ROBOTIC CAPSULE FOR SAMPLING GUT MICROBIOTA: DESIGN, DEVELOPMENT AND EVALUATION

A thesis presented in partial fulfilment of the requirements for the degree of
Doctor of Philosophy
in
Engineering
at Massey University, Palmerston North,
New Zealand.

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2022

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Abstract

In this research, a pill-sized robotic capsule was developed that can collect gut microbiota both from the gut lumen (capsule surroundings) and intestinal wall (mucosa layer). Initially, the peristaltic forces exerted on the robotic capsule inside the gut were quantified so the working environment of the capsule could be understood. Secondly, a unique sampling mechanism was developed that could gently scrape the content from the gut lining and could provide a full length assessment of microbiota after capsule retrieval. Thirdly, the design of shape memory alloy (SMA) spring actuator was realised that could apply sufficient force to overcome peristaltic and frictional forces for sample collection at the target-site. Furthermore, an actuation system was devised by tackling the high-drain current requirement of SMAs. Fourthly, a sealing mechanism was developed to secure the collected sample from cross contamination and to assure successful encapsulation. Fifthly, the robotic capsule was rigorously tested in various in vitro simulators replicating the gut environment and a dedicated gut simulator that mimicked the in-vivo environment to ensure successful and safe travel of the capsule along the gastrointestinal tract. Finally, an in vitro experimental setup that kept an intestine alive for 6 hours was used to optimise the sample collection process. The robotic capsule collected sufficient quantities of sample (more than 100 μL) for microbiota analysis from living intestines of three animal species (pig, sheep and cow) during the trials.

The study of gut microbiota is gaining increasing attention due to its direct impact on human health. Gut microbiota can provide comprehensive information about the health of a host, and it can help in the early diagnosis of diseases like cancer, diabetes, obesity, etc. The robotic capsule prototype, developed in this work, has a potential to become a vital apparatus for clinicians and scientists to sample human and animal gut in the future.

Author's Declaration

This thesis is produced with five journal publications and compiled according to the Massey University's guidelines for 'Doctoral Thesis with Publications'. This thesis is based on research that has either been published or currently in preparation. Three articles are published in IEEE journals, and one in a Wiley journal. One article is in preparation and will be submitted to BMJ journal.

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While the content is identical to the published versions, there may be stylistic differences between the enclosed work, and the published versions. Furthermore, some of the submitted chapters are relatively succinct, there is some repetition (particularly in the literature review of some chapters), and there are stylistic differences between the chapters themselves.

Acknowledgements

"Praise be to Allah for guiding us to this. We would have never been guided if Allah had not guided us" (Qur'an 7:43).

Foremost praises, thanks and gratitude to Allah who has guided me to this point where I can write this thesis to finish my PhD. It was not possible without my belief and trust on Allah that helped me to complete my thesis, Alhumdulillah (all praises to Allah alone).

I extend my sincere gratitude to my supervisor cum mentor, Ebu who has constantly helped me throughout my PhD. This journey includes various challenging times and Ebu was always there to support me which kept me moving. Ebu encouraged me to grow as an independent researcher and provided co-funding opportunities to further establish myself as an academic. I like to equally thank my other supervisor, Ibrahim Al-Bahadly, who has always extended his kind support throughout my PhD and his continued support helped me to achieve my both spiritual and intellectual goals. I cannot forgot to mention the kind support from Dave, my third supervisor, who has significantly helped me with experiments and my writing. I'm humbled to have an excellent supervisory team that allowed me to achieve a few good milestones in my PhD, so thank you Ebu, Ibrahim and Dave for your amazing support. And this thesis will not be an end, as I'll be looking forward for your continued support in future too!

I am blessed to have a caring and lovely wife who motivated me to start the PhD and continued her support throughout this journey. She helped me manage the long working hours and stressful times, and her support cannot be expressed in words. My kids, Abeera 7, Khizer 4 and Areesha 1, are the source of meditation who helped me to remain motivated throughout these years. The kids excitement about the "tummy robot" to catch bugs, kept me motivated too and helped me enhance my science communication skills.

Special thanks to my parents for continuously praying for me which made this long journey less difficult. My parents' prayers were the blessings in all the trying times and I wish to have their prayers with me forever. Thanks to my siblings who feel proud in my work, kept me motivated and supported me with their helpful prayers. I'm equally thankful to my parents-in-law who also favored me with their kind prayers. Thank you

everyone in my family for your well wishes and prayers.

I have been very well looked after by the School of Food and Advanced Technology (SFAT) specially Glenda, Dilantha and Karen from administration and Tim, Mark and Peter from the ITS team. Many thanks to all of you for helping throughout my PhD. I specially thank Tracy, Julia and Aniek from the Graduate Research School (GRS) for extending their wonderful support whenever and where-ever needed, this really made the difference in my experience as a research student. I greatly admire the support of microrobotics team - Matthew, Taimoor, Kate, Ben Lewis, Andrew (the senior), Nick, Sid, Susav, Jack, Brian, Andrew Kemp, Hamish, Martin, Ben and Victor - you all have been amazing. Thanks for all the discussion, suggestions, support and help that not just allowed me to finish the PhD but helped me gather some beautiful memories. I also extend my thank to fellow PhD students Syahmeer, Suchima, Gerald, Merit, Alex and Lilly.

A big thanks to my friends - Uzair, Haroon, Rafay, Asad, Hamid, Waseem, Waqar, Saad, Hammed, Moayad, Abdulquadri, Abdelhamid and Saiful - who provided necessary support during my PhD. The short gatherings after the daily prayers were a key to relieve the stress and improved the productivity which was not possible without these friends. Thanks to Massey Muslim Society (on-campus) and Manawatu Muslims Association (off-campus) for their assistance in facilitating the religious needs, which was a key in completing my PhD successfully. Also, thank is extended to PakNZ - Pakistani community at Palmy - for their encouragement and support. I am obliged to thank the people - Faraz Hasan, Zulfiqar Butt, Imran Muhammad, Ajmal Khan, Syed Ahmer, Wajid Hussain, Hanif Quazi, Syed Qazafi, Asif Javed, Shahnawaz Ahmed, Riaz Rehman, Mufti Saleem, Muhammad Shah and Bilal Alvi - who I admire and discussion with them helped me to achieve my PhD goals.

Finally, I again give thanks to Allah, through whom all things are possible. I pray, may Allah bestow His peace and blessings upon all His messengers, and His final messenger Prophet Muhammad, Peace be Upon Them all, both in here and hereafter.

Funding

I gratefully acknowledge the contribution of following funding organisations for their support towards the research in this thesis.

- Higher Education Commission (HEC), Pakistan for providing the scholarship for full duration of my PhD under grant 5-1/HRD/UESTPI(Batch-V)/2421/2018/HEC.
- Sir Syed University of Engineering & Technology, Pakistan for support during this PhD.
- Massey University for funding support through Massey University Research Fund (MURF) 2019 and Strategic Research Excellence Fund (SREF) 2022.
- KiwiNet for funding support through grant 2020/2021.
- Palmerston North Medical Research Foundation (PNMRF) for funding support through general grant 2021-2022.
- Massey ecentre for funding support through Grand Ideas Competition 2022.

Chapter 1

Introduction

1.1 Background and significance of this research

The human gastrointestinal (GI) tract is a 7-9 metre long passage that includes four main segments namely the esophagus, stomach, small intestine and large intestine. In an average lifetime of a human, around 60 tonnes of food passes through this passage [1]. The food is digested and absorbed by host intestinal secretions and transport mechanisms and a huge community of microorganisms (bacteria, archaea and fungi) that live inside the gut, play a major role in fibre fermentation and the synthesis of short-chain fatty acids (SCFAs), and some nutrients (e.g. vitamins) and which are collectively known as microbiota [2]. The microbiota weigh around 1.5 - 2 kilograms in a human adult and include 10^{13} to 10^{14} microorganisms which are spread along the full length of the gut and are packed within the digesta and gut lining [3]. The main segments of the gut along with the quantity of microbiota present at each segment is shown in Fig. 1.1.

This community of microorganisms has been intensively studied since the 1980s and various studies on the relationship between microbiota and human health reveal that the microbiota can act as a bio-marker for human health [4]. The gut microbiota can be informative of health status over the life of the host and can help in early diagnosis of diseases like cancer, obesity and diabetes [5–7]. Furthermore, the analysis of microbiota can help in better treatment of diseases such as coeliac disease, Crohn's disease and irritable bowel syndrome [8]. In addition, the microbiota can also help to study the relationship or interaction between nutrition and human health [8, 9]. Comprehensively, it can be inferred that the human gut microbiota is full of information related to human health.

Recent research studies suggest that the microbiota can potentially influence the mood, behaviour and several other characteristics of the host [10]. The gut microbiota have been shown to impact brain functions and behaviour, and play a role in anxiety,

depression and stress disorders [11–13]. Studies investigating the impact of microbiota on mood, have revealed that the microbiota regulates emotions and cognition because it maintains the two-way communication with the brain [14]. This growing body of evidence suggests that gut microbiota are a vital source of information on human health and well-being.

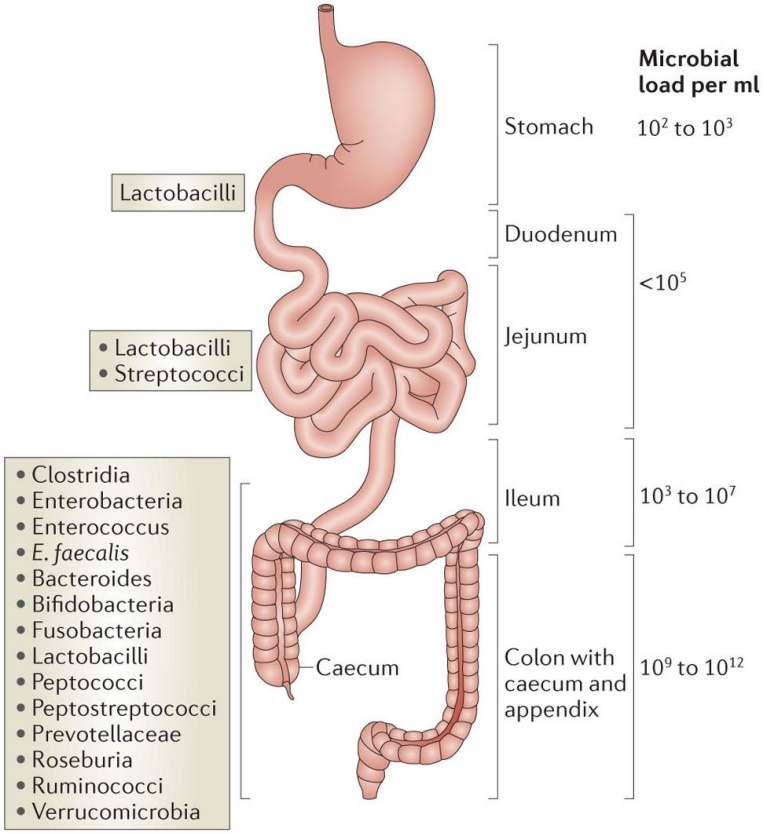

Figure 1.1: Segment-wise view of the GI tract showing the main sections of the gut. Representation of the microbial community in each section along with the major type of bacteria colonizing the mucus layer and the common metabolites are shown. (Reprinted by permission from ©2014 Nature Publishing Group [15].)

Based on the importance of analysing gut microbiota, it is critical to explore its ecology. Though greater communities of microbiota live in the colon, the small intestine is a home to a unique community of microbiota that has a potential to reveal several important linkages about human health. An abundance of nutrients are present in the small intestine and are absorbed in this region and the level of nutrients present in the digesta reduces significantly as you move in a longitudinal direction towards the colon as shown in Fig. 1.2. The opposite is true with the bacterial, archaea and fungal communities which are maximal in the colon and significantly less by comparison in the small intestinal region. Despite this, the small intestine remains a region of interest

as it is less explored in comparison to any other regions of the gut and it is a home to a significant and unique community of microbiota.

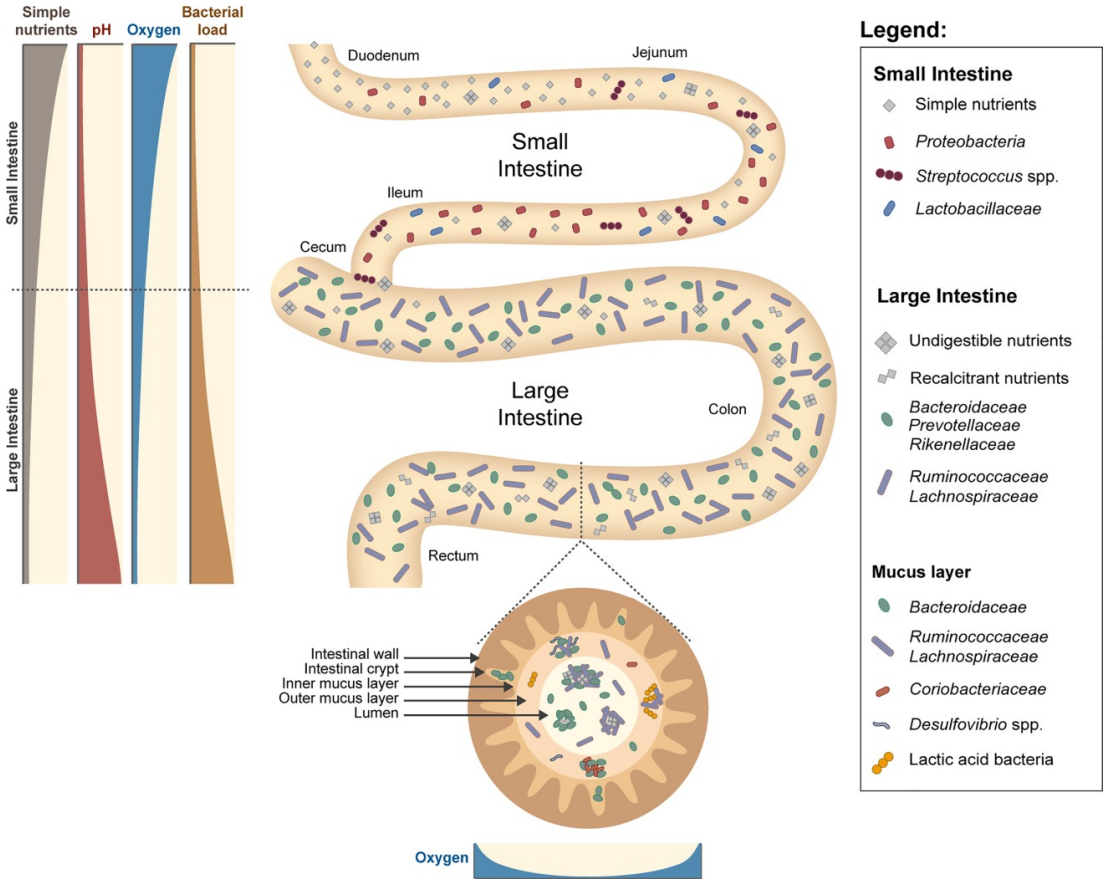

Figure 1.2: Representation of the bacterial and microbial communities across the longitudinal length of the GI tract. The circular structure shows the layers of the intestinal wall depicting the presence of microbial community at various levels. (Reproduced from [16] licensed under CC BY 4.0.)

Interestingly, the cross sectional structures of the small intestine and colon differ in various ways as depicted in Fig. 1.3. The small intestine is characterised by an additional layer of villi that significantly increases the surface area of this region which helps in nutrients absorption and also secretes digestive enzymes to support the digestion process. In addition, the small intestine has one mucus layer while the colon has two mucus layers (i.e., an inner mucus layer and outer mucus layer), and the function of the mucus layers differs in both regions. The mucus layer in the small intestine is formed by continuous secretion from goblet cells (shown in a green color in Fig. 1.3) which acts as an intestinal barrier against foreign microorganisms (pathogens) while still allowing the absorption of nutrients. This mucus layer slowly mixes with the luminal content as it moves towards the colon along with the digesta. In contrast, the

inner mucus layer of the colon is firmly attached to the surface and restricts any interaction between luminal content and the epithelium layer. The outer mucus layer in the colon is more similar to the small intestinal mucus layer, and it lubricates the left over content from small intestine and allows smooth transition of luminal content through colon which is finally moved out of body via faeces.

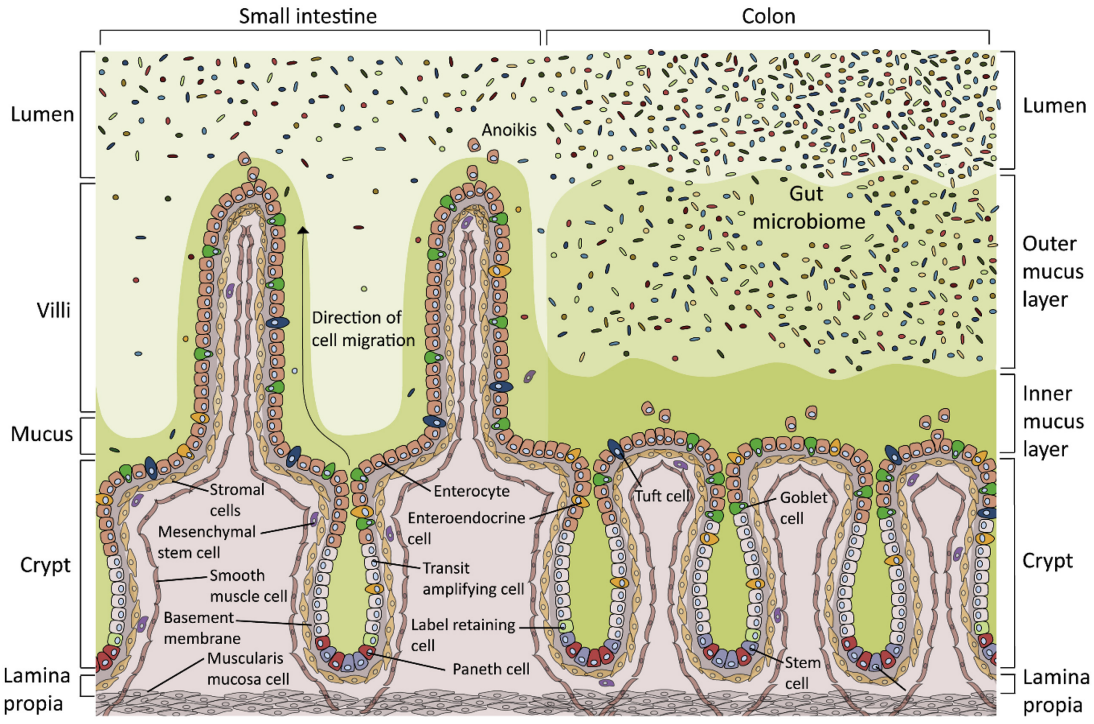

Figure 1.3: Representation of microbial community across lumen and mucosa layer in the small intestine and colon. The villi structure is only present in the small intestine along with mucus content whereas the colon contains two mucus layers. (Reproduced from [17] licensed under CC BY 4.0.)

The following facts are therefore evident from the above discussion. First, it is increasingly evident that the microbiota is pivotal in understanding human health as it can act as a bio-marker. Second, microbiota is present in various quantities longitudinally along the full length of the GI tract. Third, microbiota is packed across a cross section of the intestine, colonising the mucous layer which covers the columnar epithelium of the GI tract and the digesta within the intestinal lumen [18]. Therefore, it is critical to perform systematic longitudinal and cross sectional studies to examine the diversity of microbiota and its functions. These studies can be enabled by collecting samples of microbiota from distinct locations within the GI tract that will help establish better relationships between human health and disease.

The most common samples used as a proxy for intestinal microbiota are faecal samples, as they are non-invasive, relatively easy to collect and can be collected repeatedly.

However, faecal samples are collected at the end of the 9-metre long gut that restricts the extraction of spatial and temporal information from them as they are not collected from the actual site of digestion [19]. Furthermore, the samples are exposed to different environments throughout the gut, before collection, so they are highly contaminated [20, 21]. The faeces also mainly represent the non digested luminal content from the colon and contain no information about the microbiota living inside the mucosa layer (mucus). Another method to study gut microbiota is by the use of a 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 closest to the colon (ileum) is a home to a different community of microbiota to the hind gut [22]. The method also involves a high risk of gut perforation and bleeding, and the procedure is invasive and unpleasant for a patient [23–25]. The biopsy tools utilised collect a tissue sample but the technique is unable to fully capture microbial content. Another approach to look at the gut microbiota is by euthanasia of the animal and dissection to allow localisation of the sample collected and avoid the cross contamination. This approach provides full access to examine in detail the diversity of microbial community along the full length of the GI tract but the findings from animal models cannot be directly applied to humans. Additionally, it is considered that the behaviour of the microbes might change after the death of an animal (host), so the findings may not be accurate. Hence, the current conventional tools available to collect microbial samples from the small intestine without contamination all have limitations.

Considering the limitations of existing tools, a minimally invasive method that could collect microbial content from any location of the gut, and store or fix it to avoid cross contamination (mixing of contents from other parts of the gut), would be of great help in understanding the role of the microbiota in humans.

1.2 Motivation

The human gut microbiota is not fully explored yet. The major problem is the lack of tools to collect the microbiota from the full length of the human gut. The current gold standard for obtaining the human gut microbiota is faecal sampling but this approach has certain limitations in terms of localisation uncertainty, time constraint and carrying contamination. Likewise, flexible endoscopy with biopsy tools for sampling the GI tract is currently inadequate as its use is mainly restricted to the large intestine, poses significant risk of damaging the GI tract and is not designed to collect microbiota samples.

Hence, development of a minimally invasive tool to collect the microbial content from the small intestine would help understand a range of human health conditions. The sampling device should be small enough to completely pass through the entire gut

without damaging it. The device needs to collect sample(s) from specific location(s) of the gut and secure it from downstream contamination further down the gut. Additionally, the device should collect sample from both the intestinal lumen and mucosa layer to ensure full assessment of gut microbiota as a significant community of microbiota lives on the mucosa layer (intestinal wall).

The collected sample (microbiota) would then allow health practitioners to analyse markers for a range of human health conditions with better accuracy, insight and perspective. The assessment of gut microbiota from the full length of the GI tract will allow clinicians to perform in-depth health analysis and consequently develop better treatment methods. Furthermore, the evaluation of collected samples from different segments of the GI tract would help study the relationship of nutrition with human health. This will allow nutritionists to develop personalise diet plans that will be best suited to the host based on the requirements of host's microbes.

