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Towards Gut Microbiota Sampling Using an Untethered Sampling Device

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ABSTRACT Recent studies suggest that human gut microbiota can act as a bio-marker for human health. Also, it can function as a potential tool to understand stress and anxiety. However, the conventional tools have limitations acquiring samples of gut microbiota without contamination. In this work, an untethered robotic capsule prototype is developed that can actively collect the microbiota from the mucosa layer of the small intestine for the first time with the potential to avoid the upstream and downstream contamination. An analytical model for quantifying the peristaltic forces and developing two-way shape memory alloy spring actuator is presented. For the first time, a novel two-way shape memory alloy spring actuator (5 mm x ϕ 4 mm) is used to perform the sampling inside the gut. The spring actuator can apply 675 mN force, which is sufficient to perform in vivo sampling. A specialised experimental setup that can keep the freshly dissected intestine alive for 6 hours is utilised to test the robotic capsule. The robotic capsule prototype has collected an average of 200 μL and 112 μL sample from living pig duodenal and ileal tissues respectively i.e. in the presence of peristaltic forces. The robotic capsule was also tested on intestine of other species including cow and sheep and collected an average of 160 μL and 185 μL of content respectively from the living post-mortem tissues. The collected sample size for all the species is feasible to analyse the microbiota through next generation sequencing techniques. The experimental setup is a reliable proxy to in-vivo behaviour and the robotic capsule experimental result is promising in terms of in situ collection of microbiota.

INDEX TERMS Capsule endoscopy, GI tract, peristaltic motion, robotic capsule, shape memory alloy spring actuator.

I. INTRODUCTION

The human gastrointestinal (GI) tract contains a diversified population of microorganisms that are collectively known as microbiota [1], [2]. The GI tract microbiota have a weight of up to 2 kilograms and the population size is about 10^{14} bacteria. Gut microbiota contain lifelong information of human health and they can act as a bio-marker for disease diagnosis such as cancer, obesity, diabetes and inflammatory bowel disease [1]–[3]. Microbiota can also assist in diagnosing early stage cancer and predicting the risk of type 2 diabetes development [4]–[6]. Furthermore, microbiota are also helpful in studying the interaction between nutrition and human health [7], [8]. Moreover, some researchers believe, microbiota can even aid in improving human mood and behaviour, and can potentially help in dealing with stress, anxiety and depression [9]. This growing body of evidence suggests that gut microbiota are a vital source of information on human health and well-being.

The most common samples used as a proxy for intestinal microbiota are fecal samples, which are collected at the end of the 9 meter long GI tract. This means it is not possible to extract spacial and temporal information from these samples as they are not collected from the actual site of digestion [10].

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Furthermore, the samples are exposed to different environments throughout the gut, before collection, so they are highly contaminated. Another method to study the gut microbiota is by the use of flexible endoscopy with biopsy tools; however, this is a tethered method which limits its reach into the small intestine, and the section of small intestine close to colon (ileum) is a home to a different population of microbiota to the hind gut [11]. Secondly, this method involves a high risk of gut perforation and bleeding, and the procedure is invasive and unpleasant for a patient [12], [13]. Lastly, biopsy tools collect a tissue sample and they cannot fully capture microbial content. Hence, the current conventional tools available to collect microbial samples from the intestine without contamination have limitations.

In the early 2000s, researchers began exploring the GI tract with miniaturized robotic capsules designed to perform various functions such as endoscopy, drug delivery, locomotion, localization, sensing and tissue biopsy [14]–[24]. However, digesta sampling devices are rare in the literature [12].

Some researchers have proposed passive collection mechanisms by dissolving a seal of a chamber at the target site, and collecting the surrounding fluid [25]–[27]. But, these designs do not consider resealing the inlet after sample collection, so the samples become contaminated and resemble fecal samples after device recovery. A bi-stable mechanism has been developed which automatically sealed the inlet after sample collection, however this design did not control the upstream contamination before sample collection and hence precise sample collection at the target site was not possible [28]. Some researchers have investigated active collection mechanisms by considering diversified actuation mechanisms, like shape memory alloy (SMA) [29], motor [30], [31], and magnet [32]. These mechanisms opened and closed the inlet of chamber at the target site to avoid both upstream and downstream contamination. However, these mechanisms were based on arbitrary collection of surrounding fluid (digesta) which does not contain the full microbiome. Many microbial species are present in the mucosa layer lining the gut which cannot be collected by simple opening and closing mechanisms, rather the mechanism needs to scratch the mucosa layer to collect the microbiota [33]. Recently, a mechanical brushing concept was presented to collect the microbiota from gut lining but intestinal trials have yet to be conducted [34].

