580 plasmid [1], which has the ability of enhancing the proliferation of vascular endothelial cells, was loaded within the PEI layer located at the interface between PLGA core and MPEG shell (Fig. 1). Using DLS, the degradation behavior of the micelles was investigated in vitro. The hydrodynamic size and zeta potential of unloaded and DNA-loaded micelles indicated that they were suitable for cellular uptake and gene transfection. MTT assay and transfection efficiency experiments showed that cytotoxicity and transfection efficiency could be fine-tuned simply by changing the ratio of mPEG to PEI in mixed shell.

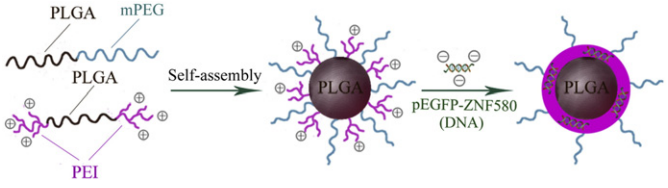

Fig. 1. Self-assembly of complex micelles and DNA-loaded micelles.

Keywords: gene vector, PEI, complex micelles, biodegradable, endothelial cells

Acknowledgments

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Electrospun macroporous fibrous scaffolds

Jun Fang[a,b], Jing Wang[b], Tong Wu[b], Anlin Yin[b], Xiumei Mo[a,b,*]
[a]*State Key Laboratory for Modification of Chemical Fibers and Polymer Materials, College of Materials Science and Engineering, Donghua University, Shanghai 201620, China*

[b]College of Chemistry and Chemical Engineering and Biological Engineering, Donghua University, Shanghai 201620, China
*Corresponding author.
E-mail addresses: yhfangjun@gmail.com (J. Fang), xmm@dhu.edu.cn (X. Mo).

Electrospinning is a simple and robust method to produce micro and nanometer diameter fibers to mimic the architecture of extracellular matrix (ECM) for tissue engineering. However, the relatively small pores of electrospun scaffolds have become a bottleneck that limits efficient cellular infiltration and tissue ingrowth [1,2]. To overcome this problem, we improved the electrospinning technique to make macroporous fibrous scaffolds. First, the composite fibrous scaffolds were formed by dual-electrospinning of PCL with PEO/NaCl salt (25–50 μm) onto a common rotating mandrel, and PCL macroporous scaffolds (pore size around 30 μm) were created after PEO/NaCl salt dissolved in water, without difficulty in washing away salt embedded deep in the scaffolds. Further, in vitro cell culture indicated that the rSMC cells cultured on the macroporous scaffolds promoted cell infiltration in 3 days (Fig. 1A and B). Moreover, a bilayer PCL fibrous vascular graft with macroporous inner layer and dense outer layer was fabricated by using the dual-electrospinning of PCL with PEO/NaCl salt and traditional electrospinning of PCL (Fig. 1C and D). The in vivo animal study for small diameter vascular is ongoing. In conclusion, this approach is a simple way to fabricate fibrous scaffolds with large pore sizes for regenerative medicine and also could be a promising strategy to make bilayer scaffold for vascular tissue repair.