1.3 Problem Statement

Gut microbiota is helpful in understanding the health of the host and it can act as a bio-marker for early diagnosis of cancer, diabetes, obesity, inflammatory bowel diseases, etc. The microbiota are beginning to reveal significant information that is important for human health conditions; however, tools for collecting a sample of microbiota from the human gut without contamination are not available to date.

The mucus is the fundamental element to study microbiota, and is secreted from the mucosa layer and forms a protective layer just above the epithelium layer to safeguard the epithelium layer from intestinal bacteria. The mucus in humans is not fully explored yet, due to the unavailability of relevant tools. It is believed that the mucus would be a few hundred microns in-depth above the epithelium layer [26]. Capturing the mucus, which includes microbiota, from living object requires a compact and efficient sampling mechanism which can collect and safeguard the sample from contamination. Some of the challenges in collecting the mucus from the GI tract are detailed below:

1. Design and development of entire capsule in swallow-able size, including sampling mechanism (sampler and storage chamber) and actuation mechanism.

2. Avoiding contamination, i.e. the collected sample should not be mixed with any content apart from the sampled location.

3. Devising a smart actuation mechanism which requires low power and takes up minimal space.

4. Fabrication of a minimally invasive capsule, which includes all the mechanical and electronic parts, with acceptable precision and tolerance.

5. Communication with the capsule to trigger the actuation of the sampling mechanism, when the capsule reaches its target site.

6. Localisation of the capsule, i.e. Position recognition inside the GI tract so that the sample can be reliably collected from a specific site.

It is not possible to capture the microbiota from the GI tract with existing tools and methods. Design and development of a minimally invasive sampling device, including actuation mechanism (actuator, on-board battery and electronic circuitry) and sampling mechanism (to collect and store microbiota - sampler and storage chamber respectively), which can go through the entire GI tract is an arduous task. Hence, the development of an efficient sampling device or a robotic capsule (which can resolve most of the mentioned challenges) is desired.

1.4 Research Goal

The main goal of this research is to design and develop a robotic capsule that has the potential to collect a microbiota sample from the GI tract without contamination. To achieve this goal, a minimally invasive robotic capsule that includes actuation and sampling mechanisms, is desired. The actuation mechanism should incorporate an actuator, an on-board battery and electronic circuitry to operate the sampling mechanism. The sampling mechanism should include a sampler to collect the microbiota from the gut lining (intestinal wall) of the small intestine and a storage chamber to secure the sample from downstream contamination. Lastly, the size of the storage chamber should include enough space to store at least 100 µL of sample so that the collected sample can be used for laboratory analysis.

1.5 Scope and limitations

The development of a mm-scale sampling device is an arduous task based on a range of challenges as laid down in problem statement section. The aim of this PhD thesis is to contribute towards the development of sampling devices that can aid in gut sample collection. The focus is to collect microbiota from the gut lining and lumen to facilitate the assessment of gut microbiota for full length of the GI tract. Emphasis is given to the design itself and then on to the development of a sampling mechanism (sampler and storage chamber) that can potentially collect a sample from the mucosa layer. Furthermore, space for a dedicated storage chamber is reserved to assure sample protection from both upstream (i.e., before sample collection) and downstream (i.e., after sample collection) contamination. The actuation system that can assist in the

sample collection process is given adequate attention specially the design of mm-scale actuator for robotic capsule or similar sized devices.

The challenges for the development of such a device can be divided in two major problem domains as follows,

- Fabrication, Instrumentation, Communication and Miniaturisation
    1. Design and development of a smart actuation system including a mm-scale actuator that can fit in the device and be capable of producing enough output force to collect a sample.
    2. A wireless communication link with the capsule to trigger the actuator for sample collection at the target-site.
    3. The capability of the sampling device to avoid contamination.
    4. The fabrication of a device with all mechanical and electronic parts.
    5. Miniaturisation of all internal components and hence the entire capsule to reduce the final product to a swallow-able size so it can transit through the entire gut.
- Localisation, Locomotion, Anchoring and Fixation of sample
    1. Localisation of the capsule, i.e., tracking of the device to determine the target-site for sample collection.
    2. Locomotion system for the capsule to manoeuvre inside the gut.
    3. Anchoring mechanism to support the capsule to stay at the target location during sample collection (if needed).
    4. Fixation (preservation) of sample from the time the sample is collected till its recovery from the faeces.

The thesis concentrates on the fabrication, instrumentation, communication and miniaturisation aspects of the capsule development while localisation, locomotion, anchoring and fixation of sample, aspects are utilised from already developed methods. The development of a commercial prototype is out of the scope of this work; however, this work will facilitate the development of such devices by reducing the research challenges.

1.6 Research contributions

This thesis has addressed several challenges as detailed earlier, and contributed in various ways towards the development of a gut sampling device. The major contributions of this thesis are detailed below.

Quantification of Peristaltic forces: The development of an analytical model and then simulation in COMSOL Multiphysics to measure the impact of peristaltic forces on the robotic capsule. Consequently, peristaltic forces were measured from living small intestines using a robotic capsule with force sensor through an embedded system. This allowed me to determine the amount of peristaltic forces applied by intestinal tissue on the robotic capsule. This further allowed the development of a smart actuation system that could overcome the intestinal forces to collect a sample from living tissue.

Design of a smart actuation system using SMA spring: Initially, two shape memory alloy (SMA) springs in an antagonistic configuration were used to produce to and fro linear motion for the proposed actuator design. Later, to reduce the design complexity and power requirements for the actuator, a novel two-way SMA spring was utilised that could produce both upward and downward movements required for sample collection. A dedicated battery with high current drain output was utilised to tackle the SMA requirement and a miniaturised wireless transceiver was used to activate the sampling process remotely.

Development of a sampling mechanism to collect gut microbiota: A unique design was fabricated that collected and stored microbiota from both the intestinal lumen and gut lining (intestinal wall). The unique design was optimised for sample collection and during experimental evaluation, it collected more than 100 μL from living intestinal tissue from three different animal species (cow, sheep and pig). Furthermore, laboratory analysis of the samples collected confirmed that the design of sampling mechanism has a potential to collect gut microbiota, mucus and digesta that will allow to perform the assessment of gut microbiota throughout the length of the GI tract.

Development of a sealing mechanism to avoid cross contamination: A purpose-built sealing mechanism was developed after extensive testing for the proposed design of robotic capsule. The robotic capsule with sealing mechanism was intensively tested in various in-vitro gut simulators to ensure the safety of the robotic capsule and ensure that the collected sample would remain secure from cross contamination. The rigorous testing of the robotic capsule allowed to provide a proof of concept case for *in-vivo* trials in the future.

1.7 Related publications and awards

This thesis has resulted in following journal publications. The research recognition and awards obtained by the work in this thesis are shown in appendix B.

1. **Rehan, M.**, Al-Bahadly, I., Thomas, D. G., & Avci, E. (2020). Capsule robot for gut microbiota sampling using shape memory alloy spring. *The International*

Journal of Medical Robotics and Computer Assisted Surgery, 16(5), 1-14. doi: 10.1002/rcs.2140.

2. **Rehan, M.**, Al-Bahadly, I., Thomas, D. G., & Avci, E. (2021). Measurement of Peristaltic Forces Exerted by Living Intestine on Robotic Capsule. *IEEE/ASME Transactions on Mechatronics*, 26(4), 1803 – 1811. doi: 10.1109/TMECH.2021.3078139.

3. **Rehan, M.**, Al-Bahadly, I., Thomas, D. G., & Avci, E. (2021). Towards Gut Microbiota Sampling Using an Untethered Sampling Device. *IEEE Access*, 9, 127175-127184. doi: 10.1109/ACCESS.2021.3111086.

4. **Rehan, M.**, Al-Bahadly, I., Thomas, D. G., & Avci, E. (2022). Development of a Robotic Capsule for *in-vivo* Sampling of Gut Microbiota. *IEEE Robotics and Automation Letters*, 7(4), 9517-9524. doi: 10.1109/LRA.2022.3191177.

5. **Rehan, M.**, Al-Bahadly, I., Thomas, D. G., & Avci, E. (2022). Smart Capsules for Sampling the Gut: Status, Challenges, and Prospects. *Gut.* (in preparation).

1.8 Thesis structure and outline

This thesis is compiled according to Massey University's Guidelines for 'Doctoral Thesis with Publications'. The subsequent chapters are based on published journal articles (four chapters) and a publishable article which is in preparation for submission to a journal. The chapter wise break-up of this thesis is organised as follows.

Chapter 2: This chapter presents an in-depth literature review of the relevant and related smart capsules developed for sampling the gut. Initially, the significance of microbiota in relation with health and disease is explained. Secondly, the benefits of collecting gut microbiota from live animals or humans are highlighted to assure that microbiota has various uses other than acting as a bio-marker to identify health problems. Lastly, the current state of the art in sampling devices and their major challenges are detailed to fully review the field of sampling devices or robotic capsules for gut microbiota sampling. This chapter discussed the existing literature on collecting gut microbiota.

Chapter 3: The small intestine moves the food in distal direction with the help of peristaltic forces and a robotic capsule needs to overcome these forces to collect a sample from the gut lining (intestinal wall). This chapter is focused on the quantification of peristaltic forces that would act on a capsule during its passage along the gut. Initially, an analytical model is presented to study the peristaltic movement of the small intestine. For the first time, finite element simulations were conducted in COMSOL Multiphysics to generate intestinal peristaltic forces, and analyse their impact on a robotic capsule. Later, a capsule prototype was developed to measure the peristaltic forces from living

small intestinal tissue, while an embedded system was used simultaneously to record the live data from the capsule - (small) intestine interaction. This chapter has provided a basis to develop a small scale (mm-size) actuator that can overcome the peristaltic forces to collect a sample from live intestinal tissue.

Chapter 4: In this chapter a unique sampling mechanism is devised to gently scrape the mucus content from the intestinal wall (mucosa layer). This design was thoroughly tested using *ex-vivo* intestinal tissue to optimise the capability of the sample collection mechanism without compromising the safety or damaging the GI tract of animal or human. The learnings from Chapter 3 were utilised to develop an actuation mechanism based on a unique combination of concentric SMA springs to act as an axial actuator. The developed actuator occupied a small space and produced sufficient output force to operate the sampling mechanism and overcome the intestinal peristaltic forces. A minimally invasive robotic capsule was tested *ex-vivo* on animal intestinal tissue, and it captured sufficient quantity of mucus and digesta for microbiota assessment. The laboratory testing of the collected samples identified an amino acid signature indicative of microbiota, mucus and digesta, which provided a proof of concept for the proposed design.

Chapter 5: This chapter presented the modeling of a unique two-way SMA spring actuator that has not been utilised before in any sampling device or robotic capsule. The temperature gap between the martensite and austenite states (hysteresis loop) was significantly reduced with the aid of a commercial manufacturer that allowed a reduction in the complexity of a previously proposed design while greatly reducing the power requirements. A specialised experimental setup that can keep the freshly dissected small intestine alive was utilised to test the robotic capsule in a realistic environment (in terms of peristaltic movements) as opposed to earlier tests with *ex-vivo* animal small intestines. The robotic capsule prototype collected sufficient quantity of sample from living porcine duodenal and ileal tissues (i.e. in the presence of peristaltic forces). The robotic capsule was also tested on living post-mortem tissues (small intestine) of other species including cow and sheep. The collected sample size for all of the species was feasible to analyse the microbiota through next generation sequencing techniques. However, the power source remain a challenge as the capsule prototype in this chapter was powered by a AAA battery that is 45 mm x ϕ 10.5 mm in size which is unsuitable for *in-vivo* ingestion.

Chapter 6: In this chapter the limitations of the power source were resolved by developing an actuation system tackling the high-drain current requirement of the two-way SMA spring actuator. Another challenge of cross contamination for assuring effective sample collection was resolved by successfully encapsulating the collection chamber which was realised by testing 3 main sealing materials. Rigorous testing of the

robotic capsule prototype was performed in a gut simulator that mimicked the *in-vivo* environment to ensure successful and safe travel of the capsule along the gastrointestinal tract. In addition, the capsule is also tested under the *ex-vivo* experimental setup to assure successful sample collection and its protection afterwards. The prototype presented in this chapter is the final prototype of this thesis and has the potential to become a vital apparatus for clinicians to sample human and animal gut in the future.

Chapter 7: This is the final chapter of this thesis and summarises the contributions of this research work. Furthermore, it provides key research dimensions that require subsequent research to further develop devices for clinical purposes.

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Chapter 2

Smart Capsules for Sampling the Gut: Status, Challenges, and Prospects

2.1 Abstract

Smart capsules are emerging at a tremendous pace with a promise to become effective clinical tools for the diagnosis and monitoring of gut health. This field emerged in the early 2000s with a successful translation of endoscopic capsule from laboratory prototype to a commercially viable clinical device. Recently, this field has accelerated and expanded into various domains beyond imaging, including sampling devices for better insight into gut health. The study of gut microbiota is gaining massive attention due to its significant impact on human health. Gut microbiota can be informative of health status over the life of the host, and it can act as a bio-marker for early diagnosis of diseases like diabetes, cancer, and obesity. Gut microbiota can also assist in better identification of inflammatory bowel diseases, ulceration, coeliac disease, Crohn's disease, and irritable bowel syndrome. In this review, the status of sampling devices is presented to highlight the broad picture of state-of-the-art devices while focusing on the technical and clinical challenges these devices need to overcome to demonstrate their value in clinical settings. The expansion of smart capsules to robotic capsules for gut microbiota collection has opened new avenues of research with great promise to revolutionise human health diagnosis, monitoring, and intervention.

2.2 Introduction

The human gastrointestinal (GI) tract is a 7-9 metre long passage and in an average lifetime of a human, around 60 tonnes of food passes through it [1]. The food is digested and absorbed by the host using a range of physical and chemical processes, while a huge community of microorganisms (bacteria, archaea and fungi) live inside the gut and play a major role in fibre fermentation and the synthesis of short-chain fatty acids, and some nutrients (e.g. vitamins) and are collectively known as microbiota [2]. This community of microorganisms have been studied for a long time, and numerous studies on the relationship between microbiota and human health reveal that the microbiota can act as a biomarker for human health [3]. The human meta-organism comprises bacterial colonies includes approximately 10^{14} prokaryotic organisms with a biomass of around 1.5 - 2 Kilograms [4]. Though the human microbiota is still not fully explored, it is pertinent that it is present at mucosa layer and in intestinal lumen [5].

The knowledge of relationships between host and its microbes has progressed significantly which suggests that the microbiota is a crucial component of study on human health. Several researchers are exploring obesity, inflammatory bowel diseases, biochemical processes, and diabetes with the help of microbiota [6–10]. It is considered that the microbiota can be informative of health status over the life of the host and can even assist in early diagnosis of diseases like cancer, obesity and diabetes [6, 7, 11]. Furthermore, the analysis of microbiota can help in better treatment of diseases such as ulceration, coeliac disease, Crohn's disease and irritable bowel syndrome [12]. Microbiota can also help to study the relationship or interaction between nutrition and human health [12, 13]. Comprehensively, it can be inferred that the human gut microbiota is full of information related to human health.

Recent research studies suggest that the microbiota can potentially determine the mood, behaviour and several other characteristics of the host [14]. Furthermore, it has been shown that gut microbiota influence brain function and behaviour, and plays a role in anxiety and mental disorders [15, 16]. It is also considered that the microbiota influences stress behaviour, anxiety and depression [17]. Studies have looked at the impact of microbiota on mood, and revealed that the microbiota regulates the emotions and cognition through the gut-brain axis which is a bidirectional communication link between the GI tract and the central nervous system [18].

Based on the importance of analysing gut microbiota, it is critical to explore its ecology. Microbiota colonise the mucous layer which covers the columnar epithelium of the GI tract and the digesta within the intestinal lumen [5]. The most common samples used as a proxy for intestinal microbiota are faecal samples, as they are non-invasive, easy to collect and can be collected repeatedly from the same individual. However, faecal samples are collected at the end of the 9-metre-long gut that restricts extraction

of spacial and temporal information from these samples as they are not collected from the actual site of digestion [19]. Furthermore, the samples are exposed to different environments throughout the gut before collection, so they are highly contaminated [20, 21]. Another method to study the gut microbiota is using 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 closest to colon (ileum) is a home to a different community of microbiota to the hind gut [22]. Secondly, this method involves a high risk of gut perforation and bleeding, and the procedure is invasive and unpleasant for a patient [23–25]. Lastly, biopsy tools are designed to collect a tissue sample, and they cannot fully capture microbial content. Hence, the current conventional tools available to collect microbial samples from the small intestine without contamination have limitations.

Commercial capsule 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, biopsy and sampling [26–28]. In this review, recent advances in the field of sampling devices are described, with a focus on understanding their benefits while addressing the limitations of these devices.

2.3 Microbiota and Human Health

Recent experiments on animals and clinical trials on humans suggest that the human intestinal microbiota is a potential candidate for pathogenesis via infection and in turn causing disease in a host. A study conducted on 40 volunteers revealed that the microbes in the intestine produce trimethylamine-N-oxide (TMAO) from the consumption of phosphatidylcholine (a chemical present in some foods), which contribute towards the physiopathology of heart disease [29]. The gut microbiota is an important tool to observe the effects on physio-pathological parameters and on controlling inflammation. A study conducted on mice observed the impact of an increase in bifidobacteria within the gut microbiota following consumption of a high fat diet which induced diabetes, suggests that the gut microbiota can be indicative of inflammation during the occurrence of diabetes and obesity [30]. Another study conducted on mice determined that artificial sweeteners induce glucose intolerance by altering the gut microbiota [9]. The same group of researchers in a related study revealed that the effect of similar diet on individual animals was different due to the response of their gut microbiota [10].

In general, obesity is co-related with the consumption of food. Studies using mice models have revealed that weight regain is highly dependent on the gut microbiota [6], suggesting that the weight regain phenomenon can be understood and resolved by targeting microbiota.

The gut microbiota of humans gets radically transformed with age and may contribute towards the development of several diseases including type 2 diabetes. To evaluate the effect of aging and diet composition in cats, a comprehensive study over 5 years has been conducted at Massey University, New Zealand [31]. The study used two groups of kittens aged 2 months and fed them with two different diets until they were 5 years of age. Faecal sampling is used to observe the effects of diet over the 5-year period. The study revealed that the faecal microbial composition was affected by both diet and age; however, insulin sensitivity was unaffected by both parameters. Body composition was affected by age, but diet had no effect. Another clinical experiment has been performed, by same group of researchers on 15 dogs, in which one group comprised of 8 dogs were fed a kibbled diet and the other group of 7 dogs were fed a raw red meat diet for 9 weeks [32], and macronutrient digestibility was assessed using faecal samples from both groups of dogs. The authors showed clear associations of specific microbial taxa with diet composition in dogs, and physiological parameters such as the digestibility of macronutrients such as protein and fat and faecal health score were also correlated with faecal microbial composition.

Faecal sampling is widely used for the analysis of different types of diseases and to check diversified markers within the GI tract. Though this method is noninvasive and easy to perform, it cannot replicate targeted samples of gut microbiota in terms of purity. The faecal sample is contaminated downstream by subsequent microbial communities and gut secretions during transit to the end of the GI tract. In addition, the sample obtained by faecal sampling does not fully represent samples from specific locations of GI tract [33]. Therefore, the latest tools for sampling gut microbiota are detailed in the next section.

2.4 Robotic Capsule for Sampling the Gut

The devices that can collect a sample from the gut are divided into two broad categories, biopsy devices that can collect a small tissue from the gut wall and sampling devices that can collect content-based samples.

2.4.1 Biopsy devices for tissue sampling

Due to the limitations of tethered endoscopy with biopsy tools in terms of their reach to entire gut and higher risk of gut perforation and bleeding, robotic capsules were developed to perform tissue biopsy. In 2003, a patent application proposed a wireless capsule system to collect a biopsy specimen in a biological body using micro-spectroscopy and/or biosensors [34]. This capsule contains two motorised blades to capture solid tissue or a liquid specimen that can be stored in two dedicated compartments to avoid

contamination until its recovery. Another design consisting of a razor connected with a torsional spring was proposed that triggered the biopsy process by melting a paraffin block which allowed the razor to rotate at high speed with the help of torsion spring to capture a tissue sample [35]. Both approaches require the capsule to be in close contact (almost rubbing) with the intestinal wall to capture the tissue. To overcome this challenge, a magnetic biopsy capsule is used and an external magnet holds the capsule's lateral hole against the intestinal wall while a cylindrical razor blade cuts the tissue with magnetic actuation [36]. However, this design lacks a triggering mechanism to effectively locate the target-site. Therefore, a design with a micro reed switch that can trigger the biopsy process based on external magnetic field was presented that included an elliptical hole to affix the target tissue and a spiral spring to produce the rotational force [37]. Once the capsule reaches the target-site, the reed switch gets triggered by an external magnetic field that heats the SMA spring resulting in cutting the special polymer string which allows the torsional spring to rotate the biopsy cutting tool, hence collecting the affixed tissue from the elliptical hole.

The biopsy tools that remain inside the capsule shell cannot guarantee collecting a tissue despite the external magnetic holding mechanism, therefore some designs are presented that actively move the tools outside the capsule shell to ensure tissue collection. A motor with rack and pinion gear system moves a biopsy tool (forceps with barbs) inside and outside of a capsule shell to perform the biopsy but this compromises the limited power available to capsule endoscopes to perform usual tasks (imaging) [38]. Another device uses two ring shaped permanent magnets to move the biopsy forceps outside the shell to actively cut intestinal tissue [39]. A shape memory alloy (SMA) actuator was used to project the biopsy razor outside of the capsule shell once the capsule was at the target-site, then the rotating magnetic field rotates the capsule prototype helping the razor to cut the tissue from the gut wall and finally two restoration magnets were used to bring the cutting tool inside capsule shell to secure the sample [40]. However, the two magnets used for restoration of the biopsy module to bring the ejected blade back inside the capsule, were too large and could not allow a telemetry system for triggering the biopsy process to fit inside traditional endoscopes. Another design uses a gear-assembly to move forceps-style blades in and out of the capsule shell which allows the biopsy system to fit inside a traditional endoscope [41].

