Mucoadhesion and Mucopenetration of Self-assembled Block Copolymer Nanoparticles

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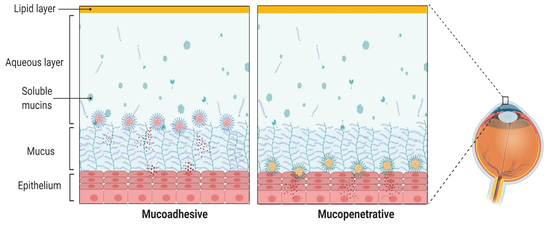
Introduction

Less than 5% of the original dose of a conventional eyedrop formulation reaches the target tissue on the front of the eye due to: (1) solution drainage via tear drainage or nasolacrimal drainage, (2) blinking or (3) systemic adsorption by the conjunctiva.

Mucoadhesive and mucopenetrative nanocarriers are promising drug delivery vehicles for the treatment of anterior segment diseases of the eye due to their small size and ability to prolong the precorneal residence time of encapsulated drugs [1].

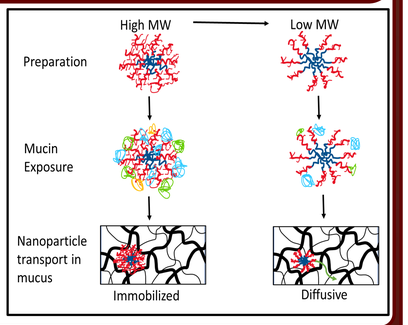
(1) Mucoadhesive nanocarriers – adhere to the tear film mucin proteins to allow for localization of drug at the site of absorption

(2) Mucopenetrative nanocarriers – penetrate through the tear film mucins to directly reach the underlying epithelium for localized drug delivery



Problem Statement

- **Self-assembled nanoparticles based on block copolymers of poly(ethylene glycol) and poly(lactic-co-glycolic acid) (PEG-PLGA)** have been widely applied across multiple drug delivery applications (including ophthalmic delivery) as effective nanoscale drug carriers [2].
- It has been previously shown that **tuning the length and density of the PEG block in self-assembled block copolymer micelles is an effective strategy for improving mucosal penetration and adhesion of nanoparticles [3]**:
  - However, the functionalization of the PEG block to include groups to improve mucoadhesion or alter drug release kinetics is challenging given the step growth nature of PEG polymerization that allows for only end group functionalization



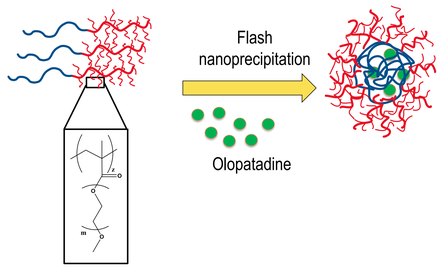
Proposed Solution

Linear-brush copolymers based on PLA and Poly(oligo(ethylene glycol) methacrylate) (POEGMA), a brush polymer derivative of PEG synthesized through free radical polymerization, **offer the capacity for easy functionalization of a PEG-based polymer** via free radical copolymerization in addition to facilitating **tunable molecular weight distributions due to the branched nature of the polymers [4].**

- Offers potential to balance mucopenetration/ mucoadhesion

We prepared nanoparticles from amphiphilic block copolymers of PLA-POEGMA **with tunable ethylene oxide side-chain lengths to facilitate mucoadhesion and mucopenetration.**

Incorporation of a poorly soluble drug (i.e., olopatadine for the treatment of allergic conjunctivitis) during nanoparticle synthesis via flash nanoprecipitation allows for facile drug loading.

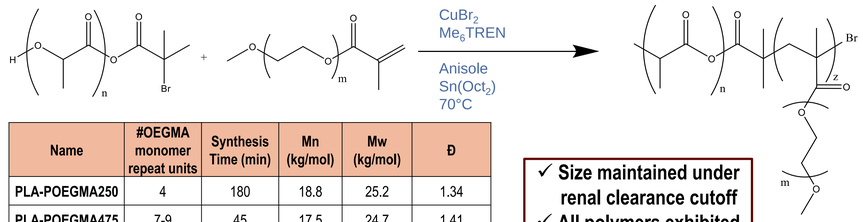


Experimental

Synthesis:

Amphiphilic copolymers based on PLA-POEGMA are synthesized via atom transfer radical polymerization (ATRP):

- A poly(lactic acid)-based macroinitiator was used both as a hydrophobic block for self assembly and an ATRP initiator enabling for the growth of the POEGMA block
- POEGMA blocks were polymerized to different chain lengths using oligo(ethylene glycol methacrylate) (OEGMA) monomers with n=4, 7-9, 20 or 40 average repeat units in the PEG brush, targeting an overall number average molecular weight (Mn) of ~20 kDa



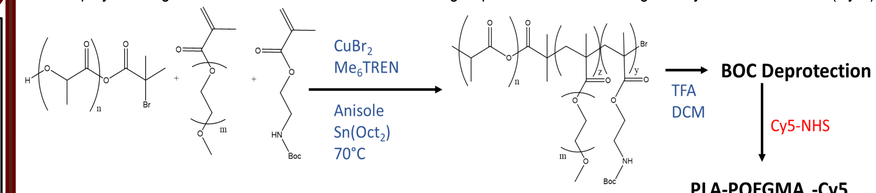
Name	#OEGMA monomer repeat units	Synthesis Time (min)	Mn (kg/mol)	Mw (kg/mol)	Đ
PLA-POEGMA250	4	180	18.8	25.2	1.34
PLA-POEGMA475	7-9	45	17.5	24.7	1.41
PLA-POEGMA900	20	40	30.8	42.9	1.40
PLA-POEGMA2000	40	15	27.3	34.1	1.25

