A NOVEL METHOD FOR COLLECTING MICROBIOME SPECIMENS Proof of Concept in Normal Healthy Volunteers

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INTRODUCTION

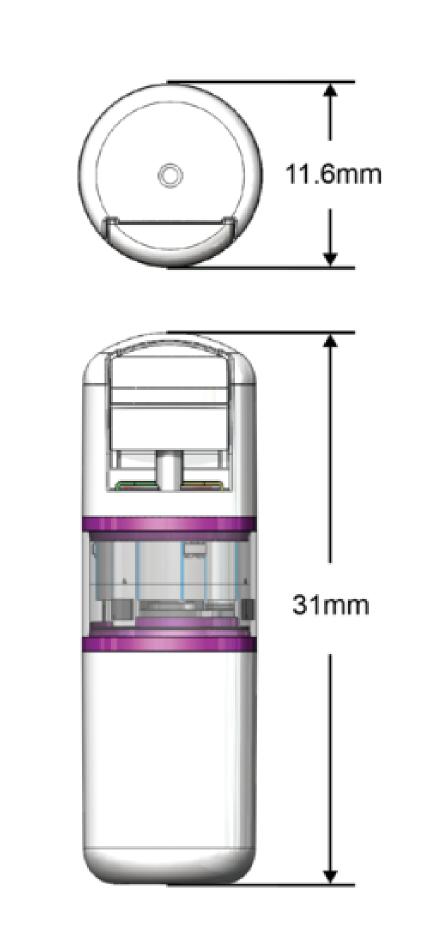
Our current understanding of intestinal microbiota is limited due to inaccessibility and lack of appropriate methods for direct sampling and analysis. Microbiome analysis using fecal samples may not provide an accurate representation of the microbial composition in the more proximal regions of the large intestine, which is likely due to the inclusion of dead organisms from the upper respiratory tract and the gut.

The Recoverable Sampling System (RSS) capsule includes proprietary localization, sampling, and preservation systems that allow for the noninvasive sampling of intestinal fluid while the capsule passes naturally through the GI tract.

Using this device, we have been able to achieve site-specific sampling of live microbiome organisms from the intestine.

RSS CAPSULE DIMENSIONS AND MODULES

- The Recoverable Sampling System (RSS) capsule is a single-use ingestible device measuring 31.0 mm in length and 11.6 mm in diameter (Fig. 1A), approximately the size of a fish oil capsule.
- The capsule's outer casing is made of inert polycarbonate and is rounded for ease of ingestion.
- The upper half of the capsule (Fig. 1B) contains the sampling chamber, and the lower half of the capsule contains the localization electronics, drive unit, and power source.
- The RSS contains proprietary localization technology that can autonomously detect five major anatomical locations, including the cecum, and trigger various functions as it transits through the gastrointestinal (GI) tract.
- The top and bottom casings are independent, allowing the top casing to extend a valve to open and allow an intestinal fluid specimen into the sampling chamber.



