Development of a Robotic Capsule for in vivo Sampling of Gut Microbiota

Muhammad Rehan, Ibrahim Al-Bahadly, *Senior Member, IEEE*, David G. Thomas, and Ebubekir Avci, *Member, IEEE*

***Abstract*—Human gut microbiota can provide comprehensive information about the health of a host but the tools to collect microbiome samples are not currently available. A standalone wireless robotic capsule that has been developed in this study, collects the microbiota both from lumen (capsule surrounding) and intestinal wall (mucosa layer) for the first time. First, a two-way shape memory alloy (SMA) spring actuation system was developed by tackling the high-drain current requirement of SMAs. The actuator can produce up to 800 mN force that was sufficient to collect samples. Second, successful encapsulation of the collected sample to avoid contamination was realised by testing 3 main sealing materials. Third, the robotic capsule was tested in a gut simulator that mimics in-vivo environment to ensure successful and safe travel of the capsule along the gastrointestinal tract. Finally an in vitro experimental setup that keeps an intestine alive for 6 hours was used to optimise the sample collection. The capsule collected 128 μL and 107 μL samples (which are sufficient quantities for microbiome analysis) from duodenual and ileal tissues of a sheep. The proposed robotic capsule has a potential to become a vital apparatus for clinicians to sample human and animal gut in the future.**

***Index Terms*—Endoscopic capsule, GI tract, peristaltic motion, robotic capsule, SMA spring actuation system.**

I. Introduction

GUT microbiota (microorganisms) can potentially act as a bio-marker to diagnose an increasing range of health problems such as cancer, type 2 diabetes, inflammatory bowel disease (IBD), and obesity [1]–[3]. Furthermore, the microbiota can be helpful in understanding stress, anxiety, mood and behaviour [4], and can assist in studying the interaction between nutrition and human health [3]. These microorganisms spread along the full length of the 7-9 m gut, weigh up to 2 kilograms and are densely packed within digesta mainly inside the gut lining [5].

The current gold standard for obtaining the gut microbiota is faecal sampling, as it is non-invasive and easy to collect. The advancement in sequencing methods has allowed to consider the faecal sample as a proxy for gut microbiota [2]. However, there is a baseline problem with this method that is its inability to segregate the longitudinal population of gut microbiota. Since the faecal samples are collected after travelling through entire gut, they represent a cumulative sample which is highly contaminated that limits to investigate the site-specific microbial population as temporal and spatial information is impossible to retrieve [6]. It is important to perform site-specific sampling and avoid contamination from other regions as the microbial population throughout the intestine varies and holds different information. Therefore, faecal sampling is not an accurate method to analyse gut microbiota [6].

Commercial endoscopes that can capture the images of the gut lining, have laid the foundation for similar sized robotic capsules that can perform monitoring, therapeutic and diagnostic functions like sensing, drug delivery and tissue biopsy [7]–[15]. In recent years, a few approaches have been proposed to collect the gut microbiota by robotic capsule which are mainly classified in two broad categories, uncontrolled (passive) and controlled (active) collection mechanisms. Uncontrolled mechanisms propose to open the inlet of a chamber at the target site to collect a sample but haven't considered the sealing of the opened gate after collection that still leads to downstream contamination [16]–[18]. A bi-stable mechanism overcame the downstream contamination problem by closing the inlet but the design couldn't stop the upstream contamination effectively and site-specific sample collection was not possible [19]. Controlled mechanisms overcame the challenges of upstream and downstream contamination and only opened at the target-site to avoid cross contamination from other regions of the gut by using a range of actuation systems like magnets, motors and shape memory alloy (SMA) materials [20]–[23]. However, most of these proposed designs were based on arbitrary collection of surrounding fluid which does not capture the full microbiome as significant populations of microorganisms live on the gut lining (wall) [5] and the sampling mechanism needs to scrape or brush the intestinal wall to capture a full microbiome sample

Manuscript received 24 February 2022; accepted 4 July 2022. Date of publication 15 July 2022; date of current version 26 July 2022. This letter was recommended for publication by Associate Editor C. Bergeles and Editor J. Burgner-Kahrs upon evaluation of the reviewers' comments. This work was supported in part by Massey University SREF 2022 and Palmerston North Medical Research Foundation (PNMRF) 2021–2022, and in part by Higher Education Commission (HEC), Pakistan, under Grant 5-1/HRD/UESTPI(Batch-V)/2421/2018/HEC. *(Corresponding author: Ebubekir Avci.)*

Muhammad Rehan is with the Department of Mechanical and Electrical Engineering, Massey University, Palmerston North 4410, New Zealand, and also with the Department of Electronic Engineering, Sir Syed University of Engineering and Technology, Karachi 75300, Pakistan (e-mail: murehan@hotmail.com).

Ibrahim Al-Bahadly is with the Department of Mechanical and Electrical Engineering, Massey University, Palmerston North 4410, New Zealand (e-mail: I.H.AlBahadly@massey.ac.nz).

David G. Thomas is with the Monogastric Research Centre, Massey University, Palmerston North 4410, New Zealand (e-mail: d.g.thomas@massey.ac.nz).

Ebubekir Avci is with the Department of Mechanical and Electrical Engineering, Massey University, Palmerston North 4410, New Zealand, and also with the MacDiarmid Institute for Advanced Materials and Nanotechnology, Wellington, New Zealand (e-mail: E.Avci@massey.ac.nz).

This letter has supplementary downloadable material available at https://doi.org/10.1109/LRA.2022.3191177, provided by the authors.

Digital Object Identifier 10.1109/LRA.2022.3191177

[24], [25]. The sampling location as well as the procedure used to collect the microbiota has critical implications on the quality of the information retrieved from sampling devices as the microbiome composition varies both longitudinally (e.g., duodenum, jejunum and ileum) and axially (e.g., lumen, epithelium, mucosa, submucosa) [26]. Recently, a magnetic actuation system was proposed to collect the microbiota from gut lining by brushing the mucosa layer but intestinal trials are yet to be realised [24].