In this article, a robotic capsule is presented that can actively collect the sample from the target site and capture the microbial population from the inner wall of the intestine. A two-way SMA spring-based actuation mechanism is used, which is small in size (5 mm $\times$ ϕ 4 mm), fits inside the robotic capsule (30 mm $\times$ ϕ 12 mm) and applies sufficient force (>516 mN) to overcome the peristaltic forces from living intestine. The robotic capsule is powered by a battery to activate the SMA spring by joule heating. A wireless transmitter is used to initiate the sampling process once the robotic capsule has reached the target site. In our previous work, a sampling mechanism was developed with an active pull-out scrapping component to collect the microbiota and a chamber to store the sample [35]. The preliminary testing of the design revealed that it collected the microbiota, mucus and digesta. However, it was tethered and was tested on ex-vivo animal intestinal tissue. Hence, the environment and the robot was not representative of the ultimate target application. Therefore, the robotic capsule in this study is untethered and tested on living intestine in vitro, which is closer proxy of the final gut conditions. This paper elaborates on the design considerations, testing and evaluation of proposed actuation and sampling mechanisms, and the feasibility of proposed design in in-vivo trials. The main novelties of this work, as compared to the current state of the art in sampling devices, are shown below:

1) The development of a unique two-way SMA spring actuator with small size (5 mm $\times$ ϕ 4 mm), low power, quick response time and low temperature requirements.
2) The optimisation of SMA spring actuator deflection to expose the sampler outside its shell to collect an optimum amount of sample.
3) The utilisation of an experimental setup to examine the robotic capsule for the first time, which maintains the intestinal tissue in vitro for 6 hours and allows the application of peristaltic forces (please see the video). This allowed testing of the robotic capsule on living tissue of three animal species i.e. pig, cow and sheep.
4) The development of an untethered sampling capsule to collect the microbiota, mucus and digesta from living intestine, with potential to avoid upstream and downstream contamination by storing the sample in a chamber.

In this work, a small SMA spring actuator, which can fit inside the robotic capsule, is designed, sourced and tested. In addition, a sampling mechanism is fabricated to collect and store microbiota from the gut. A capsule containing all components is assembled and tested in specialised experimental setup which closely replicates the in vivo environment in terms of the physiological parameters (temperature, pH and nutrition). In addition it is exposed to the peristaltic movements generated in freshly dissected intestine. The robotic capsule successfully collected samples, hence providing proof of concept for in vivo testing in the next stage of the work.

II. MATERIALS AND METHODS

A. DESIGN AND FABRICATION OF ROBOTIC CAPSULE

The design parameters of the robotic capsule in this study were selected based on clinical requirements and initial feasibility for proof of concept, and are shown in Table 1. Similar robotic capsules in clinical use are 11-13 mm in diameter and 24-32 mm in length, so a capsule with 30 mm $\times$ ϕ 12 mm dimensions will be a safe size to pass through the entire gut [36].

The sampling capsule is required to store at least 100 μL content which can be used to analyse the microbiota and digesta through next generation sequencing techniques [37].

TABLE 1. Design parameters of robotic capsule.

Description	Size
Robotic capsule (without tail)	30 mm x ϕ 12 mm
Storage chamber size	10 mm x ϕ 9.5 mm
- Capacity	500 μL
SMA spring actuator	5 mm x ϕ 4 mm
Additional space for battery inclusion in future	9 mm x ϕ 12 mm

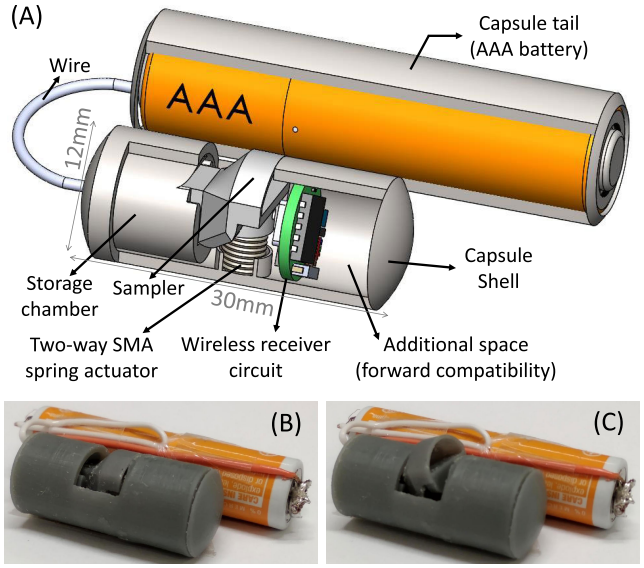

FIGURE 1. Robotic capsule prototype. (A) CAD model with AAA battery, an additional space (9 mm × ϕ 12 mm) is left to include high current drain button cell battery in future. (B) and (C) Fabricated capsule prototype with sampler closed and opened respectively.

A storage chamber with 500 μL capacity is deployed to collect sufficient sample during the experiments.

One of the major challenges is to fit all the components of robotic capsule, including battery, electronic circuitry, actuation and sampling mechanisms, within specified dimensions. In this work, a battery is attached externally to the capsule, which was made possible due to the design of in-vitro experimental setup. The battery was displaced from the main body of the capsule and attached with a 100 mm wire so the effect of peristaltic forces on robotic capsule could be assessed. These forces are likely to change with the size of the capsule if a larger battery was installed inside. An additional space of 9 mm × ϕ 12 mm is reserved inside the capsule to accommodate a button cell battery in the future.

The proposed design of robotic capsule, developed in Solidworks (Solidworks education edition 2019, Dassault Systemes SolidWorks Corporation, Waltham, MA, USA) is shown in Fig. 1(A). The fabricated capsule prototype with actuator turned off and on are shown in Fig. 1(B) and Fig. 1(C) respectively. The capsule prototype was fabricated by a 3D printer (Hunter, Flashforge 3D Printer Zhejiang, China). The SMA spring (Kellogg's Research Labs, Nashua, New Hampshire, United States) was developed based on design requirements.