All these designs presented a biopsy tool, and some also include the triggering method but most of them lack locomotion to help the capsule reach the target-site. A biopsy capsule with an active locomotion mechanism that can move flexibly within the gut and can extract a tissue sample with rotating blade mechanism from the target location by magnetic actuation was presented [42]. Later, this research group modified the blade design with a retractable biopsy punch to fit inside a traditional endoscope

so the biopsy procedure could be effectively performed with visual aid from the camera module [43].

Some designs consider obtaining a biopsy sample from the mucosa layer, as superficial collection from the epithelial layer is not always sufficient for in-depth microbiota analysis. A unique design of a micro-spike with barbs was proposed that was triggered by heating a shape memory alloy (SMA) wire that moves the micro-spike outside capsule shell by a slider crank mechanism and torsion spring to penetrate into the mucosa layer [44]. The barbs help to tear the biopsy samples which later get stored inside the capsule to avoid contamination. Another capsule for fine needle aspiration biopsy, triggers with a magnetic actuation that squeezes the capsule which allows a sharp hollow needle to penetrate into the mucosa layer to obtain a sample from inside of mucosa membrane, which was not explored previously by earlier biopsy capsules [28, 45].

One of the challenges for most of the biopsy capsules is the lack of control on their motion as they rely on peristaltic forces to reach the target-site and stopping the capsule at target-site is not possible. Some of the designs use an external magnetic field to anchor the capsule at a target-site but this increases the complexity and cost of the overall system while the capsule anchoring cannot be precisely controlled. A complete solution with a tissue monitoring module using a camera, anchoring module using SMA springs to stop the capsule at the target-site and a biopsy module using two cylindrical razors and a spiral spring to extract the tissue had shown clinical promise [46]. However, the capsule requires further miniaturisation as currently it is oversized for *in-vivo* testing and supplying the power to all modules was challenging as limited batteries can fit inside the swallowable capsule (size constraints). Another capsule uses a single magnetic actuator to drive both the anchoring mechanism and biopsy spike tool using a ratchet mechanism to overcome the power limitations, but intestinal trials are yet to be realised [47].

In order to collect biopsy samples from the stomach, one approach has been to develop a magnetically actuated capsule which releases a large number of temperature sensitive microgrippers that self-fold themselves due to change in temperature [48]. The capsule then collects the microgrippers with an adhesive patch by using its camera module.

The biopsy devices are promising tools for collecting tissue samples from the gut wall and they can overcome the limitations of tethered devices by accessing the entire gut. The biopsy tools are used with locomotion and localisation mechanisms to efficiently capture the target tissue. However, these devices cannot be used for sampling the gut microbiota as they cannot capture content-based samples.

2.4.2 Sampling devices for content-based sampling

The development of tools for sampling the gut is gaining attention based on the impact of microbiota on human health and the amount of information microbiota can reveal. The promising benefits of sampling devices for the collection of gut microbiota has resulted in many patents with an intent to produce a commercial device. A patent filed in 1957 which was later published in 1962, intended to track a capsule through x-ray to determine the target-site and open an inlet through radiant energy from outside of the subject, to collect a sample [49]. However, this design does not specify how to secure the sample from downstream contamination. Another patent suggested using a low melting point spring that can be heated from outside of the subject using high electromagnetic field, hence detaching the spring which then opens the chamber for sample collection [50]. The spring is connected to a piston inside a slider that allows the piston to move to the other end once the fluid is filled, which closes the inlet of the chamber and secures the sample from contamination. Another patent proposed to use an ether-filled bellows inside a capsule that expands on heating from an external electromagnetic field resulting in collection of the surrounding fluid, with the capsule returning to its original position once the magnetic field is removed which secures the collected sample [51].

Most of the designs use separate opening and closing mechanisms, hence making the designs complicated. Secondly, a lot of the capsules were designed for one-off use, that increases the overall cost and reduces sustainability. Therefore, SMA materials were introduced to allow use the same capsules multiple times. An SMA spring is latched inside the capsule chamber that is compressed when the temperature is changed by passing an electric current through the spring allowing the chamber to sample content from an orifice [52, 53]. Another design with a rotatable mechanism twists concentric cylinders using shape memory alloy polymer when it is heated allowing the surrounding fluid to enter through opened apertures on a circumferential wall [54]. The device moves back to its original position when heating is stopped which secures the collected sample from contamination. Another patented design uses an SMA polymer for the inlet (door) of the capsule [55]. The SMA polymer shape is designed to block the outside fluid from entering the chamber. When the SMA polymer is heated using induction heating, it allows the outside fluid to enter the capsule, once heating is stopped the polymer retain its original shape which secures the sample inside the chamber and avoids cross contamination.

However, most of these designs require a strong external (magnetic or electromagnetic) field to trigger the sampling process which requires an expensive external setup. Therefore, some internal triggering mechanisms have been proposed that rely on internal resources from within the capsule device. A capsule uses wireless communication

to trigger a set of spring-loaded concentric cylinders that are joined with a meltable thread [56]. The wireless receiver is used to ignite the heater that melts the thread and allows the spring to open the concentric cylinders which in turn collects the sample via suction from a small inlet. This capsule was designed for one time use, while another patent proposed a wireless triggering mechanism that used a motor to open the sampling chambers. The capsule consists of two motorised blades to capture solid tissue or a liquid specimen that could be stored in two dedicated compartments to avoid contamination until recovery [34].

The embedded designs that incorporate both sampling and triggering mechanisms inside the capsule, leave less room for the sample storage itself. Therefore, a simplified design was proposed that suggested collecting the content from the small intestine with both active and passive mechanisms [57]. The capsule had a vacuum compartment that is sealed, which can be dissolved by chemical reaction when it reaches the target location. For the active mechanism, the compartment's opening was covered by a magnet which was displaced by using an external magnet. However, whether active or passive, either mechanisms does not define any method to close the compartment that stops downstream contamination so targeted sampling was not possible. Another patent proposed to use a fluid sensitive membrane to cover the inlet to a chamber which get dissolved by interacting with the stomach or intestinal fluid and allows accumulation of a sample inside the chamber. The inlet then gets closed by a spring-operated valve that blocks the orifice once the chamber is filled with fluid [58]. Wrigglesworth et al. (2021) proposed an extendable mechanism to collect digesta from the ileum (small intestine) of animals to study nutrient absorption and digestion, the capsule has a mechanism to extend from the centre at the target-site to collect a sample size up to 1.5 mL [59].

The laboratory prototypes of sampling devices that are developed so far, can be classified into three major types. First, uncontrolled or passive sampling devices, as shown in Fig. 2.1, that activates the sample collection by dissolving the covering (enteric coating) over an inlet via a chemical reaction or any other method [60–65]. Second, controlled or active sampling devices, as shown in Fig. 2.2, that trigger the sampling process through wireless control (electronic or magnetic) to collect the sample at a target-site [66–73]. Third, dynamic sampling devices, as shown in Fig. 2.3, that focuses on collecting the microbiota from gut lining for in-depth analysis when the capsule reaches the target location [74–77]. The passive and active sampling approaches mainly collect the digesta fluid from the lumen whereas the dynamic sampling collects the sample from both lumen and intestinal wall.

The first sampling prototype known to the author was developed in 2008, and demonstrated simultaneous drug delivery and sampling by moving a piston that ejected

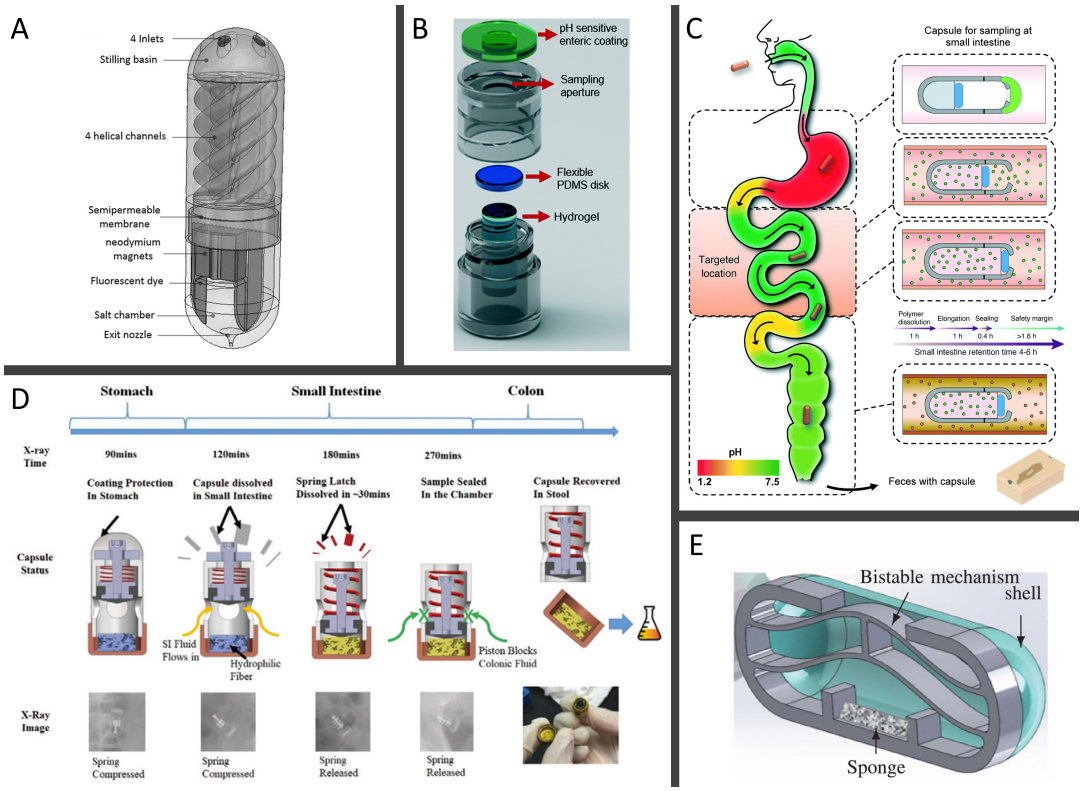

Figure 2.1: Passive sampling devices that use enteric pH coating to dissolve by reacting to the target fluid to collect microbiota and digesta sample. (A) Osmotic pill sampler that continuously samples the microorganisms throughout its passage till recovery, (reproduced from [60] licensed under CC BY 4.0.). (B) and (C) Collects the sample mainly from small intestine and secure it from contamination inside the colon by sealing the inlet through hydrogel, (reproduced from [64] with permission from the Royal Society of Chemistry.). (D) IMBA capsule explains the collection with timings in various regions throughout the gut, (reproduced from [61] © 2019 AGA Institute). (E) Bistable mechanism to collect and store the sample, (reproduced from [62] © 2018 IEEE).

a drug from the device while a small orifice at other end collected the surrounding content through suction [66]. The sampling prototype did not demonstrate a method to secure the sample as the orifice remained open after sample collection, hence led to cross contamination. Another patent proposed to use a motor to sequentially expose three storage chambers to allow collection of intestinal content from three distinct locations [67]. The motor also closed the inlets after sample collection that resolved the contamination issue. A commercial company (Biora Therapeutics inc., US formerly Progenity Inc.) patented this idea under the recoverable sampling system (RSS) name, and is in the process of carrying out clinical trials. The RSS capsule has the capability to detect five distinct sites within the gut before triggering the sampling process which reduces

the need for tracking the device from outside the subject or relying on physiological cues like pH or transit profile [68]. The localisation technology flashes LED lights that are received by photodetectors and the microcontroller based on a pre-programmed algorithm predicts the intestinal or colon location using gut anatomy. The capsule design shown in Fig. 2.2(G) and uses a motor to open the sampling aperture to expose an absorbent pad that collects the intestinal fluid [69]. The absorbent pad is soaked with preservatives that maintain the microbial community till capsule recovery from faeces that ensures better analysis after capsule retrieval.

A capsule prototype as shown in Fig. 2.2(D), has a storage chamber consisting of a flexible material that is squeezed inside the capsule and an inlet sealed with wax. Once the capsule reaches the target-site the sampling process is activated by magnetic actuation via a reed switch and a nichrome wire surrounding the inlet of chamber is heated so it melts the wax and allows the collection of fluid via vacuum suction [70]. This design did not consider resealing the inlet to avoid cross contamination. A commercial company NaviCam (AnX Robotica, US) has developed a magnetically controlled sampling capsule endoscope (MSCE) that can be manoeuvred to the target-site and its orientation can be precisely controlled using an external magnetic field [71]. The capsule contains three sampling ports sealed by a low melting point metal that is heated when the capsule reaches the target-site allowing the external fluid to move inside the chamber due to the pressure difference. The device position and orientation can be controlled by an operator using a built-in camera and external magnetic system which submerges the capsule in intestinal fluid for better sample collection, as shown in Fig. 2.2(E). The capsule uses a round shaped stopping mechanism that automatically seals the inlet once the chamber is filled with fluid.

Both the heating filament and motor require electronic circuitry and a battery that occupy most of the capsule while leaving little space for sample storage. Some capsule designs use enteric coatings which dissolve at the target-site and allow collection of the sample which reduces the components required for a triggering mechanism. An osmotic pill with four helical channels connected to a semipermeable membrane was developed that constantly passed the surrounding fluid through the channels while the membrane blocked the microorganisms inside the channels [60]. The pill was coated with a pH-sensitive enteric coating to avoid interaction with gastric juice, and the pill started sampling after the covering got dissolved in the small intestine. However, the sampling continued until the pill reached the colon as this design did not consider sealing the inlets. Another capsule prototype used a gelatin coating that dissolved in the small intestine and the inside chamber contained a hydrophilic fibre that absorbed the intestinal fluid [61]. The capsule used a spring-loaded latch that dissolved in 30 mins and moved a piston to block the chamber inlet which secured the sample from cross

Table 2.1: Sampling devices to collect gut microbiota samples

Name	Dimensions (mm)	Storage capacity (µL)	Type of Sampling	Actuation mechanism	Target location	Evaluation
Cui et al [66]	30 x ϕ 10.2	262	Active	Motor	SI	LP, *in-vivo*
Yaw et al. [67]	31 x ϕ 11	84 x 3*	Active	Motor	SI	Unspecified
RSS capsule [68, 69]	31 x ϕ 11.6	-	Active	Motor	SI and colon	CP, *in-vivo*
Du et al. [70]	20 x ϕ 14	300	Active	Vacuum suction	SI	LP, bench-top experiments
MSCE [71]	32 x ϕ 11.6	400	Active	Vacuum suction	SI	CP, *in-vivo*
Osmotic pill [60]	21.6 x ϕ 7.6	120	Passive	-	SI and colon	LP, *in-vivo*
IMBA capsule [61]	26.1 x ϕ 9.9	74	Passive	Spring loaded latch	SI	LP, *in-vivo*
Salem et al. [62]	26.1 x ϕ 9.9	200	Passive	Sponge	SI	LP, bench-top experiments
Hydrogel capsule [63, 64]	15 x ϕ 9	282.7	Passive	Hydrogel	SI	LP, *ex-vivo*
Hydrogel capsule [65]	15 x ϕ 9	282.7	Passive	Hydrogel	Colon	LP, *in-vivo*
BCMAC [72]	11 x ϕ 8	42	Active	Magnets	SI	LP, *in-vivo*
Park et al. [73]	26 x ϕ 11	15 x 3*	Active	Magnets	SI	LP, *ex-vivo*
Finocchiaro et al. [74]	30.5 x ϕ 11.5	261 x 2*	Dynamic	Magnets	SI	LP, bench-top experiments
Rehan et al. [75]	30 x ϕ 12	500	Dynamic	SMA springs	SI	LP, *ex-vivo*
Rehan et al. [77]	45 x ϕ 12	250	Dynamic	SMA spring	SI	LP, in-vitro

Markers: CP: Commercial Prototype (awaiting FDA approval), LP: Laboratory Prototype, SI: Small Intestine, * indicates multiple compartments

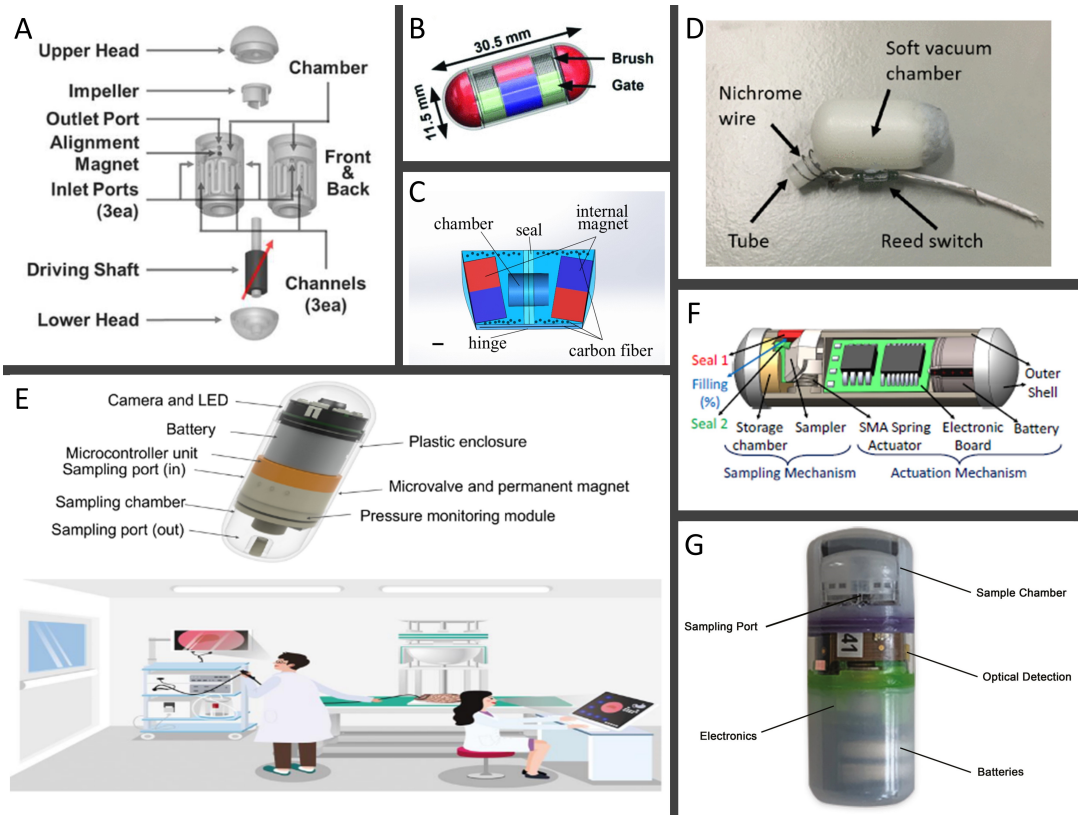

Figure 2.2: Active and dynamic sampling devices that use wireless triggering mechanism (except G) to collect microbiota and digesta sample. (A) Compact capsule with three separate channels to store the content, (reproduced from [73] © 2022 IEEE). (B) Dynamic sampling capsule that brushes the intestinal wall to collect microbiota, (reproduced from [74] © 2021 IEEE). (C) Magnetic capsule with a hinge mechanism to collect digesta and microbiota sample with blind activation based on predicted transit time, (reproduced from [72] © 2021 IEEE). (D) A flexible capsule triggered with magnet to collect the surrounding fluid with suction, (reproduced from [70] © 2018 IEEE). (E) Commercial prototype with sophisticated external magnetic control mechanism to drag the capsule to the target-site and on-board camera to visualise the collection site, (reproduced from [71] © Ding Z, et al. 2021). (F) Another dynamic sampling mechanism that scrapes the microbiota from intestinal wall. The capsule can be triggered by wireless transceiver, (reproduced from [77] licensed under CC BY-NC-ND 4.0.). (G) Standalone capsule that uses on-board camera (optical detection) to identify the target location and collect the sample based on internal microcontroller signal, (reproduced from [69] © 2021 Crohn's & Colitis Foundation.). Dynamic sampling devices that focus on collecting the microbiota from gut lining are shown in (B) and (F).

contamination, as shown in Fig. 2.1(D). Similarly, another capsule prototype based on a bi-stable mechanism also used an external enteric coating to protect the capsule from sample collection inside the stomach [62]. Once the capsule reached the small intestine, the outer covering is dissolved which exposed the inlet channel and allowed the chamber

to fill with the surrounding fluid. A twofold mechanism was designed to hold a sponge inside the chamber which swelled after absorbing the intestinal content and triggered the bi-stable mechanism to close the orifice as shown in Fig. 2.1(E), hence sealing the capsule from further collection. Another capsule prototype presented by Waimin et al. (2020) proposed a passive sampling capsule whose enteric coating also dissolved at the target-site (small intestine) and allowed the surrounding fluid to fill the capsule [63]. A dehydrated hydrogel placed inside the capsule absorbed the sampling fluid which increased its volume, resulting in pressure against a Polydimethylsiloxane (PDMS) membrane at the sampling aperture which sealed the storage chamber. The capsule design and its functionality are shown in Fig. 2.1(B) and Fig. 2.1(C) respectively, and it was tested under *ex-vivo* conditions to prove its efficacy for detecting inflammatory bowel disease [64]. Later, the design was modified with two enteric coatings on top of each other, the first coating protected the capsule from sampling inside the stomach, while the second coating protected the capsule from sampling inside the small intestinal region. Both coatings are finally removed once the capsule reaches the proximal colon where sampling starts [65]. This modification allowed the capsule to collect samples from proximal colon for detecting colonic diseases.

The capsule prototypes relying on intestinal fluid to dissolve pH based enteric coatings for sample collection have certain limitations. First, sample collection cannot start instantly, rather coating removal is passive activity that requires a long time (around 30 mins). Second, precise targeted sampling is not possible as closing the chamber for securing the sample is also a lengthy process (taking between 30 mins to 1 hour). Therefore, magnetic capsules were proposed to instantly trigger inlet opening and closing functions for targeted sample collection. A magnetic capsule is designed to blindly collect the digesta from the small intestine whereas the triggering time is estimated based on transit profile [72]. The capsule contains two small magnets embedded in the capsule shell as shown in Fig. 2.2(C), fabricated in a way that it forms hollow space between the two magnets. The external magnetic field repels the two magnets allowing the capsule to open using a hinge mechanism, and the removal of magnetic field collapses the magnets again which seals the collected digesta. Another magnetic capsule used an external magnetic field to perform locomotion and sampling [73]. First, the capsule was propelled to the target-site by a gradient magnetic field. Second, the inlet port (one of the three) was aligned with the sample collection channel by a uniform magnetic field, as shown in Fig. 2.2(A). Third, the micropump is activated by a precessional magnetic field that collects the sample in an aligned microchannel via an aligned inlet port. The design is compact and shows commercial promise, but needs to overcome the contamination issue as it uses only one sampling port through which all inlet channel to collect all of the samples hence leads to a small amount of contamination in

the second and third chambers.

