Gut organoids as a platform for evaluating delivery of nanoparticles to treat inflammatory bowel disease

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Statement of Purpose: The need for continual administration of 5-aminosalicylic acid (5-ASA) at a high dosage to suppress inflammation in inflammatory bowel disease (IBD) patients results in active side effects. Nanoparticles (NPs) can provide a timely release while keeping tissue safe. However, their exact mechanism of entrapment and release is mostly unknown *in vivo*. The following research proposes gut organoids as an *ex vivo* platform to evaluate NPs absorption, distribution, metabolism, and excretion (ADME) screening by gut epithelial cells while focusing on the effect of particle charges. Poly lactic-co-glycolic acid (PLGA) NPs loaded with 5-ASA have been coated with two different surfactants with a negative and positive charge, and the organoid entrapment was compared.

Methods: 0%, 2.5%, 5% and 7.5% w/w of 5-ASA (Sigma Aldrich) loaded PLGA (Evonik Industries) NPs were prepared using single oil-in-water (o/w) emulsion/solvent evaporation method.[1] Organoids were isolated from the small intestine of C3H/HeN wild-type mouse models, as previously stated.[2] NPs sizes and zeta potential were recorded by zeta sizer (Nano-Zs 90). NPs' interaction with organoids was recorded by optical microscopy (Leica DMi1). Using confocal fluorescent microscopy (Olympus IX2), 2.5% Rhodamine B (Acros)-loaded nanoparticles coated with alginate (negatively charged polymer) or chitosan (positively charged polymer) were mixed with cell suspension and Matrigel and tracked over time. Live/Dead cytotoxicity was performed to check the harmful effects of NP on organoids. To confirm NP's presence in the organoid's lumen, organoids were fixed and detected with laser microscope (Olympus FV1000).

Results: The particle sizes were approximately 250 nm and 350 nm for alginate coated and chitosan-coated samples, respectively. It was confirmed that the size of the NPs was not changed by increasing the ratio of 5-ASA in the system. The negative charge of alginate coated NP and the chitosan-coated NP's positive charge were confirmed using zeta sizer. Much like the control sample, none of the samples containing NP showed unusual growth behavior after 6 days. No considerable harmful NPs or entrapped 5-ASA on organoids were observed after 7 days using quantitative optical images and live/dead cytotoxicity experiments. Particles coated with chitosan got encapsulated inside the lumen more effectively than alginate coated NPs (Figure 1). NP absorption was at its maximum level on day-4, and it was reported higher quantitatively in chitosan-coated samples. Cell fixation showed that organoids' structure was untouched and, by removing the free Rhodamine B from the system, confirmed higher encapsulation for chitosan-coated NP inside the lumen compared to the alginate coated NP (Figure 2).

Conclusions: It was shown that organoids might entrap the positively charged NP better than negatively charged NPs. It was assumed that epithelial cells have more affinity to positively charged particles due to the interaction with negatively charged glycoproteins. Confirmation of higher entrapment of 5-ASA loaded positively charged NP by epithelial cells *ex vivo* is a promising finding in producing a reliable and unique delivery system for IBD treatment.

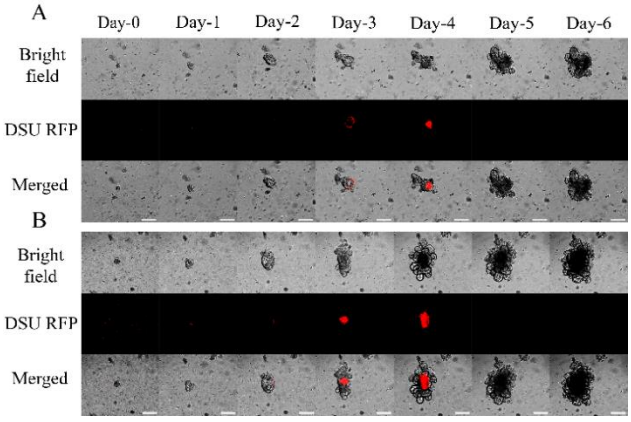

Figure 1. The mixture of 10 µl 2.5% Rhodamine B loaded nanoparticles made by A) alginate surfactant or B) chitosan surfactant, matrigel, and organoids using a confocal fluorescent microscope (1:4 V/V Nanoparticle: Matrigel suspension). Red areas are the indicators of the Rhodamine B loaded inside the PLGA nanoparticles. The magnification is 10X. Scale bars represent 200 µm.

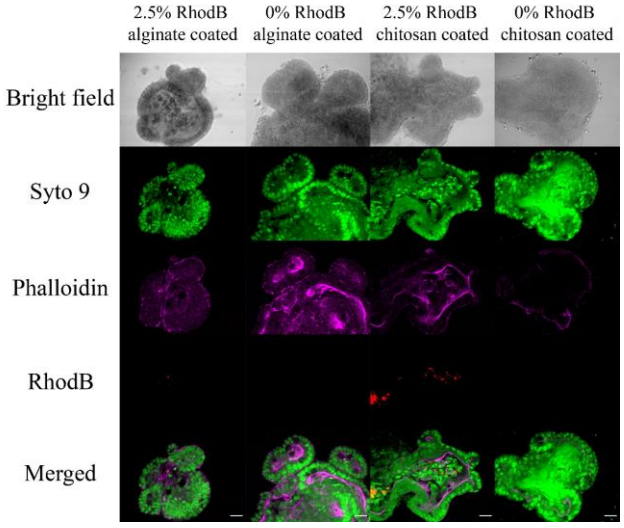

Figure 2. Fixing organoids after adding 2.5% loaded PLGA nanoparticles coated with alginate and chitosan (1:4 Nanoparticle: Matrigel). Green represents SYTO™ 9, magenta represents phalloidin, and red represents Rhodamine B. The magnification of images is 40 X with digital zoom of 1.8 and scale bars represents 20 µm.

References:

[1]Wang Q, et al. Biomaterials. 2010;31: 4980-6.
[2]Davoudi Z, et al. JBMR A. 2018;106: 876-86.