PASSIVE 3D-PRINTED FULLY ELASTIC PILL FOR SAMPLING OF GUT MICROBIOME

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ABSTRACT
Sampling of gut microbiome upstream of the colon is challenging and expensive. Research has shown that ingestible devices are effective in sampling microbiome, however existing designs are complex and suffer from high levels of contamination from untargeted regions. This paper aims to present a simple sampling design concept with a self-closing mechanism relying on swellable polyacrylate beads that close the inlet to prevent contamination. The pill is fully elastic, lightweight, biocompatible, battery-less realized using stereo-lithography based 3D-printer. Herein, it is believed that such an ingestible device will increase the understanding of spatial diversity of gut microbiome and its response to medical conditions and treatments, using a non-invasive and simple technique.

KEYWORDS
Biomedical microdevices, Gastrointestinal tract, Gut microbiome, Ingestible, Capsules, Pills, Microbiome.

INTRODUCTION
Technologies capable of noninvasively sampling the luminal content of the GI tract have the potential to improve our understanding of the role of microbiome in health and various medical conditions. Microbial conditions in the gut are typically inferred from the analysis of fecal DNA and fecal metabolites [1]. This approach is inadequate for characterizing the microbiome upstream of the distal colon [2, 3]. To gain new insights into the many beneficial functions of the gut microbiota, it is essential to sample in different locations in the gut, particularly organs located upstream of the colon. Orally ingestible medical devices for gut engineering have been reported for sensing and diagnosis [4-8]. Most devices are rigid, and some require complex fabrication and assembly techniques.

Additive manufacturing has the potential to transform the design and fabrication of ingestible devices by its ability to realize true 3D structures monolithically using biocompatible materials with wide range of properties (e.g., elastic). Such manufacturing technique involves a layer-by-layer construction of the designed pill using a 3D printer. Among the various 3D printing technologies, stereo-lithography (SLA) based 3D printing provides a balance between the print resolution, print time, build volume and cost.

Our prior work on ingestible pills for sampling the gut microbiome [3] relied on a 3D printed pill containing a battery-less osmotic pump and was validated in animal models. However, the design suffered from leakage and post-sampling contamination due to the lack of a closing valve. In this paper, we report the first elastic battery-less ingestible pill for sampling intestinal microbiome from the small intestine with a self-closing mechanism to prevent contamination and leakage. Pills elasticity allows for ease of sample intake as a result of peristaltic motion of the GI tract. The pill's sampling and locking mechanisms have been tested using different viscosity and granulation fluids. Enteric coating dissolutions and contamination tests using dyes have been performed to demonstrate the functionality of the pill and its ability to avoid leakage and contamination, respectively.

WORKING PRINCIPLE
The 3D printed elastic pill is placed inside a pH-sensitive enteric coated capsule to delay sampling until it enters the small intestine, where the coating dissolves in the higher pH environment (Figure 1). Natural peristaltic motion ensures mobility to the pill through the GI tract without any active motion. Upon exposure to the fluid from small intestine, the enteric coating dissolves, allowing lumen to freely flow through the two-side inlets. The pills elasticity ensures a natural pumping action as a result of peristaltic pressure, greatly assisting the overall sample intake. The pill contains highly hydrophilic sodium polyacrylate beads, both as an absorbent for passive suction, and for locking the intake when the beads are swollen. After the pill's reservoir reaches sufficient volume and the beads have expanded enough to close the inlet, we deem the sampling is completed. The pill then travels through the rest of the GI tract and is finally collected from the feces to study the gut microbiome.

