COLLEGE OF PHARMACY
The UNIVERSITY of OKLAHOMA HEALTH SCIENCES

Development and *in vitro* Evaluation of Topical Multi-Drug Nanomicelles for Treatment of Corneal Injury

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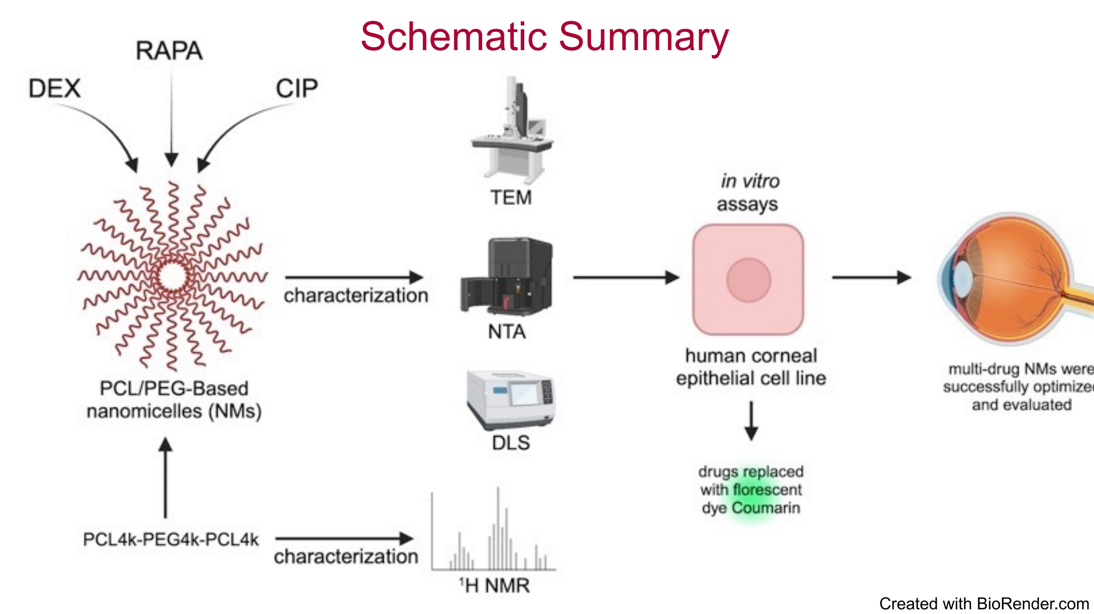
Introduction

- Corneal wound healing is critical in restoring corneal integrity and maintaining visual acuity after corneal injury. Severe injuries to the cornea have the potential to impair vision permanently and may only be treated via invasive means.[1] Therefore, this investigation has the potential to provide an effective and non-invasive treatment to regenerate corneal tissue and promote wound healing without scarring, inflammation, infection, or opacity.
- The chosen drugs and their delivery system were based on the potential for infection, inflammation, and neovascularization following corneal injury. A novel, topical, cornea-targeted drug delivery system comprised of PCL/PEG-based nanomicelles (NMs) encapsulating anti-infective, anti-inflammatory, and anti-neovascular drugs was designed to treat corneal wounds.



Objectives

- To develop amphiphilic NMs, a novel approach that can increase drug penetration, bioavailability, and the NM's ability to reach the target site.
- To characterize and evaluate the biocompatibility of PCL/PEG-based NMs loaded with dexamethasone (DEX), rapamycin (RAPA), and ciprofloxacin (CIP) for corneal treatment.

Methods

- The five different arrangements and molecular weights of block copolymers were screened for nanomicelle preparation, and PCL4k-PEG4k-PCL4k was selected for further studies based on size and loading.
- The physiochemical parameters of the three drug-loaded NMs were subjected to a comprehensive characterization process. Surface morphology, size, entrapment, and loading efficiency were studied using DLS, NTA, and TEM, and the results were further validated through *in vitro* release and kinetics studies.
- *In vitro* cell-based assays were performed on human corneal epithelial cells (HCECs) to evaluate the nano-formulation's biocompatibility. Cellular internalization was investigated *in vitro* by replacing the drugs with the fluorescent dye Coumarin.

