Poly (oligoethylene glycol methacrylate)-block-poly (D, L-lactide)-based nanoparticles as versatile hydrophobic drug delivery vehicles

Maryam Badv[1] , Omar Salem[2], Yonghong Wan[2] and Todd Hoare[1,3]
[1] School of Biomedical Engineering, McMaster University, Hamilton, ON, Canada
[2] Department of **Pathology and Molecular Medicine**, McMaster University, Hamilton, ON, Canada
[3] Department of Chemical Engineering, McMaster University, Hamilton, ON, Canada
Phone: 1-905-525-9140 ext. 24701 | E-mail: hoaretr@mcmaster.ca | Website: http://hoarelab.mcmaster.ca

Introduction

- Polymeric nanoparticle (NP) drug delivery systems, particularly NPs formulated from the block copolymer poly (ethylene glycol)-*b*-poly (lactic acid) (PEG-*b*-PLA) have shown great clinical potential given their higher stability and circulation time, improved biocompatibility, biodegradability and ease of functionalization.
- However, the single end functional group on PEG poses limitations on the use of these materials for emerging ligand-receptor targeting systems as only one ligand may be attached per polymer chain in the NP
- Herein, we address this limitation by replacing the PEG block with poly (ethylene glycol methacrylate) (POEGMA) that has a methacrylate backbone and PEG side-chains. POEGMA has shown to have similar physical and biological properties to PEG but can be copolymerized, enabling facile multi-ligand grafting.
- In addition, POEGMA can be engineered to be a "smart" polymer for drug delivery, exhibiting a lower critical solution temperature (LCST) range and a cloud point by changing the length of the oligoethylene glycol side chains.

Methods

- Drug loaded (DOX) poly (D,L-lactide) (PLA)-*b*-[poly(ethylene glycol) methyl ether methacrylate (POEGMA)-*co*-di(ethylene glycol) methyl ether methacrylate (M(EO)$_2$MA)] (PLA-*b*-P[OEGMA-*co*-M(EO)$_2$MA]) block co-polymers were prepared using PLA-Br as a macroinitiator for the Activators Regenerated by Electron Transfer (ARGET) ATRP of the POEGMA co-monomers (**Figure 1**).

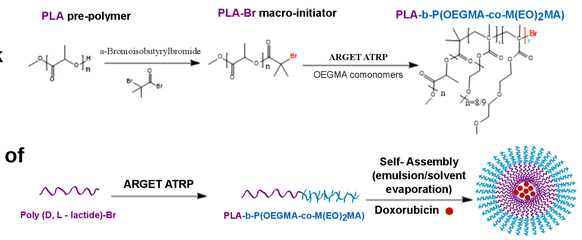


Figure 1. Block co-polymer synthesis and schematic representation of NP formation.

- DOX-loaded NPs were characterized using dynamic light scattering (DLS) and transmission electron microscopy (TEM).
- In order to determine the cloud point and the LCST range of the temperature sensitive NPs in different solutions, a Variant Cary Bio 100 UV–vis spectrophotometer was used.
- The cytocompatibility of the empty NPs was assessed using a Resazurin assay.
- NP uptake into B16F10 cancer cells and the effect of temperature in drug release from the NP was assayed via confocal microscopy.

Results

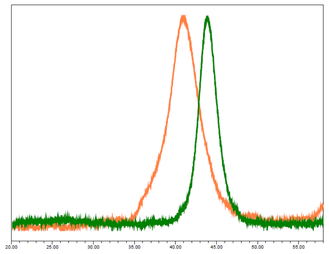
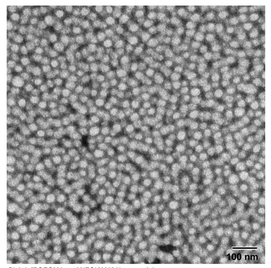


Figure 2. GPC results for the temperature sensitive block copolymers prepared by ARGET ATRP. The block copolymers exhibited narrow molecular weight distributions and well-defined compositions. The results also confirm the chain extension and polymerization of the OEGMA comonomers.

