ELECTRONIC-FREE TRACEABLE SMART CAPSULE FOR GASTROINTESTINAL MICROBIOME SAMPLING

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ABSTRACT

Non-invasive smart electronic-free sampling capsules have transformed the study of microbiome-disease interactions in distal regions of the gastrointestinal (GI) tract. Despite their benefits, a key challenge with these capsules is accurately tracking the in vivo location due to patient motility variations. To address this, a novel electronic-free smart capsule design has been proposed, incorporating a metal tracer for easy detection. The use of a stainless-steel cylinder as a metal tracer has been optimized for detection efficiency and biocompatibility, confirmed through in vitro and in vivo studies using metal detectors, X-ray imaging, and CT scans. This innovative approach offers a reliable and cost-effective solution for tracking electronic-free smart capsules, enhancing their utility in microbiome research for both human and animal studies.

KEYWORDS

Passive capsule, Gastrointestinal Sampling, *In vivo* tracking

INTRODUCTION

In recent years, there has been a growing interest in research focusing on the intricate relationship between the human gut microbiota and host health. Various studies have highlighted the significance of the gut microbial environment, termed dysbiosis, in influencing the prognosis of conditions like obesity, diabetes, inflammatory bowel diseases, and cardiovascular issues. Analyzing the gut microbiome typically involves fecal sample analysis, a non-invasive and cost-effective method to assess the microbial population in the gastrointestinal tract. However, this approach has limitations in capturing spatial and temporal information throughout the GI (gastrointestinal) tract accurately, leading to inaccurate conclusions.

To overcome the drawbacks of fecal sampling, researchers have developed innovative methods for more precise and region-specific sampling. One approach involves probe-based techniques such as endoscopy aided by biopsy tools, enabling targeted and accurate sampling, but have drawbacks of being invasive, uncomfortable and requiring skilled personnel[1]. Another emerging method is the use of ingestible capsules for localized sampling along the GI tract[2–5]. These capsules can be categorized as active, incorporating electronics for in vivo localization and sampling initiation, or passive, utilizing stimuli-responsive polymers for targeted sampling without electronics, ensuring a safer design[6].

While active capsules offer precise sampling and device localization, they come with drawbacks such as increased cost, design complexity, and concerns of safety due to the presence of electronics and batteries. On the other hand, passive capsules eliminate electronics, relying on stimuli-responsive polymers and passive mechanisms for sampling, ensuring a safer and simpler design. These advancements in sampling methods aim to address the limitations of traditional fecal analysis, providing a more comprehensive understanding of the gut microbiome's impact on gut health and physiology throughout the GI tract.

Passive capsule designs present several advantages, but a key challenge is accurately determining their location in the GI tract and the timing of excretion. The translucency of most passive capsules, which are primarily composed of polymers, poses a challenge for imaging them using common in vivo imaging methods like X-Ray and CT scans. This opacity issue is exacerbated in cases of varying motility among subjects, complicating the assessment of capsule transit and excretion times, which in turn hinders sample collection and subsequent analysis.

To overcome these challenges, researchers have explored methods such as incorporating tracers into capsule designs for electromagnetic radiation-based tracking. Tracers like radioisotopes In111 and Tc99m allow visualization through gamma scintigraphy[7], improving capsule visibility in different GI regions, but raises concerns regarding patient exposure to ionizing radiation, as well as equipment complexity and costs.

Another approach involves magnetic tracing methods, utilizing external metal tracing modalities for in vivo capsule localization. Metal detectors, originally used for detecting embedded bullets, have been repurposed for localizing swallowed metallic objects, demonstrating high sensitivity and specificity compared to x-ray radiography[8,9]. Recent applications of metal detectors in studying gastric emptying time further highlight their versatility and practicality in medical diagnostics.

By integrating a metal tracer with passive sampling capsules, a novel design has been developed to facilitate tracking using metal detectors. This modified capsule design, in conjunction with metal detection, significantly reduces the time and cost needed to confirm capsule presence in the GI tract, ensuring timely sample collection and addressing motility variations among individuals. Both in vitro tests and in vivo studies in pig models have confirmed the effectiveness of this design with metal detection, showing high accuracy in capsule localization comparable to standard imaging techniques.

