

## Elucidating the Fate of Polymer-DNA Nanoparticles in Oral Gene Delivery

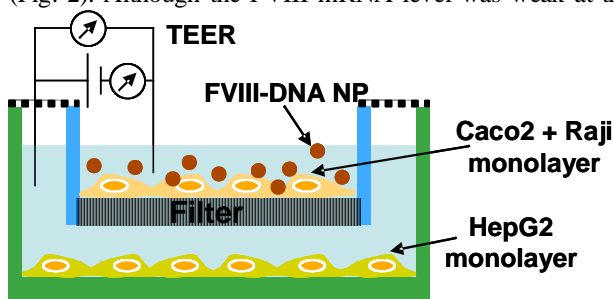
Nonviral gene delivery systems face fewer challenges than viral vectors in addressing pharmaceutical issues, such as scale-up, storage stability, and quality control. However, the gene expression level achievable by non-viral gene transfer is low and transient. For gene-based therapeutics against a disease like hemophilia, where a high expression level and persistence are desired, frequent administration would be required. Intravenous or intramuscular injection, being invasive, is unattractive and may not be better than protein replacement therapy. Oral delivery, being patient-compliant and economic, is the best administration route that can render such gene therapy practical.

Oral gene delivery for systemic medication has been perceived as impossible because of the belief that the low pH of the stomach and the harsh enzymatic environment of the gastrointestinal (GI) tract would prevent any significant gene transfer to produce systemic gene products. We have demonstrated that oral administration of DNA-nanoparticles synthesized by complexing plasmid DNA with chitosan, a natural, biocompatible polysaccharide, could lead to gene expression in the intestinal epithelium. However, the transfection efficiency has been low. We have previously applied nanobiophotonics, using quantum-dot-FRET technology, to identify and investigate the rate-limiting barriers in nonviral gene transfer by nanoparticles or nanocomplexes (NP). In the past year, we have evaluated the fate of these quantum-dot-labeled nanoparticles in a closed loop intestinal rat model. In anticipation of collaborating with Dr. James Lee's laboratory where homogeneous and well-controlled DNA nanoparticles can be synthesized by microfluidics-based electrodynamic focusing, we aspire to advance the concept of oral gene medicine with the findings in these studies.

### Transport of DNA Nanoparticles Across Caco2-Lymphocyte Monolayer

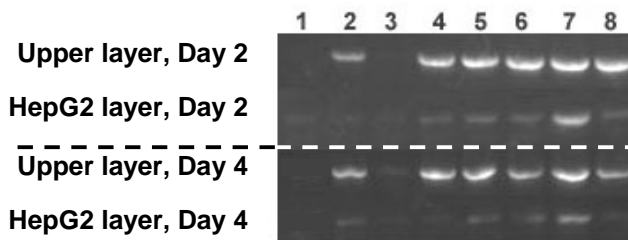
To prepare for a systematic study of gene carrier design for oral delivery, we have studied the transport of nanoparticles across a Caco-2-Raji cell monolayer. The human Caco2-Raji co-culture model produces a cellular layer that is morphologically and functionally similar to M-cells. The model can transport and transcytose both Fluospheres (0.2  $\mu\text{m}$ ) and chitosan microspheres without affecting the integrity of the monolayer. We examined the incubation of chitosan-FVIII DNA nanoparticles (390 KDa MW and 84% DA chitosan) and other PPA nanoparticles in the Caco2-Raji system (Fig. 1). The PPA gene carriers had a dipropylamine side chain (PPA-DPA) or a PEG-PPA-DPA (PEG-*b*-PPA) structure. Caco-2-Raji cells and HepG2 cells were cultured in the top and bottom chambers of the Transwell culture plates, respectively. As an attempt to simulate the scenario of DNA nanoparticles being taken up through the gut

epithelium and subsequently reaching the liver, we investigated the feasibility of the FVIII DNA nanoparticles penetrating the human intestinal model layer to transfect the underlying HepG2 cells. After confirming the integrity of the monolayer using TEER measurement after 14 days of culture, FVIII DNA nanoparticles (1  $\mu\text{g}$  of DNA) were added to the upper chamber of the Transwell insert. At days 2 and 4 post-transfection, 50 ng of total mRNA harvested from both top and bottom cell monolayers were used to run RT-PCR (45 cycles). Consistent with other studies of drug absorption through the GI tract, transport of DNA nanoparticles through the cell monolayer appears to be enhanced in the Caco2-Raji model (L2 vs. L4; L5 vs. L7) (Fig. 2). Although the FVIII mRNA level was weak at the