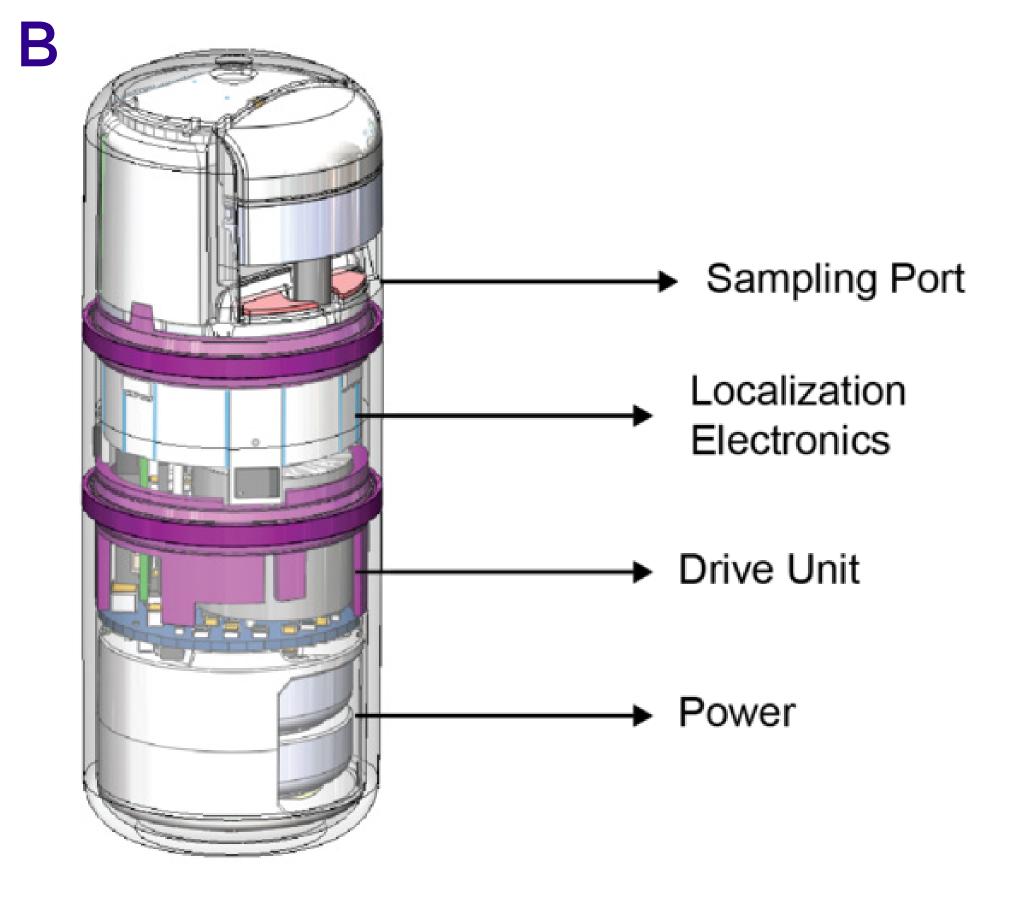


FIGURE 1. RSS capsule dimensions Rendering of the RSS capsule (A and B)

