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Layer-by-layer Self-assembly of Carbon Nanotubes with Polyelectrolytes for Biomedical Application

Lin Su[1,2,3], Peipei Zhao[1,2,3], Yunfeng Shi[1,2,3], Liting Yao[1,2,3], Wei Xue[1,2,3], Liumin He[1,2,3, *]

[1]Department of Biomedical Engineering, College of Life Science and Technology, Jinan University, Guangzhou 510632, China

[2] Key Laboratory of Biomaterials of Guangdong Higher Education Institutes, Jinan University, Guangzhou 510632, China

[3] Key Laboratory of Functional Protein Research of Guangdong Higher Education Institutes, Jinan University, Guangzhou 510632, China

*He, LM Email: jnu_lmhe@yahoo.com.cn

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Abstract. The layer-by-layer (LbL) self-assembly of carbon nanotube (CNT)/polyelectrolyte has been widely investigated for various applications. In this study, multi-walled carbon nanotube (MWCNT) was LbL assembled with three positively charged polyelectrolytes, which was PEI, chitosan and PDDA respectively. In order to investigate its potential application in biomedical field, cell biocompatibility was examined by examining the viability and morphologies of NIH-3T3 fibroblasts cultured on the glass coated with the MWCNT/polyelectrolyte assembly multi-layers.

Introduction

Carbon nanotubes (CNTs) are mainly engineered either as single-walled CNTs (SWCNTs) or multi-walled CNTs (MWCNTs). Since their discovery, CNTs have elicited considerable interest in both academic and industrial sectors for a variety of applications according to the extraordinary properties [1]. Recently, a stimulating increasing interest for CNTs is to exploit their application in biomedical engineering [2,3], in which case CNTs as well as their composites with existing biomaterials are used.

However, CNTs suffer from a limitation for practical application due to their poor solubility in either water or organic solvents. For that reason, the loadings of nanotubes in the polymer matrix are usually not high enough for special performance without compromising the homogeneity of the composite at the nanometre level [4]. Layer-by-layer (LbL) assembly by electrostatic and van der Waals interactions offers a better approach to exert control over the structure of the CNT/polymer systems from angstroms to nanometers [4,5]. Therefore, many of the issues involved of CNT/polymer composite can be evaded using electrostatic LbL self-assembly as it allows for the close-to-perfect molecular blending of the components. Until now, negatively charged CNTs have been employed to prepare multilayer films with various polycations such as poly(ethyleneimine) (PEI), chitosan (CS), poly(diallyldimethylammonium chloride) (PDDA), poly-L-lysine (PLL) and so on [4-6]. However, a question to be resolved is their biocompatibility. Thus in study, we prepared the polyelectrolyte multilayer films by employing multi-walled carbon nanotube (MWCNT) with four three polyelectrolytes, which was PEI, chitosan and PDDA respectively. Their cell biocompatibility was examined by investigating the viability and morphologies of NIH-3T3 fibroblasts cultured on the glass coated with the assembly multi-layers.

Materials and methods

MWCNT with a concentration of 0.2 mg/mL was sonicated for 2h in an ice water bath before use. The concentration of PEI, CS and PDDA was 2 mg/mL. Prior to fabrication of MWCNT/polyelectrolyte multilayer, the glass coverslips (18×18mm) were immered in PEI solution

(2 mg/mL) in water to creat a stable, positively charged surface. The multilayer build-up of MWCNT/polyelectrolyte was accomplished by immersing the PEI-activated coverslips in the charged solutions in the sequence of MWCNT (25min)/polycations (25min) until the designed multilayer was obtained. The substrates were washed in ultrapure water three times (2min per rinse) following each assembly in the charged solutions.

NIH-3T3 (mouse embryonic fibroblast cell line) cells were cultured on the MWCNT/polycation-multilayer-coated coverslips and examined after 2 days by MTS assay for cell viability and by inverted microscope for cell morphologies according to our previous study [5].

Results and discussion

The biocompatibility of MWCNT/polycation multilayer films was studied by examining the cell viability by MTS assay with the results shown in Fig. 1. Blank glass coverslip was used as control (Ctr) in this study. The absorbance reflects the number of viable cells. Compared to control group, higher absorbance was found for all the MWCNT/polycation-multilayer-coated substrates, indicating of good biocompatibility of all the MWCNT/polycation multilayer films prepared in this study. Highest absorbance was obtained when chitosan was served as polycations. It is because chitosan a structural analogue of glycosaminoglycans (GAGs) and hyaluronic acid, and thus shows excellent biocompatibility.

Cell morphologies were observed under the inverted microscope. As shown in Fig. 2, cells were well adhered on the substrates. However, more flat cells were observed on the MWCNT/polycation-multilayer-coated substrates. Combing with the cell viability results, we can conclude that the multilayer films constructed by MWCNT/polycation LbL self-assembly are biocompatible and can be potentially used in biomedical application.