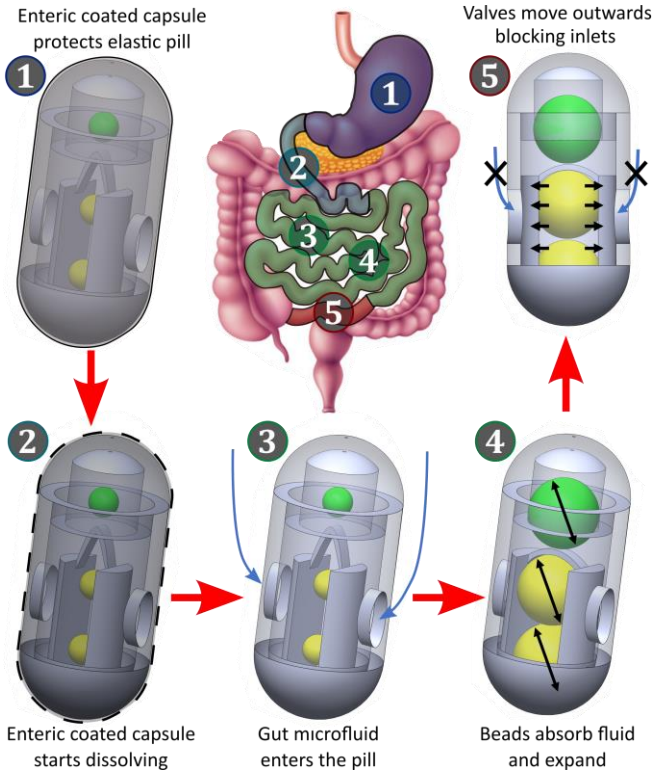

Figure 1: Sampling principle of the elastic ingestible pill. (Exact locations of the pill's status within the small intestine, will vary depending on the pill's enteric coating and pH levels through the GI tract.) 3D designed images were created using SolidWorks CAD tool.

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PILL DESIGN AND FABRICATION

The overall design of the pill is shown in Figure 2. The elastic pill consists of three main parts—an elastic case, two internal valves, and three sodium polyacrylate beads. SLA-based 3D printing was used to fabricate the ingestible device's casing using a bio-compatible/photo- curable elastic resin (Formlabs Elastic 50A V1) using a Form 2 printer (Formlabs Inc., Somerville, MA, USA).

The elastic pill comprises an external wall thickness of 1mm, an overall pill height and width of 20 and 9mm, respectively; two internal valves height and width of 9.1 and 4mm, respectively, and a gap between valves of 2.61mm (Figures 2.a and 2.b). The gap between valves is specifically designed for 3mm-diameter beads, to ensure that their position stays fixed within the pill. A 1mm gap between each valve and inlet is designed (Figure 2.c) to allow inward flow while the pill is sampling. Two elliptical holes with a height and width of 4 and 2mm, respectively, are located on two opposite sides of the pill (Figure 2.d). The two vertical valves are attached to the bottom of the pill and to each other, forming a bridge, to guarantee that the valves do not lock the inlets until beads are fully expanded.

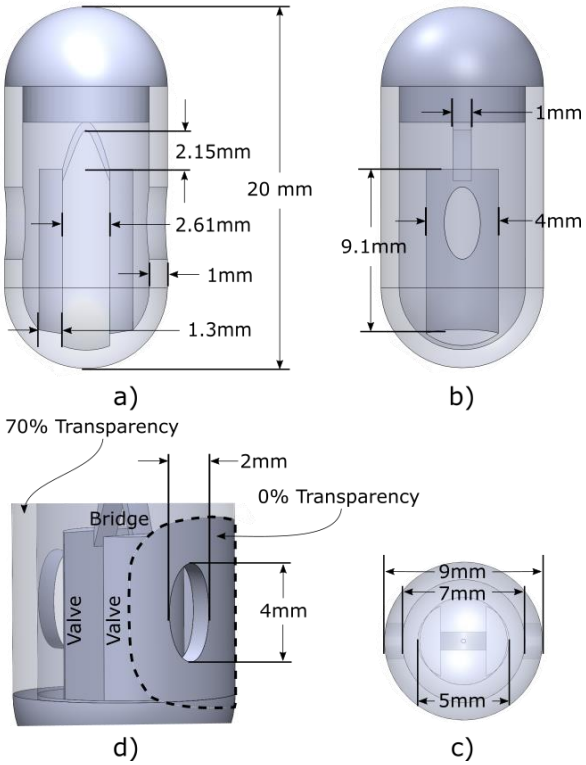

Figure 2: 3D schematic of the overall design of the elastic pill, a) side, b) front, and c) top views. d) Perspective view illustrating the side-inlets' dimensions of the elastic pill. (Partial transparencies have been added to better understand the internal parts of the pill.) 3D designed images were created using SolidWorks CAD tool.

