O1-2 **Synthesis of a linear copolymer Poly(lactic acid)-*b*-Dextran for drug delivery**

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INTRODUCTION AND OBJECTIVES

Nanomedicine, created by the fusion of nanotechnology and medicine, is one of the most promising ways to provide solutions to the challenges met by the conventional drug delivery methods. Parenterally administered drugs are prone to systemic clearance depending on the size and the properties of the drugs. The low bioavailability of the drugs caused by the clearances subsequently results in higher administration dosage, which increases the risk of side effects.

In the past decade, drug delivery systems constructed from polymeric nanoparticles (NPs) have been the cornerstone of nanomedicine due to their unique properties (Kim 2009, Ochekpe 2009, Yang 2009). The nano-sized of the drug delivery vehicles decreases the likelihood of the hepatic clearance by the liver, while at the same time increasing the life span of the encapsulated drugs. The biocompatible nature of some of the polymers used for the encapsulation matrix may also minimize the immunogenicity in the body.

In this study, a novel synthesis method was developed to conjugate two biocompatible polymers, Polylactic acid and Dextran to form a linear block copolymer PLA-Dex. A grafted copolymer PLA-Dex has already been developed by another group (Nouvel 2004). However, a grafted copolymer forms a less ordered NP structure compared to a linear copolymer, which reduces the encapsulation efficiency of drugs in the core of the NP structure. The drug loading efficiency in PLA-Dex will be assessed and compared with existing block copolymer PLGA-PEG for comparative analysis.

MATERIALS AND METHODS

Materials

The materials used in the study are Polylactic acid (PLA, Lakeshore Biomaterials), Dextran (Dex, Sigma Aldrich), *N*-Boc-ethylenediamine (Sigma Aldrich), *N*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide (EDC, Sigma Aldrich), *N*-Hydroxysulfosuccinimide (Sulfo-NHS, CNH Technologies), and sodium cyanoborohydride ($NaCNBH_3$, Sigma Aldrich). Vitamin A (MW = 286 Da, Sigma Aldrich) was used as the model drug.

Synthesis of linear block copolymer PLA-Dex

The synthesis of the linear block copolymer is divided into three stages: reductive amination between Dextran and *N*-Boc-ethylenediamine, deprotection of Boc group, and conjugation of Dextran with PLA (Figure 1).

Dextran and *N*-Boc-ethylenediamine were reacted with $NaCNBH_3$ as the reducing agent for 72 hours and purified using methanol. The deprotection of Boc group was performed by individually adding HCl and Triethyl amine (TEA). The mixture is then further purified and reacted with carboxyl-terminated PLA along with catalysts such as EDC and Sulfo-NHS for 4 hours. The resulting PLA-Dex was purified using methanol/acetone, and then dried in vacuum desiccators overnight.

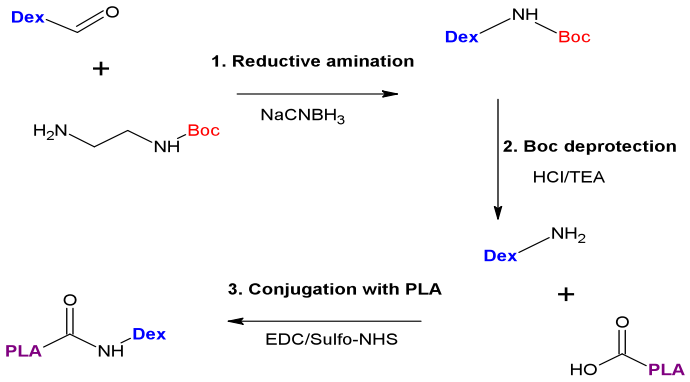

Figure 1 : Schematic illustration of synthesis of PLA-Dex

PLA-Dex's were analyzed using proton Nuclear Magnetic Resonance (H NMR) spectroscopy. Subsequently 1ml of PLA-Dex in DMSO (10mg/ml) was added in a drop-wise manner to 10 ml of water under constant stirring in order to form NPs (nanoprecipitation). After 30 minutes, the sizes of the NPs were analyzed using Dynamic Light Scattering (DLS).

Encapsulation of Vitamin_A in PLA-Dex NPs

The encapsulation of Vitamin A in the PLA-Dex NPs was accomplished using nanoprecipitation technique. PLA-Dex and Vitamin A were both dissolved in DMSO (polymer concentration of ~ 6.8 mg/ml, with varying drug concentrations). 1 ml of the DMSO solution is added drop-wise into 10 ml of water under stirring, and was left for 30 minutes. The content is then filtered through Amicon Centrifuge tubes (MW cutoff = 10 kDa) in order to remove the free drugs. The concentration of the encapsulated drug in the was measured by UV-vis microscopy with absorption at 325 nm for Vitamin A.

The Mass Yield (MY, mass of drug/mass of polymer, wt%) is then calculated with respect to various initial drug loading.

