ABSTRACT SUMMARY
A multistage nanodelivery vehicle was developed by loading of budesonide containing poly(lactic-co-glycolic acid) (PLGA) nano-particles into silicon nanoporous microparticles. The combination of micro- and nanoparticles utilizes each of the strengths of each component to be employed in oral drug delivery to inflammatory bowel disease (IBD). Drug efficiency was tested in a recently reported by us in vitro model of IBD.

INTRODUCTION
Although being the most frequently used drug formulation due to its convenience and good compliance, oral drugs for controlled delivery still have several tough challenges due to the nature of pH changes, active enzymes and interaction with resident microorganism along the gastrointestinal tract (GI).

Previous research data have shown promising results with nanoparticle formulations for oral therapeutics of IBD [1], especially for increasing drug local concentration at the site of action. However, to reach the target site, the nanoparticles have to overcome the pH gradients, and enzymatic activities in the stomach as well as physical and mechanical barrier in the intestine.

To sequentially overcome the biophysical barriers present in gastrointestinal system, we have designed a multistage system of PLGA nanoparticles loaded in porous micro-sized silicon particles reported earlier from our lab for intravenous administration[2]. In this study, we modify the application for oral delivery, making use of stability of silicon in low pH environment and its ability to degrade in neutral and basic pH, the microparticles, thus may protect the loaded nanoparticles from degradation by gastric fluid.

EXPERIMENTAL METHODS
Nanoparticles (NPs) were prepared by nano-precipitation method, PLGA and budesonide were dissolved in acetone and injected into water with 1% Poloxamer under constant stirring, acetone were evaporated overnight and the excess budesonide was washed with Vivaflow 50 filtration system. NPs were lyophilized before loading into microparticles. 1 mg of lyophilized NPs were resuspended in 40 µl PB buffer, a 10 µl aliquot was pipeted to eppendorf tube containing silicon microparticles. After thorough mixing, particles were incubated at room temperature overnight and excess NPs were removed by centrifugation.

For silicon particle degradation study, different systems, each containing 10^7 particles was resuspended in pH 1.8 simulated gastric fluid for 1 h. The buffer was replaced with pH 7.4 HBSS buffer to simulate the duodenum and jejunum pH condition for 4 h and pH of the solution was readjusted with HCl to pH 6.8, mimicking the ileum and colon pH values. Samples were taken at predetermined time points and analyzed with scanning electron microscope (SEM) and Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES).

For IBD in vitro model, macrophages and dendritic cells differentiated from peripheral blood mononuclear cells were embedded in collagen layer on transwell filter and cocultured with Caco-2 cells (Fig. 1) for 21 days before stimulated with IL-1ß for the experiment.

Figure 1. Graphical illustration of in vitro model of inflamed intestinal epithelium.

Multistage particles were added into the apical side of the in vitro model and incubated for 4 hours before washed and cultured further. Transepithelial electrical resistances were monitored and samples were taken every 24 hours for cytokine analysis.
RESULTS AND DISCUSSION

PLGA nanoparticles were successfully associated with silicon microparticles (Fig. 2(A)), causing additional reduction of silicon degradation of the multi-stage system in neutral and acidic pH environment (data not shown).

![Figure 2. (A) Scanning Electron Microscope images of APTES-modified silicon microporous particle (APTES-SMP), budesonide-PLGA nanoparticles and the loaded multistage vector (MSV). (B) Interleukin-1 beta (IL-1b) release in the coculture after 2 and 48 hours of treatment with various budesonide formulations. NP concentration was normalized to amount contained in 5 MSV/cell.](image)

This enables increased drug availability in the site of target, as the PLGA NPs are not acid resistant and therefore mostly digested in the stomach. For the amount of MSV that may reach the target site of inflamed intestine, the efficacy was analyzed via in vitro model that consist of epithelial cells and immunological compounds such as macrophages and dendritic cells to enhance the immune response. Resulting cytokine release was measured and the effect of the drug to interleukin-8 (IL-8) release reduction. The loading of budesonide NPs did not reduce the anti-inflammatory efficiency, as shown in Fig. 2(B), cells treated with the MSV formulation showed comparable, if not slightly more pronounced effect of IL-8 reduction to plain budesonide NPs. Additional reduction of IL-8 production has also been observed in the cells treated with higher MSV number per cell.

CONCLUSION

The multistage vector for oral delivery of budesonide was successfully constructed and shown to synergistically protect the system during the transport in various GI components, while enabling higher anti-inflammatory efficacy as compared to PLGA-NPs. We anticipate that higher drug concentration at the target site as well as a gradual release of budesonide, can be beneficial for IBD treatment. Animal experiments to validate the results and to evaluate in vivo toxicity of the formulation are underway.

REFERENCES


ACKNOWLEDGMENTS

We kindly thank the NCI Physical Science and Oncology Center Program and The Methodist Hospital Research Institute for the Grant support 1-U54-CA143837-01 the opportunity to explore this exciting research. We also thank Helmholtz Institute for Pharmaceutical Research Saarland and Helmholtz Center for Infection Research for providing Euro-PhD Fellowship which support Fransisca Leonard to carry out this collaborative research in The Methodist Hospital Research Institute.