B. MODELLING OF INTESTINAL FORCES

The small intestine uses two main processes (segmentation and peristalsis) to agitate and propel food towards the distal part of the gut. Segmentation mixes the food throughout the length of the intestine by producing forward and backward movements. Whereas, peristalsis occurs in a circular direction across the intestine, and moves in waves which push the food in a longitudinal direction. Therefore, peristaltic movements are responsible for pushing the food along the intestine and the robotic capsule mainly receives this force as shown in Fig. 2.

The peristaltic forces contract certain gut regions and relax adjacent regions so that the food can be pushed in a longitudinal direction, as shown in Fig. 2. In order to collect the sample from the intestine, the robotic capsule needs to satisfy (1),

$$F_A > F_P + f \tag{1}$$

where, F_A is the force applied by the actuator, F_P is the force applied by the intestine (peristaltic force) and f is the accumulated frictional force which can be defined as

$$f = f_c + f_m + f_v = \mu F_s + F_s' + \delta N_v v \tag{2}$$

where, f_c is the coulomb friction which is the stress applied by the intestine, f_m is the marginal resistance which restricts the sampler movement due to the deformation of the intestinal wall, and f_v is the viscous resistance which retards the motion due to the obstacles (e.g., digesta or mucus) between robotic capsule and intestine. The frictional forces are further elaborated on in (2), 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 forces are described in our previous work, and overall frictional forces can be considered as 200 mN [35].

The amplitude of peristaltic forces inside the small intestine is described by Miftahof on the basis of wave phenomenon and considered as 2.69 g/mm in circumferential (radial) direction [38]. The robotic capsule in this study has a diameter of 12 mm, therefore the maximum peristaltic force imposed on the capsule will be 316 mN, as calculated from (3)

$$F = mg \tag{3}$$

where, F is the force in mN, m is the mass in grams and g is the gravitational acceleration (9.807 m/s^2).

In our recent work, we have experimentally determined the radial peristaltic forces and the average value was found to be 226 mN [39]. An actuator with more than 516 mN force can comprehensively overcome both peristaltic forces (226 mN - 316 mN) and frictional forces (200 mN). Hence, with such actuator, collecting the sample from the small intestine is feasible, as depicted in Fig. 2 and (1).

C. MODELLING OF TWO-WAY SMA SPRING ACTUATOR

SMA springs, also known as Nitinol springs, are manufactured from nickel and titanium. These springs learn their

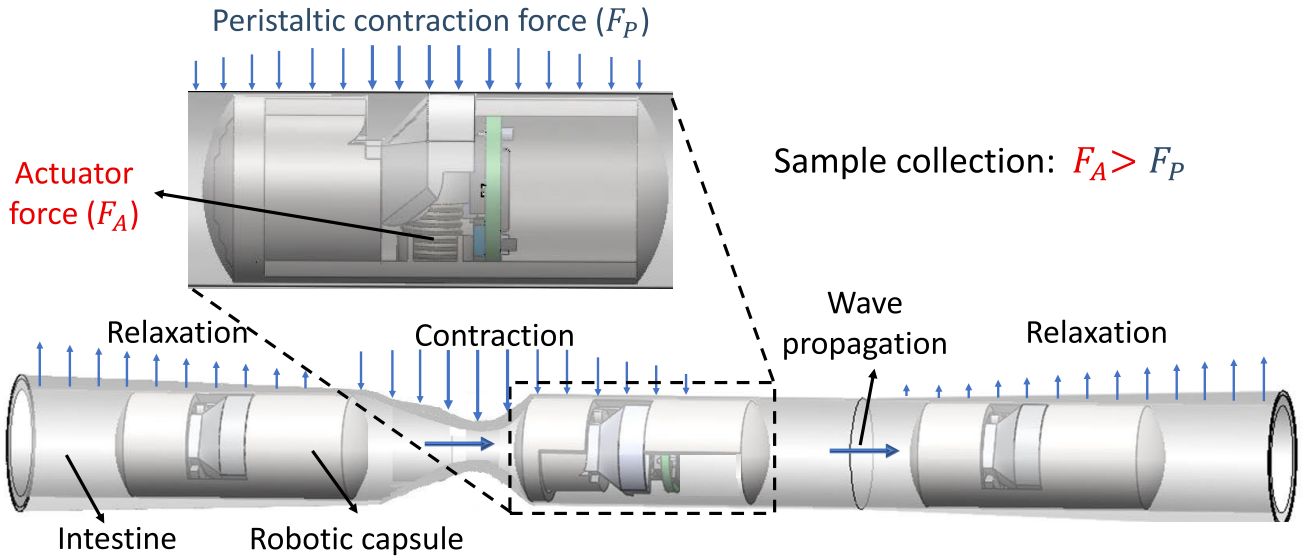

FIGURE 2. Intestinal force model.

