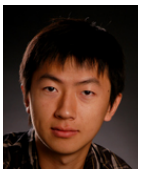
Long-lasting eye drop delivery platform for targeted ocular delivery applications



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INTRODUCTION AND OBJECTIVE

Topical ocular drug delivery remains one of the most challenging endeavours faced by pharmaceutical scientists. 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, resulting in less than 5% of the drug reaching its intended target. Therefore, current formulations must be administered frequently (i.e. twice a day) which lowers patient compliance and increases potential for adverse effects.

We hypothesize that nanoparticle (NP) drug carriers that target the corneal surface (ocular mucosa) may significantly prolong the precorneal retention of the encapsulated drugs by circumventing tear turnover clearance.

The purpose of this study is to develop a biocompatible phenylboronic acid (PBA) coated Dextran-b-poly(D,L-lactide) (Dex-b-PLA) NP drug carrier that can load a therapeutically relevant dosage, release drug at a sustained rate for a prolonged period of time, and target the ocular mucous membrane to increase the ocular retention of the drugs (
Figure 1).

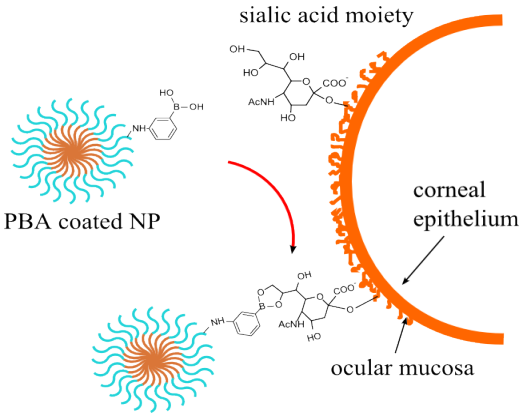

Figure 1 : Schematic illustration of PBA coated NPs binding to the ocular mucosa (Liu 2012).

MATERIALS AND METHODS

Materials Acid-terminated poly(D,L-lactide) (Mw ~ 20 kDa) were purchased from Lakeshore Biomaterials (USA). Dextran (Mw ~ 10 kDa), Cyclosporine A (CycA), 3-aminophenylboronic acid monohydrate (PBA), sodium periodate ($NaIO_4$), glycerol, sodium cyanoborohydride ($NaCNBH_3$), and Type III mucin from porcine stomach (sialic acid 0.5 - 1.5%) were purchased from Sigma Aldrich (Canada). Simulated tear fluid (STF) was prepared for the *in vitro* release experiment using a previously described formulation (Shen 2010).

Surface functionalization of NPs with PBA Dex-b-PLA was synthesized using a previously reported method (Verma 2012). Dex-b-PLA was dissolved in DMSO and added slowly into water under mild stirring. Periodate oxidation of the Dextran surface was carried out by adding 60 mg of $NaIO_4$ and stirring for an hour. Subsequently, glycerol was added to quench the unreacted $NaIO_4$. Various amounts of PBA (i.e. 40mg for Dex-b-PLA_40PBA) were added to the mixture, along with $NaCNBH_3$, for 2 hrs. All reactions were carried out in the dark. The mixture was then dialyzed in water for 24 hrs. The wash medium was changed 4 times.

Encapsulation in Dex-b-PLA_PBA NPs and in vitro release phenomena of Cyclosporine A The encapsulation of CycA in the Dex-b-PLA_PBA NPs was achieved using nanoprecipitation. Drug aggregates were filtered using syringe filters (pore size = 0.2 µm), and any free drug was further removed using Amicon Centrifuge tubes (MW cutoff = 10 kDa). The content is dried in the oven overnight, and re-suspended in acetonitrile. CycA concentration was measured by HPLC with absorption at 210 nm. The *in vitro* release phenomenon was also measured using spectroscopy. In brief, the NPs with CycA encapsulated were dialyzed in STF at 37 °C under mild stirring. 1 ml of STF was extracted at predetermined time-intervals to measure the concentration of released CycA.

In vitro mucoadhesion Mucoadhesion was calculated as the amount of mucin adsorbed per mg of NPs. Dex-b-PLA_PBA NP suspension (1ml) was mixed with 1ml of mucin solution (1mg/ml in STF) and incubated at 37°C for 1 hr. The mixture was then centrifuged at 15,000 rpm for 1 hr and free mucin in the supernatant was quantified using the periodic acid/Schiff (PAS) staining method (Lee 2006). Mucin adsorption was calculated by subtracting the free mucin concentration from the initial mucin concentration. Mucin standards (0.1, 0.25 and 0.5 mg/ml) were determined using the same procedure to obtain a calibration curve.

