Swarm Communication of Enzyme-Powered Nanobots for Inflammatory Bowel Disease Therapy

Yewon Seo, Hyunsik Choi, and Sei Kwang Hahn* (skhanb@postech.ac.kr)

Department of Material Science & Engineering, Pohang University of Science and Technology (POSTECH), Korea

Abstract

Inspired by collective behaviors in nature, synthetic swarming systems have been proposed for biomedical applications. Enzymatic micro/nanobots are promising due to self-generated chemical signaling and complex enzyme interactions. We report a tandem communication system using glucose oxidase and catalase nanobots for treating inflammatory bowel disease. After characterizing each type, we verify in vitro swarm behavior and communication on microchips. In vivo, photoacoustic imaging shows effective navigation and retention in the colon after intrarectal injection. Moreover, we observe colon recovery in a murine model, with reduced proinflammatory and increased anti-inflammatory cytokines under both preventive and delayed treatment regimens.

Introduction

This study presents a tandem enzymatic nanobot swarm system inspired by biological communication for effective treatment of inflammatory bowel disease (IBD). Glucose oxidase (GOx) nanobots decompose glucose to generate hydrogen peroxide gradients, which guide catalase (CAT) nanobots via chemotaxis. CAT nanobots then scavenge reactive oxygen species and release oxygen, which further fuels GOx nanobots. This feedback loop enables coordinated navigation, enhanced penetration, and local oxygen delivery in the intestine, addressing hypoxia and oxidative stress in IBD.

Experimental

1. Preparation of enzyme-powered nanobots.
MSNP was dispersed in PBS with enzyme solution composed of catalase (0.75 mg/mL), glucose oxidase (1 mg/mL), and NH_2-PEG-SH (2 μg/mg) and reacted for 12 hours. The resulting enzyme-functionalized nanobots were washed with PBS and sonicated for dispersion.

2. Characterization of nanobots. Nanobots size and morphology were analyzed using HR-TEM and SEM, while hydrodynamic size and surface charge were measured with DLS, FT-IR spectroscopy was used to analyze each fabrication step.

3. *In vitro* study of nanobots. Enzyme activity was assessed by quantifying enzyme concentration using a BCA protein assay kit and measuring absorbance at 670 nm after reacting with urea. Nanobot motion was analyzed under a microscope at different fuel concentrations (0, 50, 100, 250 mM) and simulated gastric fluid, with diffusion coefficient and velocity determined from MSD data.

4. *In vivo* study of nanobots. FITC-labeled nanobots were rectally administered to mice, and their distribution in the colon was visualized using PA and IVIS imaging under fuel and no-fuel conditions. colon tissues were harvested at 9 days after administration to assess retention. Histological analysis of the GI tract with H&E staining.

Results and Discussion

1. Description of enzyme-powered nanobots.

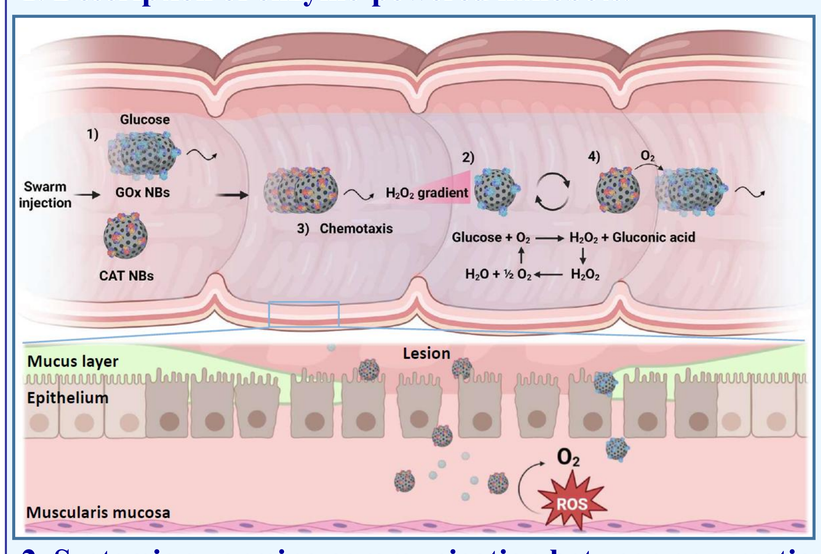

Fig. 1. Schematic of swarming communication between enzyme-powered nanobots for IBD therapy. (1) GOx nanobot (GOx NB) swarms decompose glucose, producing a hydrogen peroxide (H_2O_2) gradient, as a chemical cue. (2) Catalase functionalized nanobot (CAT NB) swarms are attracted by the gradient *via* chemotaxis while decomposing H_2O_2 to release O_2. (3) Released oxygen is than utilized by GOx NB swarm for further decomposition of glucose. This cyclic reaction allows swarms to resist peristalsis and remain at inflamed sites.