desired shape and temperature profile, and retain the memorised shape as soon as they reach the tuned temperature. Most of the designs in the literature shows single way memory, which means that the SMA spring would produce motion in one direction only. The to and fro motion of a spring is achieved by two separate springs, each working in opposite directions [35]. This adds complexity and increases the space requirement. In this study, for the first time, we have used a two-way memory based SMA spring in a robotic capsule, which can produce both forward and backward movements with a single spring. The desired amount of force and deflection can be achieved by characterising the process of heat treatment. The deflection (δ) of the spring can be computed as,

$$\delta = F\frac{8D^3n}{Gd^4} \tag{4}$$

where, F is the load, D is the mean diameter of the spring, G is the shear modulus, d is the wire diameter, n is the number of turns and can be related to length (l) of the spring as,

$$l = nd \tag{5}$$

Based on our design requirements, and considering the limited space inside the capsule, we have selected the parameters as shown in Table 2. The required actuator force should be greater than intestinal forces (516 mN) as mentioned in (1) and the deflection was considered between 1 mm and 2.5 mm to determine the optimum movement of the sampler. The amount of force produced by an SMA spring depends on its shear modulus which varies greatly with the change in temperature (T) as shown below,

$$G = \begin{cases} G_M & \text{When } T < M_f \\ G(T) & \text{When } M_f \leq T \leq A_f \\ G_A & \text{When } T > A_f \end{cases} \tag{6}$$

where, G_M and G_A are the shear modulus for martensite and austenite states respectively. M_f and A_f are martensite and austenite finish temperatures respectively and their selected values are indicated in Table 2. The term G(T) is the variable shear modulus between martensite and austenite states, and can be defined as,

$$G(T) = G_M + \frac{G_A - G_M}{2}\left[1 + sin\phi(T - T_m)\right] \tag{7}$$

where, T_m is the mean temperature and defined as

$$T_m = \begin{cases} (A_s + A_f)/2 & \text{for heating} \\ (M_s + M_f)/2 & \text{for cooling} \end{cases} \tag{8}$$

and,

$$\phi = \begin{cases} \pi/(A_f - A_s) & \text{for heating} \\ \pi/(M_s - M_f) & \text{for cooling} \end{cases} \tag{9}$$

where, M_s and A_s are martensite and austenite start temperatures respectively and their selected values are given in Table 2.

One of the major reason for using one-way SMA springs in our previous work was the requirement of larger temperature difference between the two states i.e. austenite (expansion) and martensite (contraction) as shown in Fig. 3 [35]. Based on the recent technological advancements (Kellogg's Research Labs, US), it has become possible to realise the two-way actuation with a temperature difference of 10 °C only. For the SMA spring in this study, the actuation (expansion) occurs at 52 °C and the spring returns to its original state at 42 °C. This greatly reduces the complexity and power requirement as compared to our previous design [35]. The parameters of the spring were selected on the basis of required deflection and the amount of forces (516 mN). The force analysis of the SMA spring under this study is detailed in the next section.

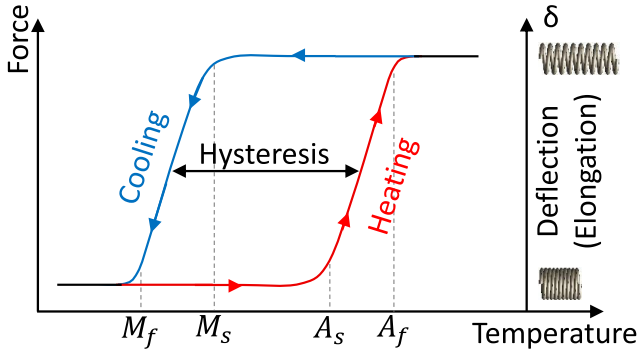

FIGURE 3. The relationship of force and deflection with different temperature states of SMA spring.

TABLE 2. Design parameters of SMA spring actuator.

Symbol	Value	Symbol	Value
D	5 mm	d	0.5 mm
n	10	l	5 mm
M_f	42 °C	M_s	45 °C
A_f	52 °C	A_s	49 °C

D. EXPERIMENTAL SETUP FOR TESTING TWO-WAY SMA SPRING ACTUATOR

The design requirement for the actuator of the robotic capsule in this study, were formulated as:

1) The actuator should produce more than 516 mN force (to overcome peristaltic and frictional forces).
2) It should produce enough deflection to expose the sampler completely against the gut wall, for collecting the sample. This is discussed in detail in section III-B-1.
3) The dimensions of the actuator should not exceed 7 mm × ϕ 5 mm (based on size constraints).
4) The actuator temperature should not exceed 55 °C, this is to ensure that the exterior temperature of the capsule remains at body temperature.

The experimental setup (TA.XT Plus, Texture Analyser, Stable Micro Systems, Surrey, United Kingdom) for testing the SMA spring is shown in Fig. 4. Power supply (HMP2020, Rohde & Schwarz GmbH & Co. KG, Munich, Germany) was used to heat the SMA spring through joules heating at different current levels, which allowed us to determine the optimum current for energizing the two-way SMA spring. Software (Exponent Connect, Stable Micro Systems, Godalming, United Kingdom) was used to calibrate the force sensor and plot the run-time force response. This analysis allowed us to determine the maximum output force of the spring, hence creating a fit for the modelling as performed in the previous sections. In addition, it helped in determining the current requirement to operate the spring at its optimum level.

E. ELECTRICAL SYSTEM FOR WIRELESS ACTIVATION OF THE ROBOTIC CAPSULE

The electrical system used to activate the sampling process in the robotic capsule is shown in Fig. 5. The wireless

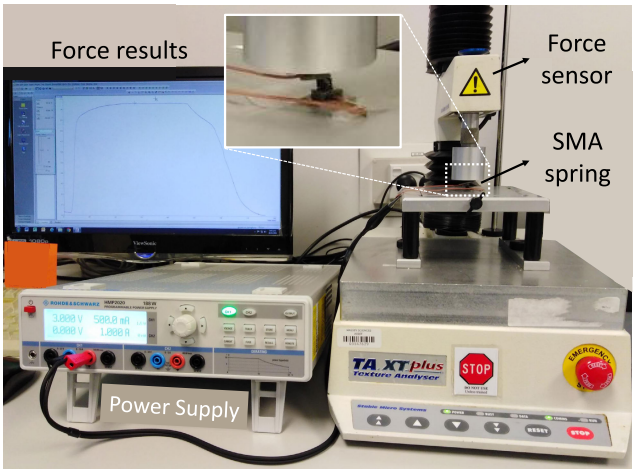

FIGURE 4. Experimental setup for force measurements of SMA spring.