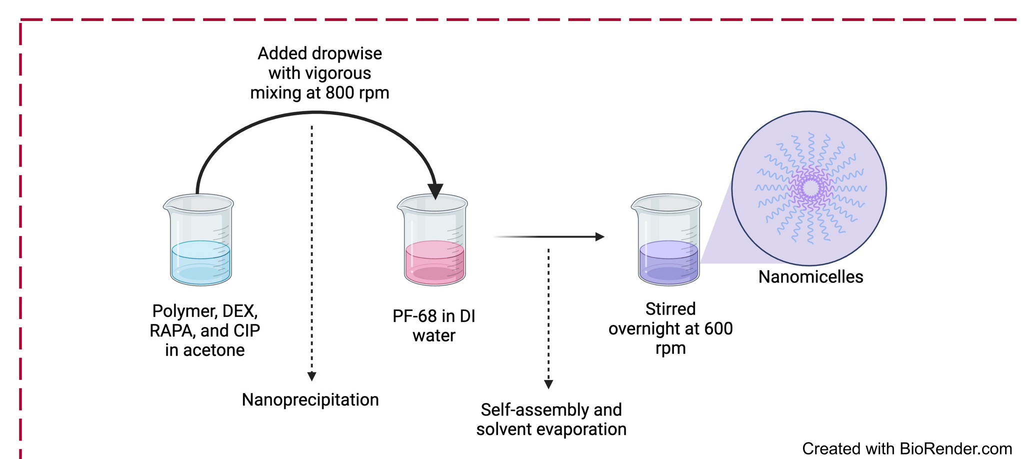


Figure 1. Schematic illustration of PCL-PEG-PCL-NMs preparation.

Results

Table 1. Screening of Block Copolymers

Polymer	Size (nm)	PDI
PCL-PEG-PCL (1000-1000-1000)	862±77.7	0.292±0.026
PCL-PEG-PCL (4674-4000-4674)	53.0±0.3	0.117±0.009
PCL-mPEG (5000-2000)	52.4±2.3	0.280±0.006
PLLA-PCL-PEG (1750-4674-4000)	169.1±1.7	0.306±0.008
PLA-PCL-PEG (1000-4617-4000)	78.0±1.9	0.184±0.004