In our previous work, we presented a sampling device that collected a microbial sample from ex-vivo sections of intestine, but the power was supplied by an external AAA battery connected as a tail (tethered device), so in-vivo testing was not possible with that design [27]. In this current study, an untethered and standalone robotic capsule is presented that can actively collect the sample from the target site and capture the full microbial population from the inner wall of the small intestine. A customised battery (10 mm x ϕ 10 mm) with a high-drain current was utilised to energise the two-way SMA spring actuator (5 mm $\times$ ϕ 4 mm). A wireless transceiver (17 mm $\times$ 10.5 mm) was integrated into the robotic capsule to trigger the sampling process at the target site during in-vivo trials. The sealing mechanism was rigorously tested so the robotic capsule can get through the acidic environment of the stomach and also protect the sample from cross contamination. This letter elaborates the design considerations, testing and evaluation of proposed standalone robotic capsule, and the feasibility of proposed design for in-vivo trials. The main novelties of this work, as compared to the state of the art sampling devices, are shown below:

1) The development of an untethered and standalone robotic capsule suitable for collection of gut microbiota from the inner wall of an intestine for the first time [16]–[23].
2) The design and development of a miniaturised actuation system by overcoming the high-drain current limitations of mm scale batteries to power the SMA actuators which was not achieved previously [27]–[29].
3) The development and extensive testing of sealing mechanism to withstand the entire gut environment and also protect the collected sample from cross contamination which is essential for in-vivo trials and was not achieved before by state of the art sampling devices [16], [17], [24].

In this work, a standalone robotic capsule with all the components was assembled and tested in a specialised experimental setup that closely replicated the in-vivo environment in terms of temperature and peristalsis. The robotic capsule was also tested in a gut simulator which replicated the stomach (acidic) and intestinal environment to determine if the capsule can withstand the challenges of an in-vivo environment and protect the sample from cross contamination. The experimental results provided a proof of concept case for in-vivo testing that is the next stage of this project.

II. Materials and Methods

A. System Configuration

The proposed robotic capsule in this study is shown in Fig. 1(a) with its internal components, and the developed capsule prototype is shown in Fig. 1(b). Whereas the sampling mechanism and the sealing options are shown in Fig. 1(c) and (d) respectively.

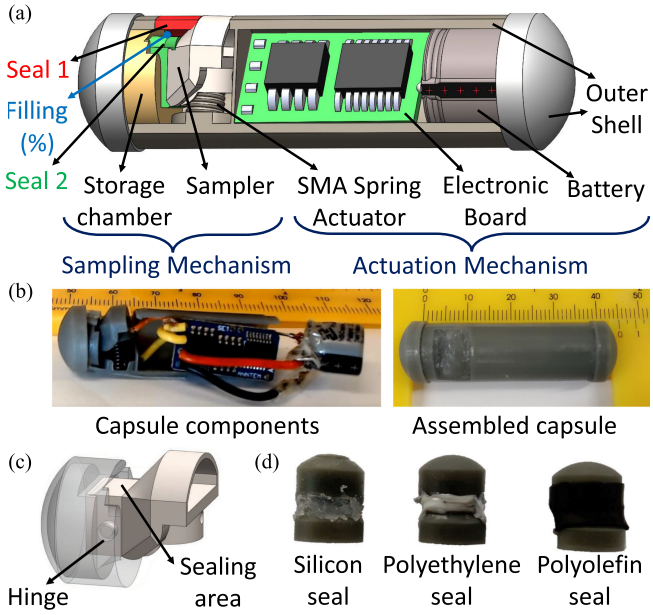

Fig. 1. Standalone robotic capsule for gut sampling. (a) CAD model. (b) Robotic capsule prototype. (c) Hinge mechanism between sampler and storage chamber. (d) Tested sealing materials at the hinge mechanism.

1) Capsule Size and Storage Capacity: The overall size of the capsule used in this work is 45 mm in length and 12 mm in diameter as shown in Fig. 1(b), which can be tested in large animals e.g., cows and horses [30]. The size of the sampling chamber in the proposed capsule is 250 μL and is sufficient to do the lab analysis after sample retrieval with the help of next generation sequencing techniques [31].

2) Bio-Compatibility and Capsule Material: Sampling the gut in-vivo requires two-way protection, first the capsule should not damage the gut during its passage and second the collected sample should be protected inside the storage chamber. Both the outer shell and sampling mechanism were fabricated with biocompatible resin to protect the gut environment and the collected sample. The actuation mechanism was encapsulated by a capsule shell to avoid any interaction with the gut fluids. Capsule shell strength is critical due to the possibility of deterioration inside the gut due to either acidic exposure in the stomach or peristaltic forces in the small intestine. Therefore, the tensile strength of three different resins, as shown below, were measured to select the optimum material for the robotic capsule fabrication.

1) FHD1200 (Flashforge, China)
2) Bioflex D60 (3Dresyn, Resyner Technologies, Spain)
3) Temp (PowerResins, 3bfab, Turkey)

The most hostile environment for the robotic capsule is the stomach that is highly acidic (pH 1.5) and can deteriorate objects if they are retained in this region for extended periods. Therefore, 30 mm $\times$ 12 mm long rectangular strips with five different thicknesses (0.5 mm, 0.75 mm, 1 mm, 1.25 mm 1.5 mm) were prepared with the three selected resins and placed in an in-vitro

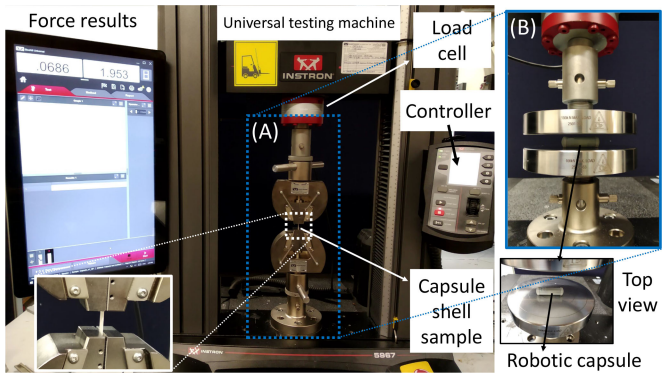

Fig. 2. Universal Testing Machine. (a) Tensile strength measurement of bio-compatible materials for capsule fabrication. (b) Actuator force output measurement of robotic capsule under various sealing mechanisms.