2. Systemic swarming communication between enzymatic swarms.

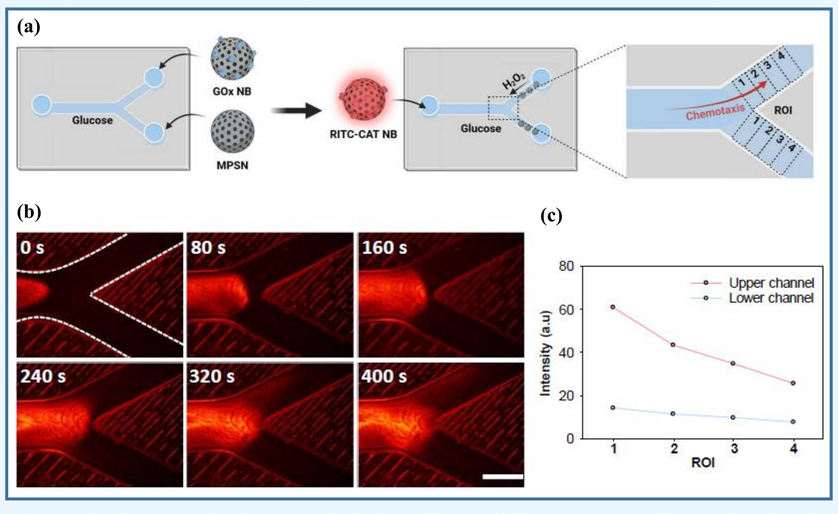

Fig. 2. Schematic illustration for the swarming communication. Swarm navigation driven by direct interactions between swarms was evaluated using a Y-shaped channel. GOx NBs and CAT NBs were initially introduced from opposite sides of the channel containing glucose solution (Fig. 2a). Red fluorescence dye labelled CAT NBs diffused from the left side of the channel and were gradually attracted toward the upper branch, where the H_2O_2 concentration was higher (Fig. 2b).

Quantitative analysis revealed that the red fluorescence intensity in the upper channel was more than twice that observed in the lower channel (Fig. 2c).

3. Collective behavior of enzymatic swarms.

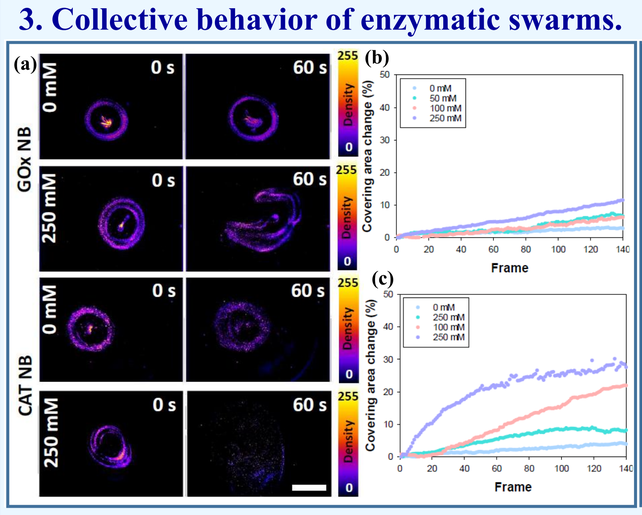

Fig. 3. (a) The density maps of swarming behavior with and without fuels (glucose, H_2O_2) for 60 s and corresponding covering area of (b) GOx NB and (c) CAT NB. And Chemotaxis behavior of CAT NBs in the middle channel after adding (d) glucose, or (e) GOx NBs with glucose in the left channel. In the presence of glucose or H_2O_2, nanobot swarms expanded rapidly, forming 3D vortices (Fig. 3b), whereas without fuels they sedimented into 2D dispersions. Swarm coverage increased with fuel concentration, as quantified by density mapping (Fig. 3b,c). CAT NB chemotaxis was tested using a non-flow microfluidic chip, showing migration toward the H_2O_2 gradient generated by GOx NBs and glucose (Fig. 3e) with complete bias within 10 min, while glucose controls showed no bias (Fig. 3d).