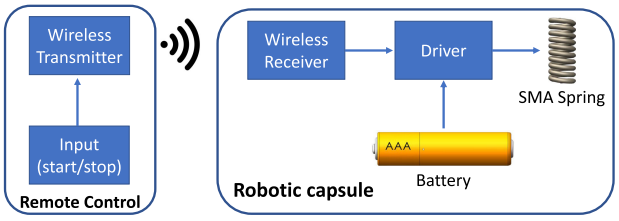

FIGURE 5. Electrical system design of the robotic capsule.

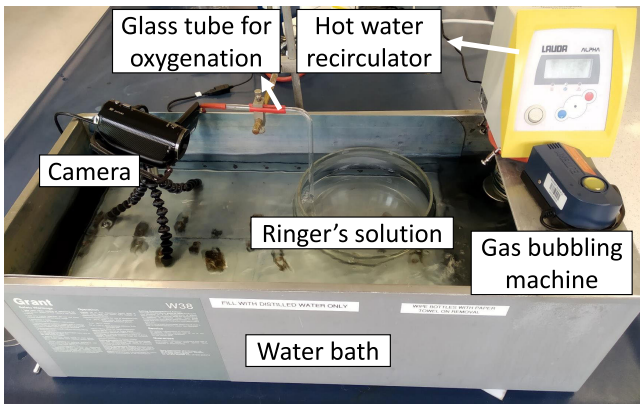

FIGURE 6. In-vitro experimental setup for postmortem tissue.

transmitter and receiver (wireless remote control, Shenzhen Anntem Technology Co. Ltd, China) operates at 433 MHz. An AAA battery was used to power the two-way SMA spring actuator (Kellogg's Research Labs, Nashua, New Hampshire, United States) through a driver circuit.

F. IN-VITRO EXPERIMENTAL SETUP

A specialised experimental setup, as shown in Fig. 6, was used to maintain the peristaltic movements of a freshly dissected animal intestine. This setup kept the post mortem tissue alive in ringer's solution by maintaining physiological parameters (eg. pH at 7.4, temperature at 39 °C). The solution

TABLE 3. Details of animal species used in experiments.

Animal species	Age ($Months$)	Weight ($Kilograms$)	Diameter of small intestine (mm)
Pig	8	$100-115$	15-30
Sheep	10	$55-70$	15-30
Cow	24	$635-725$	25-40

was oxygenated by continuous bubbling oxygen through a glass tube. A water bath and hot water recirculator were used to maintain the temperature of the entire system at body temperature.

G. SOURCING THE SMALL INTESTINES OF DIFFERENT ANIMAL SPECIES

In order to test the robotic capsule in a comprehensive manner, freshly dissected samples of small intestines from three animal species were obtained. The age and weight of each animal species along with their intestinal diameter, in which the robotic capsule was tested, are presented in Table 3. Though the age and weight of an animal affects the dimensions of the small intestine, the difference is relatively small [40]. Therefore, instead of selecting different ages and weights of the same animal species, tissues from different animal species were sourced. This allowed testing of the robotic capsule in samples with a greater range of intestinal diameters.

III. RESULTS AND DISCUSSION

A. TESTING OF TWO-WAY SMA SPRING ACTUATOR

A two-way SMA spring changed its shape (extension and contraction) with a change in temperature, which was accomplished by passing the current through the spring wire (joule heating). The force was measured by applying increasing current as shown in Fig. 7, while the voltage was fixed to 1 V. The spring didn't show any force below 300 mA current as the heat was dissipated by the surrounding air. From 300 mA the spring actuator showed progressive force and kept increasing even above 1A current; however, supplying a higher current in the robotic capsule was difficult due to space limitations. The required actuator force to overcome the peristaltic and frictional forces was more than 516 mN, which can be achieved above 460 mA as indicated by Fig. 7. The experiments were performed on three separate springs, three times each and the variation in readings are also shown in Fig. 7. The corresponding temperatures at varying currents were also recorded by thermal imaging camera (TG167, FLIR Systems Inc., USA) and the average temperature values are indicated in Fig. 7.

Response time of an actuator is another critical parameter, which was recorded against the spring deflection on varying currents during SMA spring testing. The results are shown in Fig. 8. The SMA spring took less than 5 seconds to fully deflect (extension) at higher currents i.e., above 800 mA and 5 seconds at 500 mA current for 80% deflection. The cooling time (compression) was relatively longer and it took around

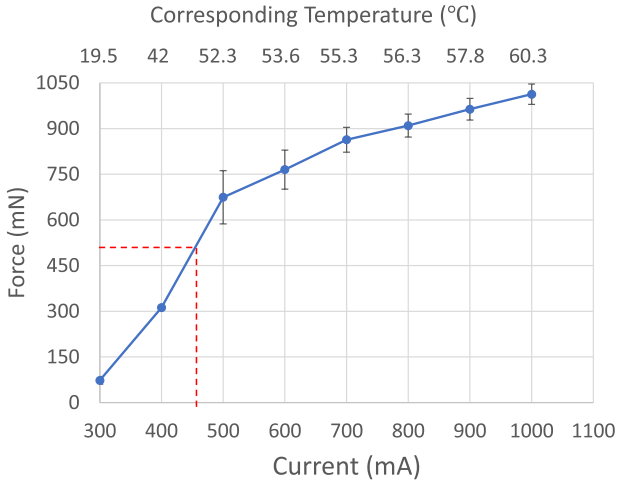

FIGURE 7. Force analysis of two-way SMA spring actuator.