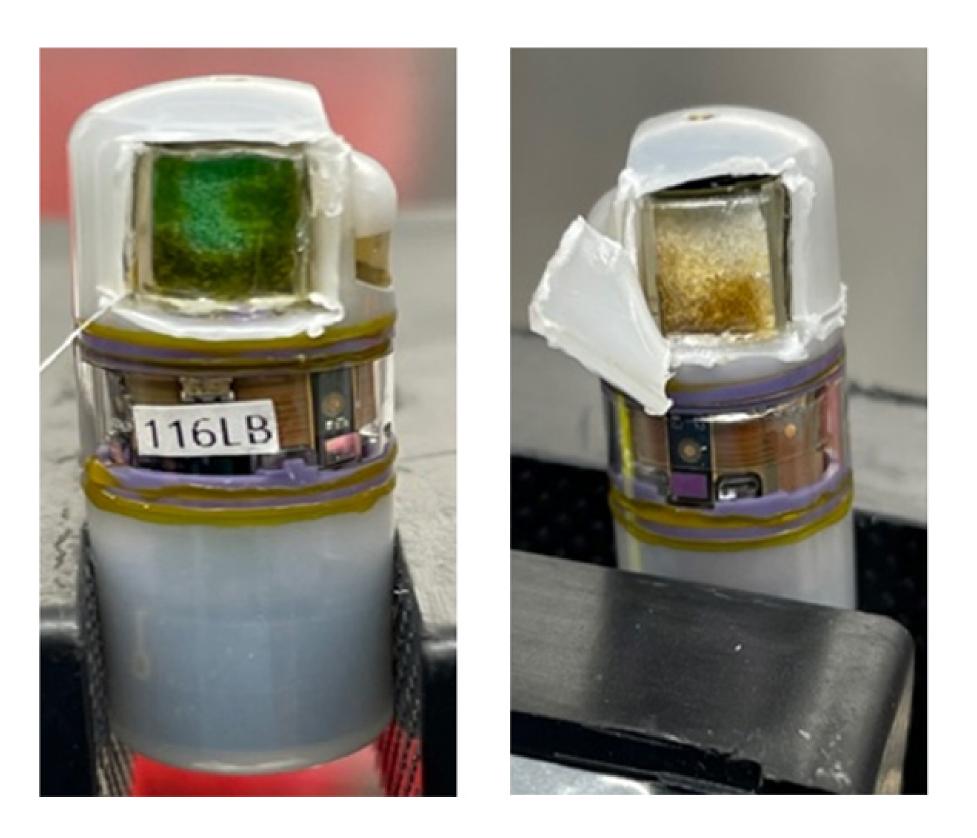


FIGURE 2. Recovered RSS capsule Aspirated specimen is visible on the sampling pads after recovery and removal of the capsule casing

METHODS

- We performed a feasibility study of microbiome collection in the cecum using the RSS capsule in four healthy subjects. Three of the subjects swallowed two capsules on two occasions several days apart while the fourth subject swallowed one capsule only.
- Intestinal fluid was collected in a sampling chamber that contains an absorbent pad impregnated with a proprietary preservative formulation developed to keep GI organisms viable for weeks.
- After ingestion and natural passage, capsules were recovered from the subjects' feces and sent to the laboratory along with a preserved fecal sample for analysis.
- The preserved intestinal fluid from each capsule was extracted and processed for quantitative culture, 16S qPCR, and 16S microbiome sequencing. Fecal samples were analyzed via 16S sequencing.

RESULTS

- All RSS capsules successfully collected an intestinal fluid specimen that reflected the correct microbial load for the specific site in the intestine.
- The amount (CFU/mL) of organisms retrieved from the RSS capsule correlates with genome copies/mL calculated by 16S qPCR (Figure 2, Table 1).
- Subject-to-subject difference of the microbiome can be observed in beta-diversity (Figure 3A).
- Beta-diversity analysis showed a distinct separation between intestinal fluid specimens and fecal samples (Figure 3B).

Capsule Serial No. Culture CFU/mL 16S Cp/μL Genome Copies/mL Subject 117LB 2.4 X 10^5 1.68 X 10^8 6.72 X 10^5 115LB Non-Viable 2.32 X 10^5 5.8 X 10^7 107LB 3.3 X 10^8 4.34 X 10^4 1.08 X 10^7 2.97 X 10^8 1.87 X 10^8 116LB 1.19 X 10^6