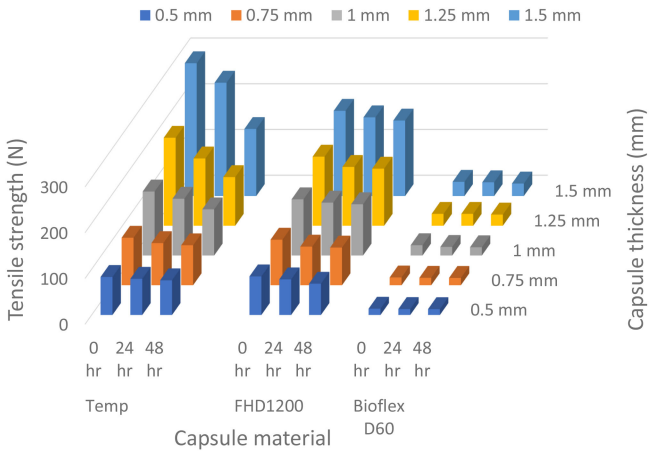

Fig. 3. Material strength testing.

stomach environment (details in next section) at 1.5 pH for 2 days. Five thicknesses were selected as different components of the capsule has different thicknesses ranging from 0.5 mm to 1.5 mm. Tensile strength of the three different bio-compatible capsule materials was measured to monitor any decay in their strength at 0 hr (without acid exposure), 24 hrs (1 day exposure) and 48 hrs (2 days exposure) using universal testing machine (UTM) (5967 Instron, US) as shown in Fig. 2(a). Each measurement was taken four times and the average value is plotted in Fig. 3.

Temp resin showed the highest strength as compared to the other test resins, however the strength reduced significantly (up to 50% for 1.5 mm) under the acidic environment as shown in Fig. 3. Bioflex D60 offered very low strength (i.e., maximum 17%) as compared to other tested resins as shown in Fig. 3 so could not be used for the robotic capsule applications at 1.5 mm or less thickness. The FHD1200 strength was similar to Temp resin for less than 1 mm thickness, but slightly less than the Temp resin for more than 1 mm thickness. FDH1200 remained more stable in the stomach environment as compared to Temp (decay up to 19% maximum in 2 days). Therefore, FDH1200 resin was selected to fabricate the capsule shell, storage chamber and sampler of the robotic capsule as shown in Fig. 1.

B. Sealing Mechanism

An important aspect for targeted sampling is to secure the storage chamber from cross contamination with gut fluids from the locations other than the target-site. The sealing mechanism should protect the storage chamber from both pre-sampling and post-sampling contamination by enabling a proper seal throughout passage along the gut. The proposed sampling system is based on a hinge mechanism that helps to scrape the microbiota from the gut lining (wall) during sample collection at the target-site. Therefore, it is critical to design an efficient sealing mechanism to stop any leakage from the hinge mechanism as shown in Fig. 1(c). At the same time, the sealant should not be too rigid otherwise it will increase the resistance force against the opening motion of the actuation mechanism that consists of the two-way SMA spring actuator. Three different materials i.e., polyolefin, polyethylene and silicon were selected based on their flexibility to ensure a proper seal. The three sealants as shown in Fig. 1(d) were examined for both the sealing efficiency and the decrease in output force of the actuator.

1) Pre-Sampling Sealing Test – Stomach Model: The robotic capsule will pass through the esophagus and stomach before reaching the small intestine (target-site). The esophagus is a straight tube and does not affect the capsule during its few seconds passage along it. The stomach is a strongly acidic environment and overstaying can result in damaging to the capsule if not constructed from a suitable material [21], [22]. The gastric juice, maintained at pH 1.5, was prepared with hydrochloric acid and pepsin, and was kept circulating by a magnetic stirrer machine (VWR, US) replicating the stomach environment as shown in Fig. 4(a) [21]. The chamber of the capsule was pre-filled with a yellow litmus letter and the capsule was placed in the stomach model environment (magnetic stirrer) for 6 hours which is twice the time expected for the capsule to stay in the stomach [32]. The litmus paper inside the storage chamber was observed afterwards to determine any leakage into the chamber. A small leak was detected in 1 out of the 5 trials using the polyethylene seal, while the polyolefin and silicon seals showed no leakage in any of the 5 trials carried out during the pre-sampling testing.

2) Post-Sampling Sealing Test - Agitation Based Intestinal Model: The robotic capsule is designed to collect a sample from the small intestine and it has to protect the sample afterwards from downstream contamination i.e., mixing of gut fluids after sample collection at the target-site. Therefore, an agitation based in-vitro intestinal model was utilised for testing the sealing mechanism after sample collection under turbulent conditions [22].

A centrifuge mixture (5702, Eppendorf, Germany) was used to agitate the collected sample inside the storage chamber of the robotic capsule by spinning the test tube at 1000 rpm for 3 hours which created a harsher environment than the peristaltic motion of the intestine and allowed testing of the sealing mechanism at a higher confidence level [22]. The storage chamber of robotic

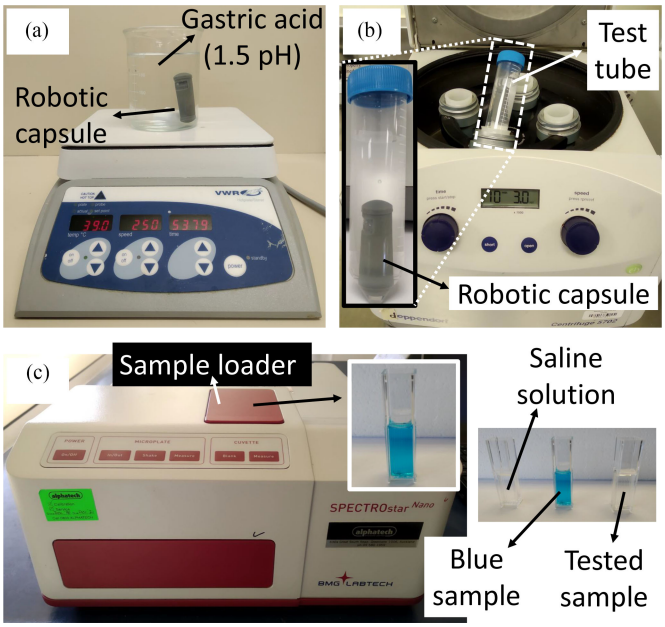

Fig. 4. (a) A magnetic stirrer machine used to replicate the stomach model and test the pre-sampling sealing mechanism. (b) A centrifuge machine to test the post-sampling sealing mechanism under agitation. (c) A spectrophotometer to analyse any potential leakage from the sealing mechanism.