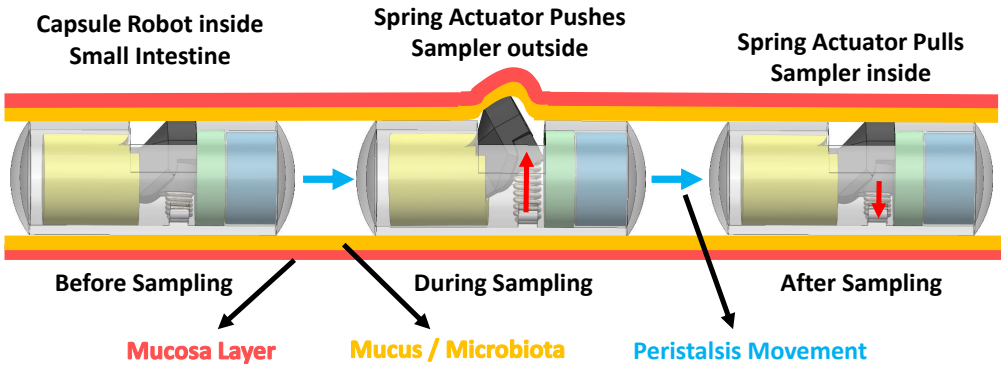

Figure 2.3: Dynamic sampling device that focus on collecting the microbiota from gut lining, image is reproduced from [75] © 2020 John Wiley & Sons Ltd.

The designs presented so far as shown in Table 2.1, collect the surrounding fluid which cannot guarantee collecting a full spectrum of microbiota, as microbiota is also present within the mucosa layer which cannot be collected by a simple opening and closing mechanism. 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 microbiota composition varies both longitudinally (e.g., duodenum, jejunum and ileum) and radially (e.g., within the lumen, epithelium, mucosa and submucosa) [78]. A magnetic capsule prototype as shown in Fig. 2.2(B) presented a brushing mechanism to collect microbiota from the gut lining (mucosa layer) that has not been explored before by the previous sampling devices [74]. First, the capsule was aligned with the intestinal wall using an external magnetic field. Second, the two gates of the sampling chambers were opened. Third, the brushing mechanism was rotated a few times to collect microbiota by rubbing on the intestinal wall. Once the brushing was complete, the gates were closed again to secure the sample inside two separate chambers. Another capsule prototype that targets sampling from the mucosa layer demonstrated a unique way of scraping the microbiota from the intestinal wall [75]. Two SMA springs connected in an antagonistic configuration eject a round channel outside the capsule shell that scraped the content from gut lining due to natural pressure from peristaltic forces and stored the sample in a connected chamber. Once the sampling was completed, the other SMA spring moves the scraping channel inside the capsule shell to secure the sample from downstream contamination. Later, the design was improved by replacing the two one-way SMA springs with one two-way SMA spring that produced both upward and downward movement in response to two different temperatures which were generated by passing the current through the spring using an on-board battery

[76, 77]. However, both the designs that collect microbiota from gut lining are yet to perform *in-vivo* trials which will demonstrate their effectiveness in terms of the quality of the sample collected.

2.5 Future perspectives and conclusion

The human gut is an interesting passage as it can be informative of health status over the life of the host. Therefore, assessment of gut microbiota is increasingly though to be a key to unlock secrets of the gut as it contains information about the host, and it can act as a bio-marker to identify health issues. Current tools have limitations in accessing the entire gut and cannot accurately collect the gut microbiota throughout the entire length of the intestines. Therefore, in this review biopsy devices are considered that are developed to collect tissue biopsy to perform targeted and site-specific analysis of gut wall. Also, sampling devices to collect the gut microbiota samples are reviewed and a critique on these devices is presented.

Various approaches have been adopted to collect gut microbiota that can be assessed after capsule recovery from faeces. Most of the prototypes in both active and passive sampling devices rely on arbitrary collection of surrounding fluid which does not capture the full microbiota as significant communities of microorganisms live on the gut lining inside mucosa layer [4] and the sampling mechanism needs to scrape or brush the intestinal wall to capture them [74–77]. A futuristic sampling device should be able to autonomously locate its target-site (longitudinal precision) and collect the sample from mucosa layer (radial precision) and guarantee the quality of the sample to study gut microbiota. Currently, most of the laboratory capsule prototypes struggle to embed all of the required functions into a tiny capsule (swallowable dimension) which is a major hurdle for further *in-vivo* testing.

The sampling devices, in the future, should focus on performing tasks in a standalone device without relying on external systems like magnetic or electromagnetic actuation. The lower dependency may simplify the operational cost and allow home testing with the capsule and personalised treatments. This may put less burden on the healthcare system and allow individuals to perform home diagnoses. Software on a mobile device may then interpret the results in a similar way to portable blood-sugar testing machines which are operated by the users at home currently. The mobile software may also update the diagnoses regularly to make and prepare weekly diet plans for optimum results. The smart sampling devices might be in use that may specify the best suited diets for each individual based on their microbiota. The rapid pace in development could determine that this happens in near future and the next generation may keep a log of their gut health from childhood to assess any drastic changes in their health to treat themselves with the aid of prescriptions provided by machine learning

algorithms and artificial intelligence, without even visiting a doctor.

The development of futuristic sampling devices may enable better treatment of gut-related problems like inflammatory bowel diseases, ulceration, coeliac disease, Crohn's disease, and irritable bowel syndrome. Furthermore, early diagnosis of diseases like cancer, obesity and diabetes might be realised which could help to treat these deadly diseases efficiently. In addition, mental health issues may also be addressed by relating the gut microbiota with relevant bio-markers. Hence, an *in-vivo* sampling device is desired that may improve the understanding of gut microbiota.

Chapter 2. References

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Chapter 3

Measurement of Peristaltic Forces Exerted by Living Intestine on Robotic Capsule

The small intestine moves the food in distal direction with the help of peristaltic forces and a robotic capsule needs to overcome these forces to collect a sample from gut lining (intestinal wall). This chapter is focused on the quantification of peristaltic forces that a capsule would observe during its passage from the gut. Initially, an analytical model is presented to study the peristaltic movement of the small intestine. For the first time, finite element simulations were conducted in COMSOL Multiphysics to generate intestinal peristaltic forces, and analyse their impact on a robotic capsule. Later, a capsule prototype was developed to measure the peristaltic forces from living intestinal tissue, while an embedded system was used simultaneously to record the live data from the capsule-(small) intestine interaction. This chapter has provided a basis to develop small scale (mm-size) actuator that can overcome the peristaltic forces to collect a sample from alive intestinal tissue.

This chapter contains content from the following article.

- Rehan, M., Al-Bahadly, I., Thomas, D. G., & Avci, E. (2021). Measurement of Peristaltic Forces Exerted by Living Intestine on Robotic Capsule. IEEE/ASME Transactions on Mechatronics. 26(4), 1803 – 1811. DOI: `https://doi.org/10.1109/TMECH.2021.3078139`.

Measurement of Peristaltic Forces Exerted by Living Intestine on Robotic Capsule

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***Abstract*—Using robotic capsules for assessing gut health has been an emerging field since the early 2000s with researchers attempting to perform diagnosis, monitoring and therapeutic functions inside the gut. The knowledge of peristaltic forces inside the intestine are crucial for designing the actuation mechanism of robotic capsules, however the impact of peristalsis on a capsule has not yet been quantified. In this work, an analytical model is presented to study the peristaltic movement of the small intestine. For the first time, finite element simulations were conducted in COMSOL Multiphysics to generate intestinal peristaltic forces, and analyse their impact on a robotic capsule. A capsule prototype (30 mm x ϕ12 mm) was developed to measure the peristaltic forces from living intestinal tissue, while an embedded system was used simultaneously to record the live data from the capsule-intestine interaction. In in-vitro experiments, the intestine applied an average axial force of 226 mN and contraction cycles of 9 times/min, while the capsule prototype experienced maximum radial force of 180 mN. A specialized in-vitro setup is developed to keep fresh ex-vivo intestine samples alive for up to 6 hours, while the capsule prototype measured the intestinal forces from the living tissue. This in-vitro experimental setup provided an excellent model for the in-vivo environment in terms of generating peristaltic movements, hence this force analysis will help in developing efficient prototypes for locomotion, anchoring, localization, biopsy, drug delivery and sampling mechanisms for robotic capsules.**

I. INTRODUCTION

The beginning of 21^{st} century was marked by a revolution in medical imaging when Iddan et al developed a minimally invasive capsule endoscope as an alternative to the tethered endoscopy [1]. A capsule endoscope moves passively inside the gut, in a similar way to food, with the help of peristaltic movement and captures images of gut lesions. One of the limitations of a capsule endoscope is its uncontrollable movement, which restricts its navigation inside the gut and it is not possible to spend significant time at the region of interest. Another limitation is its incapability to determine its precise location within the 9 meter long gut. Therefore, locomotion mechanisms have been developed to anchor at, or navigate towards, the target site [2]–[5]. Similarly, localization mechanisms were proposed to estimate the position of the capsule endoscope [2], [3], [6]. These advancements in the field of capsule endoscope laid the foundation for devices, with similar size, to perform diagnostic, monitoring and therapeutic functions like sampling [7], [8], tissue biopsy [9], sensing [2], [10] and drug delivery [11].

Advance techniques in robotic capsules such as locomotion, localization, biopsy, drug delivery and sampling, require knowledge of peristaltic forces to develop suitable actuation mechanisms. In locomotion based designs, the stopping mechanism needs to overcome the peristaltic forces to anchor at the site of interest [5]. Similarly, the contraction rate, peristaltic pressure and gas sensing can be used for localization, as this information can assist in estimating the position of the capsule [10], [12]. The biopsy tool counters the peristaltic forces in order to penetrate the gut wall, to capture the tissue [9]. Likewise, the sampling device also acts against the peristaltic forces to open its mechanism, to collect the specimen [7]. Once the peristaltic forces, applied by the intestine on the capsule endoscope or robotic capsule, are known, an accurate actuation mechanism can be designed for incorporating the additional features. Therefore, quantification of intestinal peristaltic forces can produce significant contribution for all these devices and add-on mechanisms.

Methods have been devised to measure the intestinal pressure, which can later be converted to peristaltic contraction forces based on the size of the measuring device, but they have certain limitations. Mostly, endoscopic manometry is used to measure the pressure inside the gut; however, this tethered method limits its reach into the small intestine. Secondly, this method involves a high risk of gut perforation and bleeding, and the procedure is invasive and unpleasant for a patient [2], [13]. A commercial capsule, SmartPill motility capsule (Medtronic, Minneapolis, US), and several other laboratory prototypes have measured gut pressure using minimally invasive robotic capsules with MEMS based sensors [12], [14]–[16]. These capsules have mainly recorded the intraluminal pressure within the gut, which was the cumulative pressure of each region, and it is not possible to extract the peristaltic pressure from the overall pressure signal [13], [14]. Furthermore, these sensors often capture breathing and heartbeat signals which need to be separated from the primary signal [12], [15]. Although the intraluminal pressure is helpful in treating gut related diseases, it provides less information on gut motility (peristaltic behaviour) and hence it is not possible to quantify the peristaltic forces.

Thus, this article describes how a tactile sensor based

*This research was supported by the KiwiNet grant 2020. Support was also provided by the Higher Education Commission (HEC), Pakistan under grant no. 5-1/HRD/UESTPI(Batch-V)/2421/2018/HEC.
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robotic capsule was developed to measure the peristaltic forces, directly from the living intestine. A biomechanical analytical model of the small intestine was developed for analysing the peristaltic forces. Finite element simulations are conducted in COMSOL Multiphysics, modelling the peristaltic behaviour of the small intestine, as elaborated in Fig. 1. In addition, an investigation of the impact of the peristaltic contraction forces on a robotic capsule prototype was conducted, which has not been done before. Simulation provided an in-depth analysis of peristaltic behaviour by providing the freedom to change the design parameters, such as intestinal diameter, and observe its impact on to the robotic capsule. A capsule prototype was then fabricated with a force sensor and an embedded system was devised to measure the peristaltic forces from living intestinal tissue. A specialized in-vitro setup was utilized to keep the intestine alive, while the robotic capsule recorded the peristaltic forces of the intestine. The three main contributions of this work are as follows.

1) The development of an analytical model and then simulation in COMSOL Multiphysics to measure the impact of peristaltic forces on the robotic capsule,
2) The development of a capsule prototype with a force sensor and an embedded system to measure the peristaltic forces from living intestine,
3) Rigorous discussion about quantification of peristaltic forces and its impact on robotic capsule designs.

The peristaltic motion developed in COMSOL in this study can be replicated by researchers to test advance techniques in robotic capsules like locomotion or anchoring mechanisms, by incorporating their design into robotic capsules. Similarly, the in-vitro setup in this study, which closely replicates in-vivo studies as it maintains the intestine alive for 6 hours, can be used to examine the robotic capsule under living intestinal tissue conditions. Furthermore, the impact of peristaltic forces on a capsule inside the intestine is quantified in this study and this knowledge will help in developing efficient tools for robotic capsules for biopsy, drug delivery, sampling, locomotion and localization mechanisms.

II. Materials and Methods

A. Biomechanical modelling of the small intestine

The movement of food through the small intestine is relatively poorly studied, as this region of the gut cannot be assessed through tethered tools. A pulpy fluid containing digested food and gastric juices known as chyme enters the small intestine from the stomach. The chyme is simultaneously mixed and moved along the length of the intestine using two main phenomena, namely peristalsis and segmentation. Peristalsis creates a wave-like motion by contracting and relaxing the muscles in circular direction, hence moves the chyme along the small intestine. Whereas, segmentation is mainly responsible for mixing of the chyme by creating a forward and backward motion in a longitudinal direction. The flow inside the intestine is described by the conservation of momentum and conservation of mass, which can be expressed by Navier-Stokes equations and continuity equation respectively. The

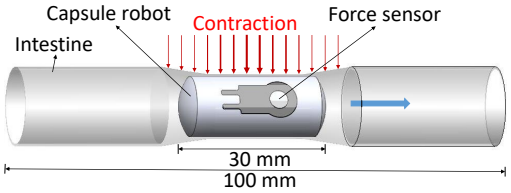

Fig. 1: Biomechanical model of the small intestine.

Navier-Stokes momentum equation in Cauchy momentum form can be defined as [17]

$$\frac{Du}{Dt} = \frac{1}{\rho}\nabla \cdot \sigma + g \tag{1}$$

Where, u is the fluid velocity, ρ represents the density, ∇ is the divergence, σ is the Cauchy stress tensor, g represents the gravity, and $\frac{D}{Dt}$ is the material derivative (Lagrangian derivative) that is the time rate of change of fluid and can be defined as

$$\frac{Du}{Dt} = \frac{\partial}{\partial t} + u \cdot \nabla \tag{2}$$

Where, $\frac{\partial}{\partial t}$ represents the change with respect to time at a given point. The Cauchy stress tensor (σ) expresses the changes in the material due to the deformation. This can be defined as a sum of deviatoric stress (changes in shape) and volumetric stress (changes in volume). Deviatoric stress can be expressed as a function of viscosity while volumetric stress can be considered as pressure, which are defined below

$$\sigma = -p + \mu(\nabla u + (\nabla u)^T) - \frac{2}{3}\mu(\nabla \cdot u)I \tag{3}$$

Where, p represents the pressure, μ is the dynamic viscosity, ∇u is the velocity gradient, $(\nabla u)^T$ is the transpose of ∇u and I is the identity matrix. The overall flow through the intestine can be described by the conservation of mass in the form of continuity equation as follows [17],

$$\frac{D\rho}{Dt} + \rho\nabla \cdot u = 0 \tag{4}$$

For incompressible fluids, the density of the fluid remains constant so (4) can be reduced to [18]

$$\rho\nabla \cdot u = 0 \tag{5}$$

Considering the assumption that the flow inside the intestine is incompressible (refer to (5)), (3) can be re-written as,

$$\sigma = -p + \mu(\nabla u + (\nabla u)^T) \tag{6}$$

Navier-Stokes momentum equation (1) can be modified by using (2) and (6), and expressed as follows [18]

$$\rho\frac{\partial u}{\partial t} + \rho(u \cdot \nabla)u = -\nabla p + \nabla \cdot \mu(\nabla u + (\nabla u)^T) + \rho g \tag{7}$$

Therefore, (7) can be described as Newton's second law of motion in which the left side defines the inertial forces wihin the fluid while right side is a sum of pressure forces, viscous forces and external forces. Equation (5) and (7) are used in COMSOL multiphysics to define the fluid flow. The effect

of gravity on fluid flow was negligible so it was ignored in simulations and the flow was considered as newtonian fluid with dynamic viscosity (μ) of $0.0014Pa \cdot s$ and density (ρ) of $1040Kg/m^3$ [18].

The peristaltic movement is generated when the intestinal muscles apply the forces inward, which results in deformation of the intestine. The original location of a point on the material is denoted by X while the new location of the point after deformation is indicated by x and the displacement vector between the two points is expressed by $w(X,t)$. The momentum conservation for an arbitrary (undeformed) volume V_0 is

$$\frac{d}{dt}\int_{V_0}\rho_0 v dV = \int_{V_0} f_V dV + \int_{\partial V_0} T dA \tag{8}$$

Where, ρ_0 is the undeformed density, v is the velocity of deformation, f_V represents the volumetric forces in the undeformed region and T is the traction of forces acting in the undeformed area. The traction using its spatial components (T_i) and normal vector using its material components (N_J) can be further defined as

$$\int_{\partial V_0} T_i dA = \int_{\partial V_0} P_{iJ} N_J dA = \int_{V_0} \frac{\partial P_{iJ}}{\partial X_J} dV \tag{9}$$

Where, small indices (e.g. T_i) are used to define spatial components, and capital indices (e.g. N_J) are used for the material components. P_{iJ} is the tensor component and X_J expresses the original location of the material particle. The velocity of deformation (v) can be represented as a function of displacement (w)

$$v = \frac{\partial w(X,t)}{\partial t} \tag{10}$$

The volume is arbitrary, so the differential form of momentum balance can be achieved by substituting (9) and (10) in (8), as shown below

$$\rho_0 \frac{\partial^2 w_i}{\partial t^2} = f_{V_i} + \frac{\partial P_{iJ}}{\partial X_J} \tag{11}$$

Where, w_i is the spatial displacement, f_{V_i} is the spatial component of volumetric forces and P_{iJ} is the spatial tensor component. The tensor form of (11) is shown below

$$\rho_0 \frac{\partial^2 w}{\partial t^2} = f_V + \nabla_X \cdot P^T \tag{12}$$

Where, P is the first Piola-Kirchhoff stress tensor that signifies the relationship between forces acting in the spatial direction to the original location (undeformed configuration). This can be related to Cauchy stress tensor (σ) as follows

$$PNdA = \sigma n da \tag{13}$$

Where, N is the normal vector before deformation, dA is the area before deformation, n is the normal vector after deformation and da is the area after deformation. The deformation will change the area, which can be computed by Nanson's formula

$$nda = JF^{-T}NdA \tag{14}$$

Where, F is the deformation gradient tensor and J is the volume factor which can be computed by the determinant of F. Hence, the (13) can be re-arranged as

$$P = J\sigma F^{-T} \tag{15}$$

Similarly, the Kirchhoff stress tensor τ can be expressed as,

$$\tau = J\sigma \tag{16}$$

Therefore,

$$P^T = \tau^T F \tag{17}$$

The density of undeformed and deformed materials can be related, based on mass conservation as,

$$\rho = J^{-1}\rho_0 \tag{18}$$

The momentum balance equation in terms of Cauchy stress tensor can be written as

$$\rho \frac{\partial^2 w}{\partial t^2} = f_v + \nabla_x \cdot \sigma \tag{19}$$

Where, ρ denotes the density of the deformed material and f_v represents the forces per deformed material. These analytical equations are used to develop a peristaltic wave model for the small intestine. The details for the modelling and simulations are shown in the next sections.

B. Design considerations for simulations

A small intestine sample 100 mm in length and 15 mm in diameter, was used in this study, as shown in Fig. 1. A robotic capsule (30 mm x ϕ12 mm) was placed inside the section of intestine. The robotic capsule was moved inside the intestine, mainly, by peristaltic movements. While interacting with a solid object, like a robotic capsule, the dominant function of intestine is peristaltic movement as compared to segmentation which was not considered in these simulations. The forces were repeatedly applied at the centre of intestine section, after an interval of 7 seconds [19]. The deformation of intestine was defined by the radial contraction ratio and was selected to be 78% based on a related study [19]. The robotic capsule received the pressure from intestinal muscles based on deformation and is recorded over the time to measure the impact of radial contraction forces on the capsule. The biomechanical model of the small intestine was simulated by considering the following assumptions.

1) The geometry of the small intestine is considered as a cylindrical shaped tube with a mean diameter of 15 mm in relaxed state.
2) A small section of 100 mm from the small intestine is modelled to reduce the computational complexity.
3) The force is applied once in each cycle, at the centre of the 100 mm section of the small intestine.
4) An incompressible Newtonian fluid flow is considered inside the small intestine.

A Finite Element Analysis (FEA) based software, COMSOL Multiphysics (version 5.5, COMSOL AB, Stockholm, Sweden), was used to develop the biomechanical model as shown in Fig. 1. The modelling parameters were selected based on related studies [18], [19].

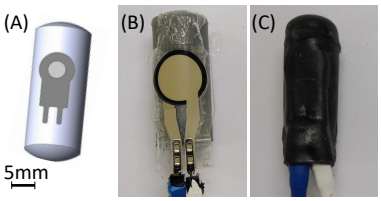

Fig. 2: Development of robotic capsule prototype. (A) CAD model (B) Capsule prototype with force sensor glued on the capsule. (C) Capsule prototype with water resistant lamination.

