Supporting information

Smart Capsule for Non-invasive Sampling and Studying of the Gastrointestinal Microbiome

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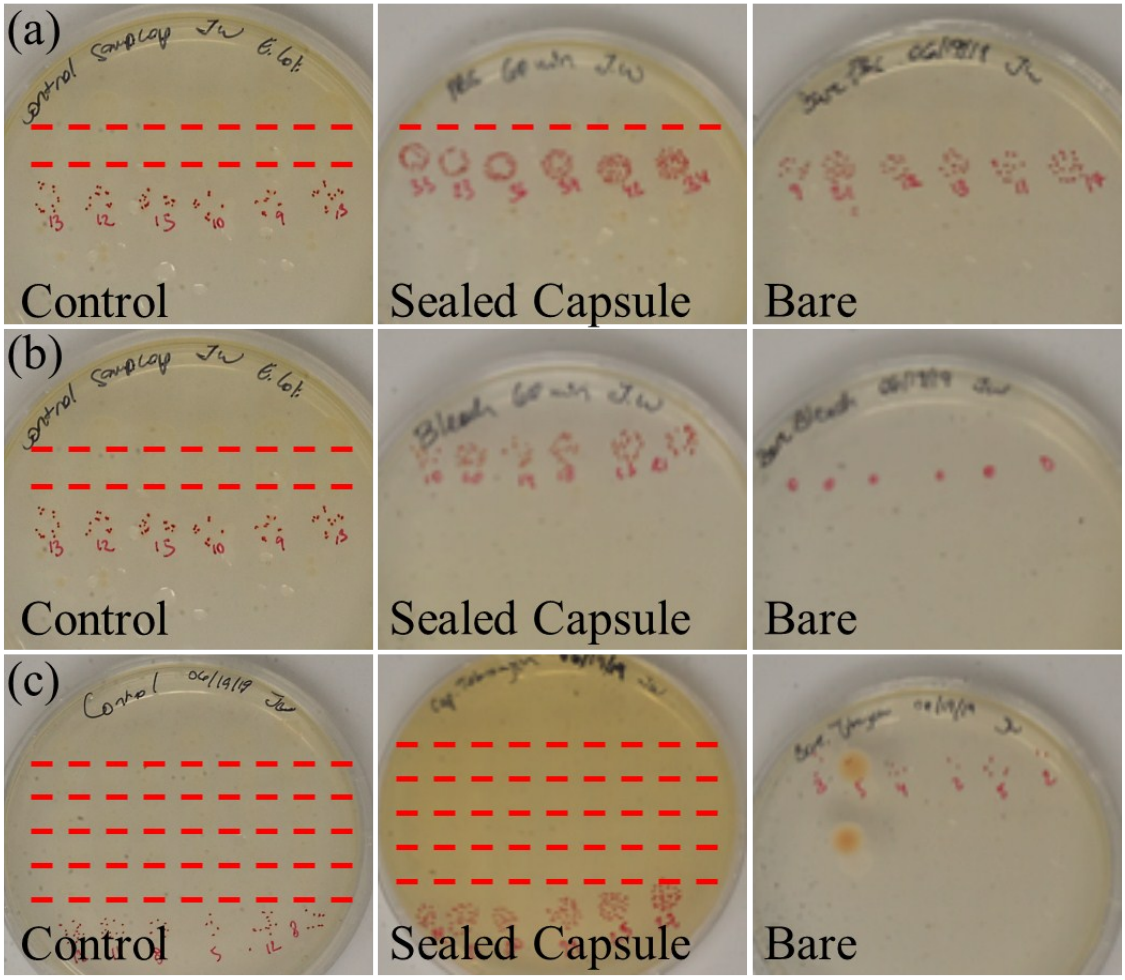

Fig. S1. Images of TSB/agar plates used for culture of *E. coli* samples captured by bare hydrogels and hydrogels within fully assembled sealed capsules after exposure to solutions of (a) PBS, (b) bleach, and (c) Tobramycin.

Bacteria captured with bare hydrogels and hydrogels within fully assembled sealed capsules were cultured following standard procedures. The viable bacterial count of samples exposed to PBS, Tobramycin, and bleach in both conditions was determined via Colony Forming Unit (CFU) counting and reported in CFU/mL, as seen in **Fig. 10** of the manuscript. **Fig. S1** shows pictures of the actual TSB/agar plates used for CFU counting procedures. The same control was used to inoculate bacterial stock solutions for experiments involving exposure to solutions of PBS (**Fig. S1a**) and bleach (**Fig. S1b**), hence the similarity between control plate. A different, more mature, stock was used for the added experiment involving exposure to a solution of Tobramycin (**Fig. S1c**). Across all conditions, there is an obvious decrease in viable bacterial counts from bacteria sampled with bare hydrogels compared to bacteria sampled with hydrogels within sealed capsules.

