Design and Development of Long-Term Therapy for Corneal Repair and Healing – 'Nanoparticles-in-Dual-Gels'

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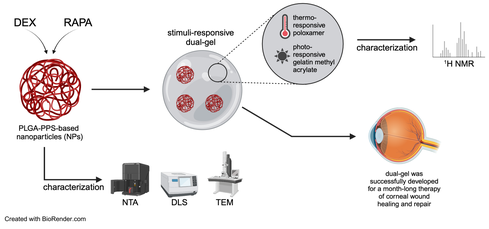
Introduction

- Severe injuries to the cornea may result in chronic inflammation that can cause **permanent vision loss**.
- In the event of corneal trauma, current treatment involves corneal transplantation using donated grafts. However, there is a limited supply of donor tissue and accompanying infrastructure to collect, store, and distribute the tissue[1, 2].
- There is a critical need to develop a topically administered formulation that fills the current treatment gap by improving safety, effectiveness, and patient adherence compared to corneal transplant surgery.

Schematic Summary



Objective

- Develop a stimuli-responsive **dual-gel** cocktail therapy consisting of **thermo-responsive poloxamer** (trPol) and **photo-responsive gelatin methyl acrylate** (prGelMa) based hydrogels embedded with PLGA-PPS-based nanoparticles (NPs) loaded with **dexamethasone** (DEX) and **rapamycin** (RAPA) that would fill the treatment gap instead of invasive procedures for corneal wound management and self-healing.

Methods

- trPol (P-407-SH) and prGelMa polymers were synthesized for hydrogel formulation.
- The purified and freeze-dried trPol and prGelMa were characterized using ^{1}H NMR. Several physicochemical parameters like gel texture, photo-induced transitions, and sol-gel transitions were determined.
- NPs were prepared using the nanoprecipitation method for DEX and RAPA and characterized for surface morphology, size, stability, entrapment, and loading efficiency using DLS, NTA, TEM, and HPLC.
- The drug-loaded NPs were embedded in the trPol and prGelMa gel combination and characterized for *in vitro* release kinetics. This formulation was collectively termed "GelPol."

Results

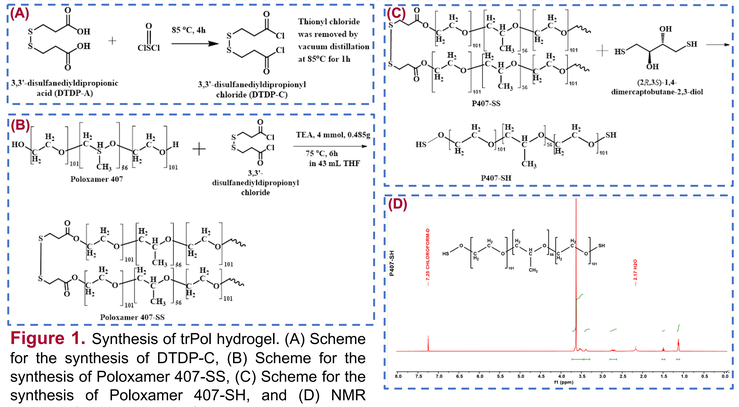


Figure 1. Synthesis of trPol hydrogel. (A) Scheme for the synthesis of DTDP-C, (B) Scheme for the synthesis of Poloxamer 407-SS, (C) Scheme for the synthesis of Poloxamer 407-SH, and (D) NMR spectra of Poloxamer 407-SH.

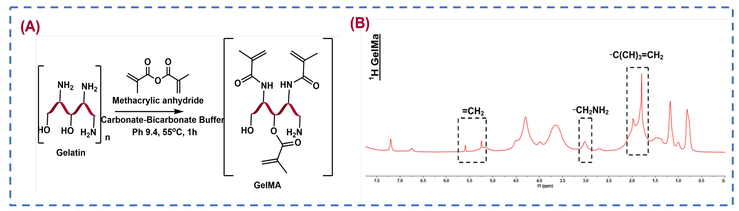


Figure 2. Synthesis of GelMa. (A) Synthesis scheme of GelMa and (B) 1H NMR spectra of GelMa.

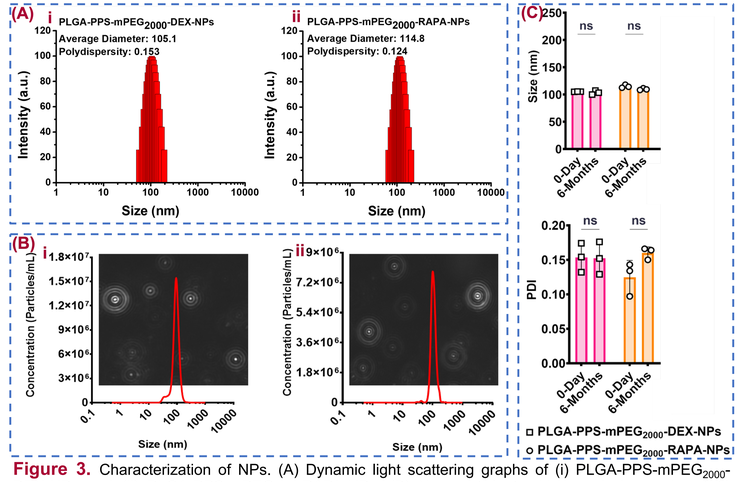


Figure 3. Characterization of NPs. (A) Dynamic light scattering graphs of (i) PLGA-PPS-mPEG$_{2000}$-DEX-NPs and (ii) PLGA-PPS-mPEG$_{2000}$-RAPA-NPs, (B) Nanoparticle tracking analysis graphs of (i) PLGA-PPS-mPEG$_{2000}$-DEX-NPs and (ii) PLGA-PPS-mPEG$_{2000}$-RAPA-NPs, and (C) Stability of the synthesized NP preparations, (i) size (nm) vs time graph and (ii) PDI vs time graph.