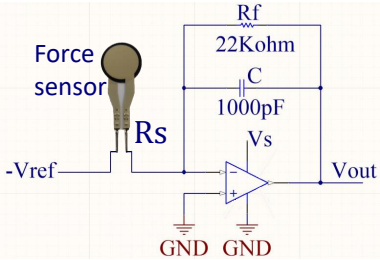

Fig. 3: Inverting Op-amp circuit for reading force measurements.

C. Design requirements and fabrication of the capsule

The dimensions of a robotic capsule should be small enough to allow transit through the entire GI tract. A capsule with size of 30mm x ϕ 12mm is considered to be safe [7], [12], [20]. The force measurement system should be sensitive to small forces, such as 10mN, so it can account for all activities related to the forces inside the intestine [21]. In addition, the measurement should be fast enough to process the force readings in less than 1s [21]. Furthermore, the robotic capsule needs to measure the contraction force in live animals or humans, hence the force sensor should operate between 35 and 40°C and in high moisture conditions. The computer-aided design (CAD) of robotic capsule with force sensor, developed in Solidworks (Dassault Systèmes SolidWorks Corporation, USA) is shown in Fig. 2 (A). The force sensor (FlexiForce ESS301 Sensor, Tekscan Inc., USA) is glued on to the outside of the robotic capsule as shown in Fig. 2 (B). The force sensor was laminated with water resistant tape to eliminate direct contact with the fluid as shown in Fig. 2 (C) and it was calibrated before each trial so its measurements were not affected. The sensor was exposed to the gut tissue; hence, the readings were obtained directly from the peristaltic forces of the intestine.

The capsule prototype was fabricated with Digital Light Processing (DLP) technique using a 3D printer (Hunter, Flashforge, China) with the resolution of 25 μm. The overall length of the capsule prototype, as shown in Fig. 2, was 30mm and its diameter was 12mm. The force sensor was calibrated to measure forces from 0 to 4N and its resolution was 5mN. The baud rate for collecting the data was set to 9600 bps, which processed the data in 5 μs. The force sensor had an operating

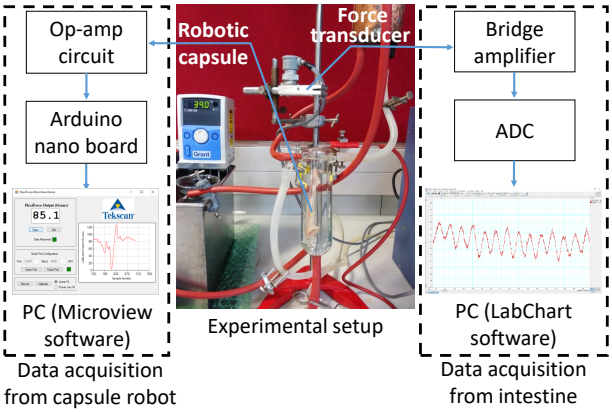

Fig. 4: Data acquisition system for peristaltic and axial force measurements. (Left side) data acquisition system of robotic capsule measures the peristaltic forces. (Right side) data acquisition system of in-vitro system measures the axial forces.

range from -40 °C to 85 °C. The force measurements were recorded by an embedded system, which is explained in the next section.

D. Data acquisition system for robotic capsule

The calibration is important in tactile sensors and it becomes more evident in this study as the gut environment is dynamic in nature. An electronic kit (FlexiForce Prototyping Kit, Tekscan Inc., USA) equipped with an Arduino nano board and an operational amplifier (op-amp) circuit was used to collect the force readings from the robotic capsule sensor. The op-amp circuit provided the flexibility required for calibrating the sensor inside the dynamic intestinal environment, by adjusting the feedback resistance and drive voltage, as shown in (20). An inverting op-amp configuration was used with a 22 KΩ resistor and 1000 pF capacitor in the feedback loop, as shown in Fig. 3. The V_{ref} signal was set to an square wave with 20 Hz frequency and 20% duty cycle. The drive voltage was made variable in the circuit, having multiple selections between 0V and 5V.

$$V_{out} = -V_{ref}\frac{R_f}{R_s} \tag{20}$$

Where, V_{out} is the output voltage, V_{ref} is the reference voltage, R_f is the feedback resistance and R_s is the resistance offered by the force sensor.

A specialized software (FlexiForce MicroView, Tekscan Inc., USA) developed for reading the force measurements, was used to record the force data. The op-amp circuit converted the force measurements to analog data, which were converted to digital form using built-in analog to digital converter (ADC) of Arduino nano board. The Arduino nano board was connected to the computer through a usb port. The MicroView software displayed the data on-screen for real-time utilization and also recorded the data for future analysis. A systematic diagram with force sensor, electronic kit and GUI view of the

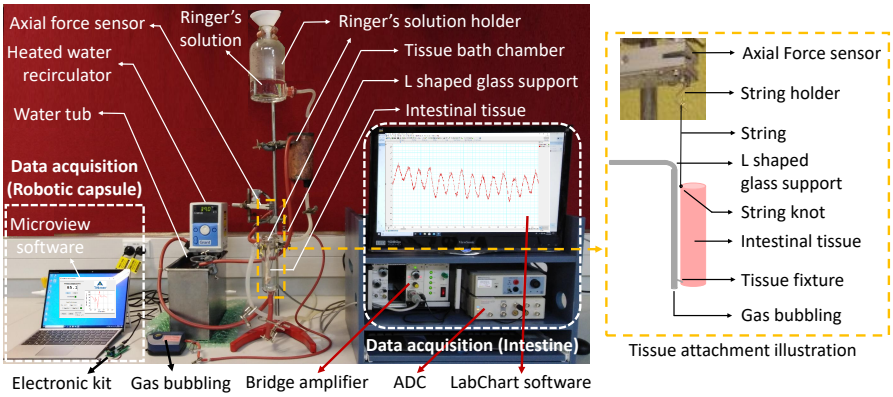

Fig. 5: In-vitro experimental setup for postmortem tissue with data acquisition systems for robotic capsule sensor and in-vitro axial force transducer. The intestinal tissue attachment inside the tissue bath chamber is elaborated seperately on right side.

MicroView software is shown in Fig. 4. Figure 4 shows two separate data acquisition systems, one for measuring the radial peristaltic forces by force sensor on the robotic capsule (left side) and a second for measuring the axial forces by force transducer in an in-vitro experimental setup (right side).

E. In-vitro experimental setup

An in-vitro experimental setup, which kept fresh intestinal tissue alive for up to 6 hours inside a tissue bath chamber, was used to test the capsule prototype. The in-vitro experimental setup is shown in Fig. 5. A test tube shaped tissue bath chamber was filled with ringer's solution, which provided the nutrients to keep the tissue alive [22]. The ringer's solution was kept oxygenated from an L shaped glass tube as shown in Fig. 5. A heated water recirculator was used to continuously circulate the heated water through the tissue bath chamber and the ringer's solution holder, which maintained the temperature of the entire system at the body temperature of lamb i.e. 39 °C. The ringer's solution holder was used to store the ringer's solution at body temperature, which was added to the tissue bath chamber as needed during the experiment.

The in-vitro setup also included a force transducer (MLT0420, ADInstruments Ltd., Dunedin, NZ) which measured the axial forces from the intestinal tissue, while the tissue was placed inside the tissue bath chamber. The force transducer was connected to the PC through a bridge amplifier (Bridge Amps, ADInstruments Ltd., Dunedin, NZ) and ADC (PowerLab 4/16, PowerLab, ADInstruments Ltd., Dunedin, NZ) as shown in Fig. 4 (right side). A dedicated software LabChart (LabChart, ADInstruments Ltd., Dunedin, NZ) was used to record and plot the data of axial forces from the living intestinal tissue.

III. Results

A. Simulations of interaction between peristaltic forces and robotic capsule

The analytical model, developed in this work, is implemented in COMSOL Multiphysics. A 2D-Axisymmetric model was developed and its mesh consisted of 3112 domain elements and 504 boundary elements. The internal domain (inside the intestine) was defined with laminar flow while the robotic capsule was also moving inside this domain. Whereas, the outside domain (intestine wall) was defined with the structural mechanics module and it goes under the deformation in order to exert the peristaltic force on the internal domain. Equation (5) and (7) were used to define the internal domain, while (12) and (19) were used for outside domain. A repetitive peristaltic wave of 7 seconds per cycle was generated across the cross section of an intestine as shown in Fig. 6 (A)-(C).

The progressive wave applied the force on the capsule inside the intestine based on its deformation. The peristaltic force was applied through the boundary load function through a built-in Heaviside function in COMSOL as shown below.

$$F_{A_r} = L_{max} \cdot load(zs, ts) \tag{21}$$

Where, F_{A_r} is the force in radial direction, L_{max} is the maximum load applied from the intestinal wall, $load$ is the Heaviside function, zs and ts are the dimensionless arguments for the Heaviside function.

The literature on intestinal motility suggested that the peristaltic force applied by the intestine is 1.72 g/mm in a longitudinal direction and 2.69 g/mm in a radial direction [23]. This study was considered by various researchers to design their locomotion [5], localization [24], drug delivery [25] and sampling [7] mechanisms. Based on these studies, the overall load for the 100 mm section of small intestine was calculated as 1.7 N in an axial direction and applied through the load function in (21). The peristaltic wave driven by the $load$ function, deforms the intestine and pressurizes the capsule inside the intestine. The interaction between the intestine and the robotic capsule can be seen in Fig. 6 (D)-(F).

The small intestine exerted force in an axial direction, which resulted in the deformation of the intestine in a radial direction. The robotic capsule records the pressure and Fig. 6 (G) shows an example of pressure values for an instant time. The radial pressure applied to the capsule is 2.69 g/mm [23] which will

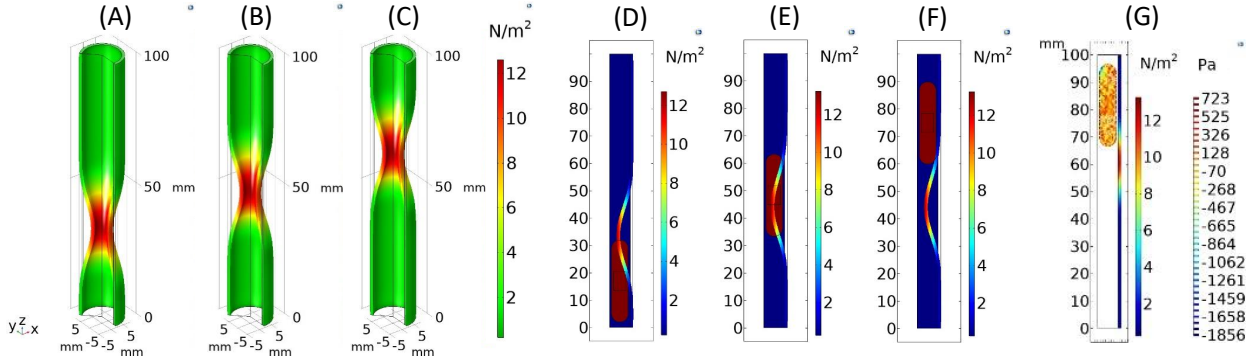

Fig. 6: Simulations performed in COMSOL Multiphysics. (A)-(C) 3D view of repetitive peristaltic wave of 7 seconds/cycle generated across the 100 mm intestine (A) t = 0.5 sec, (B) t = 3.5 sec, (C) t = 6.5 sec. (D)-(F) 2D-axisymmetric view of robotic capsule interacting with the intestine due to the repetitive peristaltic movement (D) t = 0.5 sec, (E) t = 22 sec, (F) t = 32 sec. (G) Pressure experienced by the robotic capsule due to radial contraction of the intestine.

result in 160 mN force which is equivalent to 890 Pa. In these simulations, the robotic capsule received an average pressure of 500 to 1065 Pa which is equivalent to a force of 90 mN to 192 mN.

B. Experimental results

1) Sample preparation for contraction force measurements: Fresh intestines of 5 lambs, dissected 1 hour before the experiments, were obtained on different days. The duodenum region of the small intestine was selected for experiments, as this region produces the highest frequency of contractions as compared to the other regions of the small intestine. The intestine was cut in to 100 mm long tissue samples, and immediately stored in chilled ringer's solution, to maintain its physiological properties until the start of the experiments. During the experiment, one end of the tissue sample was affixed to L shaped glass tube's support and the other end was tied with suture material. This material was stretched and fixed at the string holder of the axial force sensor as shown in Fig. 5. The intestinal tissue samples were kept under tension so the axial movements along the vertical axis were detected by the force transducer of the in-vitro system. The environment inside the tissue bath chamber maintained the postmortem tissue which continued to behave like living intestine, and started to produce peristaltic forces. The radial forces compressed (deformed) the tissue, which resulted in shrinking of the tissue in axial direction, which were recorded by the LabChart software through axial force transducer as shown in Fig. 4 (right side). Simultaneously, the robotic capsule measured the radial peristaltic forces, which were recorded in MicroView software through peristaltic force sensor as shown in Fig. 4 (left side).

2) Calibration of force sensor for capsule prototype: The force sensor inside the capsule prototype was calibrated each time before recording the peristaltic forces from the intestine. The tethered capsule prototype was inserted inside the tissue bath chamber and linearly increasing loads were applied to the capsule and the data was measured through the electronic kit as shown in Fig. 4 (left side). The data was simultaneously recorded and displayed through the MicroView software. Similarly, dynamic loads were also applied and radial force sensor (robotic capsule) readings were recorded. The radial force sensor (robotic capsule) showed less than 2% error for values between 0 and 200 mN, while 6% for readings greater than 250 mN.

3) Axial and peristaltic force results: In-vitro experiments were conducted on 13 intestinal samples from 5 lambs. The range of axial force measurements from each sample, measured by the in-vitro force transducer, is shown in Fig. 7. The Fig. 7 shows the minimum force (bar at bottom) and maximum force (bar at top) applied by each intestinal tissue sample. This allows to visualize the variation among different range of forces, applied within each sample. The (rectangular) box for each intestine sample between the minimum and maximum bars represents the 25^{th} percentile (first quartile) and 75^{th} percentile (third quartile), which is used to determine the effective range of forces applied by each intestinal tissue sample. The Figure shows the axial force behaviour of each sample under the in-vitro system in more detail. The mean forces recorded by the in-vitro axial force transducer are also shown in Table I along with the maximum readings observed by the robotic capsule under each intestine sample. The axial force transducer recorded the accumulated axial force measurements from the intestine, as any radial contraction throughout the 100 mm section of the intestine leads to shrinking of the longitudinal muscles. However, the robotic capsule only detected the radial (peristaltic) forces exerted exactly on the body of the capsule. The peak values of the peristaltic forces detected by the robotic capsule are shown in Table I. Peristaltic forces recorded by the robotic capsule are less than those recorded by the axial force transducer, because the capsule only recorded radial contractions which occurred directly on the face of the sensor. See supplementary video for further details about simulations and experiments.

Mean axial forces measured by the axial force transducer

TABLE I: Peristaltic forces recorded by the force transducer and robotic capsule

Intestine sample	1	2	3	4	5	6	7	8	9	10	11	12	13
Mean axial force measured by in-vitro force transducer (mN)	216	248	229	232	239	310	290	292	346	113	92	183	152
Peak radial force detected by robotic capsule (mN)	140	60	45	180	0	120	30	155	135	90	0	55	65

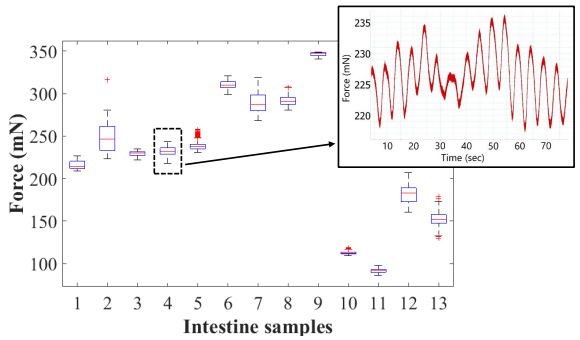

Fig. 7: Variations in axial forces generated by 100 mm intestinal tissue samples measured by the axial force transducer. In each sample, top and bottom bars show the range of axial forces, rectangular box indicates first and third quartile, red line shows the median and red plus sign shows the outliers. Window at top right shows a sample of force measurement data, plotted by LabChart software.

(in-vitro system) and peak peristaltic forces detected by the radial force sensor (robotic capsule), for each trial, are shown in Table I. The average axial force generated by the 13 selected samples was 226 mN while the average of peak peristaltic forces detected by the robotic capsule was 83 mN. Data was recorded for at least 30 minutes from each sample and the average is computed from 10 minutes of stable force output. The mean contraction cycle rate was 9 cycles per minute. The contraction rate used in simulation was 8.57 cycles per minutes and both contractions are in accordance with the relevant studies [5], [23].

IV. Discussion

The analytical modelling of the small intestine provides an understanding of gut motility. The model presented in this work is used as a base to simulate the contractile motion of the small intestine in COMSOL Multiphysics. The assumptions made in modelling and simulation are common to similar motion related studies [18], [26]–[28]. Simulation of a robotic capsule to study the impact of peristaltic forces, is a unique contribution which would help researchers to evaluate the designs of locomotion, localization, biopsy, sampling and drug delivery mechanisms. The simulation of peristaltic waves allows to study the impact of various parameters on a robotic capsule, such as change in radius of the intestine or the robotic capsule. The force analysis on the robotic capsule in this work can be used to study the pressure locations, which would allow installation of the actuation mechanism at the least exposed areas of the capsule, or optimize the tools to increase the efficiency of the device. Although literature [23] suggested that the robotic capsule in this study will experience a pressure of 890 Pa, it didn't specify which part would be exposed less. The simulations results revealed that the robotic capsule, as shown in Fig. 6 (G), will be exposed to a range of pressure levels and this information will determine the best location for installing the tools for performing diagnosis, monitoring and therapeutic functions inside the intestine. Furthermore, this study shows that the robotic capsule would be exposed to a range of pressures from 500 Pa to 1065 Pa, which is equivalent to a load of 90 mN to 192 mN.

In order to verify the findings of our analytical modelling and simulations, a specialized in-vitro setup was utilized in this work, which keeps a fresh intestine alive for up to 6 hours. The in-vitro system recorded the intestinal contractions through an axial force transducer and plotted the axial forces through LabChart software, which ensured that the intestinal tissue was alive. The average force recorded by the axial force transducer in 13 trials of tissue from 5 different lambs was 226 mN and the range of axial force measurements were from 92 mN to 346 mN, which agrees with previous work based on an in-vivo measurement from anaesthetize pig of 215 mN to 328 mN [12]. Another study suggested that a 100 mm section of intestine will generate 198 mN contraction (peristaltic) forces in a radial direction [23], which is also within the range of forces shown in Table I. The axial force transducer was fixed at the stretched ends of the intestinal tissue, hence it recorded the accumulated contraction forces of the entire section in axial direction. However, the robotic capsule only observed the peristaltic (radial) forces applied to its body. The peristaltic forces experienced by the capsule prototype were in the range of 30 mN to 180 mN, whereas the simulations predicted the range from 90 mN to 192 mN, which shows that the efficiency of predicting the peak peristaltic force was 94%. In two trials, the capsule prototype didn't recorded any force and this was either due to wrong calibration or a lack of peristaltic wave contracting at the position of the capsule. The capsule prototype recorded comparatively low peristaltic forces in some of the trials, this could occur when the peristaltic force applied to the capsule body wasn't captured fully by the sensor. Furthermore, we tested the robotic capsule by attaching the force sensor at 2 more locations, as compared to the Fig. 2 (B), one at the front face of the capsule and second on the front edge of the capsule. The capsule with front face sensor resulted in measuring less forces while the capsule on front edge detected almost similar results as the case shown in Fig. 2 (B). The experimental results verified, similar to simulations, that the radial peristaltic forces occur more at the side walls of the robotic capsule as compared to the front side, which could allow us to determine the low pressure points to place our tools for performing diagnosis,

monitoring and therapeutic functions.

The robotic capsule effectively measured the peristaltic forces from the living tissue. Although the robotic capsule under this study was tethered, the force sensor was calibrated before each trial. Calibration ensured that the wire did not affect the results from the force sensor. The force sensor was pasted on the exterior of the capsule, to record the peristaltic forces directly from the intestinal tissue. This method was specially adopted based on the availability of the in-vitro setup in this study, but for in-vivo trials this will not be possible and a proper encapsulation will be required. To further ensure results, in future works the robotic capsule will be tested in-vivo after incorporating a telemetry system.

V. Conclusion

This paper presented a novel method of measuring the impact of peristaltic forces on a robotic capsule in gut tissue. An analytical model was developed and simulated in COMSOL Multiphysics. The model measured the impact of peristaltic forces from the intestine on the robotic capsule. Later, an in-vitro system, which maintained intestinal tissue for up to six hours, was used to directly measure the forces from the intestine. The forces generated by the intestine were recorded by the axial force transducer and robotic capsule sensor simultaneously. The axial force transducer measured all the contraction forces, which can be understood as an accumulated force of the entire tissue. While, the robotic capsule recorded the radial forces acting directly on its body. Both forces were well-aligned with the simulation results and the related literature. These results will be useful in understanding the gut motility and quantification of peristaltic forces. In addition, knowing the impact of peristaltic forces on the capsule will enable the development and optimization of novel robotic capsules for locomotion, localization, biopsy, sampling and drug delivery purposes.

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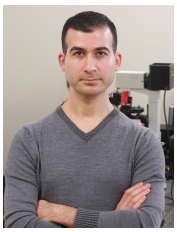

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3.6 Calibration of force sensor

The calibration of flexiforce sensor was carried out using both linear and dynamic calibration methods as explained below.

3.6.1 Linear calibration

Five-point linear calibration was achieved by a default program of MicroView software (FlexiForce MicroView, Tekscan Inc., Boston, MA, USA) that is specially designed to calibrate the force sensors used in this study. The robotic capsule was manually placed inside the 100 mm long post-mortem intestinal tissue. First, linearly increasing known loads (98mN, 196mN, 294mN, 392mN and 484mN) were applied on the capsule and the readings were obtained by the data acquisition system, as shown in Fig. 3.8. Each value is measured three times to ensure that the reading is correct. On the application of external load, the force sensor changes its resistance, which was converted to digital value by the ADC converter in Arduino nano board. The Arduino nano board (program) calibrated the resistance to the known load and MicroView software recorded and displayed the force readings. The temperature during the entire calibration and testing process was maintained at 39 °C and the drive voltage used was 2.2 V.