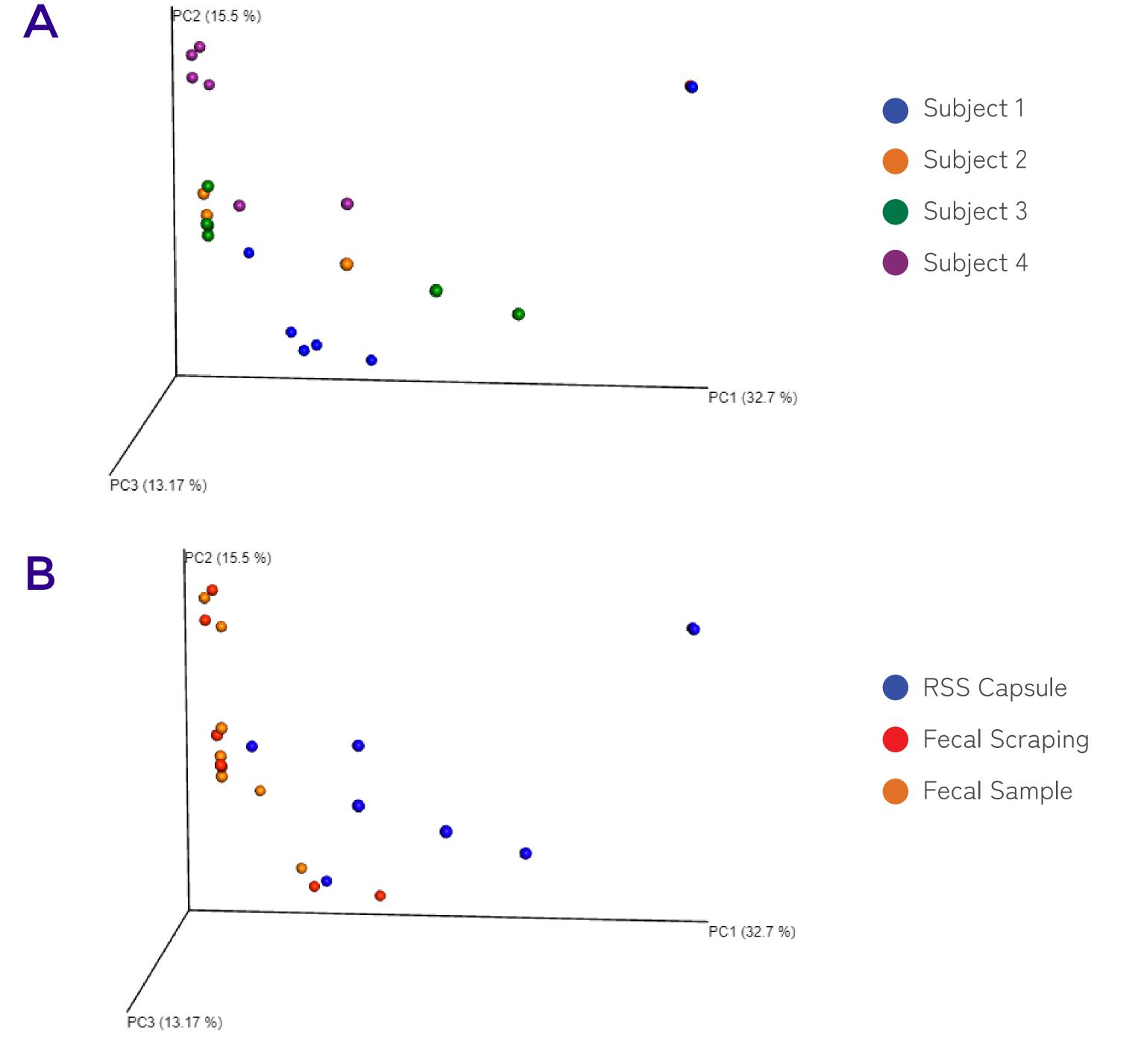


FIGURE 3. Beta diversity of 16S sequencing

Comparison of unweighted UniFrac distance A. between subjects and B. between sample types (RSS capsule: blue, fecal: red and orange)



SUMMARY

- The RSS capsule is an electromechanical device that can recognize anatomical features within the GI tract and samples intestinal fluid at a pre-programmed, targeted location, such as the proximal large intestine.
- Both an intestinal fluid specimen and a fecal sample were collected from each subject. Comparison of quantitative culture results, 16S qPCR, and 16S sequencing between the intestinal fluid specimens and conventional fecal samples showed:
 - Successful collection of intestinal fluid specimens by the RSS
 - A distinct separation of microbial diversity between the intestinal fluid specimens and the fecal samples, indicating that the RSS is collecting a unique, site-specific sample

CONCLUSION

This study demonstrated proof of concept that the RSS capsule can successfully collect a site-specific sample in the intestine. The study also suggests that samples from distinct locations in the GI tract can be different from fecal samples, and may offer unique insights into microbiome research.

This device has potentially enormous implications for the microbiome field as a non-invasive, anatomical, site-specific collection method which may provide more insight on specific drug targets in the intestine.

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