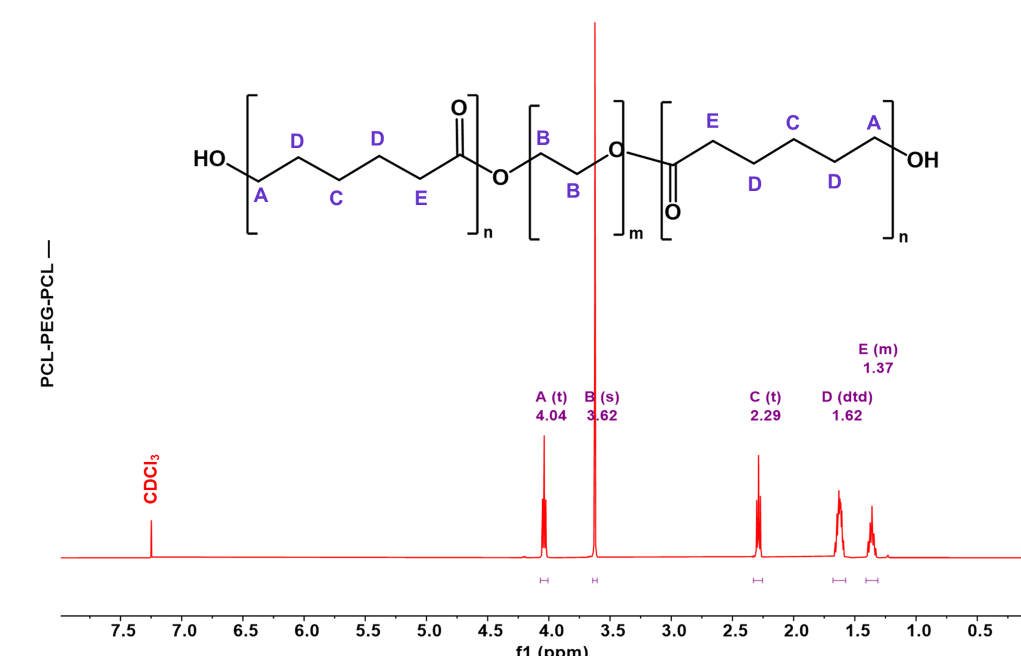


Figure 2. NMR Spectra of PCL-PEG-PCL.

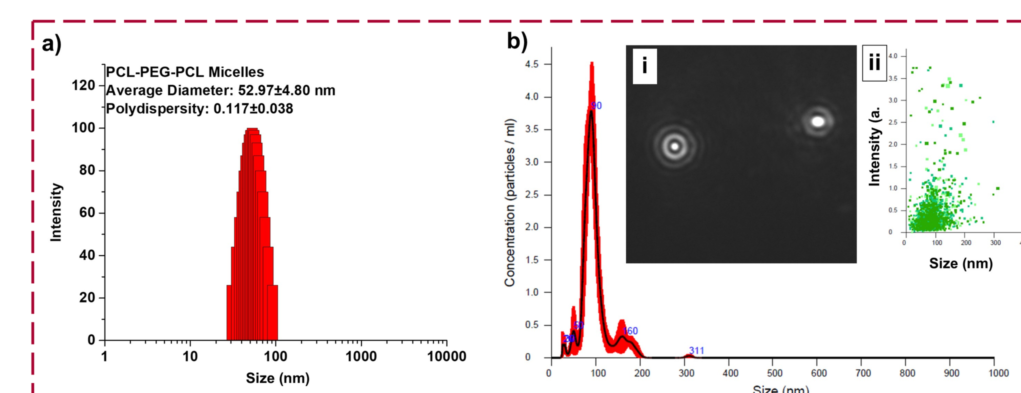


Figure 3. Characterization of blank NMs. (a) Dynamic light scattering graph of blank NMs. (b) Nanoparticle tracking analysis graph. (i) The captured image of NMs by the NTA camera, and (ii) the size distribution graph showing the size of individual NMs.

