Developing Non-viral Gene Delivery System For Intestinal Mucosal Tissue

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Background: Gene delivery to intestinal mucosa would enable treatment of local disorders such as IBD and colon cancer effectively. Successful mucous-penetrating delivery system should carry contradictory surface properties, where negative charge aids to avoid entrapment in mucous; positive charge promotes cellular uptake by epithelial cells. Different strategies have been implemented to solve this charge dilemma including; coating particles surface with densely positive and negative charge group, coating with hydrophilic or neutral muco – inert polymer and zetapotential changing systems. In this project, cationic peptide nanoparticles of glycosaminoglycan binding enhanced transduction (GET) system for non-viral gene therapy were coated with hydrophilic co-polymer of hydroxyethyl acrylamide and β-alanine acrylamide (pHEA) to enhance the mucous penetration and achieve improved gene delivery to intestinal In vitro model then delivery of IL-10 vector for treatment of IBD.

Methods: Nanoparticles formed by mixing of GET peptides and pDNA at volume ratio 1:1, then coated with different concentration of pHEA polymer at ratio of 1:2. The formed nanoparticles were characterized with DLS and encapsulation of pDNA were determined with YOPRO-1 assay. The luciferase expression of encoded GLuc reporter gene were tested on co-culture model of Caco2 and HT29-MTX cells. The mucous diffusion of the nanoparticles were investigated using stomach mucin suspension by quantitative measurement of fluorescently labelled nanoparticles penetration across Transwell. Also microfluidic slide filled with mucin were optimized for mucous diffusion measurement as well as diffusion on mucous producing cells.

Results: Coating DNA/GET nanoparticles with pHEA derivative B and C with ratio of 10 and 20 of β-alanine acrylamide respectively, generated negatively charged nanoparticles with size range between 200-500nm and zetapotential between (+16mV – -18mV). These nanoparticles were able to increase the transfection efficiency of luciferase reporter gene with 2 folds compared to uncoated nanoparticles on intestinal model of differentiated Caco2 cells and mucous producing cells (HT29-MTX). Although the coated particles did not show significant enhancement of Rhodamine labelled GLuc uptake but generally the cellular uptake of differentiated cells is low even with Lipofectamine 2000 and PEGylated nanoparticles. The coated particles were able to protect the encapsulated DNA from Dnase enzyme (up to 0.05U/µl) compared to uncoated particles when run on agarose gel and maintain the size and charge over storage at 4°C for 3 weeks. The mucous diffusion tested on Transwell showed that coated nanoparticles diffuse quicker with 47% relative nanoparticles diffusion after 4hrs compared to 35% of uncoated nanoparticles and similar to 40% PEGylated nanoparticles (42%). Also the confocal images of nanoparticles diffusion on mucous producing cells showed less entrapment of coated nanoparticles on the mucous layer compared to uncoated nanoparticles.

Conclusions: GET peptide system can be used as non – viral gene delivery system for intestinal mucosal model, and coating with pHEA derivative generates negatively charged nanoparticles capable of enhancing the transfection and mucous penetration as well as protecting the encapsulated DNA from Dnase activity. So pHEA polymer can be used as an alternative to PEGylation and can be used for gene delivery for treatment of local disorder such as IL-10 vector for IBD.