The incorporation of metal detection in passive capsule designs presents a promising solution to the persistent challenge of traceability within the GI tract. This innovation not only assists in medical decision-making but also has broad applications in microbiome analysis across human, livestock, and veterinary research.

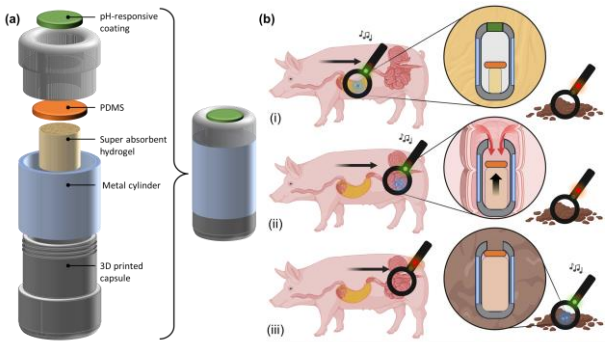

Figure 1: a) Schematic of capsule design and components. b) Illustration of in vivo capsule sampling mechanism, (i) No sampling in stomach, (ii) dissolution of pH responsive polymer in small intestine, sampling initiated, and hydrogel swells (iii) sealed capsule is passed and detected with metal detector.

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printer with clear resin from Formlabs inc. The DOW Sylgard 184 silicone elastomer kit, employed for creating the polydimethylsiloxane (PDMS) disks, was sourced from DOW Corning corporation. Various other chemicals and reagents, such as acrylamide, ammonium persulfate (APS), N,N′-methylene bisacrylamide (MBA), and phosphate-buffered saline were procured from Sigma–Aldrich (USA). Eudragit L100-55, a methacrylic acid-ethyl acrylate copolymer (1:1), was obtained from Evonik (USA) and applied as a pH-responsive coating on the capsule aperture. Hollow brass and stainless steel (SS 316L) cylinders, featuring a thickness of 0.35 mm and an outer diameter of 9.5 mm, were purchased from McMaster-Carr (USA) and adjusted to fit the 3d printed capsule designs. Corn starch and gelatin, utilized in synthesizing tissue-mimicking solutions, were acquired from Walmart (USA). Lastly, a portable MD-300 handheld metal detector from Walmart was utilized for all experimental capsule tracking procedures.

Briefly, the two-part capsule shell was designed using Autodesk Inventor CAD software to dimensions (inner diameter, outer diameter, and height) of 7, 9.5, and 20 mm respectively. The design was fabricated using SLA methods using clear resin and Formlabs Form-3 3D resin printer. The PDMS discs were created by combining 1:10 ratio of curing agent: PDMS base and curing overing at 70°C. The final assembled capsule and the corresponding components are shown in Fig. 2a, b.

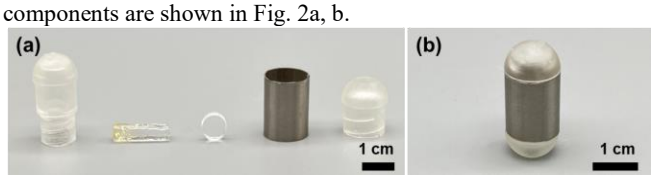

Figure 2: a) Image highlighting the individual capsule components used in the design, from left to right, bottom housing, hydrogel, PDMS disc, metal tracer and top housing. b) Fully assembled capsule design.

To ensure that the capsule design would maintain integrity upon exposure to relevant physiological pH ranges in the GI tract, a simulated accelerated *in vitro* corrosion test was performed using different metals by conducting open circuit potential (Eoc) and potentiodynamic polarization scans. The corrosion rate for each metal studied in both acidic (pH 1.2) and basic (pH 7.5) environments, using a Gamry instrument (Reference 3000 Potentiostat/Galvanostat/ZRA,USA) controlled by framework data acquisition software. This test will be able to simulate the metallic corrosion in the extreme pH environments found in the GI tract.