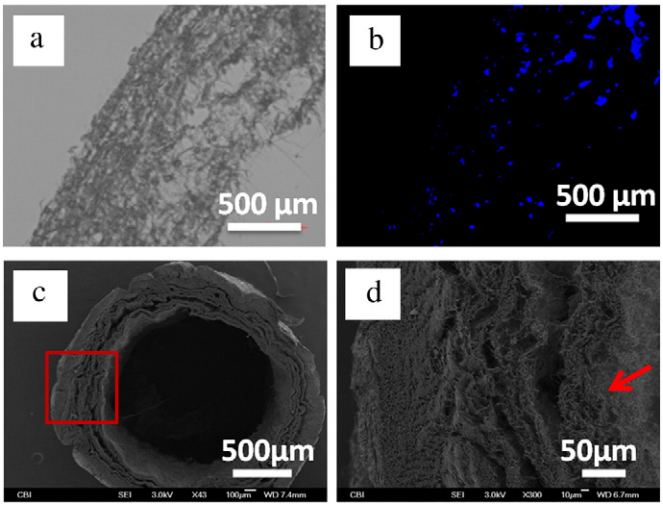

Fig. 1. Bright field (A) and DAPI staining (B) of rSMC cell infiltration into macroporous PCL fibrous scaffolds after 3 days seeding (blue: nuclei). SEM micrographs of bilayer PCL graft (ID = 1.3 mm) (C) and increased magnification of the red rectangle (D). The red arrow indicates the lumen of the graft.

Keywords: electrospinning, porous scaffold, vascular graft, tissue engineering

Acknowledgments

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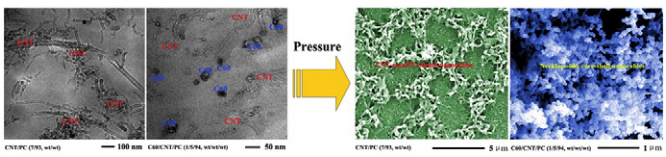
Pressure-crystallized carbon nanotube-core/polymer-sheath nanocables for drug delivery

Zhongping Li[a], Xin Chen[a], Chong Lu[a], Pengfei Tian[a], Jun Lu[a,c,*], Rui Huang[b], Lingyi Zhao[d,*]
[a]School of Materials Science and Engineering, Southwest Jiaotong University, Key Laboratory of Advanced Technologies of Materials, Ministry of Education of China, Chengdu 610031, China
[b]College of Polymer Materials Science and Engineering, Sichuan University, Chengdu 610065, China
[c]State Key Laboratory of Molecular Engineering of Polymers, Fudan University, Shanghai 200433, China
[d]Affiliated Hospital of Chengdu University, Chengdu 610081, China
*Corresponding authors.
E-mail addresses: junluprc@hotmail.com (J. Lu), zh00103@163.com (L. Zhao).

Carbon nanotubes (CNTs) have attracted tremendous attention as potential multifunctional drug delivery systems, owing to their enhanced cellular uptake, and high surface area that allows attachment of multiple copies of cell targeting molecules [1]. Nevertheless, pristine CNTs are limited in their applications as drug delivery vehicles due to their high hydrophobicity that causes aggregation and toxicity [2]. Compared with their single-component counterparts, nanocables, initially referring to the coaxial structures of several layers of nanotubes and/or clusters, may provide the possibility of synergistic functionality and properties [3]. Herein, unique nanocable assemblies, with CNT cores and crystalline polymer sheaths, were fabricated in situ by a feasible approach, showing promising features for safe drug delivery (Scheme 1). Binary CNT/polycarbonate (PC) and ternary fullerene C60/CNT/PC composites, both with an overall good dispersion of carbonaceous fillers, were crystallized at high pressure, respectively. After dissolving the amorphous parts with dimethylacetamide, isolated CNT-core/crystalline PC sheath nanocables were exposed in the recovered pressure-crystallized CNT/PC samples. Especially, with the increase of CNT loading, interpenetrating three-dimensional (3D) hybrid networks, constructed by such nanocables, were formed. Furthermore, by the simultaneous introduction of C60 and CNT, the synergistic action of the zero- and one-dimensional carbon materials finally promoted the large scale growth of a novel necklace-like nanocable and its 3D network under specific conditions, with nano-scaled C60-core/PC shell crystalline granules attached on CNT-core/PC-sheath wires along axial directions. Combining the properties of the nanostructured carbon allotropes and the crystalline polymer, nanocables may permit a niche application in targeted drug delivery to reduce short- and long-term toxicity, sustain drug release and prevent aggregation.



Scheme 1. Pressure-induced growth of CNT-core/crystalline polymer-sheath nanocables.

Keywords: carbon nanotube, controlled release, drug delivery, nanocable, xenobiotic toxicity