The case of the pill was designed using a CAD tool such as SolidWorks (Dassault Systems SolidWorks Corporation, Waltham, MA). It is composed of two main sections: The bottom case has two-sided elliptical inlets and two internal microvalves for closing of the inlets autonomously. The top case is utilized as a shield for the pill once the beads are inserted.

The assembly process of the pill is as follows (Figure 3): Two sodium polyacrylate beads are inserted in the bottom section of the pill, in between the two valves. Afterwards, one sodium polyacrylate bead is placed inside the top section of the pill. The two sections are then attached using the same elastic resin (Formlabs Elastic 50A V1) and cured in the FormCure photocuring device (Formlabs Inc.) for 20 min. at 60°C. The pill is finally inserted in a pH-sensitive enteric coated capsule, to prevent it from sampling in the stomach's acidic environment.

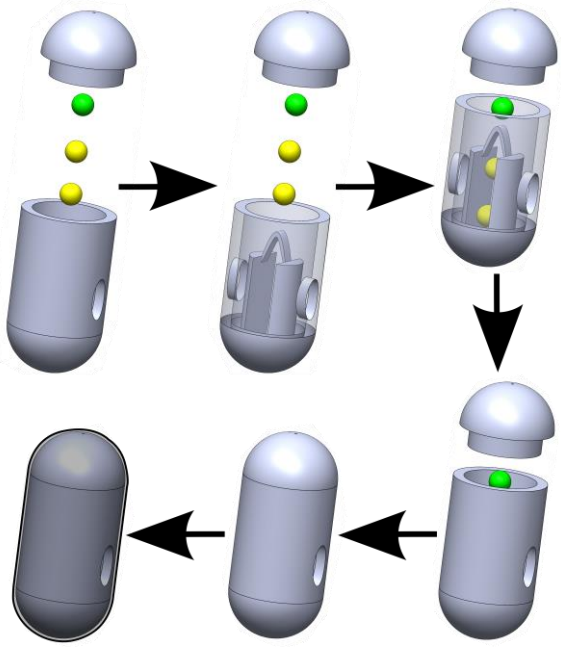

Figure 3: Assembly scheme of an elastic ingestible pill. (Some transparencies have been added to better illustrate the internal cavities of the proposed pill design.) 3D designed images were created using SolidWorks CAD tool.

RESULTS AND DISCUSSIONS

The sampling and locking mechanisms were first tested in-vitro, by inserting two red beads in the bottom section of the pill, in between the two valves. The pills were then placed vertically in a container filled with DI water, as shown in Figure 4.a (approximated viscosity of water was 8.90×10^{-4}Pa·s). DI water started flowing into the pill through the two side inlets, making the red beads expand. The internal gap between valves started increasing, until the valves fully locked the inlets, as shown in Figures 4.a and 4.b. A second experiment was performed with a higher viscosity sample (0.8Pa·s) and granulation, such as banana puree, to test sampling and locking performance. The pills' sampling performance was tested by measuring the time required to acquire the sample from the DI water and banana puree, until the valves fully locked the inlets. Results showed that within 20- and 90-minutes minutes, the beads were fully swollen, and inlets locked, preventing additional acquisition of DI water and banana puree, respectively (Figures 4.b and 4.c). The viscosity increment and granulation of the banana, in comparison to DI water, are the two main factors for the sample rate decrement. Yet, in both experiments, the beads fully expanded, pushing the valves outwards, and fully locking the elastic pills' inlets.

This work did not study the variation in size of the inlet-holes or valves. Yet, several deductions were obtained from preliminary design studies: sampling rate could be improved by increasing the cross-sectional area of the inlet, increasing the gap between inlets and valves, and by reducing the valve's overall width. Nevertheless, these variables are limited by the overall external

and internal sidewalls of the pill, overall height of the pill, and the required leakage and contamination performance level. Therefore, a trade-off between sampling rate, pill size and leakage/contamination performance needs to be met, to obtain the desired elastic ingestible device.