capsule was pre-filled with a blue coloured sample and the capsule was placed in a test tube filled with a colourless solution (saline) as shown in Fig. 4(b). The blue coloured sample was prepared by adding a blue food colour liquid to water that allowed to test the sealing mechanism rigorously as liquid sample has more chances of leakage as opposed to a gel type viscous sample which will be the actual targeted material (mucus/microbiota) during in-vivo trials.

The saline solution inside the test tube was observed after the agitation for any traces of blue colour using a spectrophotometer (SPECTROstar Nano, BMG Labtech, Germany) as shown in Fig. 4(c), which could determine any leakage from the storage chamber into the surrounding saline solution. The spectrophotometer measured the optical density (OD) of the blue sample to act as a reference for tested samples, and it determine an OD above 3 as shown in Fig. 5. Samples of surrounding fluid from the test tube were taken from each trial with respective seals (polyolefin, polyethylene, silicon) and measured for any potential traces of blue colour mixing using spectrophotometer. Each sample returned zero OD as shown in Fig. 5, which shows all the three seals performed well under turbulent conditions.

C. Actuation Mechanism

An active actuation mechanism was designed as shown in Fig. 6 that triggers the sampling process once the capsule is at the target-site. The wireless transmitter (Shenzhen Anntem Technology Co. Ltd, China) operates at 433 MHz, activates the sampling process when the capsule is at the target-site and the wireless receiver inside the capsule activates the SMA spring actuator through a current driver circuit. The two-way SMA spring actuator is a bi-directional spring that applies force in

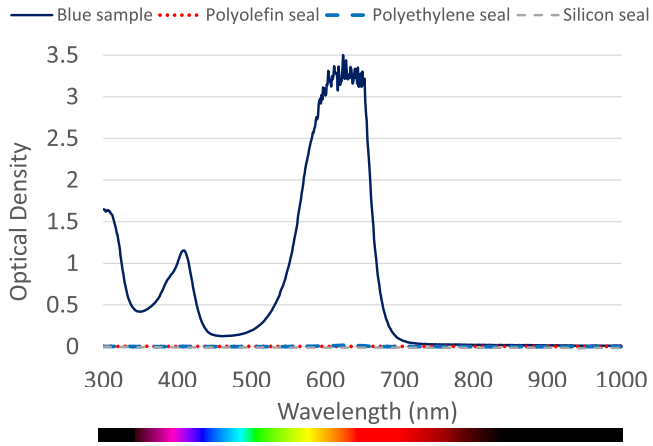

Fig. 5. Spectrophotometer results for the post-sampling sealing test under agitation conditions. The blue sample shows OD above 3 while all the three tested samples returned zero OD showing there was no leakage from the storage chamber.

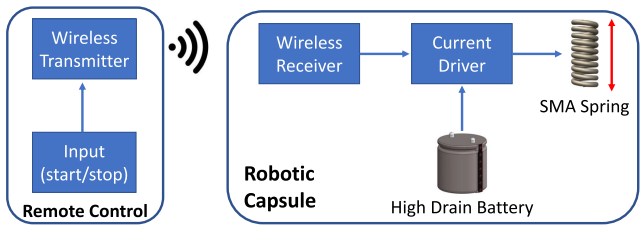

Fig. 6. Actuation mechanism of robotic capsule.

both directions with the changes in the temperature. The spring expands (increases in length) when the temperature reaches 52 °C and move back to its original length (compress) when temperature falls below 42 °C. The temperature difference between the expansion and compression states of the SMA spring is due to the hysteresis effect between austenite and martensite states of the SMA material which is explained in our previous work [27]. The temperature change in the spring is produced by passing the current through the spring which increases the temperature of the spring due to joule heating. The wireless activation and bi-directional movement of the SMA spring for opening and closing the sampler is shown in the supplementary video.

1) Battery Design and Testing: The two-way SMA spring actuator (Kellogg's Research Lab, US) was specially designed for the proposed robotic capsule. This is a compact actuator (5 mm x ϕ 4 mm) but it required higher amounts of current (500 mA - 1 A) as it was activated by joule heating. Fulfilling the requirement of high current is an arduous task due to the scarcity of high-drain small-size batteries. Therefore, systematic experiments were conducted to test both commercially available and custom-made batteries considering high-drain current requirements. Total sampling duration was 5 minutes and the battery was required to supply the power to the actuator during this time. The battery size can be calculated by (1)

$$C(mAh) = I(mA) * t(h) \tag{1}$$

TABLE I
BATTERIES TESTING ON ACTUATOR

Name	Dimensions (mm)	Capacity (mAh)	Actuator Response Time (s)	Current Drain Range (mA)
LR44/SR44 (Sony, Japan)	5.4 x ϕ 11.6	150	-	15-35
675 (Eclipse, US)	5.4 x ϕ 11.6	620	-	20-100
M10100 (Custom made, Friendly Store, China)	11.4 x ϕ 10	60	-	10-35
1/3AAA (Custom made, Shop910, China)	16 x ϕ 10	170	-	370 – 460
1/3AAA (Custom made, Shop910, China)	14 x ϕ 10	200	-	200 – 300
2/3AAA (Custom made, Friendly Store, China)	28.2 x ϕ 10.1	400	-	250 – 350
2/3AAA (Custom made, Friendly Store, China)	28 x ϕ 10	400	60	350 – 450
2/3N (Custom made, Friendly Store, China)	28.5 x ϕ 12	300	80	200 – 350
NSC1010 (Huahui New Energy, China)	10 x ϕ 10	40	2	800 – 1400
HCC1015 (Huahui New Energy, China)	15 x ϕ 10	70	1.8	2000 – 3000
ICR10180 (Custom made, Friendly Store, China)	18 x ϕ 10	150	1.5	1500 – 3500
S10 (Custom made, Shop910, China)	18 x ϕ 10	100	1.2	2500 – 3000
HCC1020 (Huahui New Energy, China)	20 x ϕ 10	90	1	3000 – 3500

where, C is the battery capacity, I is the required amount of current and t is the actuation time (battery life).