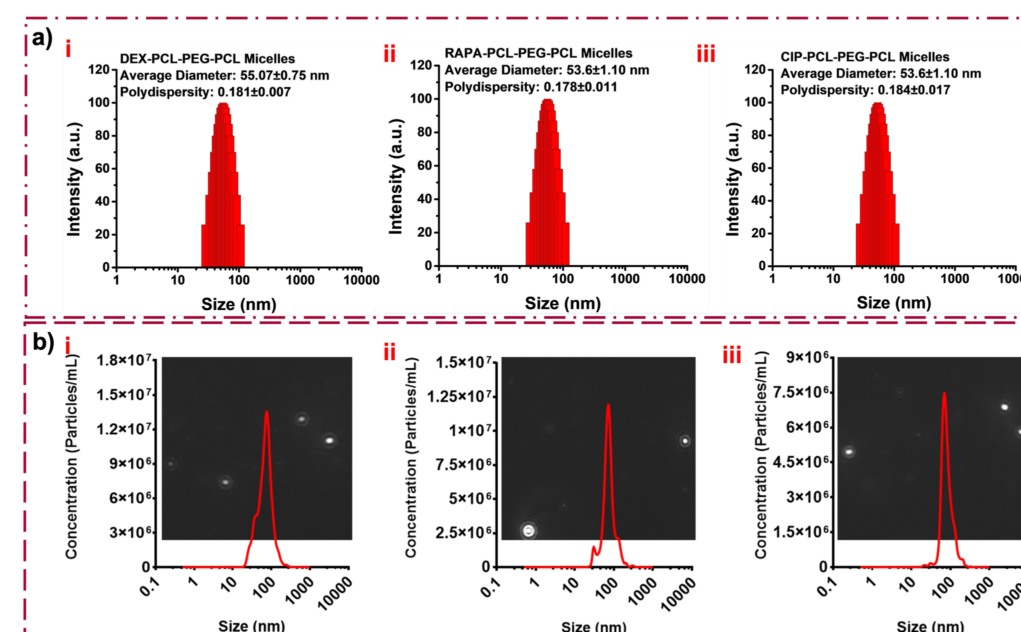


Figure 4. Characterization of drug-loaded NMs. (a) Dynamic light scattering graphs of (i) DEX-PCL-PEG-PCL-NMs, (ii) RAPA-PCL-PEG-PCL-NMs, and (iii) CIP-PCL-PEG-PCL-NMs. (b) Nanoparticle tracking analysis of (i) DEX-PCL-PEG-PCL-NMs, (ii) RAPA-PCL-PEG-PCL-NMs, and (iii) CIP-PCL-PEG-PCL-NMs.

Table 2. Characterization of DEX, RAPA, and CIP-loaded NMs.

Parameters	DEX-PCL-PEG-PCL- NMs	RAPA-PCL-PEG-PCL- NMs	CIP-PCL-PEG-PCL-NMs
Size (nm)	55.07±0.75	53.6±1.10	53.6±1.10
PDI	0.181±0.007	0.178±0.011	0.184±0.017
EE (%)	86.6±7.5	87.3±6.8	81.1±8.6
DL (%)	7.87±0.68	7.94±0.62	7.37±0.78