RESULTS AND DISCUSSION

Figure 2 shows a typical set of H NMR spectra on the three stages of the synthesis of PLA50-Dex6 (MW_{PLA} = 50 kDa, MW_{Dex} = 6 kDa). The reductive amination is confirmed by the presence of peak at 1.44 ppm (Bottom spectrum in the figure), which disappears in the next stage – the deprotection of Boc (middle spectrum). Finally, the conjugation of Dextran with PLA is shown with the presence of multiple peaks near 5.2 ppm for PLA (top spectrum).

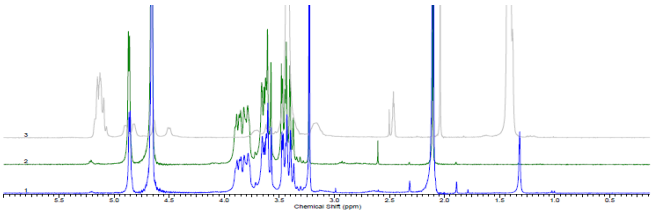

Figure 2 : H NMR spectra of three stages of synthesis of PLA50-Dex6 (from bottom to top)

The DLS result (Figure 3) shows that the increase in MW_{Dex} reduces the particle sizes, while the increase in MW_{PLA} increases them. Higher MW of PLA increases the size of the core structure of the NPs. In contrast, higher MW of Dex has higher chance of "folding-down" onto the surface of the NPs, thus reducing the overall size of the NPs. However, the trends must be further validated by investigating more PLA-Dex's with different MW's.

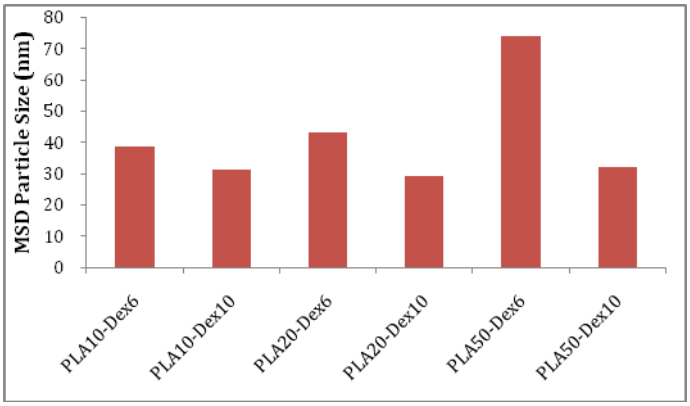

Figure 3 : DLS results of PLA-Dex with different MW combinations

The amount of encapsulated Vitamin A was calculated for three different block copolymers: PLA20-Dex6, PLA20-Dex1.5, and PLGA-PEG. At low initial drug loading (up to ~ 6 wt%), all three polymers show similar MY. At higher initial loadings, the MY was higher for PLA20-Dex6 compared to that of PLA20-Dex1.5. It is hypothesized that Dex with higher MW on the surface of NPs creates a denser polymer shell matrix reducing the diffusivity of the drugs, and thereby increasing the encapsulation efficiency. At this point, PLA20-Dex6 seems promising in terms of drug encapsulation since they show higher MY than that of PLGA-PEG. However, more encapsulation studies with different MW's of PLA and Dex must also be investigated to fully understand the potentials of using PLA-Dex for drug delivery application.

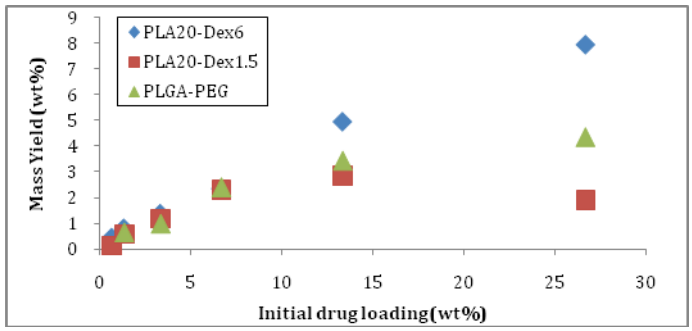

Figure 4 : Mass Yield of Vitamin A in three block copolymers

CONCLUSION

A novel method of synthesizing a linear block copolymer PLA-Dex was developed. Sizes of less than 100 nm were observed for the PLA-Dex NPs. Encapsulation of Vitamin A in PLA-Dex of different MW's was studied. Although, PLA20-Dex6 shows higher MY compared to that of PLGA-PEG, further studies are required to arrive at a more vigorous conclusion. In the future, encapsulation of various types of anticancer drugs in PLA-Dex should be studied to assess the potentials of PLA-Dex NPs in cancer therapy. Understanding the release profiles of drugs from the NPs are also key aspects in the development the drug delivery system. Finally, the interaction of the drug encapsulated NPs with the human immune system and the cell-uptake efficiencies by cancer cells should also be analyzed in the future.

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