Immobilization of gene vectors on Maleic Anhydride-Grafted-Poly(D,L-Lactide-co-Glycolide) surfaces for Localized Gene Delivery

Guilei Ma, Cunxian Song

The Tianjin Key Laboratory of Biomaterials, Institute of Biomedical Engineering, Peking Union Medical College & Chinese Academy of Medical Sciences, Tianjin 300192, China. e-mail: bmemgl@126.com

Introduction: Conventional strategies for localized gene therapy using endovascular stents result in suboptimal localization and potentially dangerous distal spread of genes. As an alternative, immobilizing gene delivery vectors onto the material surfaces followed by implantation into target regions (i.e., substrate-mediated gene delivery) can potentially lead to effective and localized gene transfection while preventing systemic vector spread. Successful approaches have been explored to immobilize gene vectors on the surface of biomaterials and stents by our groups [1-3] and others. In this study, we present a new strategy for fabricating localized gene delivery systems by producing poly(D,L-lactide-co-glycolide) (PLGA) as stent coatings and subsequently immobilizing gene vectors on its surface for enhanced substrate-mediated gene delivery. Thus, the goal of the present study was to synthesize and characterize the PLGA modified with functional carboxylic groups and to examine their localized gene delivery characteristics.

Methods: PLGA (75:25, Mw = 71,134), branched poly ethylenimine (PEI, 25kDa), 1-ethyl-3-(3-dimethyl amino-propyl)-carbodiimide hydrochoride (EDC) and *N*-hydroxy succinimide (NHS) were purchased from Sigma-Aldrich Corporation. Maleic anhydride (MA) and benzoyl peroxide (BPO) purchased from Tianjin Chemical. The method is described briefly as follows: First, PLGA was functionalized using free-radical grafting of MA groups onto the polymer backbone. Subsequently, bound MA groups were converted to carboxyl groups. Then, nano-sized particles of cationic polyethylenimine (PEI)/DNA complexes were covalently immobilized onto the modified PLGA. PEI can condense with DNA by self-assembly and form complexes with DNA spontaneously as a result of electrostatic interactions. A scheme of the reactions involved in the protocol is shown in Figure 1.

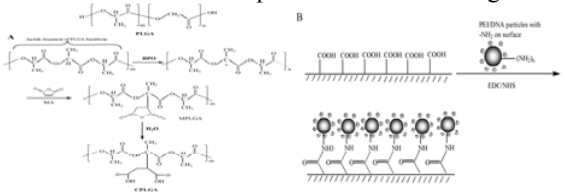

Figure 1. (A) Synthesis of MA-grafted-PLGA based functional film. (B) Scheme of the reactions involved in the immobilization of PEI/DNA particles onto the MA-grafted-PLGA films.

Results: In this study, carboxyl acid groups were introduced into the PLGA by MA grafting reactions. FTIR and NMR spectra indicated that MA was successfully grafted onto the PLGA (Figure 2) backbone. Rhodamine tests showed that the graft content of MA to PLGA was 2.06 %. The carboxylic acid groups on the surface of the MA-grafted-PLGA films were further assessed by ATR-FTIR (Figure 3). The density of carboxylic acid groups on the surface of MA-grafted-PLGA films was 0.892±0.11 nmol/cm^2.

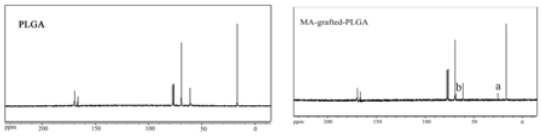

Figure 2. ^{13}C NMR spectra of PLGA and MA-grafted-PLGA.

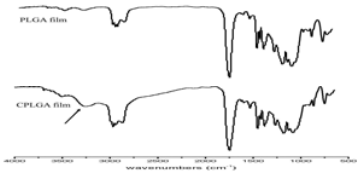

Figure 3. ATR-FTIR spectra of PLGA films and MA-grafted-PLGA films.

The immobilization of PEI/DNA complexes on the surface of film was evaluated by SEM. SEM images showed the conjugation of PEI/DNA complexes on the surface of MA-grafted-PLGA films (Figure 4). It can be seen that PEI/DNA complexes were irregularly aggregated on the MA-grafted-PLGA films in the control groups by the physical adsorption method, while they are uniformly deposited on the chemically activated CPLGA film preserving their spherical morphology and size.

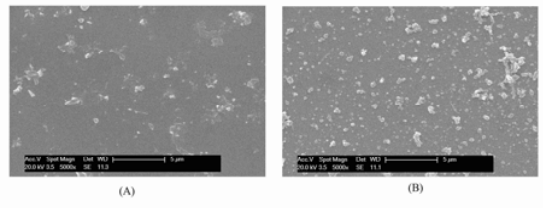

Figure 4. SEM micrographs of PEI/DNA complexes immobilized onto the surface of MA-grafted-PLGA films films. (A): physical adsorption method; (B): chemical immobilization method.

Conclusions: This method has the potential to be an effective tool in surface-mediated gene delivery. These gene-activated PLGA film surfaces are being evaluated for their efficacy in localized gene delivery both in cell culture and *in vivo*. Our group will further investigate the hypothesis that this technique could deliver gene vectors in a site-specific manner and provide relatively high levels of local gene expression with minimal distal spread.

References:

[1] Mei L. J Gene Med. 2006;8:690-698.
[2] Jin X. J. Gene Med. 2008;10:421-429.
[3] Bao JB. Song. Biomed. Mater. 2009;4:011002.