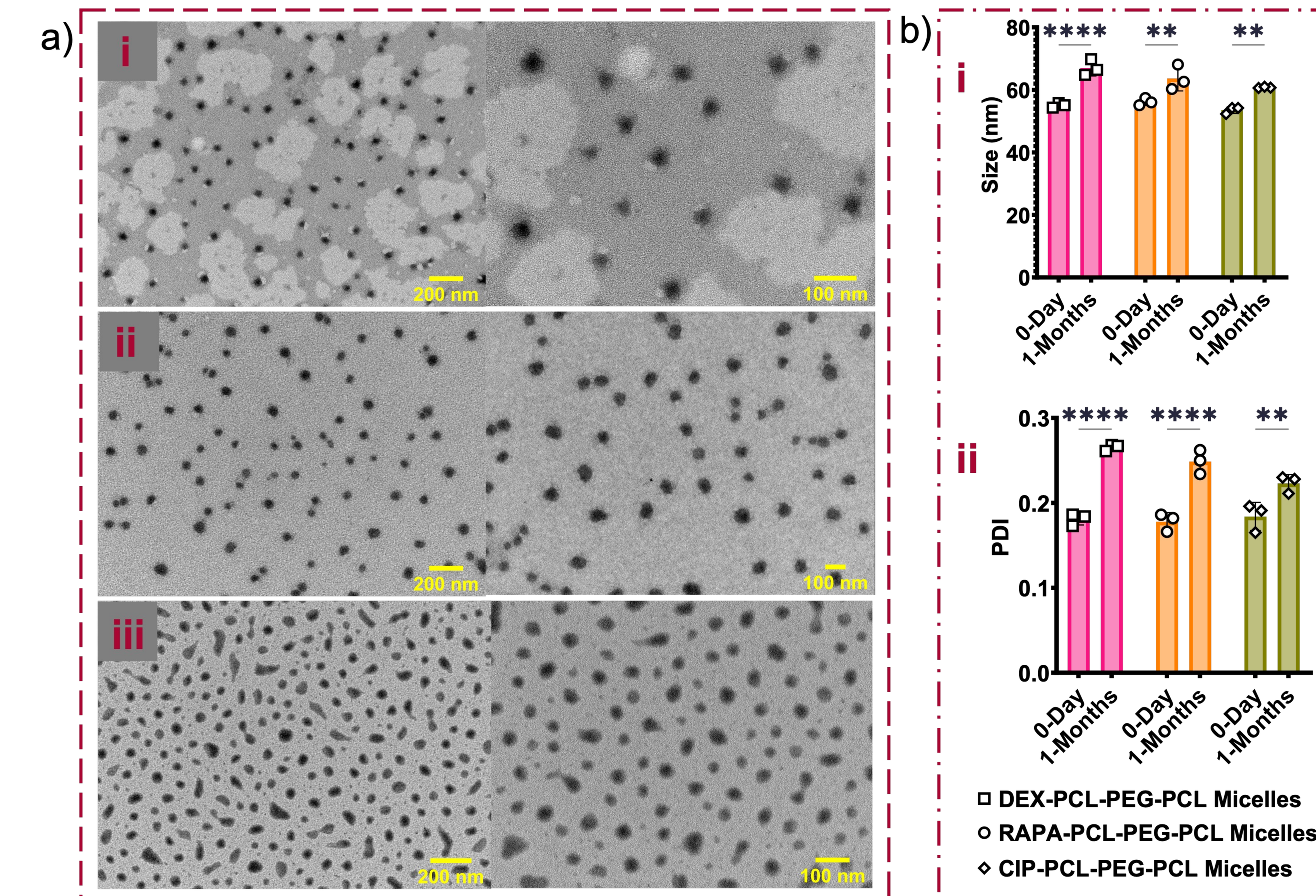


Figure 5. Morphology and stability of NMs. (a) TEM images of (i) DEX-PCL-PEG-PCL-NMs (images with scale bar 200 and 100 nm), (ii) RAPA-PCL-PEG-PCL-NMs (images with scale bar 200 and 100 nm), and (iii) CIP-PCL-PEG-PCL-NMs (images with scale bar 200 and 100 nm). (b) Stability of the synthesized NM preparations, (i) size (nm) versus time graph, and (ii) PDI versus time graph.