RESULTS AND DISCUSSION

The degree of PBA functionalization on the NPs was quantified using UV-vis absorption at 291 nm. The

particle sizes and morphology were characterized by DLS and TEM respectively (
Figure 2). PBA functionalized NPs with diameters less than 30 nm and spherical morphologies were obtained.

Formulation	PBA:Dex[a)] (mol%)	Diameter (nm)
PLA-Dex	0	47.9 ± 0.5
PLA-Dex_10PBA	2.85 ± 0.03	27.5 ± 0.9
PLA-Dex_40PBA	12.2 ± 0.2	26.7 ± 0.1
PLA-Dex_160PBA	22.9 ± 0.3	25.2 ± 1.0
PLA-Dex_320PBA	34.6 ± 0.2	28.1 ± 0.3

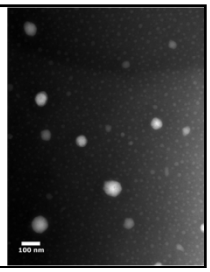

Figure 2 : PBA surface modification and NP size (Left) and TEM image (Right).

CycA, commonly used for treating dry eye syndrome, was used as a model drug to evaluate the pharmacokinetic properties of using the NPs. Clinically relevant dosing was achieved using these NPs (drug load in NP: up to 13.7 wt%). Both the PBA modified and unmodified NPs showed total release up to 5 days (
Figure 3); much longer than previously reported values (~1 day). The prolonged release suggests that these NP carriers may be able to reduce the topical administration frequency.

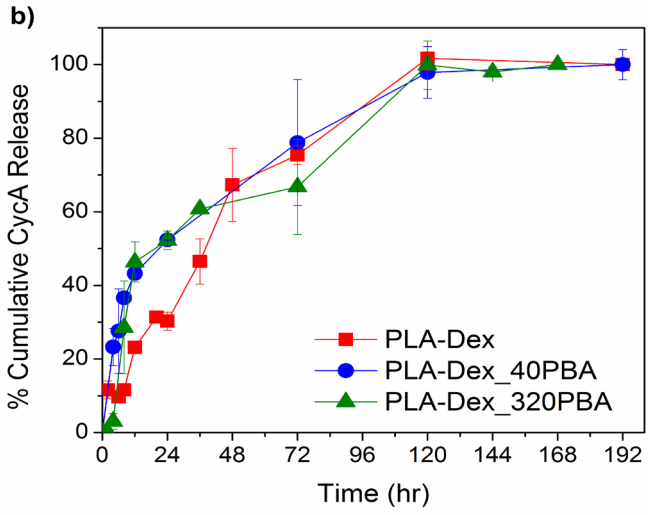

Figure 3 : *in vitro* CycA release

In vitro colorimetry with PAS staining was used to quantify mucin-NP interaction. PBA modified NPs showed improved mucin-NP interaction compared unmodified NPs (
Figure 4). Excess PBA (Dex-b-PLA_320PBA) on the NP surface showed a decrease in mucin-NP interaction, likely due to the colloidal instability that could cause the NPs to aggregate amongst themselves rather than binding with mucin.

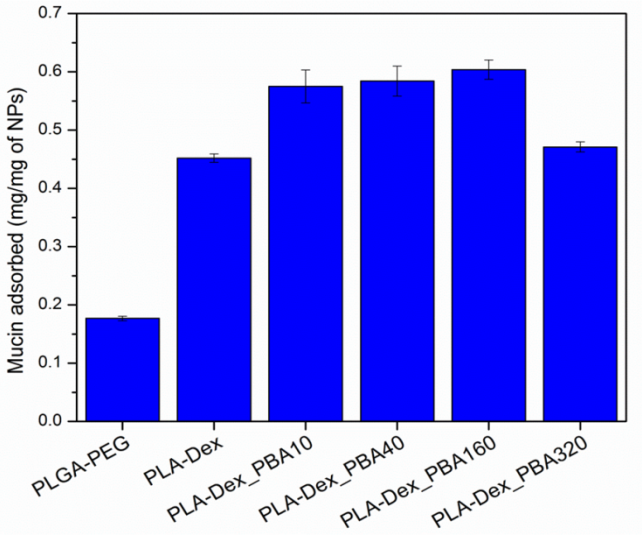

Figure 4 : *in vitro* mucoadhesion study

CONCLUSIONS

The surface of the NP composed of linear block copolymers Dex-b-PLA was modified with PBA to form a mucoadhesive targeting drug carrier. NPs with sizes less than 30 nm and spherical morphology were able to achieve therapeutically relevant dosage of CycA and release them at a sustained rate for up to 5 days *in vitro*. PBA surface functionalization facilitated *in vitro* mucin-NP interaction. The current provides promising results for the use of Dex-b-PLA_PBA NPs to improve the bioavailability of ocular drugs intended to target the anterior segment of the eye.

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