4. Enhanced navigation of enzymatic swarms in the colon.

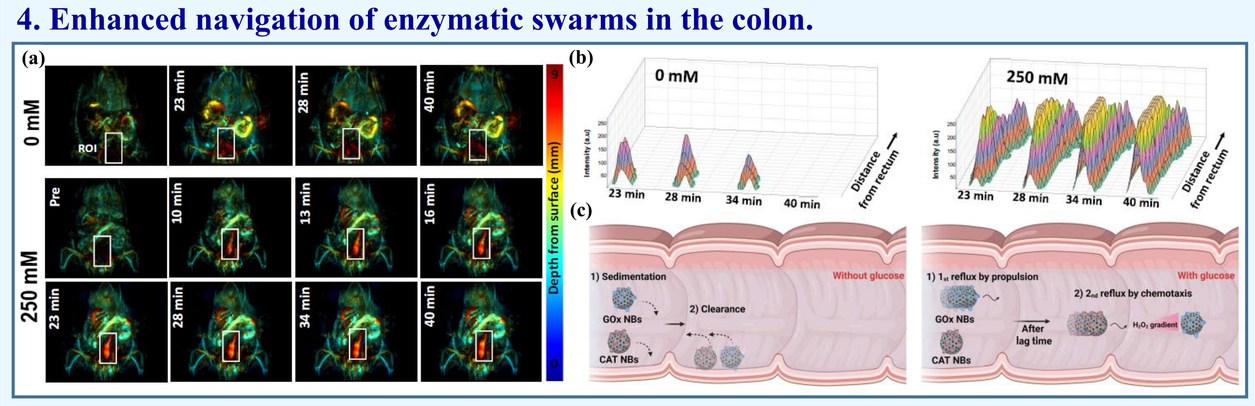

Fig. 4. PA imaging of enzymatic swarm distribution and retention after intrarectal injection. PA imaging was performed for 40 min after intrarectal instillation of ICG labelled swarms (Fig. 4d). Without glucose, the PA signal moved toward the rectum and disappeared, whereas glucose sustained and enhanced the ROI signal (Fig. 4e). This suggests peristaltic clearance without glucose and upstream reflux with glucose via GOx propulsion and CAT chemotaxis (Fig. 4f).

5. Therapeutic effect of enzymatic swarms on IBD.

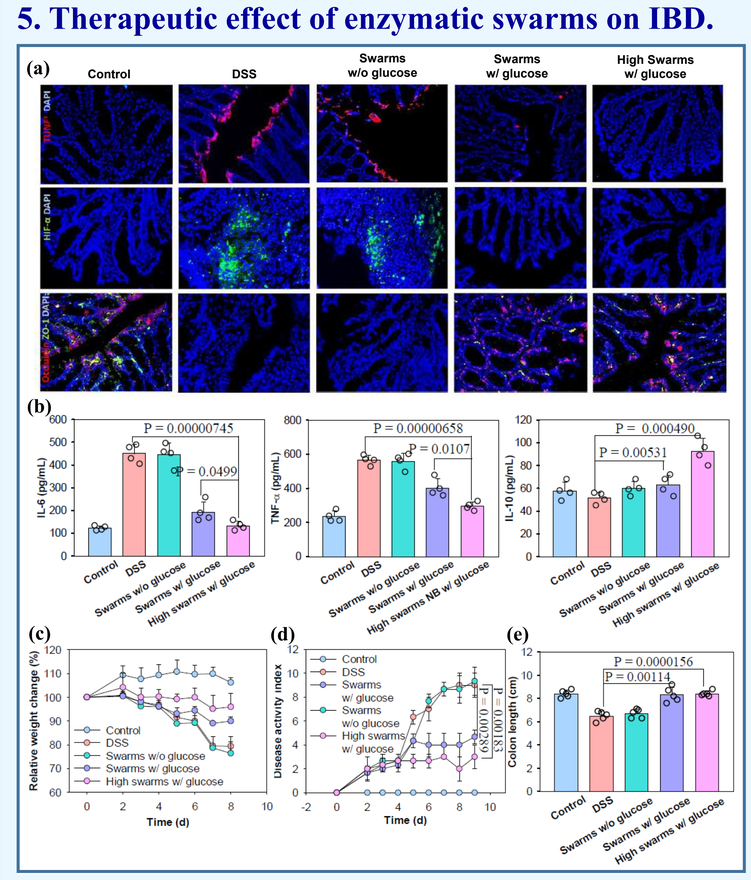

Fig. 5. (a) TUNEL assay (b) ELISA analysis (c) Weight change and (d) disease activity index monitoring of mice during treatment. (e) Colon length collected from the mice at 9 d. To evaluate therapeutic efficacy, enzymatic swarms were tested in a DSS-induced IBD mouse model. Histological analysis confirmed marked tissue restoration and alleviation of hypoxia, with substantially reduced apoptosis and increased tight junction protein expression (Fig. 5a). Swarms with glucose effectively mitigated weight loss, reduced DAI, and preserved colon length compared to controls (Fig. 5c–e). High-dose swarms exhibited clear concentration -dependent benefits, yielding the lowest proinflammatory and highest anti-inflammatory cytokine levels (Fig. 6b). Importantly, histopathological analysis consistently confirmed no significant abnormalities in major organs, thereby strongly supporting the therapeutic safety of enzymatic swarms.

Conclusions

This study demonstrates, for the first time, that two distinct enzymatic nanobot swarms can communicate through a cascade reaction, producing emergent behaviors reminiscent of biological collectives. By linking glucose oxidase (GOx) and catalase (CAT), we established a feedback loop: one swarm generates a chemotactic signal, while the other reinforces propulsion via oxygen release. This interaction converts simple enzyme-driven motion into systemic swarm communication, evident both in vitro and in vivo, where the swarms accumulate at inflamed sites, scavenge ROS, and deliver oxygen to alleviate symptoms. Importantly, these therapeutic effects were achieved without conventional drugs, underscoring the potential of swarm-based nanomedicine as a stand-alone strategy. Beyond IBD, enzymatic swarm communication could be adapted to disorders involving hypoxia, oxidative stress, or localized inflammation, though challenges remain for clinical translation.

References

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