**Figure 1.** Schematic of transfection experiment in the Caco2-Raji model.

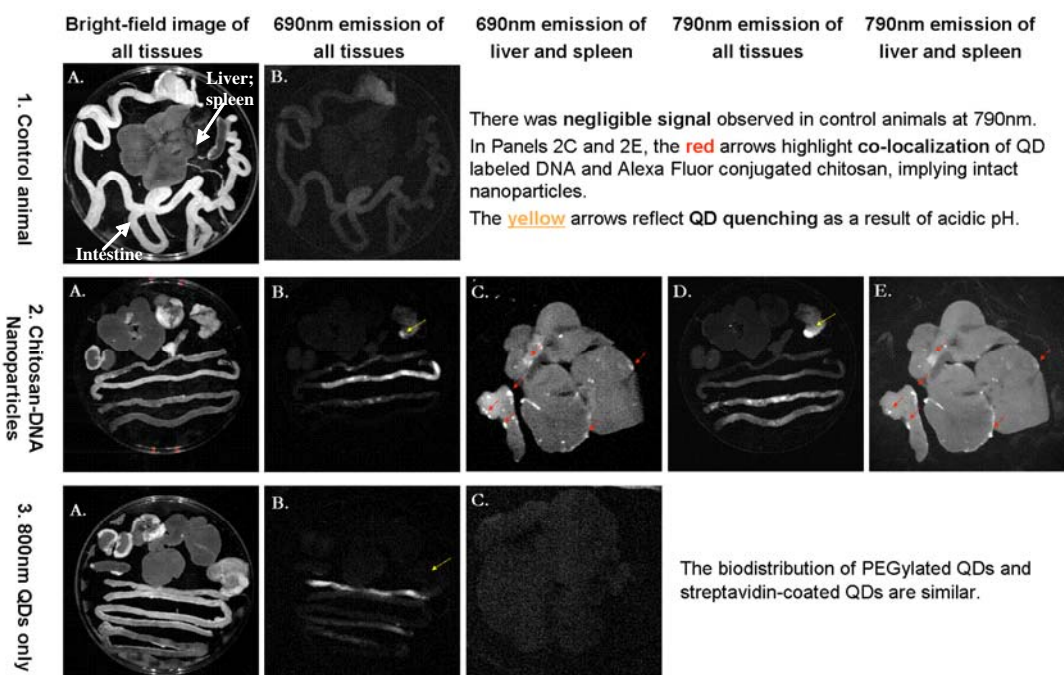
day 2 time point (L4), the detectable expression in the bottom HepG2 cells in three separate experiments was reproducible. Interestingly and unexpectedly, the PPA-DNA nanoparticles performed better than the chitosan DNA



**Figure 2.** FVIII mRNA expression in upper monolayer and bottom HepG2 cell layers, at Day 2 and 4 post-transfection. Amount of beta-actin mRNA was comparable across all samples (data not shown).

Lane # : sample ID

- 1 : Caco2 monolayer, Control
- 2 : Caco2 monolayer, + Chitosan/hFVIII NP
- 3 : Caco2-Raji monolayer, Control
- 4 : Caco2-Raji monolayer, + Chitosan/hFVIII NP
- 5 : Caco2 monolayer, + PPA-DPA/hFVIII NP
- 6 : Caco2 monolayer, + PEG-*b*-PPA/hFVIII NP
- 7 : Caco2-Raji monolayer, + PPA-DPA/hFVIII NP
- 8 : Caco2-Raji monolayer, + PEG-*b*-PPA/hFVIII NP



**Figure 3.** Ex vivo imaging of chitosan DNA nanoparticles (quantum dot-labeled DNA, Alexa Fluor-labeled chitosan) delivered to the closed intestinal loop of rats. Panel 1: Controls, in which GIT of animals was only flushed with saline. Very low background signals were observed at 690nm and 790nm; Panel 2: Animals to which chitosan-DNA nanoparticles were administered. Co-localization of 690nm and 790nm signals was observed in the liver and the spleen, suggesting presence of intact nanoparticles in these tissues; Panel 3: Animals to which only “naked” 800nm QDs were administered. Signals were observed only in GI tract but not liver and spleen, indicating that the QD did not enter hepatic circulation and were stable in the lumen of GIT.

nanoparticles (L4 vs. L7). Even at day 4, the mRNA level was significantly above background (L7). These results show an exciting proof of concept.

### Biodistribution of Chitosan-DNA nanoparticles in a closed intestinal loop rat model

The nanoparticle suspension was injected into the relevant compartments, 2mL into the stomach and 1mL each in the duodenum, jejunum and ileum. 4h post-administration of the nanoparticles, the animals were euthanized, and different tissues excised for *ex vivo* imaging in a Kodak In Vivo Imaging System. The AlexaFluor680 labeled chitosan was visualized under 630nm excitation/690nm emission. The QD-labeled DNA was visualized under 495nm excitation/790nm emission.

		690 nm Emission (Alexa Fluor 680-conjugated chitosan)	
		+	-
790 nm Emission (800nm QD labeled DNA)	+	Co-localization of signals reflects intact nanoparticles	Nanoparticles dissociated
	-	QD signal quenched, or nanoparticles have dissociated	Components of nanoparticle have been completely degraded

Signals observed at different excitation and emission wavelengths reveal the different states of the nanoparticles,

as shown in Table 1. Most importantly, the co-localization of the two signals enabled us to ascertain that the nanoparticle did not dissociate *in vivo*, and that the DNA did not travel independently to the liver following uptake.

Results shown in Figure 3 indicate that intact chitosan-DNA nanoparticles were taken up by the small intestine and transported to the liver. Presumably, chitosan was able to protect the DNA and facilitated the opening of tight junctions in the epithelium, as suggested by other work in the literature. Noteworthy is that quantum dots, by themselves, were not transported to the liver or the spleen. Nanoscale size alone is therefore insufficient for efficient absorption.

### Publications

1. Kadiyala I, Loo Y, Rice J, Roy K, and Leong KW. *Physicochemical properties modulating the transport of Chitosan-DNA nanospheres in human intestinal M-cell model versus normal intestinal enterocytes*. Acta Biomaterialia, submitted.
2. Loo Y and Leong KW. *Elucidating the fate of Polymer-DNA Nanoparticles in Oral Gene Delivery*. Cold Spring Harbor Conference: In Vivo Barriers to Gene Delivery, New York, 2007.