In order to demonstrate the adequacy of the proposed hydrogel in sampling different bacterial strains, we designed an experiment using three different bacteria grown in both aerobic and anaerobic conditions. The bacteria used in this experiment are *Escherichia coli, Staphylococcus aureus, and Lactobacillus casei*. *E. coli* is a gram-negative bacterium commonly found in the gut and in stool [1]. *S. aureus* is a gram-positive bacterium which can cause infections in the gut and is commonly found in infants [2]–[4]. *L. casei* is a gram-positive bacterium, also commonly found in the gut and often used as a probiotic due to its positive impact on the gut microbial flora [5], [6]. All of these bacteria are facultative anaerobes, as they can proliferate in both aerobic and anaerobic environments. Tryptic Soy Broth (TSB), Brain Heart Infusion (BHI), and De Man, Rogosa and Sharpe (MRS) were used as culture media for *E. coli*, *S. aureus*, and *L. casei* respectively. Growth in aerobic conditions was achieved by growing bacteria in their respective media in an anaerobic chamber at 37 °C overnight. Growth in anerobic conditions was achieved by growing bacteria in their respective media in an anaerobic chamber at 37 °C overnight. All subsequent hydrogel exposure and CFU plating was performed in the respective chambers. In order to sample the bacteria with the hydrogel, 1 mL of the bacterial stock was added to a 1.5 mL centrifuge tube containing a dried hydrogel. Hydrogels were allowed to swell for three hours before extraction. Hydrogels were then immersed into 100% PBS solutions for extraction of the sampled material. The total sampled volume was estimated to be close to 250 μL by measuring the decrease in volume after 3 hours of swelling. To serve as a control, 250 μL of stock solutions were directly inoculated into 100% PBS. Both solutions containing the hydrogel and control were allowed to shake in a rocking platform for 15 minutes. The resulting concentration of viable bacteria counts was quantified via CFU counting.

Results from **Fig. S2** show the number of viable bacteria sampled with a hydrogel compared to the control. In bacterial samples cultivated in aerobic conditions (**Fig. S2a**), sampling with the hydrogel resulted in no loss of viable bacteria in the case of *E. coli*, a 1-log reduction in viable *S. aureus* sampled, and a 1-log reduction in *L casei* sampled. In bacterial samples cultivated in anaerobic conditions (**Fig. S2b**), a 1-log reduction in viable bacteria was observed across all three strains. The reason for a 1-log reduction across almost all bacterial strains and conditions could be attributed to an overestimation of the total amount of fluid sampled with the hydrogel or due to a loss of viable bacteria due to strong attachment to the hydrogel surface. Nonetheless, these results

show high affinity of different bacterial strains to the hydrogel matrix used in the sampling capsule and suggest that the hydrogel is a more than adequate sampling agent for bacteria found in the gut.

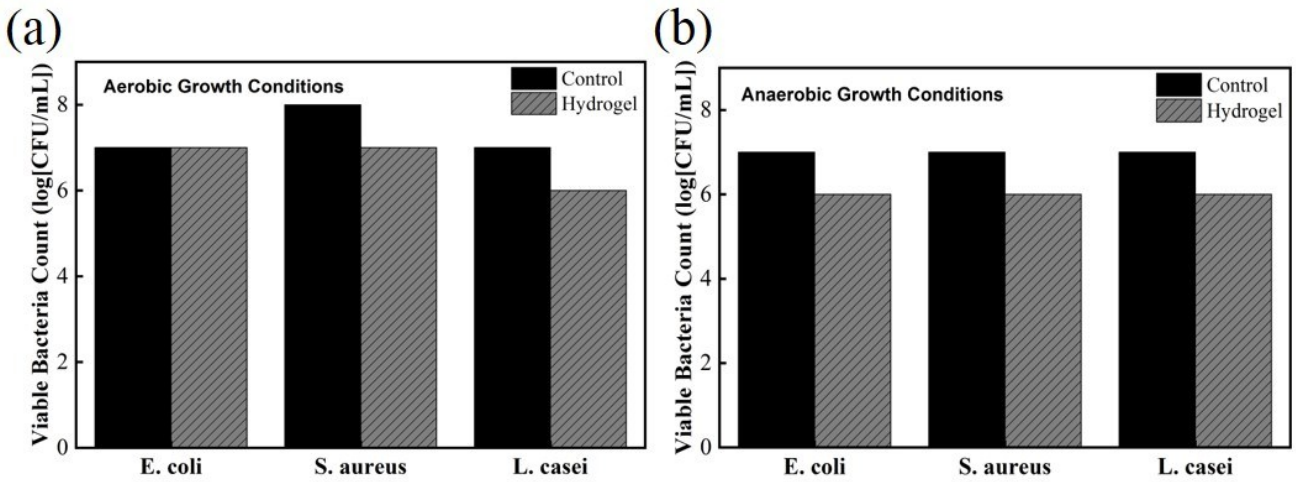

Fig. S2. Total number of viable bacteria counts in log[CFU/mL] of E. coli, S. aureus, and L. casei sampled using a hydrogel cultivated in both (a) aerobic and (b) anaerobic conditions. Direct inoculation using 250 µL of stock solution was used as control.

Furthermore, the positive results in anaerobic conditions suggest that there should be no loss due to changes in growth conditions from aerobic to anaerobic (gut) conditions.

References

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