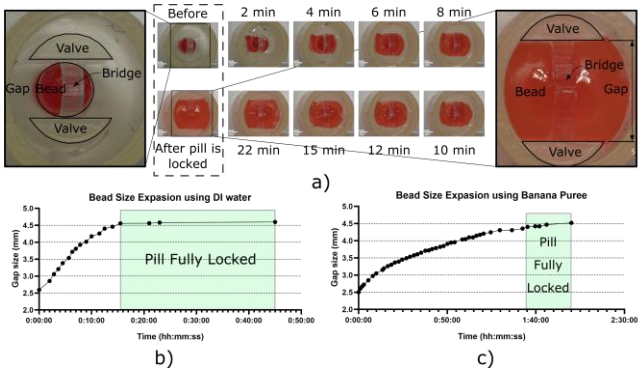

Figure 4: The locking mechanism performance of the elastic pill. a) Sequence of microscope images from pre-sampling stage of the bead inside of pill, until pill is fully locked with bead swollen. Images are captured with a Keyence VHX digital microscope, using -1° tilt angle, X30 magnification, and E20 lens. Top case of the pill is removed for sake of clarity during experiment. b) Graph illustrating the bead's (gap between valves) expansion progress over time using DI water. c) Graph illustrating the bead's (gap between valves) expansion progress over time using banana puree.

The elastic pill is covered with a pre-defined pH-sensitive enteric coating to delay sampling until it enters the small intestine, where the coating dissolves in the higher pH environment. To validate this initial sampling concept of the pill, the enteric coating performance was tested. The dissolution profile for the enteric coating can be controlled by an appropriate choice of polymer(s). The fabrication and testing of lab-made enteric coatings was not the focus of this study. Nonetheless, the elastic pills were inserted in commercial enteric capsules that could withstand up to pH 5. The enteric coating dissolution was tested by immersing enteric coated pills in a pH 2 buffer and kept in motion with a magnetic stirrer. Pills with gelatin-based capsules were also immersed and stirred in a neutral buffer for comparison purposes. Results showed that the enteric coated pills remained intact even after 2 hours of immersion and motion, where the gelatin-based pills' capsules dissolved withing 10 minutes as expected. The enteric coated pills were then transferred to an undyed pH 6 buffer, and stirred, where the coating dissolved exposing the inlets for sampling.

The results showed that the enteric capsule protects the pill from the stomach's acidic environment until it reaches the small intestine where it dissolves, allowing gut microbiome to enter the elastic pill. Farther studies and redesigns of the enteric coating could provide the pill with a more accurate initial sampling point. This, combined with a user-defined pill design, could more precisely define the start and finish sampling location within the small intestine, to better treat and diagnose gut conditions.

Finally, rigorous contamination tests were performed (Figure 5) by inserting post water sampling in a solution containing Rhodamine B dye and measuring any color change with a UV-Vis spectroscopy (Figure 5.a). We expect the content of the pill not to change color to prove no contamination.

