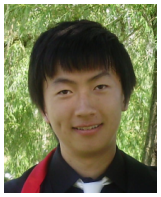
Mucoadhesive nanoparticles for topical ocular drug delivery



Liu S., Verma M. and Gu F.
University of Waterloo, Waterloo, Canada (sandy.liu@uwaterloo.ca)

INTRODUCTION AND OBJECTIVE

Ocular drug delivery remains one of the most challenging endeavors faced by the pharmaceutical scientists due to the various pharmacological barriers that exist around the eye. Topically applied drugs (i.e. eye drops) are constantly washed off from the eye by various mechanisms such as lacrimation, tear dilution, and tear turnover. Similarly, systemic and periocular administration of drugs are hindered by barriers such as blood-retina barrier and sclera. The numerous barriers in the eye result in low bioavailability of the drug in the organ.

Nanoparticles have recently attracted much attention as drug carriers to delivery ocular drugs for specific targeting and prolonged retention to overcome the low bioavailability of the conventional methods (Diebold 2010).

In this study, Cyclosporine A (commonly used for the treatment of dry eye) is used as a model drug to study the potential of using Dextran-Poly(D,L-lactide) (PLA-Dex) nanoparticles (NPs) as drug carriers for targeted and prolonged therapy.

MATERIALS AND METHODS

Materials

The materials used in the study are Polylactic acid (PLA, MW = 20 kDa, Lakeshore Biomaterials), Dextran (Dex, MW = 10 kDa, 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). Cyclosporine A was purchased from Sigma Aldrich.

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). The resulting PLA-Dex was purified using methanol/acetone, and then dried in vacuum desiccator overnight.

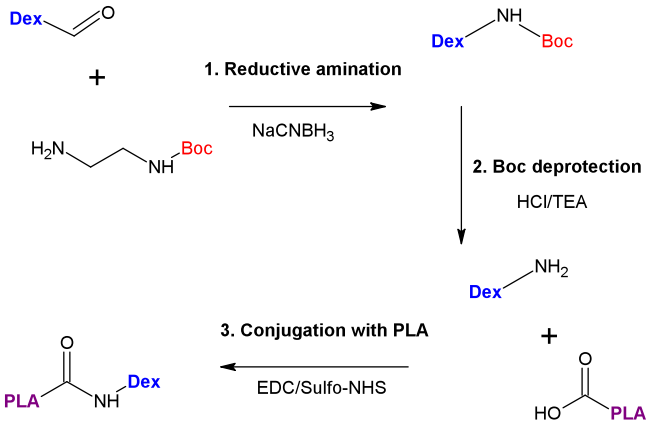


Figure 1 : Schematic illustration of synthesis of PLA-Dex

Each stage of the synthesis was analyzed using proton Nuclear Magnetic Resonance (^{1}H NMR) spectroscopy. 1ml of PLA20-Dex10 in DMSO (10mg/ml) was added in a drop-wise manner to 10 ml of water under constant stirring in order to form nanoparticles. After 30 minutes, the sizes of the NPs were analyzed using Dynamic Light Scattering (DLS).

Encapsulation in PLA-Dex NPs and In vitro release phenomena of Cyclosporine A

The encapsulation of Cyclosporine A in the PLA-Dex NPs was achieved using nanoprecipitation technique. PLA20-Dex10 and Cyclosporine A were dissolved in DMSO (polymer concentration of ~ 6.8 mg/ml, with varying drug concentrations). After nanoprecipitation, the content is filtered using syringe filters (pore size = 0.2 µm) to remove aggregates, and subsequently filtered using Amicon Centrifuge tubes (MW cutoff = 10 kDa) in order to remove the free drugs. The content is dried in oven overnight, and re-suspended in acetonitrile. Cyclosporine A concentration was measured by High Performance Liquid Chromatography (HPLC) with absorption at 210 nm. The drug loading (mass of drug/mass of polymer, wt%) is then calculated with respect to various initial drug loading ratios.

The *in vitro* release phenomenon was also measured using spectroscopic method. In brief, the nanoparticles with Cyclosporine A encapsulated were dialyzed in phosphate buffer saline (PBS, pH 7.4) at 37 °C under mild stirring. 1 ml of PBS was extracted at

predetermined time-intervals to measure the concentration of released Cyclosporine A.

RESULTS AND DISCUSSION

Figure 2 shows a set of ^{1}H NMR spectra on the three stages of the synthesis of PLA20-Dex10 (MW_{PLA} = 20 kDa, MW_{Dex} = 10 kDa). The reductive amination is confirmed by the presence of peak at 1.44 ppm (bottom spectrum), 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).