To ensure that the materials used in the construction of the capsule do not negatively impact the gut health, a series of *in vitro* tests were conducted for verification of bioinert and biocompatible properties. Biocompatibility testing was conducted using HCT-8 cells obtained from ATCC, USA, for 3 days following a standard protocol for MTT assay. Briefly, the cells were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with l-glutamine, sodium pyruvate, and fetal bovine serum (FBS). The cells were then seeded in 12-well plates and allowed to form a monolayer, wherein the test material was placed in the center and incubated for 3 days. Cell viability was quantified as the ratio of sample absorbance to control absorbance. Further, live dead staining was performed using ethidium homodimer-1 and calcein-AM to visualize the live and dead cells.

To further validate that the capsule materials do not interfere with the microbiome present in the gut, a series of experiments were performed by exposing the different metals to different bacteria found commonly in the GI tract. Cultures of Gram-positive Enterococcus faecalis ATCC 29 212 (*E. faecalis*) and Gram-negative Escherichia coli ATCC 25 922 (*E. coli*), at 10^9CFU/mL, OD_{600nm}=0.4, had the metal tracers placed in them and samples collected and plated at regular time intervals.

To ensure that the capsule design would be successfully imaged within the GI, a set of tests were conducted to simulate the conditions and conduct metal detection and X-ray imaging testing. The tests simulated the i*n vivo* tracking of the capsule with the help of 1% w/w agarose gel tissue mimicking solution, prepared by dissolving agarose in Phosphate buffered saline (PBS).

The tests involved embedding varying lengths of these capsules within the tissue mimicking agarose solution with increasing quantities of corn starch as a scattering agent. Each test condition was X-ray imaged using a Del Medical X-ray universal system, and the metal tracers in each image were analyzed for image intensity and contrast v/s the background media using ImageJ software.

To assess the efficacy of capsule tracking utilizing the tracer, an in vivo investigation was carried out on three porcine subjects. This research was approved by the Purdue University Animal Care and Use Committee, protocol number 1911001975. The pigs, weighing between 45 and 55 kg, were provided a diet equivalent to three times their daily energy maintenance requirement (4% of body weight per day), predominantly consisting of corn and soybeans. Prior to the commencement of the trial, the pigs underwent a 5-day acclimatization period in their enclosures measuring 2 m × 1.3 m, with unrestricted access to water. On the sixth day, after a 6-hour fasting period, each pig was administered a single capsule. Daily tracking of the capsules was conducted using a handheld metal detector. To verify the capsule's location, X-ray imaging (Del Medical X-ray universal system) and CT-Scan (GE Healthcare GmbH, Germany) were employed at various intervals during the study. Analysis of all X-ray radiographs was performed utilizing VetRocket software.

RESULTS AND DISCUSSION

The first design element to be considered was the type of metal used in the metal tracer. The combined density of the capsule would need to be greater than that of gastric fluid, to overcome in the stomach flotation and reduce gastric residency time. To achieve this, 3 different metals of varying lengths were tested, and only SS316L and Brass at 9 and12mm provided a much larger difference than the 1.004g/cm^3 density of gastric fluid as seen in Fig. 3a-c. A further concern of metal corrosion within the relevant pH ranges in the GI tract required corrosion resistance testing.

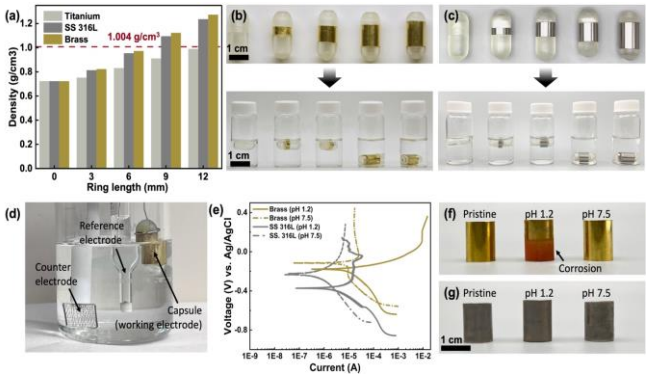

Figure 3: a) Calculated values for total capsule density with metal tracers differing lengths and material. Test for capsules sinking with different b) brass and c) SS316L lengths. d) Image of Potentiodynamic test setup for characterizing the corrosion rate of metal tracers. e) Potentiodynamic polarization curves obtained from the brass and SS in acidic (pH 1.2) and slightly alkaline (pH 7.5) solutions. f) Image of Brass and g) Image of metal tracers