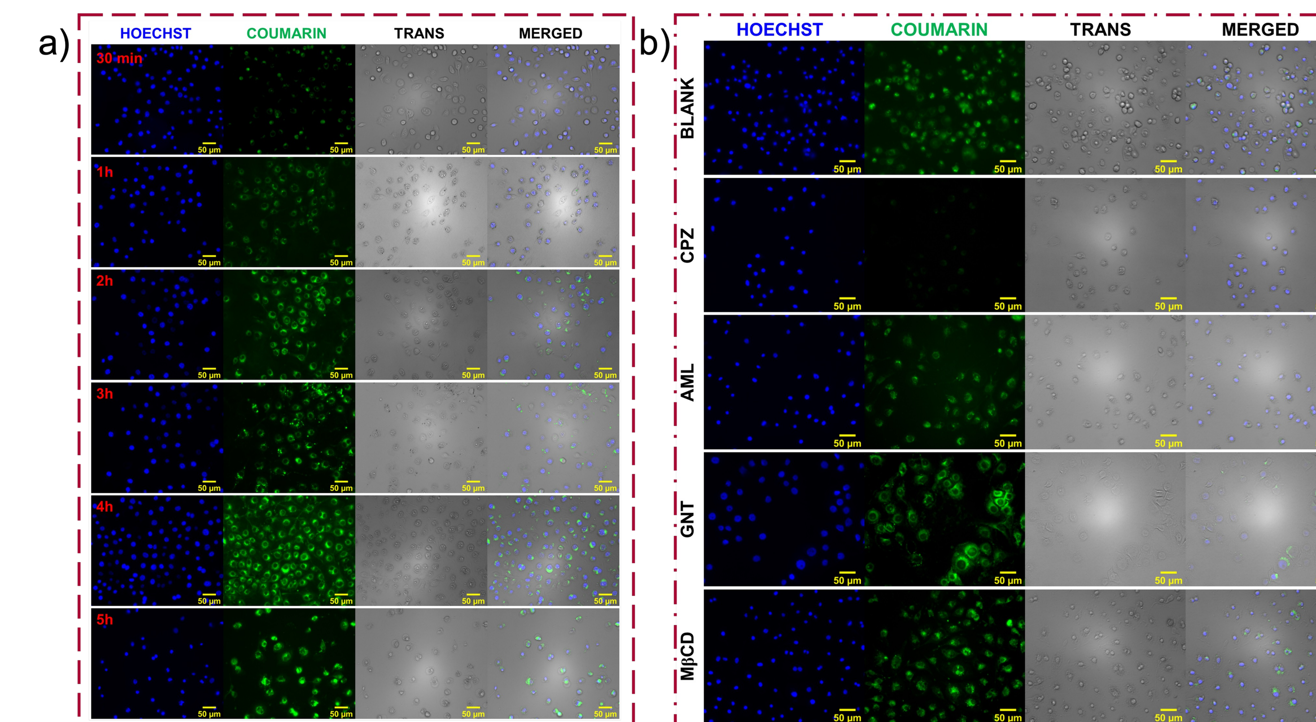


Figure 6. Cellular internalization study of PCL-PEG-PCL-NMs on HCECs. (a) Time-dependent cellular internalization of coumarin-6 (C-6) loaded PCL-PEG-PCL NMs from 30 minutes to five hours. (b) Internalization of C-6 loaded PCL-PEG-PCL-NMs in the presence of various pathway inhibitors. (CPZ; chlorpromazine, AML; amiloride, GNT; genistein, MβCD; Methyl-β-cyclodextrin). The study suggested there was enough cellular internalization observed within five hours of incubation, and that the most probable pathway for internalization is clathrin-dependent endocytosis.

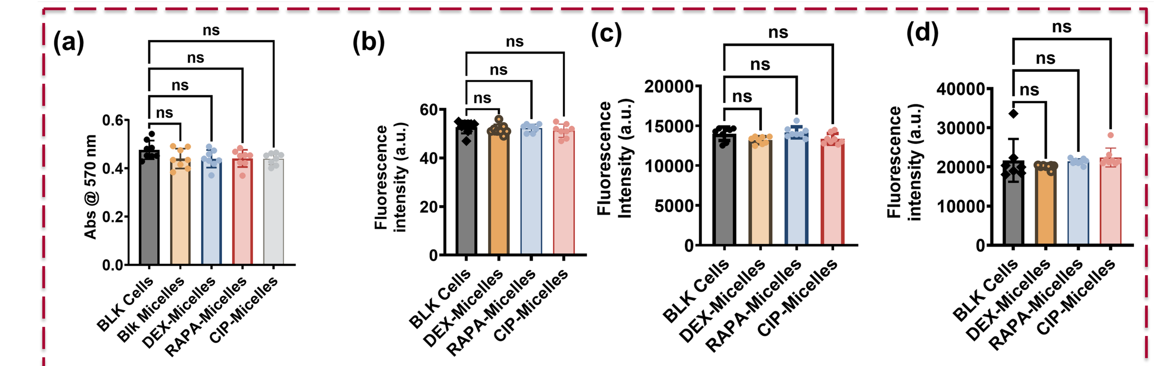


Figure 7. Cellular compatibility of the developed formulations. (a) The viability of HCECs after treatment with DEX, RAPA, and CIP-loaded NMs individually determined by MTT assay. No significant (p>0.05) changes in cell viability were observed. (b) Live-dead cell determination was conducted using propidium iodide (PI) nucleus staining. No significant (p>0.05) change in the PI staining was observed compared to blank cells, suggesting a significant quantity of surviving cells after treatment with the drug-loaded NMs. (c) Intracellular reactive oxygen species (ROS) generation in the HCECs after treatment with NMs was determined using DCFH-DA assay. No significant (p>0.05) changes in DCF fluorescence were observed compared to blank cells, which suggests negligible generation of intracellular ROS. (d) Apoptotic state was assessed using caspase 3/7 assay. No significant fluorescence associated with the caspase tag was observed suggesting that the cells did not undergo apoptosis after treatment with the NMs.

