Impact of Surface Charge on Toxicity, Permeability and Cellular Interaction of Biodegradable Nanogels

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Statement of Purpose: Nanocarriers show great promise for transporting drugs across biological barriers, reducing drug clearance and improving the bioavailability of drugs at the target. Over the years, extensive research has been done to functionalize the nanocarriers to construct systems that could deliver drugs more efficiently. Modification of the surface charge of nanoparticle is one of the factors along with size, composition, and shape that affects cellular interactions and the efficiency of drug loading and release. The objective of this work is to study the effects of the surface charges of nanogels on the toxicity, *in vitro* permeability and intercellular trafficking of the nanogels.

Methods: Thermoresponsive and biodegradable nanogels, based on Dextran-polycaprolactone-hydroxyethyl methacrylate (Dex-PCL-HEMA) macromer and N-isopropylacrylamide (NIPAAM) monomer were synthesized at 45 °C using Irgacure® 2959 as an UV initiator [1]. To render nanogels negatively, positively or not charged, 0 or 2%, 5%, or 10 mol % of acrylic acid or 2 amino-ethyl methacrylate with respect to NIPAAM was added to the reaction mixture. The nanoparticles were characterized with respect to size, zeta-potential, yield. The 24 h cytoxicity of nanoparticles synthesized with different charges was assessed in ARPE-19 cells by an MTT assay at concentrations of 0, 0.1, 0.2, 0.5, 1, 2, 5 and 10 mg/mL. The *in vitro* permeability of Fluorescein isothiocyanate (FITC) labeled nanogels across the ARPE-19 cell membrane on a transwell plate and the sclera and cornea of 10 days old piglets placed in the spherical joint of a Valia-Chien Cell apparatus at a concentration of 1 mg/mL and 37 °C/32 °C, respectively, was investigated. FITC labeled 4k-dextran was used as a control. ARPE-19 cells were incubated with 1 mg/mL of FITC-labeled nanogels for 2 h. Hoechst 33342 was used to stain the nucleus of ARPE-19 cells and 4% paraformaldehyde was used to fix the cells. After washing several times with PBS, the cells were analyzed via Leica DMi8 microscope and SH800 Cell Sorter to observe cellular interaction.

Results: The nanogels showed a z-average diameter of 50-300 nm, zeta potential of -10-10 mV depending on their surface charges. MTT assay results showed that the nanogels were not toxic to ARPE-19 cells at concentration up to 10 mg/mL, 2 mg/mL, 1 mg/mL for 10% AA, bland and 10% AM nanogels, respectively. The nanogels with 10% acrylic acid were more permeable than 4k-dextran across the ARPE-19 cell membrane and the porcine sclera and cornea. The surface charges of the nanogels significantly affected the permeability of the nanogels across the *in vitro* ocular barriers. Immunofluorescence and flow cytometry data will need to be analyzed further to determine whether there is a direct correlation between the charge of nanogel and intracellular cell trafficking.

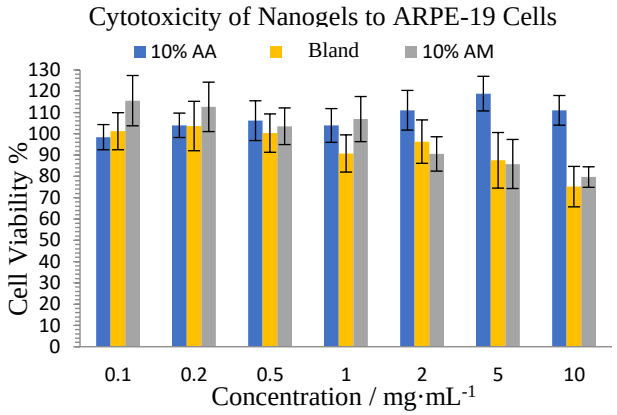

Figure 1. Cytotoxicity of 10% AA, 10% AM and bland nanogels to ARPE-19 cells at a series of concentration after 24 h of incubation (n = 4).

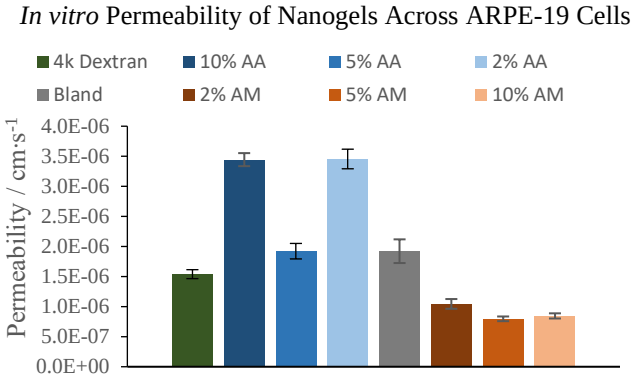

Figure 2. *In vitro* permeability of FITC-labeled 4 kDa, FITC-labeled 10% AA, 10% AM and bland nanogels across ARPE-19 cells (n = 4).

Conclusion: The functionalized thermoresponsive and biodegradable nanogel system was successfully synthesized and the surface charges played an important role in the size, zeta potential, toxicity, permeability, and cellular interactions. Completion of this project will have a significant impact on constructing an ideal drug delivery system for delivering various drugs to ocular tissues.

Reference:

[1] X. Huang, T.L. Lowe, Biodegradable Thermoresponsive Hydrogels for Aqueous Encapsulation and Controlled Release of Hydrophilic Model Drugs, Biomacromolecules, 6 (2005) 2131-2139.