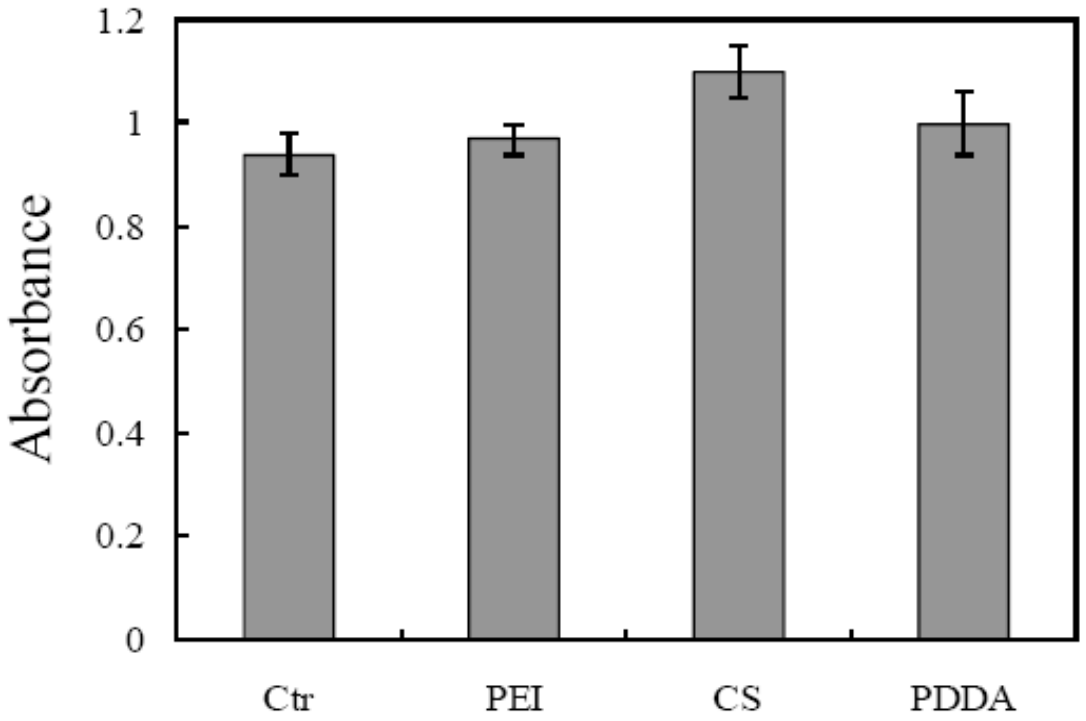

Fig. 1 The viability of NIH-3T3 cells cultureed on different substrates for 2 days.

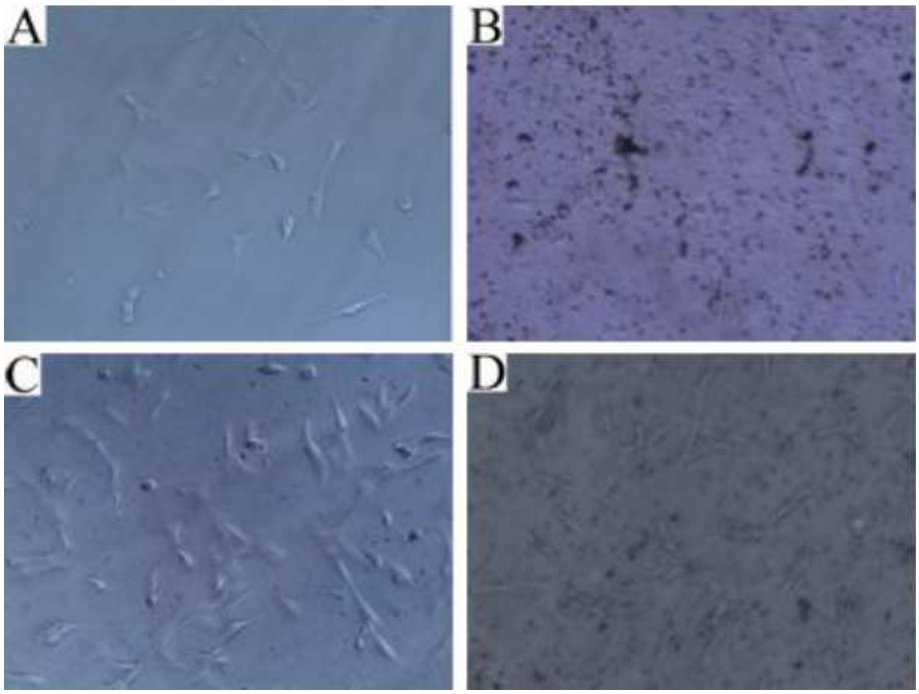

Fig. 2 Morphologies of NIH-3T3 cells cultureed on different substrates for 2 days. A:blank glass as control group, B: MWCNT/PEI-multilayer-coated substrate, C: MWCNT/CS-multilayer-coated substrate, MWCNT/PDDA-multilayer-coated substrate.

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