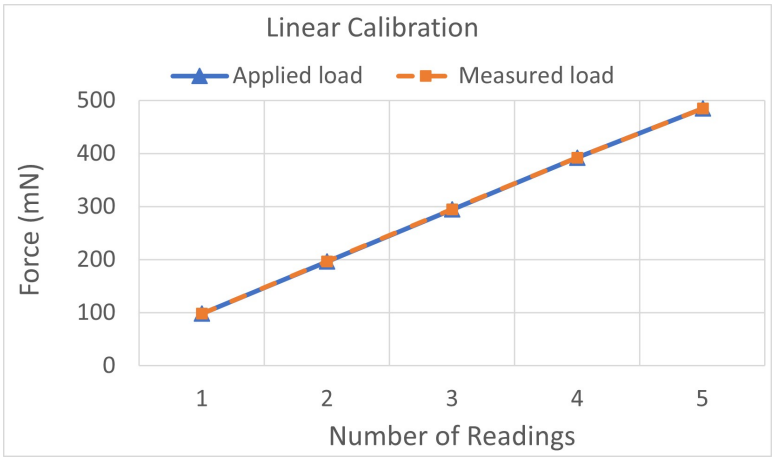

Figure 3.8: Linear calibration of the force sensor.

3.6.2 Dynamic calibration

After linear calibration, dynamic calibration was also performed as the peristaltic movements are dynamic in nature. Both increasing and decreasing known loads were applied to the robotic capsule and the readings were simultaneously recorded by the data acquisition system and displyed in MicroView software. Like linear calibration, each value was recorded three times to ensure that the sensor and known weights are properly

aligned. The graph in Fig. 3.9 show the dynamic calibration to specify the error after calibration.

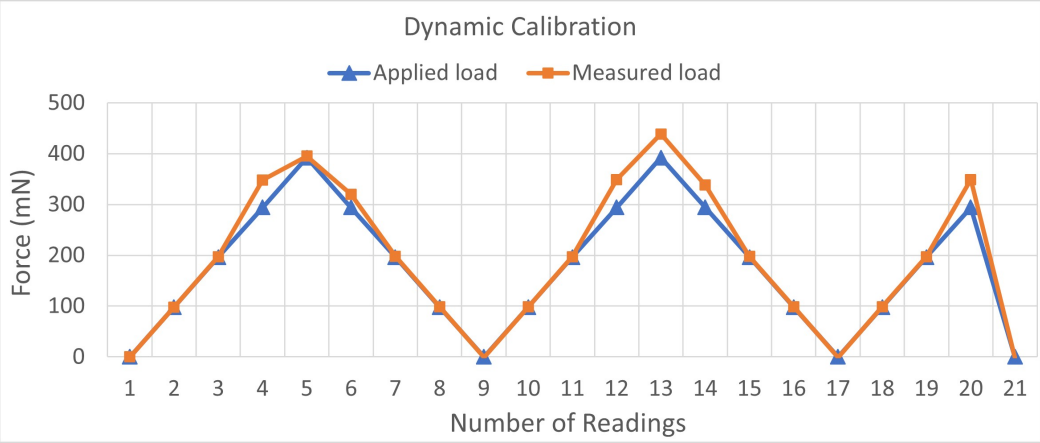

Figure 3.9: Dynamic calibration of the force sensor.

3.7 Additional details

3.7.1 Limitation of force sensor

The Flexiforce sensor (ESS301) used in this study is sluggish in nature and require around 7 seconds of recovery time. This mean that the measurement of peristaltic forces should allow at least 7 seconds to let the sensor recover to its original resistance, before measuring the next reading. Fortunately, the peristaltic cycle is also around 7 seconds that allowed enough recovery time to the sensor during the experiments. Additionally, the peristaltic forces applied only on top of the sensor are recorded which allowed further time to the sensor for its recovery.

3.7.2 Peristaltic and radial forces

The published paper includes the terms ”peristaltic forces” and ”radial forces” which should be considered same in Chapter 3.

3.7.3 Table I: Additonal results

The Table I in the published paper include mean axial forces while the robotic capsule shows peak radial forces. For design perspective, peak forces are important, therefore peak axial forces are also added in Table 3.2 to provide further information on the experiments carried out during the in-vitro trials.

Table 3.2: Peak forces recorded by the force transducer and robotic capsule

Intestine sample	1	2	3	4	5	6	7	8	9	10	11	12	13
MAF (mN)	216	248	229	232	239	310	290	292	346	113	92	183	152
PAF (mN)	227	316	235	244	258	321	319	308	349	119	98	207	179
PRF (mN)	140	60	45	180	0	120	30	155	135	90	0	55	65

*MAF: Mean axial force measured by in-vitro force transducer, PAF: Peak axial force measured by in-vitro force transducer, PRF: Peak radial force detected by robotic capsule.

Chapter 4

Design of Sampling Mechanism for Robotic Capsule to Collect Gut Microbiota using SMA Springs Actuator

In this chapter a unique sampling mechanism is devised to gently scrape the content from small intestinal wall (mucosa layer). This design is thoroughly tested under *ex-vivo* small intestines to optimise the capability of sample collection mechanism without compromising on the safety of the GI tract of animal or human. The learning from previous chapter (i.e., chapter 3) were utilised to develop an actuation mechanism based on a unique combination of concentric SMA springs to act as an axial actuator. The developed actuator occupies a small space and produces sufficient output force to operate the sampling mechanism for overcoming the intestinal peristaltic forces. A minimally invasive robotic capsule was tested *ex-vivo* on the animal small intestine, and it captured sufficient quantity for microbiota assessment. The laboratory testing of collected sample discover an amino acid signature indicative of microbiota, mucus and digesta, which provided a proof of concept for the proposed design.

This chapter contains content from the following article.

- Rehan, M., Al-Bahadly, I., Thomas, D. G., & Avci, E. (2020). Capsule robot for gut microbiota sampling using shape memory alloy spring. The International Journal of Medical Robotics and Computer Assisted Surgery, 16(5), 1-14. DOI: `https://doi.org/10.1002/rcs.2140`.

Received: 3 April 2020 | Revised: 18 June 2020 | Accepted: 2 July 2020

DOI: 10.1002/rcs.2140

ORIGINAL ARTICLE

The International Journal of Medical Robotics and Computer Assisted Surgery WILEY

Capsule robot for gut microbiota sampling using shape memory alloy spring

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Funding information
Massey University Research Fund (MURF) 2019; Higher Education Commision, Pakistan, Grant/Award Number: 5-1/HRD/UESTPI (Batch-V)/2421/2018/HEC

Abstract

Background: Human gut microbiota can provide lifelong health information and even influence mood and behaviour. We currently lack the tools to obtain a microbial sample, directly from the small intestine, without contamination.

Methods: Shape memory alloy springs are used in concentric configuration to develop an axial actuator. A novel design of sampling mechanism is fabricated for collecting the sample from the gut. Storage chamber (500 µl) is used to protect the sample from downstream contamination.

Results: The developed actuator occupies a small space (5 × Ø5.75 mm) and produces sufficient output force (1.75 N) to operate the sampling mechanism. A non-invasive capsule robot was tested ex vivo on the animal intestine, and it captured an average of 134 µl content which was sufficient for microbiome assessment.

Conclusions: Laboratory testing revealed that the collected sample had an amino acid signature indicative of microbiota, mucus and digesta, which provided a proof of concept for the proposed design.

KEYWORDS
capsule robot, GI tract, gut microbiota, intestine, micro robot

1 | INTRODUCTION

A significant population of microorganisms (bacteria, archaea and fungi) live inside the gastrointestinal (GI) tract, play a major role in fibre fermentation and the synthesis of short-chain fatty acids, and some nutrients (e.g., vitamins), and are collectively known as microbiota.[1] Human intestinal microbiota consist of 10^{13}–10^{14} microorganisms which can act as biomarkers.[2] The microbiota, present in the GI tract, contain lifelong information on the health of an individual and can assist in early diagnosis of diseases such as cancer, diabetes and obesity.[3–5] Several researchers believe that analysis of microbiota could be helpful in predicting obesity, diabetes and inflammatory bowel disease.[3–6] Microbiota can also help to study the relationship or interaction between nutrition and human health.[3]

Given the significance of gut microbiota, it is critical to explore its ecology. Microbiota colonise the mucous layer which covers the columnar epithelium of the GI tract and the digesta within the intestinal lumen.[7] The population of microorganisms (microbiota) inside the intestine has been studied using various methods. The most common samples used as a proxy for intestinal microbiota are fecal samples; however, they do not categorise spatial inhabitants, and it is not possible to localise them. Similarly, they lack temporal information and cannot reciprocate real-time gut environment as they are examined after travelling through the entire GI tract, which exposes the sample to contamination.[8] Some efforts have been made in obtaining samples from the human gut with biopsy; however, it is a tethered method which largely restricts its use to the large intestine.[9,10] Furthermore, tethered methods involve a high risk of gut perforation, bleeding and require sedation, and the procedure is also invasive and unpleasant for a patient in terms of comfort.[10] Most importantly, the sample obtained by biopsy is a tissue sample, and it cannot fully capture microbial content from the intestine.

Based on the limitations of fecal sampling and biopsies using tethered methods, several researchers have begun to use non-invasive

capsule robots to navigate the GI tract. Capsule robots use natural peristaltic movements to move along the GI tract, and their small size enables them to pass through the GI tract without getting stuck. The capsule robots have been used for various applications such as a replacement for traditional endoscopy,[11] for performing therapeutic functions via drug delivery[12] and to obtain biopsy samples[9,10,13]; however, sampling the luminal content remains a challenge.[9] Gu et al.[14] and Sprenkels[15] proposed the concept of sealed chambers, which can be passively dissolved by a chemical reaction at the target site to allow the surrounding fluid to enter the apparatus. Stoltz[16] proposed a temperature-sensitive shape memory alloy to open and close the inlet of the chamber by heating through inductive coils. Tang et al. patented the idea of using an active sampling mechanism (e.g., motor) to capture the content.[17] Similarly, a capsule robot designed to perform drug delivery with simultaneous sampling was described by Cui et al.[18] Du et al. proposed a capsule robot with a sealed vacuum chamber whose inlet was sealed with wax. An external magnet is then used to trigger the actuation of nichrome wire, which melts the wax, and the surrounding fluid enters the chamber due to vacuum suction.[19] A bi-stable mechanism to seal the capsule reservoir using a sponge-based passive actuator is presented by Salem et al.[20] The main focus in these designs was to capture the surrounding digesta; however, the microbiota are also present on the mucosa layer. Moreover, resealing the inlet of the storage chamber to avoid downstream contamination was not considered by most of these designs.

Considering the importance of sampling the microbiota from the intestine using non-invasive methods, we present a shape memory alloy (SMA) spring-based capsule robot that can capture digesta, mucosal cells and mucous content from the small intestine. The small intestine is the region of interest in this study as it is the major site of digestion and absorption and is not fully explored currently due to the limitations of relevant sampling tools. A device which samples tissue and digesta in the small intestine will also help in studying digestion directly as most of the nutritional studies to date are based on indirect fecal sampling.[21] The main contribution of this study is the development of a sampling mechanism, which can collect microbial content from the small intestine. The collected sample will assist in understanding the relationship of microbiota with human health at the site of digestion. The other major contribution is the development of an actuation mechanism which occupies a small space and produces sufficient output force to operate the sampling mechanism inside the small intestine. Detailed analysis of SMA springs actuator is presented in this work, which has a potential to help researchers for developing small-scale (millimetre size) actuators.

2 | MATERIALS AND METHODS

2.1 | Capsule design and fabrication

The capsule robot enters the GI tract through the mouth. It is swallowed and passes through the oesophagus, stomach, small intestine and large intestine and is finally excreted from the body, along with feces. A capsule robot, which can pass through the entire GI tract without getting stuck at any location, is desired. Capsule robots of varying sizes have been developed, and some of them have been launched commercially for different operations.[9] In commercially available capsules, the maximum length is 35 mm (e.g., 35 mm × Ø10 mm, InteliSite capsule; Innovative Devices),[22] and the maximum diameter is 13 mm (e.g., 28.4 mm × Ø13 mm, Omom capsule; Jinshan group).[23] The capsule size will be based on these capsule dimensions, so that our capsule will safely pass through the entire GI tract of an adult human.

The computer-aided design (CAD) based concept design of the capsule robot in this study is shown in Figure 1. The capsule is mainly composed of an actuation mechanism and a sampling mechanism. The actuation mechanism uses SMA springs in a concentric configuration as an axial actuator, a printed circuit board and a button cell battery. The sampling mechanism includes a sampler and a storage chamber. The overall length of the capsule is 30 mm, and its diameter is 12 mm.

The capsule prototype (i.e., capsule encapsulation or outer shell, sampler and storage chamber) was fabricated with a Digital Light Processing (DLP) technique using a FlashForge Hunter three-dimensional (3D) printer. DLP uses a liquid resin, similar to a stereolithography apparatus, to print 3D objects using UV light. In the DLP technique, the object is vertically manufactured layer by layer, and the resolution of the printer is 0.025 mm. The main objective of the capsule is to collect microbiota from the small intestine; therefore, more than half of the volume of the capsule is dedicated to the sampling mechanism. The sampling mechanism consists of two parts; a sampler and a storage chamber. The sampler is a tool designed to gently scratch the microbiota from the mucosa layer of the small intestine (collecting digesta adhering to this layer as well), and then slide the captured content into the storage chamber, as shown in Figure 2. The overall length of the sampler is 9 mm (effective length 7 mm), and the outer dimensions of storage chamber are 10 mm × Ø9.5 mm, and it has a capacity of almost 500 µl. The sampler is fixed at the gate of the storage chamber with pivots, so it can rotate to move inside and outside of the capsule shell, as shown in Figure 3. The sampler is required to rotate θ degrees to collect the sample from the intestine, and the actuation mechanism provides the required rotation. The SMA spring moves linearly with deflection δ to ensure θ degrees rotation of the sampler as shown in Figure 3 and expressed by Equation (1).

$$\text{Sin}\,\theta = \frac{\delta}{L_s} \tag{1}$$

where θ is the angle of rotation of the sampler, δ is the deflection of the spring and L_s is the length of the sampler from the pivot to the joint.

The dimensions of each part were selected after a rigorous process of optimisation, and their finalised dimensions are shown in Table 1. The fabricated capsule with its parts is shown in Figure 4.

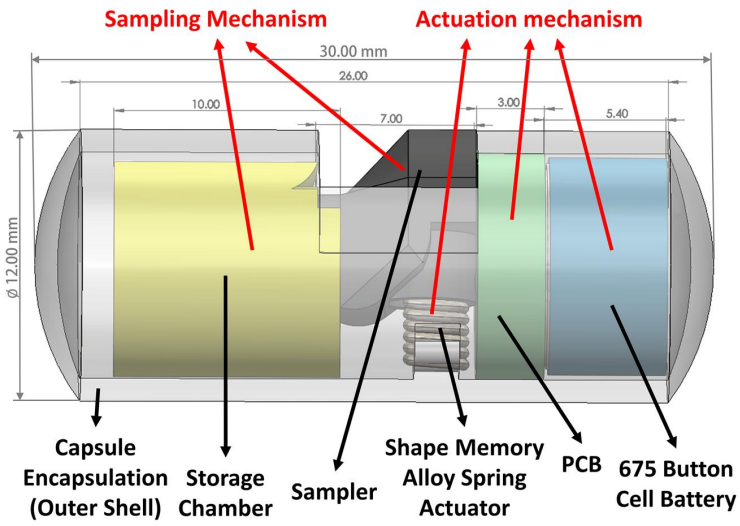

FIGURE 1 CAD-based concept design of capsule robot. PCB, printed circuit board

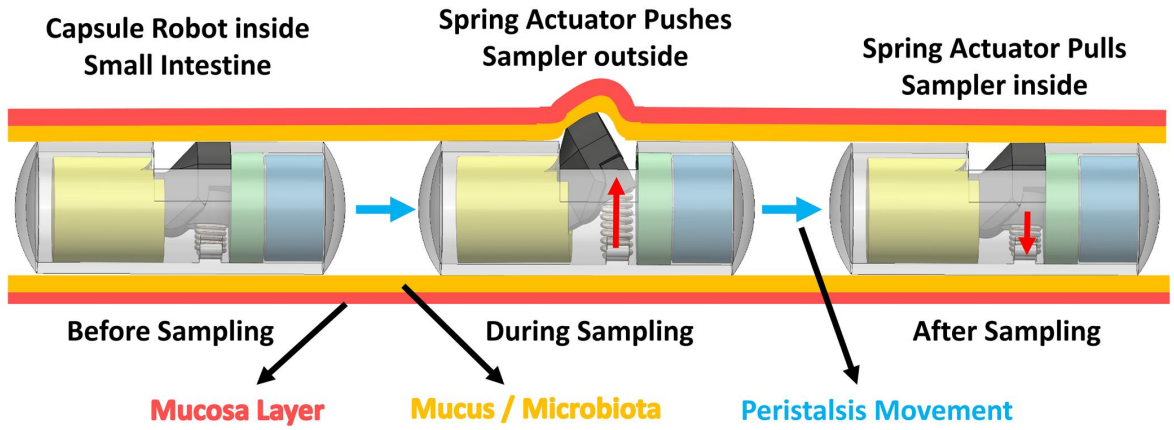

FIGURE 2 Sampling mechanism of capsule robot

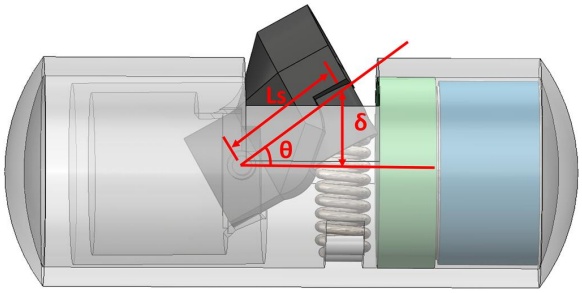

FIGURE 3 Mathematical relation between sampler and actuator

2.2 | Modelling of intestinal forces

The capsule robot enters the gut through mouth and is propelled along the gut naturally using peristaltic forces. Once it reaches the small intestine, it is designed to collect a sample by pushing the sampler outside while peristaltic motion occurs. The push force produced by the actuator (F_A) would be resisted by the peristaltic force (F_p) and frictional force (f) as shown in Equation (2). The frictional forces inside the intestine are threefold which are shown in Equation (3); coulomb friction (f_c) is the stress applied by the intestinal wall, marginal resistance (f_m) is the hindrance caused by the intestinal deformation and viscous resistance (f_v) is the interference due to the mucus and digesta above the epithelium layer.[24–26] The

TABLE 1 Components of capsule robot and their dimensions

Component	Dimensions (mm)
Capsule shell	30 × Ø12
Storage chamber	10 × Ø9.5
Sampler	9 × 10 × 8
SMA spring 1 (S1)	5 × Ø3.4
SMA spring 2 (S2)	5 × Ø5.75

Abbreviation: SMA, shape memory alloy.

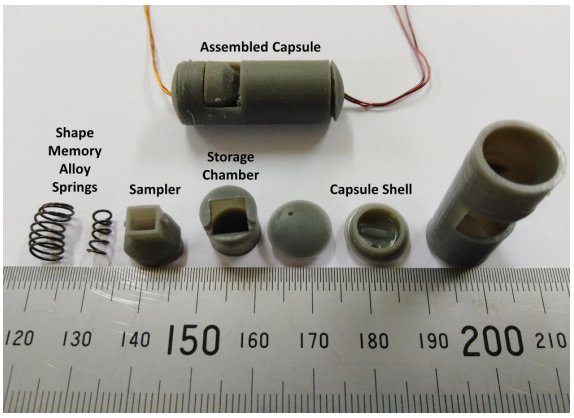

FIGURE 4 Capsule robot with 3D printed parts and shape memory alloy springs

forces acting on the sampler while moving against the intestinal wall are shown in Figure 5.

$$F_o = F_A - F_p - f \quad (2)$$

$$f = f_c + f_m + f_v \quad (3)$$

The coulomb friction comprises static and dynamic friction, as shown in Equations (4) and (5), respectively. The peristaltic forces act in the form of waves and appear between 9.4 and 11 times per minute.[27] When the peristaltic force is absent, then the capsule would remain idle and static frictional force would resist the sampler movement as expressed in Equation (4). When the peristaltic force acts on the capsule, it propels the capsule and dynamic frictional force restricts the sampler movement as shown in Equation (5).

$$f_{C_1} = \mu_s F_{top} \quad (4)$$

$$f_{C_2} = \mu_d (F_{top} + F_{centre}) \quad (5)$$

where f_{C_1} and f_{C_2} are the static and dynamic coulomb frictional forces, μ_s and μ_d are the static and dynamic frictional coefficients and F_{top} and F_{centre} are the intestinal forces applied from the regions as shown in Figure 5.

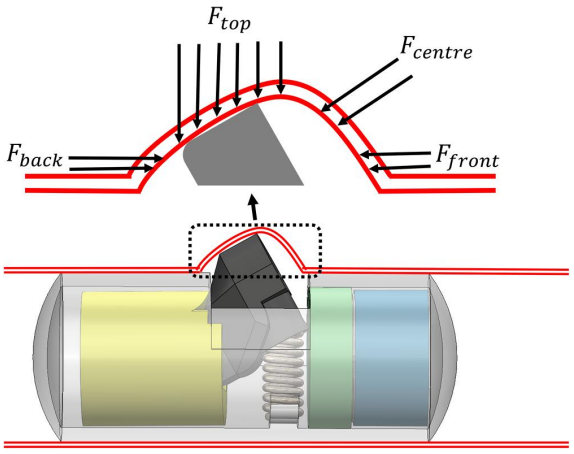

FIGURE 5 Intestinal forces on the sampler

Marginal friction restricts the sampler movement due to deformation of the intestine from the front, back and centre areas, as shown in Equation (6). Viscous resistance depends on the speed of the sampler and is expressed in Equation (7).

$$f_m = F_{front} + F_{centre} + F_{back} \quad (6)$$

$$f_v = \delta(N_v)v = \delta(F_{top} + F_{centre})v \quad (7)$$

where, v is the velocity of the sampler, δ is the coefficient of the viscosity and N_v is the radial stress exerted on the sampler.