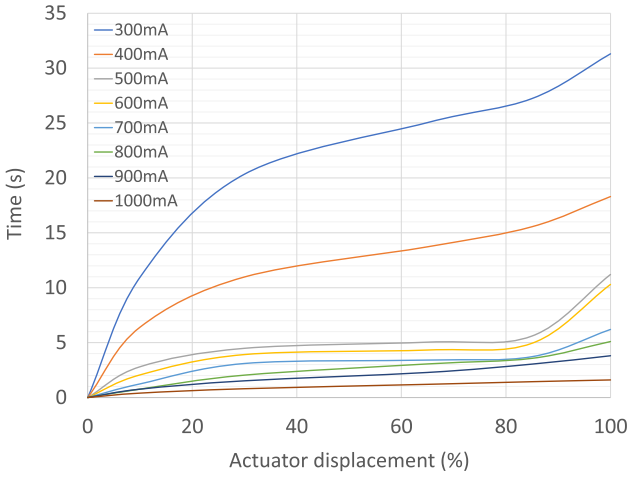

FIGURE 8. SMA spring actuator response time on varying currents.

30 seconds to fully return to its original state. The response time of SMA actuator is not fast but for sampling application it does not pose a potential problem.

A thermal imaging camera was used to determine both internal (inside capsule) and external (outside capsule shell) temperatures after energizing the SMA spring actuator in the robotic capsule. The temperature profile both from inside and outside the capsule on varying currents is shown in Table 4. This analysis allowed us to determine if any potential harm could occur to the intestine of animal and/or human in future. The current for the actuator in our experiments was 500 mA, which means that the inside temperature of the capsule was 53.2 °C while the outside temperature was 34.4 °C, as shown in Fig. 9. As the body temperature of the animals used in our experiments was approximately 39 °C, this demonstrates that the capsule will not harm the intestine. The temperature testing was carried for 7 minutes, as compared to actual experimental trials in which we turned the actuator on for 5 minutes. This further shows that the actuator will not

TABLE 4. Variation in internal and external temperatures of the capsule due to varying actuator current.

Current (mA)	Temperature inside °C	Temperature outside °C
400	47.4	32.5
500	53.2	34.4
600	54.9	34.5
700	57.5	35.9
800	58.6	37.0
900	60.7	37.7
1000	64.9	38.9

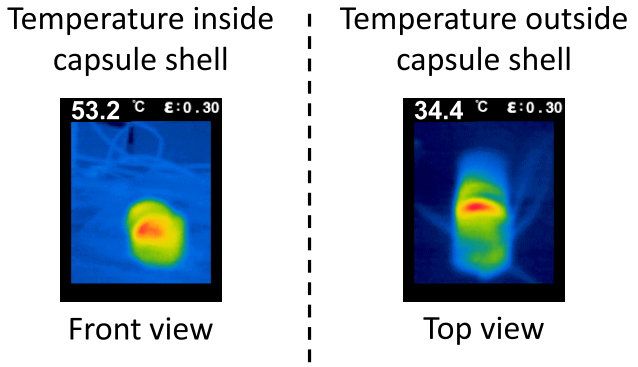

FIGURE 9. Temperature profile of robotic capsule at 500 mA current recorded by thermal imaging camera.

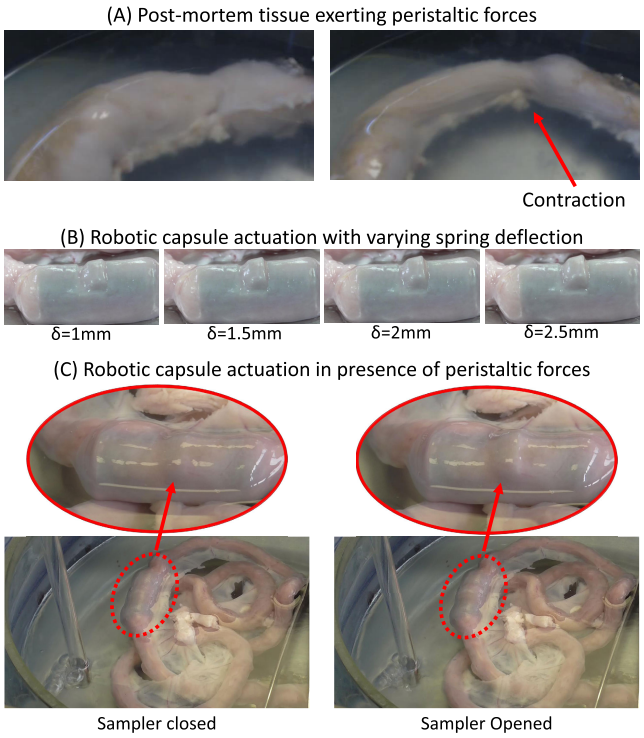

FIGURE 10. Sample collection process under in-vitro experimental setup.

damage the intestine even if the sampling process is extended to 7 minutes.