Polymer type	Mn	Mw	Polydispersity
PLA-Br	7800	8376	**1.074**
PLA-*b*-[POEGMA-*co*-M(EO)$_2$MA]	14378	19196	**1.33**



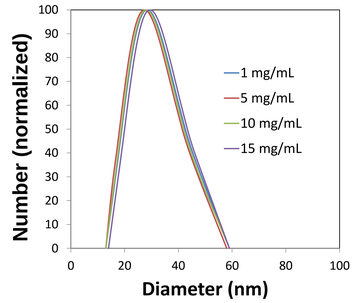
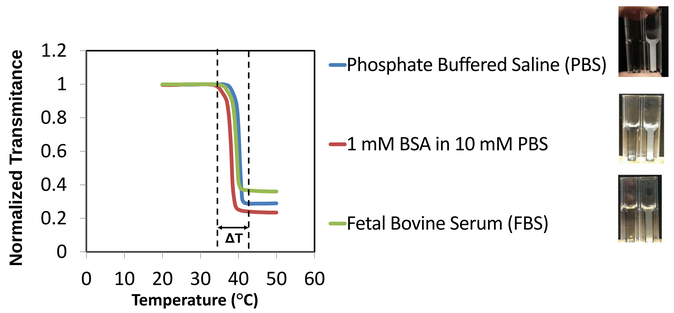


Figure 3. DLS (right) and TEM (left) results of the NPs. According to the DLS results, DOX loaded NPs had an effective diameter of 40 ± 1 nm with a polydispersity of 0.03. The TEM results were also aligned with the results obtained from DLS.



Solution	Cloud point (°C)	ΔT (°C)
10 mM Phosphate Buffered Saline (PBS)	**38.47**	**3**
Fetal Bovine Serum (FBS)	**37.47**	**2.5**
1 mM BSA in 10 mM PBS	**35.72**	**5**

Figure 4. Plots of transmittance as a function of temperature measured for temperature sensitive NPs in different solutions. When the NPs are placed in biological environments such as FBS and BSA, their cloud point decreases by 1 to 3 °C. The NPs also exhibit a narrow LCST range (ΔT) in all three solutions.

Results

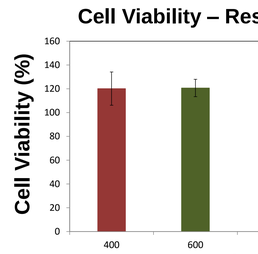


Figure 5. *In vitro* cytotoxicity assay of blank NPs against 3T3 fibroblast cells. Results of the Resazurin assay demonstrate that PLA-*b*-[POEGMA-*co*-M(EO)$_2$MA] NPs do not show cytotoxicity up to 1000 mg/mL of polymer concentration, which is a high concentration of material to be screened via an *in vitro* cell-based assay.

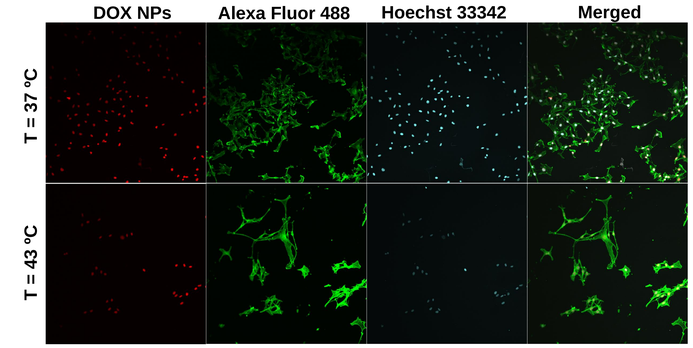


Figure 6. Cellular uptake and drug release of DOX loaded NPs (red) at 37 ºC or 43 ºC after incubation with B16F10 cells for 2 h. Cells were incubated with 15 µg/mL of DOX loaded NPs. Alexa Fluor 488 phalloidin (green) and Hoechst 33342 (blue) were used to stain F-actin and cell nuclei, respectively. Incubation of NPs at a temperature higher than the cloud point (43 ºC) resulted in NP aggregation and ultimately drug release which resulted in cell death. Cell concentration is significantly lower at 43 ºC compared to 37 ºC which is caused by the release of the cytotoxic chemotherapeutic drug at higher temperatures.

Conclusions

- PLA-*b*-P[OEGMA-*co*-M(EO)$_2$MA]-based NPs offer a more versatile delivery platform compared to conventional PEG-PLA NPs with the potential to enable environmentally-responsive targeting and drug release.
- The results obtained indicate high efficacy of the NP preparation process and the viability of our approach in tailoring PLA-*b*-[POEGMA-*co*-M(EO)$_2$MA] based NPs as a versatile drug delivery system.
- Future work will focus on active targeting of the NPs *in vivo* to further increase the efficacy of NP uptake at the site of disease.

Acknowledgements and References

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