Both commercially available and custom designed high drain batteries were tested on two-way SMA spring actuator to check the performance of the actuation mechanism. The size and capacity of each battery along with current drain are shown in Table I. The battery with the lesser response rate (t $<$ 5 s), higher drain current (I $>$ 500 mA) and smallest size was selected (NSC1010) (Huahui New Energy, China).

2) Force Measurement: A two-way SMA spring actuator was used to open and close the sampler with the help of hinge mechanism as shown in Fig. 1(c). The amount of force required to open the sampler at the target-site is defined in (2)

$$F_O > F_P + f + f_S \tag{2}$$

where, F_O is the opening force applied by the sampler, F_P is the force applied by the intestine (peristaltic force) which was determined to be 226 mN (average) in our previous work [33], f_S is the resistance produced by the sealing mechanism and f is the accumulated frictional force which can be further defined as

$$f = f_c + f_m + f_v = \mu F_s + F_s' + \delta N_v v \tag{3}$$

where, f_c is the coulomb friction which is the stress on the capsule due to intestinal motion, f_m is the marginal resistance that happens due to deformation of intestinal wall which restricts the sampler movement, and f_v is the viscous resistance that restricts the capsule motion due to obstacles (e.g., chyme or digesta). The frictional forces are further elaborated on in (3), where μ is the coefficient of friction, F_s is the normal force on the sampler, F_s' is the force due to the deformation of the intestine, δ is the coefficient of viscosity, N_v is the radial stress on the sampler and v is the velocity of sampler. These frictional forces were described in our previous work, and overall frictional forces can be considered as 200mN [25].

The sealing mechanism has increased the amount of resistance (f_S) hence increasing the required force to open the sampler at the target site as shown in (2). Therefore, we have measured the overall actuator force generated before and after the sealing mechanism was added as shown in Fig. 7 to choose the optimum

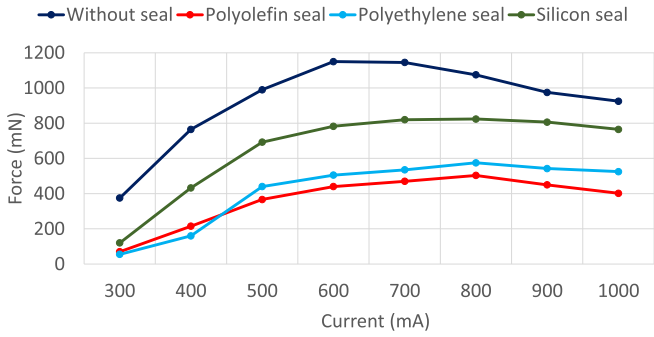

Fig. 7. Force analysis before and after sealing mechanism.

sealant for our capsule. A UTM as shown in Fig. 2(b) was used to measure the accumulated sampler force.

The two-way SMA spring actuator generates more than 1 N force for currents between 500 mA to 900 mA without the sealing mechanism, which can be supplied by the selected battery (NSC1010). The polyolefin seal and polyethylene seal offered higher resistance to the actuator, hence overall output force with these seals was less than 600 mN. The silicon seal showed greater flexibility at the hinge mechanism and the actuator applied around 800 mN force after this seal was added. Since the required force to collect a digesta sample is more than 426 mN (F_P is 226 mN and f is 200 mN), all three materials can be used as sealants.

Based on pre-sampling sealing test, post-sampling sealing test and force measurement experiments, silicon seal was selected as it offered greater output force while stopping the leakage into the storage chamber completely.

D. Development of a Gut Simulator

A customised in-vitro gut simulator was designed to replicate the overall gut environment in terms of pH, temperature, enzyme activity, digesta content and retention time to allow the capsule to experience gut transit in-vivo and determine any potential failure point. A 15 g dog food sample along with robotic capsule were placed in a specimen container on 15 place hotplate magnetic stirrer (IKA RO 15, Bio-strategy, New Zealand) as shown in

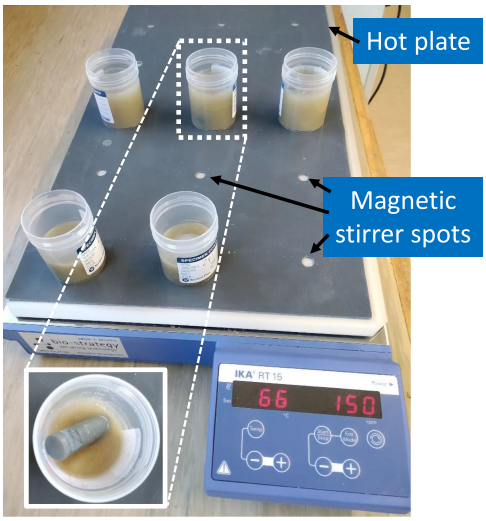

Fig. 8. In-vitro gut simulator.

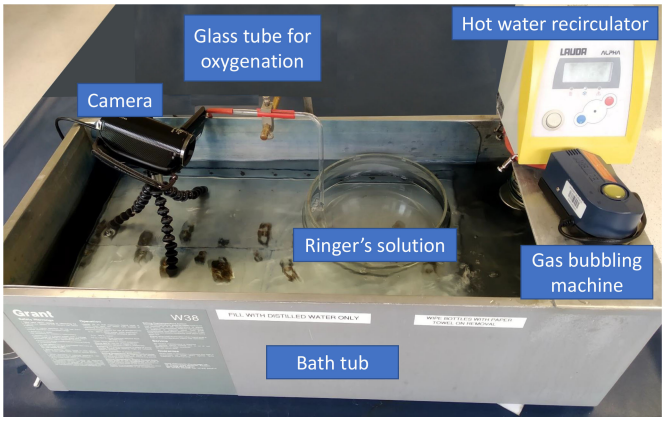

Fig. 9. In-vitro experimental setup to maintain peristaltic movements of ex-vivo intestine.