B. IN-VITRO SAMPLING TRIALS

Freshly dissected animal intestines were sourced from the Post Mortem Room in the School of Veterinary Science (Massey University, Palmerston North, New Zealand) and a 300 - 400 mm section of small intestine was separated from the rest of the small intestine. This isolated section was placed in the experimental setup, as shown in Fig. 10(A) and (C), which kept the intestinal tissue alive for at least 6 hours. The robotic capsule along with external battery was manually placed inside the section of small intestine. When the intestine started applying peristaltic forces, the sampling process was initiated wirelessly. The two-way SMA spring actuator opened the sampler against the peristaltic forces which started collecting microbiota from the surrounding digesta and intestine wall (mucosal layer) respectively. The scratching from the intestinal wall by the sampler potentially captured intestinal villi tissue from the gut lining. However, no damage to the intestinal wall was seen and this tissue naturally regenerates over a short period of time [41].

The sampler remained open against the intestinal wall for 5 minutes during which the capsule was moved by the peristaltic motion, aiding sample collection. After 5 minutes the actuator was turned off by the wireless transceiver. The actuator closed the inlet by pulling the sampler back to its primary position. The sample collection process under in vitro experimental setup is shown in Fig. 10 and can be seen in the supplementary video.

1) OPTIMISATION OF SMA SPRING ACTUATOR DEFLECTION FOR OPTIMUM SAMPLE COLLECTION

Varying spring deflections (1 mm, 1.5 mm, 2 mm and 2.5 mm) for the SMA spring actuator were tested on two sections of pig small intestine (duodenum and ileum), to analyze the impact on sampler opening, as shown in Fig. 10(B). This allowed us to determine the optimum length of deflection to collect the maximum amount of sample. The sample collection results with varying spring deflections are shown in Fig. 11. A spring deflection of 1 mm slightly opened the sampler and collected an average of 26 μL content with 40% failure rate in the duodenal tissue. A 1.5 mm spring deflection collected an average of 136 μL sample from duodenal tissue, however the average collection was only 20 μL from ileal tissue due to more viscous digesta, which restricted entry into the sampler. Actuator deflection of 2 mm and 2.5 mm collected 200 μL and 210 μL content respectively from the duodenal site and 112 μL each from ileal site, which is a feasible quantity for microbiome assessment through next generation sequencing techniques. Since a 2.5 mm spring deflection didn't produce significant differences compared to 2 mm, and both deflections (2 mm or 2.5 mm) fully exposed the sampler, we selected the 2 mm deflection for our actuator as an optimum deflection.

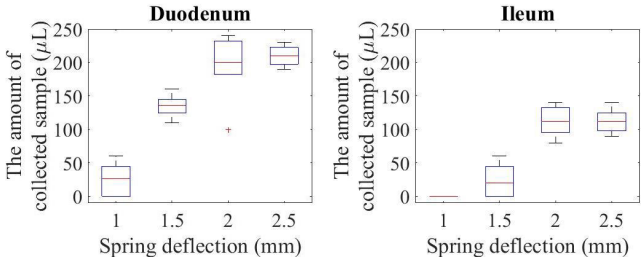

FIGURE 11. Variation in sample size collection by varying spring deflection on pig duodenal and ileal tissue.

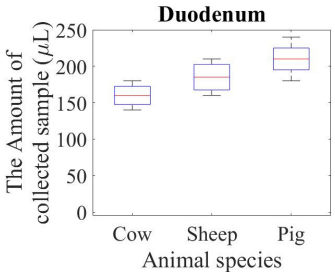

FIGURE 12. Variation in sample size collection on different animal species.

2) SAMPLE COLLECTION FOR DIFFERENT ANIMAL SPECIES

A robotic capsule with a 2 mm spring deflection was tested on small intestine (duodenum) of two further species to ensure the effectiveness of developed capsule prototype. The average quantity collected from each animal, i.e. cow and sheep, in five trials were 160 and 185μL respectively. Collected sample size in in vivo trials will ensure successful lab analysis through next generation sequencing techniques. The results are shown in Fig. 12.

C. DISCUSSION

A two-way SMA spring actuator is a good candidate for small scale applications like robotic capsules. The spring offers more than 1 N force at higher current levels (1 A). As the combination of peristaltic and frictional forces inside the small intestine are 516 mN, a sampling capsule is required to overcome these forces. Although this can be achieved at 460 mA current, the values shown in Fig. 7 are average values and to accommodate the minimum force offered by the SMA spring, 500 mA current is a realistic choice from a design perspective as it offers minimum force of 580 mN and an average force of 675 mN.

A 675-button cell battery (5.4 mm $\times$ ϕ 11.6 mm) has a capacity of 650 mAh but the drain current is relatively low i.e. around 50 mA. The SMA spring actuator requires 500 mA current with 1 V to achieve the required motion but commercially available batteries with this size limit cannot drain high enough current. Therefore, in the existing design it is achieved by a separate battery connected as a tail. In future, a current booster circuit will be used with a commercially available button cell battery to achieve the required current and a dedicated space (9 mm $\times$ ϕ 12 mm) is already left in capsule prototype to accommodate this futuristic inclusion. Alternatively, a super-capacitor can also be used to store the charge for certain time and then discharge it to the actuator. Lastly, wireless power transmission can also be considered as this approach has recently achieved the required power ($>$ 500 mW) in robotic capsule designs [42]. The space reserved for the on-board battery inclusion can be increased to 15 mm $\times$ ϕ 12 mm, if needed, by reducing the storage chamber capacity to 105 μL. Currently, the storage chamber capacity is 500 μL with a dimensions of 10 mm $\times$ ϕ 9.5 mm which can be reduced to 4 mm $\times$ ϕ 9.5 mm for the lower capacity as the minimum desired sampling quantity is 100 μL.