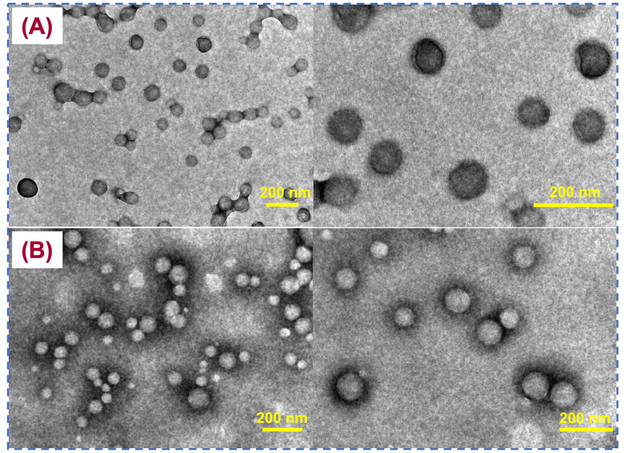


Figure 4. Transmission electron microscopy (TEM) images of (A) PLGA-PPS-mPEG$_{2000}$-DEX-NPs (scale bar 200 nm) and (B) PLGA-PPS-mPEG$_{2000}$-RAPA-NPs (scale bar 200 nm).

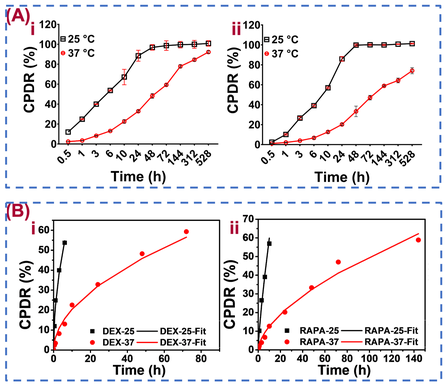


Figure 5. *In vitro* release study. The graphs show the cumulative percent release of (i) DEX and (ii) RAPA at 25 and 37 ºC. (A) Cumulative percent release of drugs recorded up to 528 hours showing sustained release of DEX and RAPA at 37 compared to 25 ºC. (B) The release kinetics of DEX and RAPA determined using the Korsmeyer-Peppas model showed non-Fickian release of the drug-loaded NPs from the gel matrix.

Table 1. Characterization of DEX and RAPA loaded PLGA-PPS-mPEG$_{2000}$-NPs.

Model parameters	PLGA-PPS-mPEG-DEX-NPs	PLGA-PPS-mPEG-RAPA-NPs
Size (nm)	105.2±0.2	114.9±2.93
PDI	0.153±0.021	0.124±0.024
EE (%)	87.27±2.15	88.11±5.44
DL (%)	7.95±0.20	8.00±0.50

Table 2. *In vitro* release kinetics of DEX and RAPA from hydrogel formulation.

Model parameters	PLGA-PPS-mPEG-DEX-NPs		PLGA-PPS-mPEG-RAPA-NPs	
	25 °C	37 °C	25 °C	37 °C
K_1	22.20	6.24	10.08	3.20
n	0.50	0.52	0.77	0.57
R^2	0.9951	0.9953	0.9950	0.9953

Conclusions

- The P-407-SH and GelMa polymers were successfully synthesized and characterized using NMR.
- DEX and RAPA loaded PLGA-PPS NPs were synthesized using the nanoprecipitation solvent evaporation method.
- The size (nm)/polydispersity index of the prepared DEX and RAPA-NPs determined by DLS was found to be 105.2±0.2 /0.153±0.021, and 114.9±2.93/0.124±0.024, respectively.
- NTA and TEM confirmed their monodispersity and spherical morphology. The encapsulation efficiencies of DEX and RAPA were found to be 87.27±2.15% and 88.11±5.44%, respectively. The NPs were found stable at 4 ºC storage for six months. The release kinetics of the drugs determined by the Korsmeyer-Peppas model suggest a non-Fickian diffusion mechanism *(0.45<n<1)*.
- The drug-loaded NPs, stimuli-responsive hydrogels, and nanoparticles-in-dual-gels were successfully developed for a month-long therapy of corneal wound healing and repair.
- Further investigations are reasonable to evaluate the in vivo performance, biocompatibility, and therapeutic efficacy of the GelPol formulation in relevant animal models.

References

1. Ocul. Surf. 19 (2021) 290–306. https://doi.org/10.1016/j.jtos.2020.10.006.

2. Surv. Ophthalmol. 52 (2007) 375–396. https://doi.org/10.1016/j.survophthal.2007.04.008.

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