before and after the potentiodynamic corrosion test in pH 1.2 and 7.5

After conducting an accelerated potentiodynamic corrosion testing, Fig. 3d-f, SS316L showed a much higher corrosion resistance potential in both pH 1.2 and pH 7.5, Using Tafel slope analysis, the brass exhibited a corrosion current of 9.52 µA at the corrosion potential, resulting in a corrosion rate of 0.112 mm/year in acidic conditions. In contrast, SS 316L indicated a high corrosion resistance in low pH environments, where the corrosion current and rate were notably lower at 1.6 µA and 0.017 mm/year respectively as compared to brass. In alkaline conditions, both SS 316L and brass formed passive layers on their surfaces, with low corrosion currents of 0.5 µA and 3.5 µA, and estimated corrosion rates of 0.006 mm/year and 0.039 mm/year for SS 316L and brass respectively. The results validated that the optimal design was a 12mm SS316L metal tracer to use on the final capsule design.

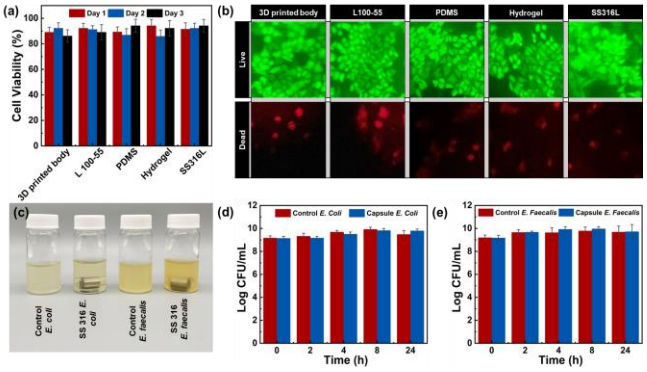

Figure 4:Biocompatibility and Bioinert characteristics of capsule design: a) Cell viability graph showing the percentage of viable cells for each material over the 3 days. b) Live/ dead (green/red) staining images on day 3. c) Image of experimental setup for both bacteria in control and fully assembled capsule conditions. Bacterial population for all test conditions over 24h for d) E. coli and e) E. faecalis.

As seen in Fig. 4a, the results of the MTT assay, biocompatibility test shows that all the materials used within the construction of the capsule had >85% cell viability over a 3-day period. Fig. 4b, displays the live/dead cells that were found for each material, highlighting a significantly higher proportion of live cells that were found at the end of the test. None of the materials used in the capsule were found to be immediately toxic. To then assess, the bioinert properties, to ensure that the capsule does not alter the microbiome in the gut, Fig. 4c shows the testing environment, the control vial without the capsule was used to compare against bacterial environment with the capsule for both *E. coli and E. faecalis*. Fig. 4d shows that the *E. coli* in the capsule condition remains at 9.84 ± 0.18 Log CFU mL^{-1} over the 24h period, with no observable change as compared to the control (9.46 ± 0.35 Log CFU mL^{-1}). For the gram-positive *E. faecalis* in Fig. 4e, the population in the test condition was found to be 9.70 ± 0.60 Log CFU mL^{-1} for the 24h, similar to the control conditions, (9.74 ± 0.67 Log CFU mL^{-1}). The results of this study show that the capsule materials are both biocompatible and also bioinert, without having any adverse effects on the surrounding microbiome.

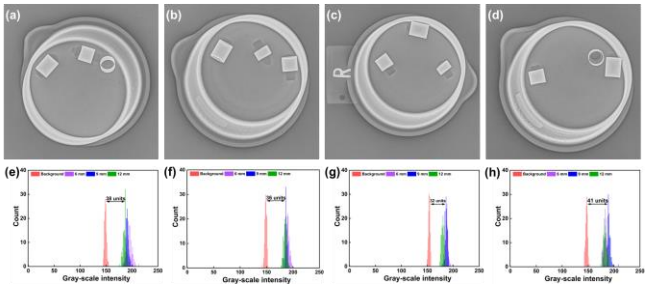

Figure 5:X-ray imaging for increasing lengths of SS316L metal tracer (6, 9 and 12mm) inside tissue mimicking solutions. a) DI water control setup, and agarose gel containing b) 0.2%, c) 0.4%, and d) 0.8% cornstarch content. Image intensity graphs comparing metal tracer intensity to background media for e) DI, agarose gel containing (f) 0.2%, (g) 0.4%, and (h) 0.8% cornstarch filler.