Fig. 8. The bench has a built-in hot plate which kept the temperature at 37 °C and the magnetic stirrer continuously rotated the sample to replicate the gastric motion which is common to digestion [34]. Water, HCl and pepsin were added to maintain the pH at 2.0 as per the specified quantities in [34] and the mixture along with the capsule was stirred at 150 rpm for 3 hours to simulate the gastric environment [32]. Afterwards, pancreatin, $NaHCO_3$ and Na maleate buffer solution were added to neutralise the pH at 7.0 as per the specified quantities in [34] before stirring for another 3 hours to simulate the intestinal environment. The robotic capsule retention timings for each phase, e.g., stomach and small intestine, were obtained from a related study on beagle dogs [32].

E. Living Intestinal Trials for Sample Collection

A customised in-vitro experimental setup was designed as shown in Fig. 9, that can maintain the peristaltic movements of a freshly dissected animal intestine [35]. The experimental setup keeps the intestinal tissue alive for 6 hours at a pH of 7.4 using ringer's solution under 39 °C [27]. The system acted as a proxy for in-vivo trials and the sampling capability of the wireless robotic capsule was tested using living intestinal tissue exhibiting peristaltic movements as shown in supplementary video.

III. Results and Discussion

A. Gut Simulator – in Vitro Study

The storage chambers of three capsules were pre-filled with a yellow litmus paper and these capsules were not actuated (opened) during entire journey in the gut simulator to check the sealing capability. After passing through both stomach and intestinal environments, the colour of litmus paper did not change, as shown in the supplementary video, which indicates the seal worked successfully during the trials. The aim of these trials was to rigorously test the sealing mechanism in a proxy in-vivo environment.

In addition, two separate capsules without litmus paper (empty storage chambers) were also tested which were actuated (opened) during intestinal simulation phase. After the trials, the storage chamber were checked and they were filled with the content, which demonstrated the capsule had collected the surrounding fluid (sample) during these trials.

B. Sampling Trials on Living Intestines

The fresh small intestines of sheep were obtained from a local butcher and cut into 300 mm long sections. The robotic capsule was inserted into the intestinal tissue manually and placed in the ringer's solution (in vitro experimental setup). Once the intestine started applying the peristaltic forces that can be seen in the supplementary video, the robotic capsule was actuated wirelessly to collect the sample. The sample collection process was stopped after 5 minutes through wireless transceiver and the collected quantity of digesta was measured afterwards.

1) Seal Optimisation for Optimum Collection: Initial trials resulted in collecting an average of 68 μL content which was less than the targeted (desired) quantity of 100 μL [36] (the amount for the assessment of full microbiome using next generation sequencing methods [31]). This mainly occurred due to the seal which stopped the sampler from fully opening against the intestinal wall. Therefore, the filling percentage of the silicon seal between seal 1 and seal 2 as shown in Fig. 1(a) was optimised to allow the sampler to open wider and collect more sample. The silicon seal was filled at 3 levels i.e., 100%, 75% and 50% and they all ensured a proper seal without any leakage during pre-sampling and post-sampling trials as detailed in the previous sections. The robotic capsule collected an average of 108 μL sample at 75% filling and 133 μL sample at 50% filling as shown in Fig. 10 which shows that lesser filling makes the sealing area more flexible allowing the sampler to open fully. Therefore, 50% seal was considered as an optimum level of filling which allows collection of more than the 100 μL content (desired quantity [36]) and also ensured the proper encapsulation (no leakage). Each trial was performed three times and the standard

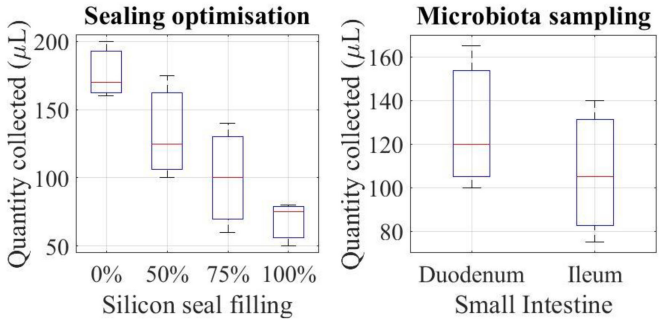

Fig. 10. (Left) Optimisation of filling percentage for the silicon seal of robotic capsule under intestinal trials. (Right) Sample collection from duodenal and ileal tissue after optimisation.

deviation was 16.9, 31.1, 34.7 and 13.1 for 0%, 50%, 75% and 100% seal fillings respectively.

2) Sample Collection Trials: The robotic capsule was tested using duodenal and ileal tissues which are the beginning and ending regions of small intestine to ascertain effective collection throughout the small intestine during future in vivo trials. The capsule collected an average of 128 μL and 107 μL of digesta from duodenal and ileal regions as shown in Fig. 10. Each trial was performed three times and the standard deviation was 27.1 and 26.5 for duodenal and ileal regions respectively. The duodenal tissue resulted in higher collection as compared to ileum region possibly due to its smaller diameter that resulted in better collection from the mucosa layer (inner wall of intestine).

C. Discussion

Most of the capsules described in the literature focus on arbitrary collection of surrounding fluid which cannot guarantee collecting a representative microbiome sample from a gut region. The developed robotic capsule has a capability to collect a full microbiome sample with the help of its unique design, which scrapes the content from the intestinal wall as a significant population of microbiota reside on the mucosa layer [5]. During intestinal trials the opened sampler was facing the intestinal wall and the peristaltic forces pushed it forward aiding to the sample collection. During in-vivo trials capsules may not enter the intestine in forward direction, so the capsule was also tested in a backward orientation (sampler facing the intestinal wall from the back) and it collected an average of 85 μL content in two trials which is less than the desired quantity of 100 μL, but still sufficient to perform the microbiome assessment [36]. Though the targeted (desired) sampling quantity of 100 μL allows the assessment of full microbiome using next generation sequencing methods, samples between 18 μL and 61 μL were successfully analysed in a related study that shows lesser quantities can be used for microbiota analysis [22]. If needed, the direction the capsule faces as it enters the intestine can be controlled during in-vivo trials with magnetic locomotion methods to collect more than 100 μL sample size [8], [12].