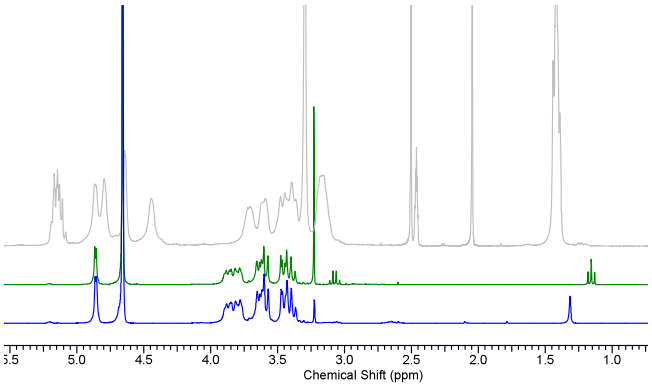


Figure 2 : ^{1}H NMR spectra of three stages of the synthesis of PLA20-Dex10 (from bottom to top)

At 20 and 40 wt% initial feed of Cyclosporine A in the nanoprecipitation method, the PLA20-Dex10 NPs showed drug loading of 7.48 and 4.23 wt% respectively. The drop in the drug loading from 20 to 40 wt% initial feed may be due to the increased chance of drug aggregation with higher concentration. The drop in the drug loading also corresponded to the drop in the NP size between the same samples, from 52.1 nm to 39.2 nm (Figure 3).

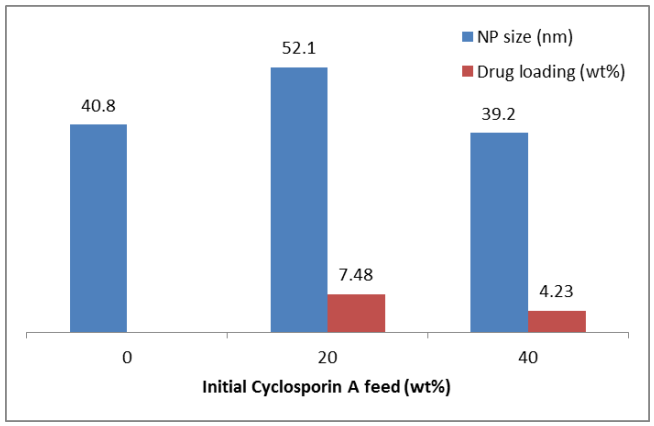


Figure 3 : NP particle sizes of PLA20-Dex10 with various amounts of Cyclosporine A drug loading

The release of Cyclosporine A from the PLA20-Dex10 showed three distinct regions. The initial burst release was observed up to 24 hrs, and it is mostly likely contributed by the free drug in the system that may be bound on the surface of the nanoparticles. The second region showed a steady release for the next 7 days, which is likely due to the diffusion of the drug from the NP matrix. The last region showed a slight increase in the rate of release until 14th day, which can be attributed to the process of the degradation of the NP matrix. The study showed a controlled and steady release up to 2 weeks from the NPs. However, further studies using *in vivo* models must be used to analyze the actual concentration profile of the drug in the eye.

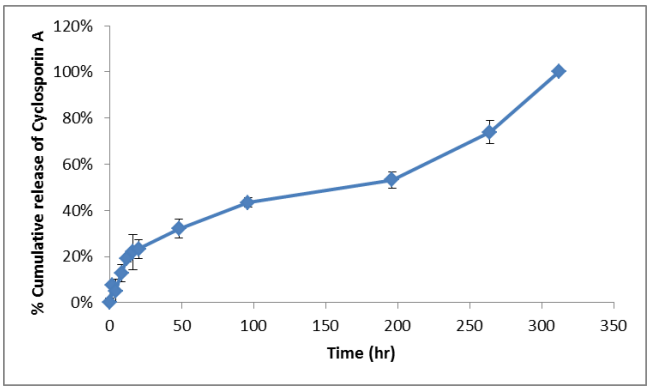


Figure 4 : *In vitro* release phenomena of Cyclosporine A from PLA20-Dex10

CONCLUSION

Sizes of less than 100 nm and drug loading of up to 7.48 wt% were achieved for Cyclosporine A in PLA-Dex NPs. *In vitro* release study showed controlled release of the drug for up to 2 weeks, but however, understanding the release profile of drugs in the *in vivo* environment is a key aspect in the development the drug delivery system. Although the development of nanoparticle ocular drug delivery system at its infant stage, results thus far show that the ocular medicine will benefit enormously from the application of nanotechnology.

REFERENCES

- Deibold Y. et al. (2010) *Applications of nanoparticles in ophthalmology*. Progress in Retinal and Eye Research (29) 596-609