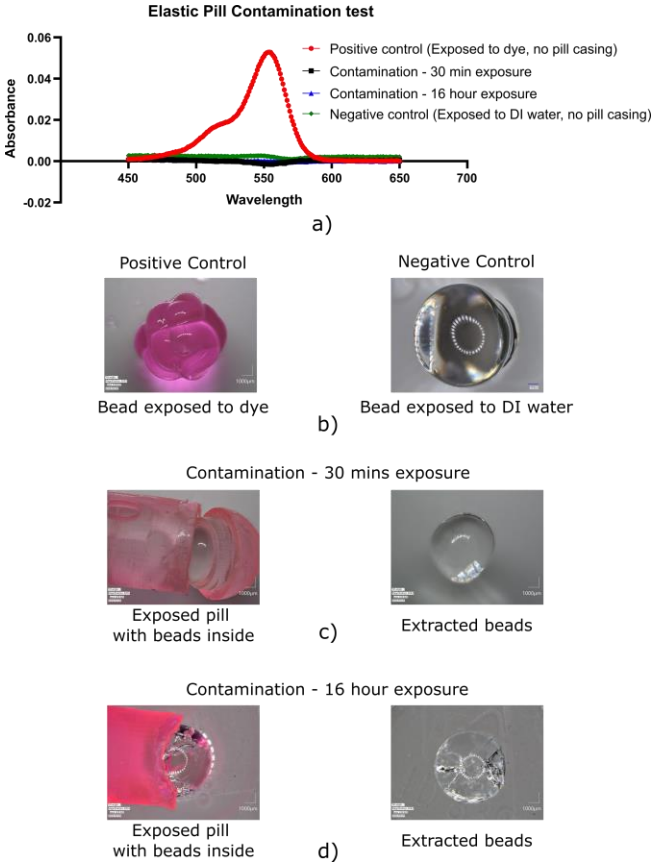

Figure 5: Contamination test performance of the elastic pill. a) Graph illustrating UV-Vis absorption spectrum results from the pills contamination experiments. Images are captured with a Keyence VHX digital microscope, using -1° tilt angle, X20 magnification, and E20 lens. Microscope images are taken to illustrate the difference in color between b) the positive and negative controls, where beads where directly exposed to dye and DI water, respectively; and contamination tests, where fully locked elastic pill were exposed to dye for c) 30 minutes and d) 16 hours.

A negative and positive control were first tested, by submerging beads in DI water and 20nmol Rhodamine/mL aqueous solution, respectively. Once beads were fully expanded (Figure 5.b), they were individually soaked in 3mL of DI water for 30 min. and their absorbance measured using UV-Vis spectroscopy.

Two contamination tests were then performed by submerging post-sampled and fully locked pills in 20nmol Rhodamine/1mL DI water for 30 minutes and 16 hours, as shown in Figures 5.c and 5.d, respectively.

The UV-Vis Spectroscopy showed a positive control with an absorbance peak of 554nm (Figure 5.a). Yet, the negative control and the contamination tests did not show any absorbance peaks at the same wavelength. Therefore, no measurable contamination was detected, indicating that the automatic closing valve worked as expected.

CONCLUSIONS AND FUTURE WORK

The proposed elastic ingestible pill can be realized using a SLA based 3D printer using an elastic resin. The fabrication process is simple and does not require any labor-intensive fabrication steps as would be required using a cleanroom or machine shop. Moreover, the design is scalable in size, which it can be used for sampling in small to large animals. Results from in-vitro experiments show that

the proposed elastic pill design is capable of sampling reliably with negligible leakage and contamination using different viscosity and granulation fluids.

Future research will focus on adjusting the composition of the enteric coating to control the initial sampling location in the GI tract. Further experiments will also be performed on the pill's side-inlets and valves by adjusting its size and design to alter the sampling rate and farther decrease potential leakages. Finally, validation of the elastic pills will be performed in ex-vivo sections of the GI track and in-vivo, in pig animal models.

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REFERENCES

[1] J. Qin, R. Li, Jeroen Raes, et al., "A human gut microbial gene catalogue established by metagenomic sequencing" *Nature*, 464, 03 (2010).

[2] D. Kumral, and A. Zfass, "Gut movements: A review of the physiology of gastrointestinal transit", *Digestive Diseases and Sciences*, 63, 10 (2018).

[3] H. R. Nejad, B. Oliveira, et al., "Ingestible osmotic pill for in vivo sampling of gut microbiomes", *Advanced Intelligent Systems*, 1, 07 (2019).

[4] N. Mandsberg, J. Christfort, et al., "Orally ingestible medical devices for gut engineering", *Advanced Drug Delivery Reviews*, 165-166, 05 (2020).

[5] W. Weitschies, L. Muller, et al., "Ingestible devices for studying the gastrointestinal physiology and their application in oral biopharmaceutics", *Advanced Drug Delivery Reviews*, 176 (2021).

[6] C. Steiger, A. Abramson, et al., "Ingestible electronics for diagnostics and therapy", *Nature Review Materials*, 83-98, 4 (2019).

[7] M. Koziolek, M. Grimm, et al., "Investigation of pH and temperature profiles in the GI tract of fasted human subjects using the Intellicap® System", *J. Pharm. Sci.,* 2855-2863, 104 (2015).

[8] C. Cheng, Y. Wu, et. al., "A wireless, ingestible pH sensing capsule system based on iridium oxide for monitoring gastrointestinal health", *Sensors and Actuators B: Chemical*, 130781 (2021).

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