The sampler in the proposed design needs to overcome peristaltic forces to scrape the microbiota population from the intestine wall, hence it requires an actuator with a higher output force. A two-way SMA spring actuator is designed for the proposed prototype which is rarely used before by any other robotic capsule studies. The major limitation of SMA actuators is a high current requirement and hence small batteries are not suitable. In the proposed actuation system, by evaluating various customised and commercially available high drain batteries, a suitable candidate has been selected, which was small enough (10 mm x ϕ 10 mm) to fit into a robotic capsule. Both inside and outside temperatures of the robotic capsule were measured using a thermal imaging camera (TG167, FLIR Systems Inc., USA) during actuation which determined that the inside temperature was 53.2 °C while the outside temperature was 34.4 °C. This shows that the high-drain battery or the SMA spring (when hot) will not pose any harm during in-vivo studies. The wireless transceiver used for initiating the sampling process will be useful during in vivo trials as targeted sampling can be achieved using imaging devices, e.g., X-ray or ultrasound [37].

Sealing is very important to avoid contamination both before and after the sample collection which is designed, optimised and extensively tested to protect the storage chamber during in vivo trials. The sealing also makes sure that the sample does not leak out after collection and the sample is ready for analysis after retrieval.

The current capsule prototype (45 mm x ϕ 12 mm) can be tested on large animals e.g., cows and horses but will require further miniaturisation for human testing. The length of current wireless board is 17 mm which can be reduced to 5 mm by miniaturising it on a round PCB which will reduce the capsule size to 33 mm x ϕ 12 mm that will be similar to the acceptable capsule size for human trials approved by FDA (PillCam UGI 32.3 mm x ϕ 11.6 mm) [38].

IV. Conclusion

A standalone robotic capsule was developed to collect the microbiota from the gut during in-vivo trials. A small size high-drain current battery (necessary for SMA actuators) has been utilised to develop a standalone robotic capsule. The challenges of safety, both for the host and the capsule itself are resolved by fabricating the capsule with bio-compatible materials that neither deteriorates nor harms the host. Three sealing materials were tested for the proposed capsule to secure the sample from contamination and the selected material (silicon) was optimised to ensure optimum sample collection at the target-site. A special gut simulator was designed to replicate the different stages of in-vivo environment and the capsule underwent full testing to ensure safe passage during future in-vivo trials. Lastly, the capsule has successfully collected more than the target amount of 100 μL sample under proxy in-vivo environment (living intestine), which provides enough confidence to perform in-vivo studies in the next study.

Animal Ethics

The animal intestines were sourced from a butcher and it was not required to kill any animal for our research. Massey University Animal Ethics Committee only require animal ethics approval if the animal is killed for the research itself, which was not the case in this study. Therefore, the study is exempt from animal ethics approval.

REFERENCES

[1] Y. Fan and O. Pedersen, "Gut microbiota in human metabolic health and disease," *Nature Rev. Microbiol.*, vol. 19, no. 1, pp. 55–71, 2021.

[2] M. Kim *et al.*, "Fecal metabolomic signatures in colorectal adenoma patients are associated with gut microbiota and early events of colorectal cancer pathogenesis," *Mbio*, vol. 11, no. 1, 2020, Art. no. e03186-19.

[3] M. Jayachandran, S. S. M. Chung, and B. Xu, "A critical review of the relationship between dietary components, the gut microbe akkermansia muciniphila, and human health," *Critical Rev. Food Sci. Nutr.*, vol. 60, no. 13, pp. 2265–2276, 2020.

[4] K. Rea, T. G. Dinan, and J. F. Cryan, "Gut microbiota: A perspective for psychiatrists," *Neuropsychobiology*, vol. 79, no. 1/2, pp. 50–62, 2020.

[5] G. P. Donaldson, S. M. Lee, and S. K. Mazmanian, "Gut biogeography of the bacterial microbiota," *Nature Rev. Microbiol.*, vol. 14, no. 1, pp. 20–32, 2016.

[6] H. Wu, Y. Xing, H. Sun, and X. Mao, "Gut microbial diversity in two insectivorous bats: Insights into the effect of different sampling sources," *MicrobiologyOpen*, vol. 8, no. 4, 2019, Art. no. e00670.

[7] K. Kalantar-Zadeh *et al.*, "A human pilot trial of ingestible electronic capsules capable of sensing different gases in the gut," *Nature Electron.*, vol. 1, no. 1, pp. 79–87, 2018.

[8] S. Yim and M. Sitti, "Design and rolling locomotion of a magnetically actuated soft capsule endoscope," *IEEE Trans. Robot.*, vol. 28, no. 1, pp. 183–194, Feb. 2012.

[9] V. Iacovacci *et al.*, "A fully implantable device for intraperitoneal drug delivery refilled by ingestible capsules," *Sci. Robot.*, vol. 6, no. 57, 2021, Art. no. eabh3328.

[10] S. Miyashita, S. Guitron, K. Yoshida, S. Li, D. D. Damian, and D. Rus, "Ingestible, controllable, and degradable origami robot for patching stomach wounds," in *Proc. IEEE Int. Conf. Robot. Autom.*, 2016, pp. 909–916.

[11] C. Wang, V. R. Puranam, S. Misra, and V. K. Venkiteswaran, "Snake-inspired multi-segmented magnetic soft robot towards medical applications," *IEEE Robot. Automat. Lett.*, vol. 7, no. 2, pp. 5795–5802, Apr. 2022.

[12] D. Son, H. Gilbert, and M. Sitti, "Magnetically actuated soft capsule endoscope for fine-needle biopsy," *Soft Robot.*, vol. 7, no. 1, pp. 10–21, 2020.

[13] N. K. Mandsberg, J. F. Christfort, K. Kamguyan, A. Boisen, and S.K. Srivastava, "Orally ingestible medical devices for gut engineering," *Adv. Drug Del. Rev.*, vol. 165, pp. 142–154, 2020.

