Amphiphilic Dendrimer Hydrogels for Enhanced Antiglaucoma Drug Delivery

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Statement of Purpose: For incurable chronic ocular diseases, such as glaucoma, patients must apply therapeutic drug solutions up to 3 times daily. Considering lifelong treatment, such dosing regimens are staggering. With the advancement of biomaterial sciences, traditional approaches to ocular drug delivery have evolved with aims to increase patient compliance by reducing dosing frequency. Gel formulations, with improved mucoadhesive and absorption properties, promise to provide sustained therapeutic efficacy via controlled release and enhanced drug adsorption. Recently, we synthesized a novel class of hydrogel, namely polyamidoamine (PAMAM) dendrimer hydrogels, which possesses a number of hydrophobic dendrimer cores and a hydrophilic PEG network.[1] This new platform uniquely integrates the properties of nanoparticles and hydrogels. The objective of this project was to examine amphiphilic dendrimer hydrogels for antiglaucoma drug delivery.

Methods: *Gel preparation:* Dendrimer hydrogels comprising cross-linked PAMAM dendrimer G3.0 coupled with PEG diol (12000 $g \cdot mol^{-1}$) were synthesized according to our previous work.
Drug solubility test: To examine the solubility of the hydrophobic antiglaucoma drug brimonidine, an excess of drug was added to PBS containing non-cross linked G3.0-PEG acrylate at 7.5% w/w. Sample solutions were vortexed, equilibrated overnight, centrifuged to remove undissolved drug particles, and then subjected to UV-Vis spectrophotometry at 248 nm for quantification of drug concentrations.
Toxicity assay: The cytotoxicity of dendrimer hydrogels to human corneal epithelial cells (HCECs) following 24 h-incubation was assessed using the MTT assay.
Drug release kinetics: Hydrophobic brimonidine was loaded into dendrimer hydrogel alone or with hydrophilic timolol. The in vitro release kinetics of these two drugs in pH 7.4 PBS at room temperature was determined.
Cell uptake studies: Cellular uptake studies were performed using 0.5% timolol maleate and 0.1% brimonidine within a hydrogel solution (7.5% w/w). PBS solution containing 0.5% timolol maleate and 0.1% brimonidine was used as control. HCECs were incubated with gel formulation or PBS solution for 1 hr. The drug contents inside the cells were determined via HPLC-MS.
Results: Detailed characterization and discussion of dendrimer hydrogels can be found in our previous publication.[1] It was observed that uncross-linked 7.5% (w/v) G3.0-PEG-Acrylate increased the solubility of the hydrophobic drug brimonidine by 77.5% as compared to PBS solution. The MTT assay showed HCECs remained nearly 100% viability after overnight incubation with dendrimer hydrogels. In vitro drug release kinetics demonstrated that gel formulations of timolol maleate and brimonidine were released for 2.5 days and 3 days respectively compared to 1.5 hours for co-drugs in PBS. A 7-day sustained release of brimonidine was achieved when dendrimer hydrogel was formulated to contain 7-doses (Figure 1). Through HPLC-MS analysis, HCECs were shown to uptake 76% more brimonidine and 69% more timolol by hydrogel formulation versus PBS solution.

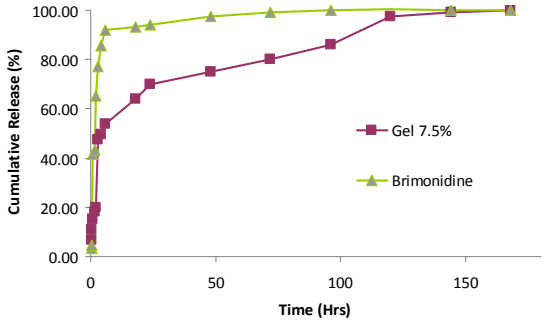

Figure 1. Sustained release of brimonidine over 7 days.

Conclusions: This work has shown that the dendrimer hydrogel has good biocompatibility with HCECs. A sustained release profile of drug molecules from the hydrogel was achieved. In HCEC uptake studies, increased drug uptake with the hydrogel was attributed to the hydrogel adsorption to cell surfaces. These findings validate either improved cellular uptake and/or sustained delivery due to mucoadhesiveness of the gel. Mucoadhesiveness leads to longer drug residence time, increased absorption of drugs, and decreased dosing. This preliminary *in vitro* work confirms incorporation of dendrimers with PEG to form a crosslinked network which improves the properties of two antiglaucoma drug molecules. In the near future, *in vivo* rabbit studies will be planned for study of sustained efficacy of dendrimer hydrogel drug formulations.
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References:
1.Desai, P. N.; Yuan, Q.; Yang, H., Synthesis and characterization of photocurable polyamidoamine dendrimer hydrogels as a versatile platform for tissue engineering and drug delivery. *Biomacromolecules* **2010,** 11 (3), 666-73.