The activation time of the SMA spring actuator (sampler opening) was 5-11 seconds with 500 mA current which is reasonable for a sampling application. The sampler closing time was relatively longer and it took around 30 seconds to fully close the sampler under idle conditions which would potentially be shortened under the presence of external forces from the intestine (peristaltic forces). The longer closing time will be helpful in securing samples from the gut and there is no need to add an additional cooling or heat dissipation component. The heating of the SMA spring actuator did not increases the temperature of exterior shell of the capsule above body temperature, as recorded by thermal camera, so it will not damage the intestine tissue. Similarly, the SMA spring actuator is well confined inside the sampler and the increase in temperature did not affect the inside space of the storage chamber hence it would not damage the collected sample. However, a careful set of experiments will be conducted before future in vivo trials to confirm this. For this in vitro study, no significant problems were observed due to heating and cooling of the SMA spring either inside (storage chamber and collected sample) or outside (capsule shell and intestine) of the capsule, which was verified by the thermal camera.

The chyme inside the small intestine was viscous and didn't entered the storage chamber via the smaller opening when the sampler was not fully exposed. The in vitro trials revealed that the 2 mm outward push from the SMA spring actuator fully exposed the sampler and resulted in maximum collection, although less sample was collected from the ileum compared to the duodenum. It appeared that the duodenum had a smaller lumen diameter that allowed the sampler to scrape the content from the wall, while the ileum contained more viscous food particles which blocked the entrance of the sampler, hence resulted in the lower sample collection. In either case the microbiota and digesta can be analysed as the average collection was more than 100 μL for the cases where spring deflection was 2 mm and above. A few trials were conducted without activating the SMA spring actuator (zero spring deflection i.e. no sampler opening). The storage chamber didn't collected any content without sampler opening which verified the sample collection was not aided by any potential leakage. Sample collection from various locations of small intestine (such as duodenum and ileum) have potential to reveal the relationship between gut related diseases and location specific microbiota composition.

In the current experimental setup, the capsule was visible and the sampling process was initiated through a wireless device; however, for in vivo trials it will be essential to determine the capsule location. One of the potential methods is to track the capsule with ultrasound imaging and trigger the capsule wirelessly in a similar way to the current method [32]. Identifying the precise location via ultrasound will be difficult but adding transit time information will help in targeted sampling during in vivo trials. A similar weight and size dummy capsule will be fed to the animal to develop personalised transit profile. After this the robotic capsule can be fed and triggered at the target site with the help of ultrasound imaging and developed personalised transit times. Alternatively, capsule positioning information can also be obtained using a pH sensor which can distinguish between different regions of the gut (e.g. stomach, small intestine and colon) as they have different pH levels.

The robotic capsule was also tested on small intestine of different species to demonstrate efficacy. The capsule collected least digesta content from the cow intestine as its lumen diameter was the largest and the capsule floated inside the thick muscle without making consistent contact with the intestinal wall. Since peristaltic motions contract (squeeze) the normal diameter of the intestine by up to 78%, this should allow the robotic capsule to collect samples of microbiota from larger diameter intestines as well. However, the capsule design is most effective for smaller gut diameters as it samples the microbial population from both the lumen and walls of the gastrointestinal tract. The overall difference in gut dimensions between each species is relatively small, and the capsule collected an average of more than 100 μL content from each animal species, which is the desired sampling quantity.

The active collection of the sample by opening and closing the sampler at the target sites has potential to avoid contamination. One of the major challenges during in vivo testing will be to secure the sample by properly sealing the inlet of the sampler. Prior to the in vivo work the robotic capsule will be tested in stomach and intestine digestion models to validate the sealing mechanism of the robotic capsule. In the current study, an ideal sealing mechanism was not investigated as the capsule was mainly tested for its actuation mechanism in terms of overcoming the intestinal forces and collecting more than 100 μL sample from small intestine.

IV. CONCLUSION

This paper reports the development of a robotic capsule that can collect a microbiota sample from the gut. Analytical modelling to quantify the intestinal peristaltic forces and development of a two-way SMA spring actuator is explained. The SMA spring actuator can apply significant force to overcome both peristaltic and frictional forces, during sample collection. A wireless transceiver is used to initiate the sampling process and an external battery is used to energize the actuator, which will be replaced by on-board battery for in vivo testing in next stage of development. The robotic capsule is tested in an in vitro experimental setup, which maintains the freshly dissected tissue alive for at least 6 hours. The robotic capsule has been tested using tissue from different animal species and has successfully collected more than the desired sample size of 100 μL for each test. The intestinal tissue model used replicates in vivo conditions in terms of peristaltic movements, and provides enough confidence to perform in-vivo studies in future.

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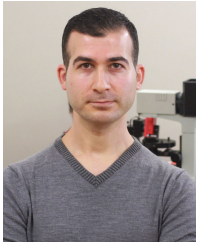

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