[14] M. Mau, S. Sarker, and B. Terry, "Ingestible devices for long-term gastrointestinal residency: A review," *Prog. Biomed. Eng.*, vol. 3, no. 4, pp. 1–18, 2021.

[15] S. Yim and M. Sitti, "3-D localization method for a magnetically actuated soft capsule endoscope and its applications," *IEEE Trans. Robot.*, vol. 29, no. 5, pp. 1139–1151, Oct. 2013.

[16] H. Rezaei Nejad *et al.*, "Ingestible osmotic pill for in vivosampling of gut microbiomes," *Adv. Intell. Syst.*, vol. 1, no. 5, 2019, Art. no. 1900053.

[17] X. Du, K. F. Chan, X. Xia, P. W. Y. Chiu, and L. Zhang, "A magnetically-triggered soft capsule for on-demand mucus collection," in *Proc. IEEE Int. Conf. Cyborg Bionic Syst.*, 2018, pp. 250–254.

[18] T. McKnight and D. Wrigglesworth, "Sampling device," U.S. Patent 0,038,086, Feb. 2016.

[19] B. Salem, G. Aiche, L. Rubbert, P. Renaud, and Y. Haddab, "Design of a microbiota sampling capsule using 3D-printed bistable mechanism," in *Proc. 40th Annu. Int. Conf. IEEE Eng. Med. Biol. Soc.*, 2018, pp. 4868–4871.

[20] Z. Ding *et al.*, "Novel scheme for non-invasive gut bioinformation acquisition with a magnetically controlled sampling capsule endoscope," *Gut*, vol. 70, pp. 2297–2306, 2021.

[21] J. F. Waimin *et al.*, "Smart capsule for non-invasive sampling and studying of the gastrointestinal microbiome," *RSC Adv.*, vol. 10, no. 28, pp. 16313–16322, 2020.

[22] P. Shokrollahi *et al.*, "Blindly controlled magnetically actuated capsule for noninvasive sampling of the gastrointestinal microbiome," *IEEE/ASME Trans. Mechatronics*, vol. 26, no. 5, pp. 2616–2628, Oct. 2021.

[23] G. Jin, G. Wang, X. Liu, H. Cao, and B. Wang, "977 – Intestine microbiome aspiration (IMBA) capsule: A new autonomous and minimally invasive device for whole gut microbiome sampling and mapping," *Gastroenterol.*, vol. 156, no. 62019, Art. no. S–205.

[24] M. Finocchiaro *et al.*, "Design of a magnetic actuation system for a microbiota collection ingestible capsule," in *Proc. IEEE Int. Conf. Robot. Automat.*, 2021, pp. 6905–6911.

[25] M. Rehan, I. Al-Bahadly, D. G. Thomas, and E. Avci, "Capsule robot for gut microbiota sampling using shape memory alloy spring," *Int. J. Med. Robot. Comput. Assist. Surg.*, vol. 16, no. 5, 2020, Art. no. e2140.

[26] G. Cummins, "Smart pills for gastrointestinal diagnostics and therapy," *Adv. Drug Del. Rev.*, vol. 177, 2021, Art. no. 113931.

[27] M. Rehan, I. Al-Bahadly, D. G. Thomas, and E. Avci, "Towards gut microbiota sampling using an untethered sampling device," *IEEE Access*, vol. 9, pp. 127175–127184, 2021.

[28] M. A. Messerschmidt *et al.*, "ANISMA: A prototyping toolkit to explore haptic skin deformation applications using shape memory alloys," *ACM Trans. Comput. Hum. Interaction*, vol. 29, no. 3, pp. 1–34, 2022.

[29] M. Boyvat, J.-S. Koh, and R. J. Wood, "Addressable wireless actuation for multijoint folding robots and devices," *Sci. Robot.*, vol. 2, no. 8, 2017, Art. no. eaan1544.

[30] M. Steinmann, R. J. Bezugley, S. L. Bond, J. S. Pomrantz, and R. L. èguillette, "A wireless endoscopy capsule suitable for imaging of the equine stomach and small intestine," *J. Vet. Intern. Med.*, vol. 34, no. 4, pp. 1622–1630, 2020.

[31] D. W. Fadrosh *et al.*, "An improved dual-indexing approach for multiplexed 16s rRNA gene sequencing on the illumina MiSeq platform," *Microbiome*, vol. 2, no. 1, pp. 1–7, 2014.

[32] M. Koziolek *et al.*, "Characterization of the GI transit conditions in beagle dogs with a telemetric motility capsule," *Eur. J. Pharmaceutics Biopharmaceutics*, vol. 136, pp. 221–230, 2019.

[33] M. Rehan, I. Al-Bahadly, D. G. Thomas, and E. Avci, "Measurement of peristaltic forces exerted by living intestine on robotic capsule," *IEEE/ASME Trans. Mechatronics*, vol. 26, no. 4, pp. 1803–1811, Aug. 2021.

[34] S. Mishra, J. Monro, and D. Hedderley, "Effect of processing on slowly digestible starch and resistant starch in potato," *Starch-Stàrke*, vol. 60, no. 9, pp. 500–507, 2008.

[35] E. Bülbring, A. Crema, and O. B. Saxby, "A method for recording peristalsis in isolated intestine," *Brit. J. Pharmacol. Chemotherapy*, vol. 13, no. 4, pp. 440–443, 1958.

[36] S. Claassen, E. D. Toit, M. Kaba, C. Moodley, H. J. Zar, and M. P. Nicol, "A comparison of the efficiency of five different commercial DNA extraction kits for extraction of DNA from faecal samples," *J. Microbiol. Methods*, vol. 94, no. 2, pp. 103–110, 2013.

[37] T. Wang, W. Hu, Z. Ren, and M. Sitti, "Ultrasound-guided wireless tubular robotic anchoring system," *IEEE Robot. Autom. Lett.*, vol. 5, no. 3, pp. 4859–4866, Jul. 2020.

[38] *Medtronic: Pillcam UGI System*, Accessed: Jul. 20, 2022. [Online]. Available: https://www.medtronic.com/covidien/en-us/products/capsule-endoscopy/pillcam-ugi-system.html