The peristaltic forces are not steady forces and appear at intervals; hence, the sampler will be exposed to ripple-based forces from the intestinal wall. Therefore, the force required for opening the sampler as expressed in Equation (2) observes two situations. When peristaltic motion occurs, the required opening force is expressed in Equation (8), while in the absence of peristaltic forces the expression is shown in Equation (9).

$$F_o = F_A - F_p - (\mu_d(F_{top} + F_{centre}) + (F_{front} + F_{centre} + F_{back}) + \delta(F_{top} + F_{centre})v) \quad (8)$$

$$F_o = F_A - (\mu_s F_{top} + (F_{front} + F_{centre} + F_{back}) + \delta(F_{top} + F_{centre})v) \quad (9)$$

An actuator which can operate in the presence of peristaltic forces would also work in the absence of peristaltic motion. Therefore, an actuator which can produce a force greater than the F_p and f, as shown in Equation (8), is desired. To estimate the force required by the actuator, various studies conducted on a similar area are considered.[28–30] A study using a capsule robot suggests that the axial peristaltic force exerted on the capsule is 450 mN and its radial component is 700 mN.[28] Another study on the wave phenomena of the intestine specifies that the force applied in the axial and radial directions is 17.2 and 26.9 g/mm, respectively[29]; therefore, the capsule robot in this study would encounter a force of 516 mN in axial direction and 807 mN in the radial direction. The sampler applies the force against the radial peristaltic force whilst performing

sampling, so it needs to overcome the force in the radial direction which is approximately 800 mN. Other research on the destructive force of the small intestine reveals that the force exerted by the small intestine of humans is between 0.8 and 1.2 N.[30] Capsules with different breakage forces (e.g., 0.8, 1, 1.2 and 1.6 N) were passed through the gut, and the results were determined based on the breakage of capsules in the intestine. Since, these capsules were passed through the stomach before reaching the intestine, the actual destructive force was likely to be lowered.[30] Therefore, the actual force applied by the small intestine was less than 1 N. Based on these studies, we can estimate that the peristaltic force applied by the small intestine is less than 1 N.

Similarly, a study on frictional forces determined that the force exerted on the capsule robot range between 0.1 and 0.4 N depending on diameter of capsule and intestine.[24] This study revealed that the average frictional force on the capsule is between 100 and 200 mN.[24] Another study specified that the total frictional force imposed on the capsule is 56.9 mN.[25] A detailed study of frictional forces imposed on a capsule robot determined that the viscous friction inside the small intestine is 0.74 mN, the coefficient of friction varied between 0.008 and 0.018 throughout the small intestine and the cumulative frictional force fluctuated between 22.68 and 35.09 mN inside the small intestine.[26] Therefore, based on the above studies,[24–26] we can estimate that the frictional resistance between the small intestine and capsule robot is less than 200 mN.

Based on these considerations, we focused on developing an actuator which provided at least 1 N force, so that it could overcome both peristaltic forces and frictional forces. This assumption is a starting point to develop a miniaturised actuator for the capsule robot, which can be tested on intestinal tissue for practical realisation.

2.3 | Actuation mechanism

The desired actuator is required to occupy a limited space, consume low power and deliver enough force to operate the sampler. A major portion of the internal space of the capsule robot was dedicated to the sampling mechanism; hence, less room was left for the actuation mechanism. Therefore, an actuator which can fit inside the capsule and occupy a small space is desired. The capsule robot has size restrictions, but it can carry a button cell battery which has a limited supply of power in the limited space. Lastly, the actuation mechanism should apply enough force to push and pull the sampler to allow collection of the sample at the desired location.

Several actuators were considered for our system, based on the actuator specifications, as shown in Table 2. The criteria for actuator selection was as follows:

- The length should be a maximum of 10 mm
- The output force should be at least 1 N
- It should be driven by a button cell battery

TABLE 2 Comparison of selected actuators

Actuator	Dimensions (mm)	Output force	Power requirement
DC motor	8.1 × Ø3.9	Low	Low
Stepper motor	7.9 × Ø4.7	Low	Low
Solenoid	10.4 × 7 × 5	Medium	High
SMA spring	5 × Ø5.75	Medium	High

Abbreviation: DC, direct current; SMA; shape memory alloy.

The overall size constraints of the capsule robot limit the use of several micro-electro-mechanical system (MEMS)-based actuators. The smallest possible-sized direct current (DC) motor (LD320802002-B1; Leader Microelectronics) and stepper motor (6H16; Sanyo Denki) were selected for analysis. The motors were small and had the lowest power requirements compared to other options; however, their torque was low as compared to SMA springs. A solenoid actuator (C21; EndlessParts) was slightly oversized, and the linear force generated was low as compared to SMA springs. SMA springs (5-NiTi-0.5-4.75; Kellogg's Research Labs) offer flexibility in their size, and they can be as small as 3 mm × Ø1 mm at the expense of comparatively low output force. SMA springs require comparatively higher power but they can be activated by joule heating, that is, the thermal energy generated by the flow of current through the spring wire, which can be supplied by the button cell battery. Hence, SMA springs were selected based on the optimal choice considering the examined actuators as detailed in Table 2.

The SMA material is a mixture of nickel and titanium, commonly known as Nitinol. This material can learn any shape and transform to its learned shape when heated. SMA springs trigger on temperature difference, and we selected 45°C for actuation in our system. The normal human body temperature is 37°C, so 45°C seems a reasonable value for the spring actuation.[31] Choosing a higher temperature than this may be dangerous as it might damage the GI tract. The temperature of the SMA spring can be raised by applying the current through the spring, which is known as joule heating. The flow of current increases the internal temperature of the SMA spring, hence actuates the SMA spring, that is, the spring moves to its learned shape.

In our capsule robot design, we need two-way actuation, a push force for moving the sampler outside the capsule shell and a pull force to bring the sampler back into its original position inside the capsule shell. However, SMA springs which have a two-way actuation feature require a large temperature gap between each actuation. If the spring extension (push effect) happens at 45°C, then spring compression (the pull effect) should trigger at not less than 75°C, which might damage the intestine.[32] Therefore, we have chosen two SMA springs with different diameters with both designed to actuate at 45°C; however, they are triggered at a different time to perform the sampling. The push effect is generated by SMA spring (S1) while the pull effect is produced by SMA spring (S2) as shown in Figure 6.

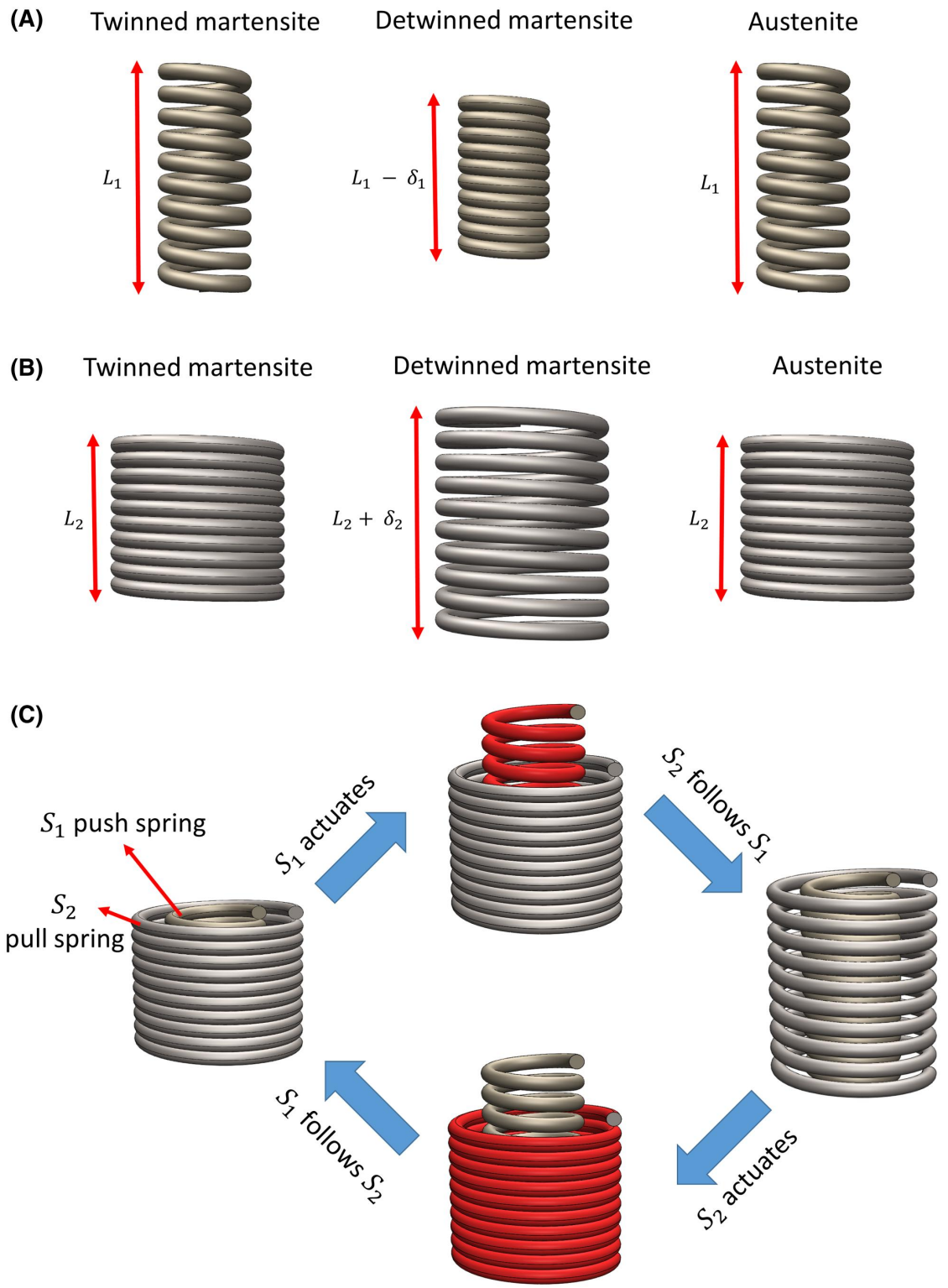

FIGURE 6 Shape memory alloy spring actuator. (A) Push spring S1. (B) Pull spring S2. (C) Antagonistic con□guration with spring S1 and S2

SMA springs exist in a twinned martensite state at room temperature, which is their learned state. Springs are reshaped by squeezing or expanding their lengths (detwinned martensite) as shown in Figures 6A and 6B. Once they are heated and they exceed their threshold temperature, they move to austenite state which is their learned shape, that is, initial state as of twinned martensite. In this actuator design, two separate springs are used together in the antagonistic configuration as shown in Figure 6C. The push spring is squeezed by δ before installation in the capsule and once it moves above its threshold temperature by joule heating, it expands as shown in Figure 6A. Hence, it pushes the sampler outside the capsule shell as shown in Figure 2. The pull spring, in contrast, shrinks when it is heated, as shown in Figure 6B, which will pull the sampler back inside the capsule shell, as shown in Figure 2. Hence, push (S1) and pull (S2) springs attached in an antagonistic configuration allow the sampler to move outside and back inside the capsule to perform sampling as shown in Figure 2 and Figure 6C. The length of the spring 'L' is determined using Equation (10). The spring constant or spring stiffness is related to the amount of deflection as shown in Equation (11).

$$L = n\,d \tag{10}$$

$$k = \frac{F}{\delta} = \frac{G\,d^4}{8D^3 n} \tag{11}$$

where k is the spring constant, F is the load, δ is the deflection by the spring, G is the modulus of the rigidity, d is the diameter of the wire, D is the mean diameter of the coil and n is the number of coils in the spring.

The spring actuator is used in antagonistic configuration with two SMA springs as shown in Figure 6C. The push effect is generated by deflection of S1 in austenite state 'δ_{1A}' while the secondary spring S2 resists the movement in martensite state 'δ_{1M}', and the amount of force 'F_1' is expressed by Equation (12). The pull effect is produced by compression of S2 in austenite state 'δ_{2A}' with S1 acting as a secondary spring which resists the movement in martensite state 'δ_{2M}' and the amount of force shown in Equation (13).

$$F_1 = F_{1A} - F_{2M} = \delta_{1A}\frac{G_A\,d_1^5}{8\,D_1^3 L_{1A}} - \delta_{2M}\frac{G_M\,d_2^5}{8\,D_2^3 L_{2M}} \tag{12}$$

$$F_2 = F_{2A} - F_{1M} = \delta_{2A}\frac{G_A\,d_2^5}{8D_2^3 L_{2A}} - \delta_{1M}\frac{G_M\,d_1^5}{8\,D_1^3 L_{1M}} \tag{13}$$

where F_{1A} is the force produced by S1 in austenite state, F_{2M} is the resistive force offered by S2 in martensite state, F_{2A} is the force produced by S2 in austenite state, F_{1M} is the resistive force offered by S1 in martensite state, G_A is the modulus of rigidity in austenite state and G_M is the modulus of rigidity in martensite state. L_{1A} and L_{2A} are the lengths of the spring S1 and S2 in austenite state, respectively. L_{1M} and L_{2M} are the lengths of the spring S1 and S2 in martensite state, respectively.

The diameter of wire is a key parameter in Equations (12) and (13) for the spring design. We selected four different wire diameters (i.e., 0.25, 0.5, 0.75 and 1 mm) for initial testing having part numbers 5-NiTi-0.25-1.6, 5-NiTi-0.5-2.4, 5-NiTi-0.75-1.6 and 5-NiTi-1-0.5. The results of testing the SMA springs with different wire diameters are shown in Figure 7. The threshold value for triggering the actuation of the SMA spring increases with increasing wire diameter. This occurs because the wire diameter increases the overall volume; therefore, a higher current is required to raise the temperature via joule heating. In these trials, the force is measured, as detailed in the next section, by increasing the amount of current. Preliminary criteria indicated that the potential actuator should apply at least 1 N force. The SMA wire with 0.25 mm diameter did not provide sufficient force, particularly at lower currents. However, as the wire diameter increased, the amount of output force also increased, albeit at the expense of higher currents. The actuator is driven by a button cell battery so the amount of current available for actuation is limited to 650 mA. Therefore, the SMA springs with wire diameter 0.75 mm and 1 mm cannot be used in the capsule robot as they require a current greater than a button battery can provide. Therefore, based on optimum design parameters, a 0.5 mm wire diameter was selected for the desired actuator. An SMA spring with 0.5 mm wire diameter can be actuated by Zinc-air 675 button cell battery and produce more than 1 N force as shown in Figure 7B.

Another important parameter to consider in designing the SMA spring is the identification of optimum output force.[32] The SMA wire increases its internal temperature by joule heating which is effective within a specified range of applied current, with smaller or larger current than the optimum range would result in reduced output force.[32] The maximum output force using the 0.5 mm wire diameter is attained at 800 mA, and a further increase in current would result in similar or lesser output force (see Figure 7B).

A key parameter in spring designing is the coil diameter. A wire diameter of 0.5 mm was selected based on results shown in Figure 7, and it had a G_A and G_M of 61.9 and 7.5 GPa, respectively, which are determined by experiments. Initial criteria that the actuator should apply at least 1 N push force indicated that the diameter of the push spring S1 should be at most 4.1 mm as determined by Equation (12). Based on this, a 2.9 mm diameter was selected for S1 which could produce 2.83 N of force for the push effect. The pull spring is used in a concentric configuration with the push spring, and a 5.25 mm mean diameter was selected for S2 which allowed a 1.35 mm gap for insulation between the two springs. The pull force produced by S2 was 668 mN as calculated by Equation (13).

The SMA springs used in the actuator were Nitinol springs part number 5-NiTi-0.5-2.4 and 5-NiTi-0.5-4.75, and their parameters are shown in Table 3. The major constraints for the actuator in the capsule robot, as mentioned earlier, are size and power. The resulting force for these springs at different levels of current was tested and is shown in the next section.

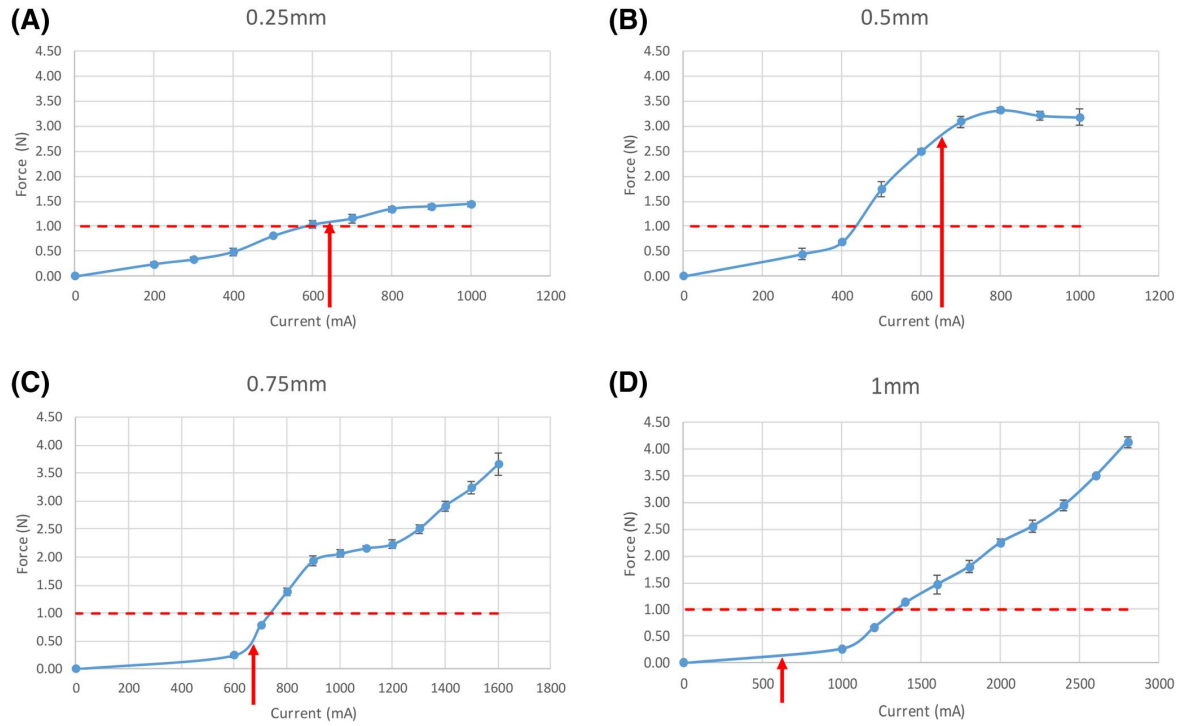

FIGURE 7 Force analysis for shape memory alloy springs with different wire diameters. Red arrow on *x*-axis indicates the maximum supply of current by a button cell battery (i.e., 650 mA). Red line on *y*-axis indicates the minimum required force to overcome intestinal forces (i.e., 1 N)

TABLE 3 Parameters of shape memory alloy springs

Type	Force direction	Length (mm)	Diameter, D (mm)	Wire diameter, d (mm)
S1	Push	5	2.9	0.5
S2	Pull	5	5.25	0.5

2.4 | Power and force analysis for SMA springs

The experimental setup for testing the springs is shown in Figure 8. The digital spring balance (Portable Electronic Scale 40 kg/10 g) is hung vertically, and the SMA spring is attached to it. Variable DC power supply (Rohde & Schwarz Programmable Power Supply model HMP2020) is used to supply the current to the spring. The current generates heat, and the spring applies the force to acquire its learned shape. The spring balance measures the force applied by the spring. The experiments were carried out on a vibration isolation table (Nexus ThorLabs) to avoid any external influence.

Two 5-mm-long SMA springs, S1 and S2, were tested. The digital spring balance displayed the amount of weight which was applied by the SMA spring. As the spring was aligned vertically, the force was calculated by using Equation (14).

$$F = w\,g \tag{14}$$

where F is the calculated force applied by the spring, w is the weight measured by the spring balance and g is the gravitational force, that is, 9.807m/s^2.

The force of springs S1 and S2 was measured by increasing the amount of current, and the experimental results are shown in Figure 9. The spring S1 exerted its optimum force at 800 mA and a further increase in current, resulted in a lower output force as shown in Figure 9. S1 demonstrated a higher output force as compared to S2, as it had greater stiffness. The time taken by each spring to produce the respective force was also measured, and it is shown in Figure 10. S1 took a shorter time in actuation as compared to S2, which was due to the difference in the wire length of each spring. The wire length 'L_w' can be calculated by Equation (15).

$$L_w = \pi\, n\, D = \frac{\pi\, L\, D}{d} \tag{15}$$

The lengths of S1 and S2 are 128 and 165 mm, respectively, which are calculated using Equation (15) and parameters in Table 3. The experimental results in Figure 10 showed that the time in actuation of both springs was slightly different. The difference between the mathematical and experimental results was due to systematic errors in the manufacturing or experimentation of the spring. While taking the measurements, ideally, the power supply and spring

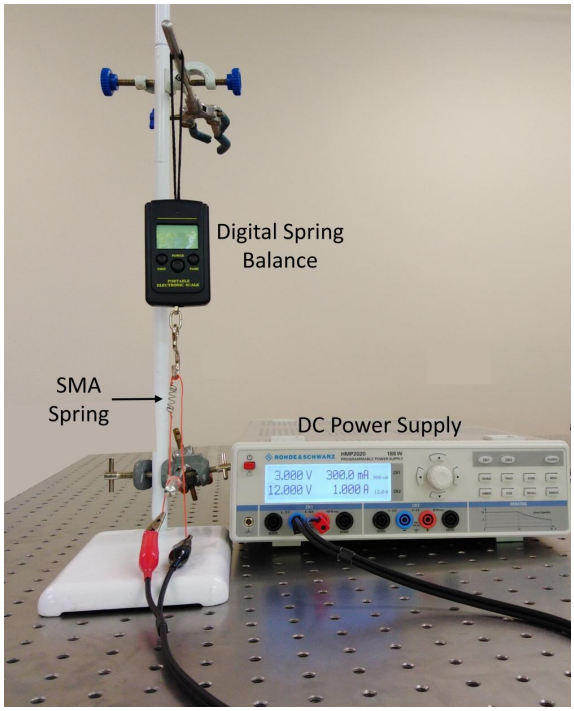

FIGURE 8 Experimental setup for force measurement

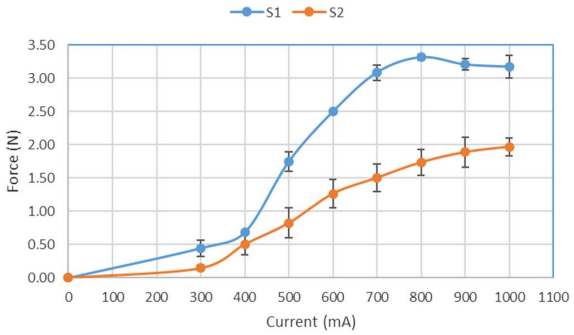

FIGURE 9 Force measurement of shape memory alloy springs

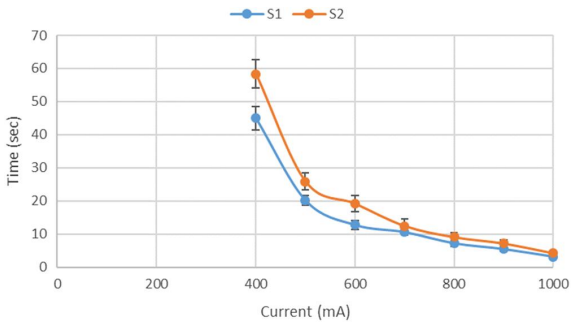

FIGURE 10 Actuation time for shape memory alloy springs

balance are required to be connected at the edges of each spring. However, in the actual experiment, the measuring wire was fixed between the spring edge and first loop. Hence, the length of each spring in each trial was not exactly the same, which lead to some systematic error.