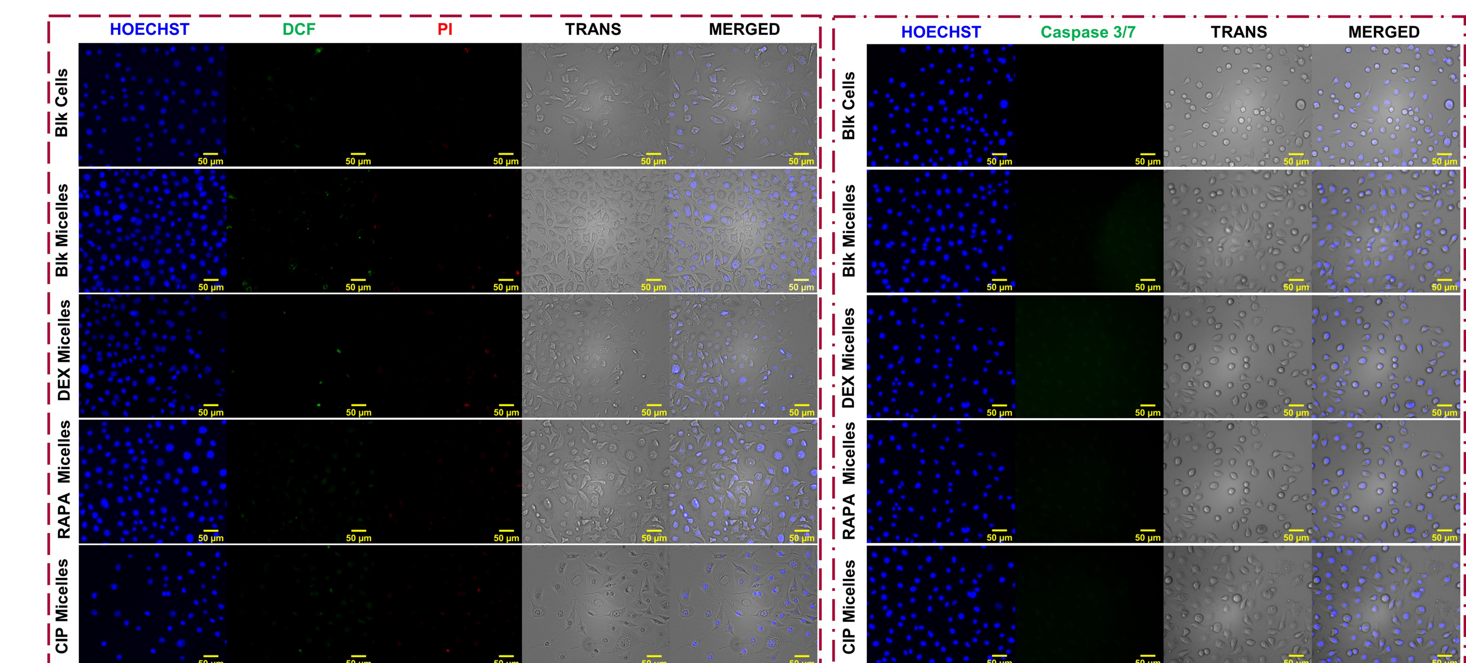


Figure 8. Fluorescence microscopy of treated and untreated cells stained with HOECHST, DCFH-DA, and PI. No significant DCF or PI fluorescence generation was observed in the untreated and treated cells, confirming that the developed NMs are highly biocompatible with the HCECs.

Figure 9. Fluorescence microscopy of HCECs treated with developed NMs and stained using caspase 3/7 marker to assess the apoptotic state. No significant fluorescence of caspase tag was observed in the microscopy images, confirming that most of the cells survived normally after treatment with drug-loaded NMs.

Conclusions

- PCL4k-PEG4k-PCL4k block copolymer was characterized by NMR. The multi-drug NMs were successfully optimized.
- The range of drug encapsulation in the NMs was about 80-90% for all three drugs. The NMs were biocompatible and evaluated for cellular uptake, which occurs via the endocytic pathway. The multi-drug NMs were evaluated for biocompatibility and cellular internalization on HCECs.

References

1. Exp Eye Res. 2020 Aug;197:108089. doi: 10.1016/j.exer.2020.108089. Epub 2020 Jun 15. PMID: 32553485; PMCID: PMC7483425.

Acknowledgments

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