✓ **Size maintained under renal clearance cutoff**
✓ **All polymers exhibited a low PD**

Fabrication of Fluorescent Cy-5 Labeled polymer:

Fluorescent PLA-POEGMA polymers are synthesized to enable particle tracking studies by:

- Copolymerizing POEGMA blocks with amine functional groups for co-functionalizing with cyanine5 NHS ester (Cy-5)

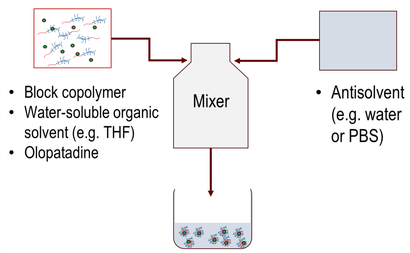


Name	#OEGMA monomer repeat units	Synthesis Time (min)	Mn (kg/mol)	Mw (kg/mol)	Đ	% functionalization with Cy5
PLA-POEGMA250-Cy5	4	180	18.8	18.7	1.31	5%
PLA-POEGMA475-Cy5	7-9	45	17.5	20.2	1.28	7%

Nanoparticle Fabrication:

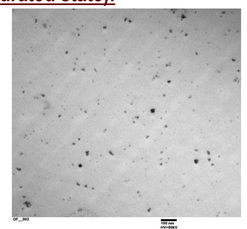
Fluorescent and nonfluorescent nanoparticle fabrication through flash nanoprecipitation:

- Nanoparticle size can be tuned through (1) choice of organic solvent and (2) injection flow rate through mixer



	Average Eff Diameter	Average Polydispersity
PLA-POEGMA250	217 ± 1.37	0.15 ± 0.04
PLA-POEGMA475	56 ± 0.55	0.24 ± 0.01
PLA-POEGMA900	80 ± 0.99	0.16 ± 0.01
PLA-POEGMA2000	82 ± 0.67	0.15 ± 0.02

TEM Image of PLA-POEGMA 475 NPs (dehydrated state):



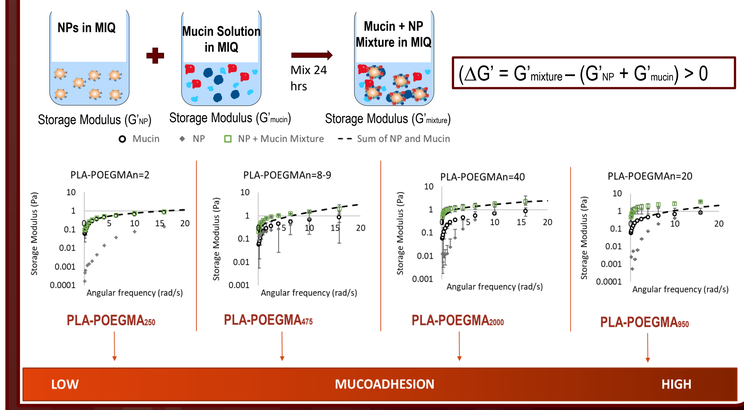
✓ **Monodisperse and colloidally stable NPs**

Mucoadhesion

Rheology:

Assessing mucoadhesive properties of PLA-POEGMA NPs through rheology.

- **The rheological response of the NP-mucin mixture should be greater than the contributions from the NPs and mucin alone for mucoadhesive polymers:**

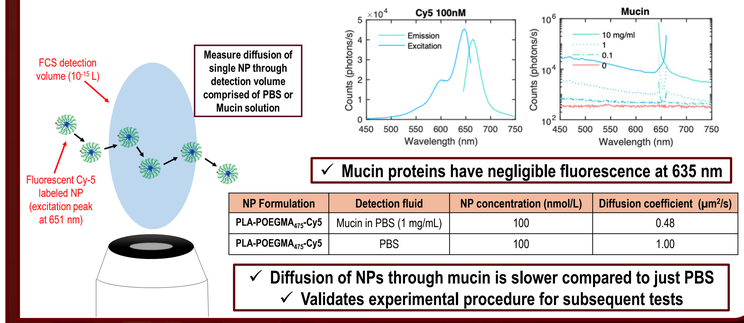


Mucopenetration

Fluorescence Correlation Spectrometry (FCS):

Assessing mucopenetration properties of PLA-POEGMA$_n$ NPs through FCS.

- **The diffusion coefficient of mucopenetrative NPs in a mucin mixture (1 mg/mL) should be greater compared to non-mucopenetrative counterparts**
- Preliminary study focused on:
  - Determining autofluorescent properties of mucin solution
  - Comparing diffusion coefficients for PLA-PEOGMA$_n$ NPs in phosphate buffer saline (PBS) and mucin solution to determine which are more penetrative



✓ **Mucin proteins have negligible fluorescence at 635 nm**

NP Formulation	Detection fluid	NP concentration (nmol/L)	Diffusion coefficient (μm²/s)
PLA-POEGMA475-Cy5	Mucin in PBS (1 mg/mL)	100	0.48
PLA-POEGMA475-Cy5	PBS	100	1.00

✓ **Diffusion of NPs through mucin is slower compared to just PBS**
✓ **Validates experimental procedure for subsequent tests**

Conclusion

- We fabricated amphiphilic block copolymers composed of PLA-POEGMA$_n$ exhibiting low PDI and offers control over size of polymer.
- Monodisperse and colloidally stable NPs can be fabricated through flash nanoprecipitation.
- PLA-POEGMA950 and PLA-POEGMA2000 polymers exhibit rheological synergism.
- Mucin proteins have negligible autofluorescence and the diffusion coefficient of NP in PBS is greater than the diffusion coefficient of NPs in mucin solution.

Acknowledgements and References

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