To validate that the metal tracers can be effectively imaged with the use of X-ray radiography, a series of *in vitro* tests was conducted by imaging varying tracer lengths embedded in tissue mimicking solution with corn starch for increased scattering. The obtained images can be seen in Fig. 5a-d with increasing percentages of corn starch filler. By analyzing the image intensity for the metal tracers, Fig. 5e-h, the peak intensity of the tracer was compared to the peak intensity of the background. The obtained peak intensities for the tracers show a large difference in gray scale values between them and the average background intensities of 38, 36, 32, and 41 units respectively for increasing percentages of corn starch. The values obtained illustrate the ease of distinguishing the capsule design once ingested and placed under X-ray imaging for tracking.

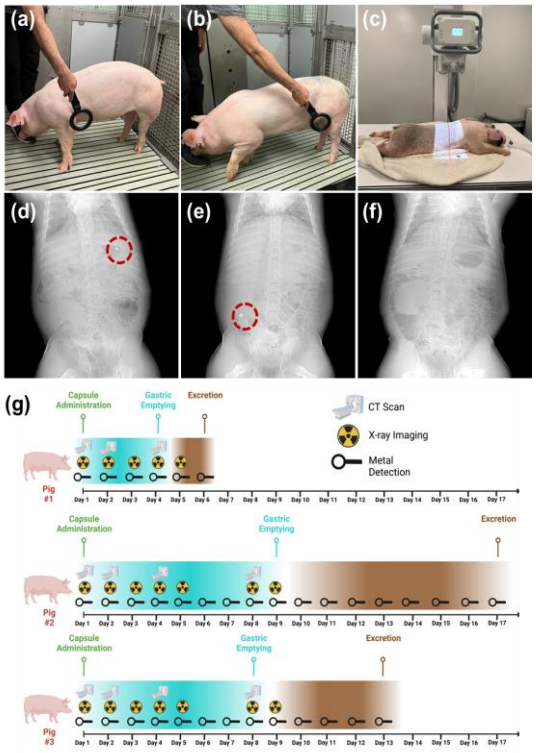

Figure 6: Capsule tracking and administration in a pig model, a) Metal detection in the upper GI tract, near the stomach and b) capsule detection in the colon. c) Anesthetized pig being X-ray imaged to confirm findings from metal detection. d) X-ray radiograph confirming capsule localization in the stomach and e) in

the colon. f) X-ray image showing absence of capsule, once it has been passed. g) Transit time of capsule in each of the pigs, and the detection/imaging modalities being performed on each respective day, being either metal detection, X-ray imaging or CT scans.

As a final verification study, the capsule was tested within *in vivo* conditions with the help of 3 pig models. The passage of the capsule was tracked as per the timeline shown in Fig. 6g, where handheld metal detection was conducted daily, and the accuracy of the procedure was compared against X-ray imaging and CT scans. During the first days after administration, the metal detector indicated that the capsule still resided in the stomach, which was then corroborated with the use of X-ray imaging. Fig. 6a-b shows the metal detection that was conducted to determine capsule localization in the GI tract, which was then further verified with the help of X-ray imaging as seen by Fig. 6d-e. The obtained results show that the capsules resided in the gastric environment for 3, 8, 7 days for Pig 1-3 respectively. This prolonged gastric residency can be attributed to the anatomical differences between porcine and human stomach. Pigs have a U-shaped stomach, for which the passage of non-disintegrating large doses is not assisted by gravity through the stenotic torus pylorus at the end of the stomach[10–13]. Whereas humans have a J-shaped stomach which facilitates easier passage through the gastric region. However, despite the difference in transit and motility, all capsules passed through the GI tract of the pigs successfully at day 6, 17 and 13 respectively for Pigs 1-3. The results from the *in vivo* study confirm that handheld metal detection as a tracking modality is accurate, cost-effective and provides a rapid response time. The overall capsule design is one that is compliant with the intestinal tract for ingestion without any concern of adverse effects occurring.

CONCLUSION

Here we have demonstrated a novel approach to track and localize passive ingestible capsule without needing to rely on electronics or expensive and time-consuming imaging modalities. The use of smart sampling capsules provides a new insight into the gut microbiome which was previously associated with challenges. The overall capsule design was verified to be both biocompatible and bioinert through a series of *in vitro* studies, and the materials chosen were tested to ensure that they had a high degree of corrosion resistance in physiologically relevant pH ranges. The incorporation of the metal tracer onto the sampling capsule design successfully facilitated the use of rapid metal detection with a high degree of accuracy as shown in the *in vivo* study. This novel method introduced provides a new approach for both healthcare professionals and researchers to quickly study the microbiome within a subject of interest while alleviating concerns of exact capsule localization and motility. Finally, the introduced design provides a new and important research tool that can be used to further the understanding of the gastrointestinal microbiome and gut health.

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REFERENCES

[1] E. J. Kuipers, J. Haringsma, *J Surg Oncol* **2005**, *92*, 203.

[2] M. Rehan, I. Al-Bahadly, D. G. Thomas, E. Avci, *IEEE Access* **2021**, *9*, 127175.

[3] R. N. Sharaf, A. K. Shergill, R. D. Odze, M. L. Krinsky, N. Fukami, R. Jain, V. Appalaneni, M. A. Anderson, T. Ben-Menachem, V. Chandrasekhara, K. Chathadi, G. A. Decker, D. Early, J. A. Evans, R. D. Fanelli, D. A. Fisher, L. R. Fisher, K. Q. Foley, J. H. Hwang, T. L. Jue, S. O. Ikenberry, K. M. Khan, J. Lightdale, P. M. Malpas, J. T. Maple, S. Pasha, J. Saltzman, J. A. Dominitz, B. D. Cash, *Gastrointest Endosc* **2013**, *78*, 216.

[4] S. Park, H. Lee, D. Kim, H. Kee, S. Park, *IEEE/ASME Transactions on Mechatronics* **2022**, *27*, 4384.

[5] P. Shokrollahi, Y. P. Lai, S. Rash-Ahmadi, V. Stewart, M. Mohammadigheisar, L. A. Huber, N. Matsuura, A. E. H. Zavodni, J. Parkinson, E. Diller, *IEEE/ASME Transactions on Mechatronics* **2021**, *26*, 2616.

[6] S. Nejati, J. Wang, U. Heredia-Rivera, S. Sedaghat, I. Woodhouse, J. S. Johnson, M. Verma, R. Rahimi, *Lab Chip* **2022**, *22*, 57.

[7] S. Senekowitsch, P. Schick, B. Abrahamsson, P. Augustijns, T. Gießmann, H. Lennernäs, C. Matthys, L. Marciani, X. Pepin, A. Perkins, M. Feldmüller, S. Sulaiman, W. Weitschies, C. G. Wilson, M. Corsetti, M. Koziolek, *Pharmaceutics* **2022**, *14*, DOI 10.3390/pharmaceutics14040801.

[8] A. Kessler, A. Yellin, A. Kessler, J. Kronenberg, *J Laryngol Otol* **1990**, *104*, 435.

[9] S. P. ROS, F. CETTA, *Pediatr Emerg Care* **1992**, *8*, 134.

[10] C. Suenderhauf, N. Parrott, *Pharm Res* **2013**, *30*, 1.

[11] L. J. Henze, N. J. Koehl, H. Bennett-Lenane, R. Holm, M. Grimm, F. Schneider, W. Weitschies, M. Koziolek, B. T. Griffin, *European Journal of Pharmaceutical Sciences* **2021**, *156*, 105627.

[12] R. L. Oberle, H. Das, *Pharm Res* **1994**, *11*, 592.

[13] M. Hossain, W. Abramowitz, B. J. Watrous, G. J. Szpunar, J. W. Ayres, *Pharm Res* **1990**, *7*, 1163.

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