Figure 9 shows that the amount of force increased with the amount of current; however, the capsule robot has a limited supply of current. The maximum current supplied by the zinc-air 675 button cell battery is 650 mA. Considering this constraint, we restricted the actuator to 500 mA current which produced a 1.75 N force for S1 and a 0.83 N force for S2. S1 exerts more force than S2; hence, it is used for pushing the sampler outside the capsule against the peristaltic force. The actuation time at 500 mA for S1 was 20 s and for S2, it was 26 s, which can be a limiting factor in instantaneous actuation. The actuation time can be reduced by designing a current booster circuit to apply high current; however, at this stage, it is not required.

3 | RESULTS

3.1 | Simulations for sampling mechanism

The entire capsule is designed and packaged inside a capsule shell, as shown in Figure 1, on SolidWorks Education Edition 2018. Simulations for the sampling and actuation mechanisms were carried out to visualise the relationship between the springs-based actuator and the sampler. The conversion of translational output (δ) from the SMA springs-based actuator to rotational input (θ) for the sampler is shown in Figure 11. The impact of changes in the length of sampler (ΔL_s) and the deflection of spring ($\Delta\delta$) is shown in Table 4. Greater sampler rotation assures better exposure of the sampler to the intestinal wall; however, it would be produced at the expense of larger spring deflection and/or lesser sampler length. The spring itself is 5 mm long and more than 2 mm deflection reduces its output force gradually. In addition, a sampler with less than 7 mm length increases the rotational friction progressively. Therefore, based on simulations, we selected the length of the sampler and the deflection of the spring as 7 and 2 mm, respectively. This combination provided angular movement of 16.6° for the sampler, as shown in Table 4. The simulations showed this rotation was sufficient to fully expose the inlet of the sampler to the outside environment for scrapping the microbiota from the wall of the small intestine.

These results were further investigated through real-time ex vivo trials of the small intestine, as shown in the next section.

3.2 | Ex vivo sampling trials

The capsule robot was tested on a 100-mm post-mortem section of small intestine from a lamb, as shown in Figure 12A. The small section was separated from the rest of the small intestine and the capsule was manually inserted inside it, as shown in Figure 12B. The

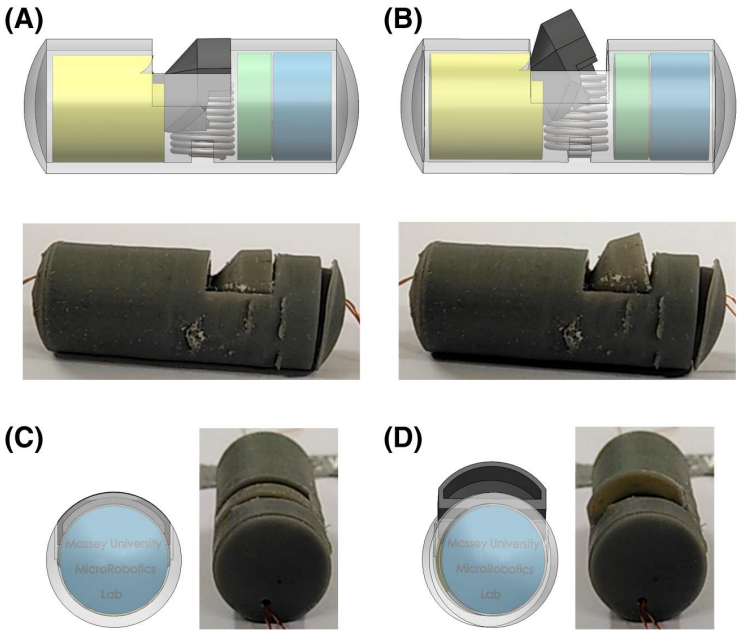

FIGURE 11 Sampling and actuation mechanisms of the capsule robot. A spring actuator triggers, and the sampler moves outside capsule shell to collect the sample. The side view of the capsule robot when sampler is (A) closed and (B) open. The front view of capsule when sampler is (C) closed and (D) open

TABLE 4 Impact of spring deflection and sampler length on the rotation of sampler

		Sampler length (L_s) (mm)			
Sampler rotation (θ)		**6**	**7**	**8**	**9**
Spring deflection δ (mm)	1	9.6°	8.2°	7.2°	6.4°
	2	19.5°	16.6°	14.5°	12.8°
	3	30°	25.4°	22°	19.5°

peristaltic motion was generated manually by pushing the capsule gently. The capsule inside the intestine is visible and can be seen in Figure 12C. The capsule then exited the small intestine (Figure 12D), and the sampler and storage chamber were dismantled from the capsule shell (Figure 12E), with the content inside the sampling chamber visible in Figure 12E. The content from the storage chamber was transferred to an Eppendorf measuring tube and can be seen in Figure 12F. By following the same procedure, seven capsules were tested. The quantity of sampled content collected in each trial is shown in Table 5. At least 100 µl of content was captured in each trial, which is the sufficient quantity to analyse the microbiota. A supplementary video is attached to show the overall procedure.

3.3 | Laboratory analysis of the collected sample

The sample collected using ex vivo trials was sent to the Nutrition Laboratory (School of Food & Advanced Technology, Massey University) for analysis. Amino acid analysis was carried out, which quantitatively indicates the presence of amino acids in a sample. The sample was freeze-dried and hydrochloric (HCl) hydrolysis was performed followed by reverse-phase high-performance liquid chromatography separation using AccQ Tag derivatization.

The amino acid profile of the sample collected is shown in Table 6, which showed that 51.15% of the sample composed of amino acids. This in turn strongly suggests that the remainder of the sample consisted of digesta containing cellulose plant material which diluted the amino acids in the sample.

The laboratory test revealed that the amino acid signature of the sample was indicative of a mixture of mucus and microbial protein, with fibrous digesta comprising the remainder, which provided a proof of concept for the proposed design. These trials will now be replicated in vivo, with the aim of collecting microbiota which will be sequenced using standard next-generation techniques.[33]

4 | DISCUSSION

A novel capsule robot was designed in this work, which comprises sampling and actuation mechanisms. The major portion of the capsule is dedicated to the sampling mechanism, which includes a dedicated portion of storage chamber to store up to 500 µl of digesta or mucus content. The storage chamber collects the content at target site and closes its inlet after sampling to avoid the downstream contamination. The sampling mechanism was designed for this work and tested with simulation software. Subsequently, the design was 3D printed and tested ex vivo on post-mortem animal intestinal tissue. The results show that the sampling mechanism could effectively capture more than 100 µl of intestinal content in a 100 mm section of small intestine. The storage chamber was not completely filled in any of the trials due to small travelling distance; however, the volume collected would be sufficient to assess the microbiome using next-generation

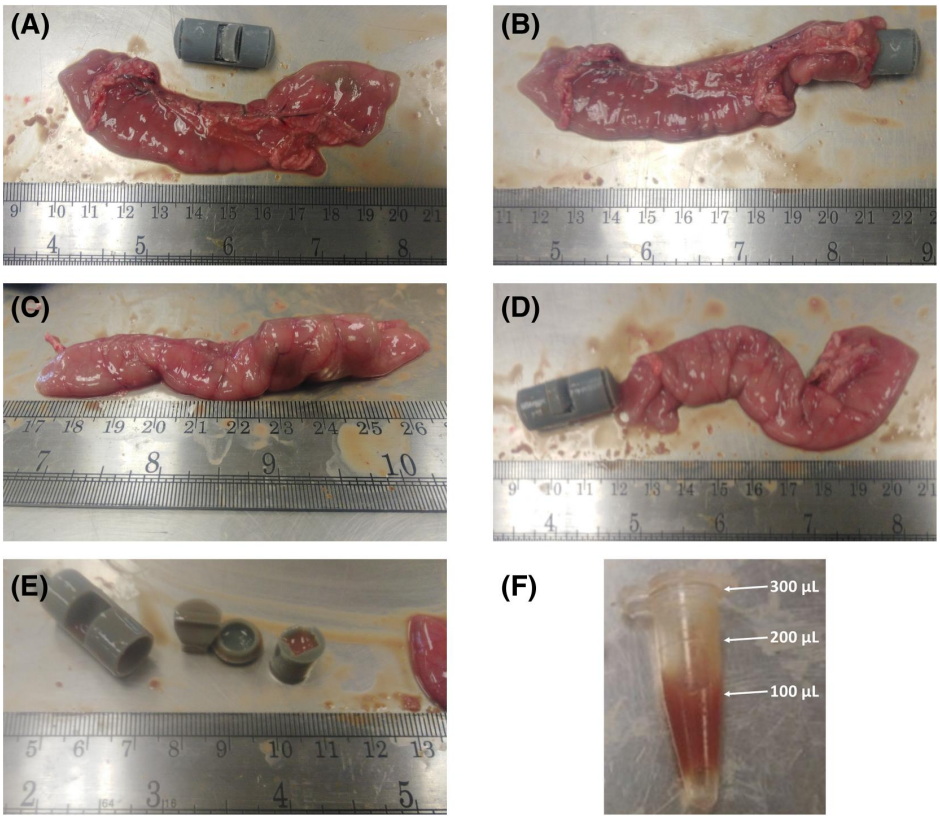

FIGURE 12 Ex vivo experiments on the small intestine of a lamb. (A) The capsule robot and 100 mm section of small intestine, (B) the capsule entering the section of small intestine, (C) the capsule inside the small intestinal tissue, (D) the capsule exiting the section of small intestine, (E) the disassembled capsule parts and collected sample visible inside the storage chamber and (F) the sample is removed to an Eppendorf measuring tube

sequencing technology.[33] If the sampling distance is increased from a small 100 mm section of small intestine to the full extent of the organ, then the sample size collected should increase. In ex vivo trials, we restricted our travelling distance to 100 mm to demonstrate the effectiveness of the system for short term actuation. Overall, it can be inferred that the sampling mechanism can capture a signi cant amount of digesta from a short (100 mm section) of the 6-m-long small intestine of an adult human.

The actuation mechanism of the capsule robot was designed using SMA springs which occupy less space than traditional MEMS-based actuators. The time and force analysis of the SMA springs suggest that they can be powered by a button cell battery to produce suf cient force. SMA springs S1 and S2 produced 1.75 and 0.83 N force, respectively, at 500 mA, which is appropriate to operate the sampling mechanism. Actuator needs to push the sampler against the peristaltic force and friction, which is around 1 N. Therefore, a 1.75 N force push effect is suf cient to open the sampling mechanism against the peristaltic force and the 0.83 N force pull effect is also reasonable to close the sampling mechanism which is assisted by natural intestinal forces. The results clearly show that the SMA springs are a reasonable choice for the actuation mechanism, considering an on-board battery with suf cient output current is available.

At this stage, we have powered the capsule using an external power supply. In future work, zinc-air 675 button cell battery will be used which has a rated capacity of 650 mA. The zinc-air battery will be installed in capsule robot after air-up, which allows the battery to boost the power by reacting to the surrounding oxygen from air. The SMA spring actuator requires current for few minutes only, which will be provided by the battery, even in the absence of oxygen. Zinc-air battery needs 5% oxygen to provide output power ef ciently.[34] The oxygen in small intestine varies from 10% to 20% depending on the diet.[35] Therefore, zinc-air battery can boost power within intestine if needed. A gas permeable membrane can be used to access the gas in intestine without imposing any damage to the intestinal lumen or environment.[35]

The sampler design presented in this work, collected the digesta from surroundings as well as from the wall by scratching it gently while moving in forward direction along with the peristaltic movement as shown in Figure 2. When the capsule entered the intestine in backward direction, then the capsule mostly captured

TABLE 5 Measured quantity of sampled contents

Trial number	Quantity (µl)
Capsule 1	225
Capsule 2	100
Capsule 3	125
Capsule 4	150
Capsule 5	100
Capsule 6	110
Capsule 7	125
Cumulative average	134

TABLE 6 Mucin amino acid composition

Amino Acids		
Name	Abbrev	Composition %
Glutamic acid	Glx	6.43
Asparagine	Asx	4.94
Leucine	Leu	4.72
Lysine	Lys	4.36
Arginine	Arg	3.84
Valine	Val	3.55
Alanine	Ala	2.89
Glycine	Gly	2.86
Threonine	Thr	2.70
Proline	Pro	2.60
Phenylalanine	Phe	2.55
Isoleucine	Ile	2.51
Serine	Ser	2.33
Tyrosine	Tyr	2.14
Histidine	His	1.37
Methionine	Met	1.37
Hydroxy-proline	Hydroxy-pro	0.00
Total		51.15
Units		mg/100 mg

the digesta from surrounding instead of scooping the digesta from the wall. In either case, the sampler captured the digesta; however, the quantity varied a lot between the two scenarios. An active locomotion technique can be added to control the direction of capsule.[9,10,12] In addition, the damage caused by the sampler to the intestinal wall was nominal. The top surface of the sampler was smooth, so it posed no observable damage to the intestinal wall. Furthermore, cutting the intestinal villi and cellular material from the gut lining is not considered harmful as these are naturally recovered in short period of time.[36] In future, a simulation model to study the potential damage to the intestine will be developed to nullify the impact of any possible harm to intestine during in vivo experiments.

In future, in vivo experiments will require a triggering mechanism for the proposed actuator to start sampling process. The pH level can act as a marker for this (e.g., pH level jumps from 2 to 7 between the stomach and small intestine). Similarly, oxygen sensor can also be used to determine major segments of the GI tract.[35] Alternatively, wireless communication can also be used to trigger the actuation by visualizing the capsule from outside through imaging devices.[9] Our proposed capsule has sufficient space to accommodate additional components in the future. Furthermore, more space can be created inside the capsule by reducing the volume of storage chamber from 500 to 200 µl.

The components of the capsule robot were 3D printed for rapid prototyping; however, they lack rigidity. To make 3D printed parts stronger, a 1 mm layer was used (e.g., external diameter of capsule shell was 12 mm while internal diameter was 10 mm) which reduces the available space of the capsule. Furthermore, the air gap of 0.2–0.5 mm was needed between each part (e.g., storage chamber and outer shell) to ensure smooth assembly and disassembly, and also to avoid physical damage to the parts. In future work, we will manufacture parts using a micro-milling machine to increase rigidity. This would also allow more space to accommodate components needed in in vivo trials.

The capsule robot in this study was similar in shape and size to commercially available capsule endoscopes that moves with the help of natural peristaltic motion. This strongly suggests that our proposed capsule has potential to move and sample utilising peristaltic movements. The procedure was tested with both pushing and pulling forces, where capsule orientation was also different in each trial. This is a standard practice for ex vivo experiments.[37,38] In future, a model of mechanical intestine will be developed to effectively mimic the peristaltic contraction forces and motion.[39]

5 | CONCLUSION

This study presents a novel design of a capsule robot to collect microbiota from the small intestine. An actuation mechanism has been developed, which uses SMA springs due to their small size and good output force. Experimental results show that the SMA springs can operate the sampling mechanism, and they can be powered by a button cell battery. The devised sampling mechanism has a capacity to collect and store 500 µl of content from the target site. Ex vivo trials on post-mortem animal intestinal tissue successfully collected more than 100 µl sample (digesta, mucus and microbiota), which is a feasible quantity for microbiota analysis. Lab analysis of the collected sample indicated that the proposed design of the capsule robot shows promise for sampling microbiota, mucus and digesta from the GI tract.

ACKNOWLEDGEMENTS

The authors would like to thank Bryce Ilton and Kirsty Anderson for their support in conducting the intestinal trials. This research is supported by the Massey University Research Fund (MURF) 2019. Support is also provided by the Higher Education Commission (HEC), Pakistan under grant no. 5-1/HRD/UESTPI(Batch-V)/2421/2018/HEC.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Rehan M, Al-Bahadly I, Thomas DG, Avci E. Capsule robot for gut microbiota sampling using shape memory alloy spring. *Int J Med Robot*. 2020;16:e2140. https://doi.org/10.1002/rcs.2140

Chapter 5

Development of Actuation Mechanism for Robotic Capsule to Sample Gut Microbiota using two-way SMA Spring Actuator

This chapter presented the modeling of a unique two-way SMA spring actuator that has not utilised before in any sampling device or robotic capsule. The temperature gap between the martensite and austenite states (hysteresis loop) is significantly reduced with the aid of a commercial manufacturer that allows reduction of the complexity of the previously proposed design while greatly reducing the power requirements. A specialised experimental setup that can keep the freshly dissected small intestine alive is utilised to test the robotic capsule in a realistic environment (in terms of peristaltic movements) as opposed to earlier tests with *ex-vivo* animal small intestines. The robotic capsule prototype has collected sufficient quantity of sample from living porcine duodenal and ileal tissues i.e. in the presence of peristaltic forces. The robotic capsule was also tested on living post-mortem tissues (small intestine) of other species including cow and sheep. The collected sample size for all the species was feasible to analyse the microbiota through next generation sequencing techniques.

This chapter contains content from the following article.

- Rehan, M., Al-Bahadly, I., Thomas, D. G., & Avci, E. (2021). Towards Gut Microbiota Sampling Using an Untethered Sampling Device. IEEE Access, 9, 127175-127184. DOI: `https://doi.org/10.1109/ACCESS.2021.3111086`.

IEEE Access
Multidisciplinary ⋮ Rapid Review ⋮ Open Access Journal

Received August 20, 2021, accepted September 1, 2021, date of publication September 9, 2021,
date of current version September 20, 2021.

Digital Object Identifier 10.1109/ACCESS.2021.3111086

Towards Gut Microbiota Sampling Using an Untethered Sampling Device

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This work was supported in part by the KiwiNet Grant 2020/2021, and in part by the Higher Education Commission (HEC), Pakistan, under Grant 5-1/HRD/UESTPI(Batch-V)/2421/2018/HEC.

This work involved human subjects or animals in its research. The authors confirm that all human/animal subject research procedures and protocols are exempt from review board approval.

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].

The associate editor coordinating the review of this manuscript and approving it for publication was Zheng H. Zhu.

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

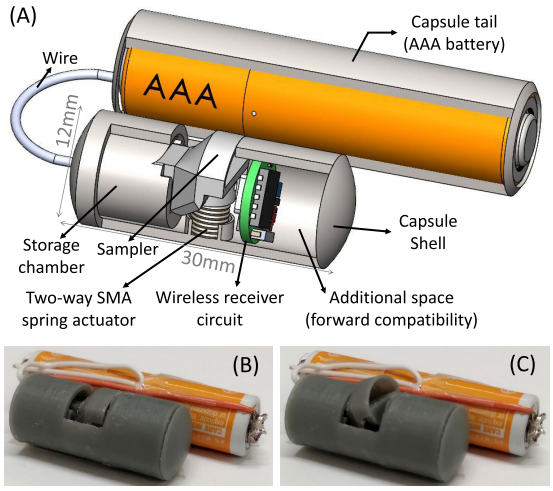

FIGURE 1. Robotic capsule prototype. (A) CAD model with AAA battery, an additional space (9 mm × ϕ 12 mm) is left to include high current drain button cell battery in future. (B) and (C) Fabricated capsule prototype with sampler closed and opened respectively.

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

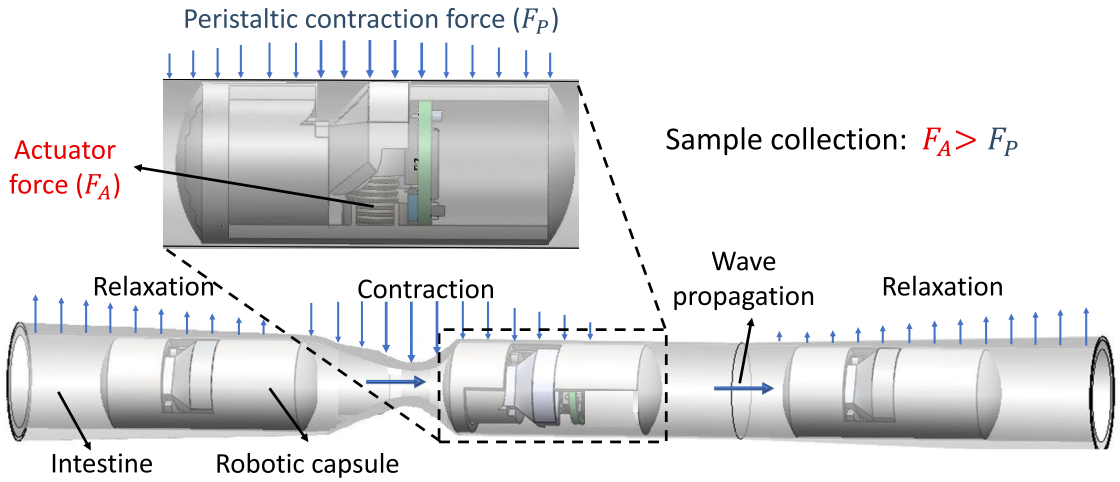

FIGURE 2. Intestinal force model.

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.

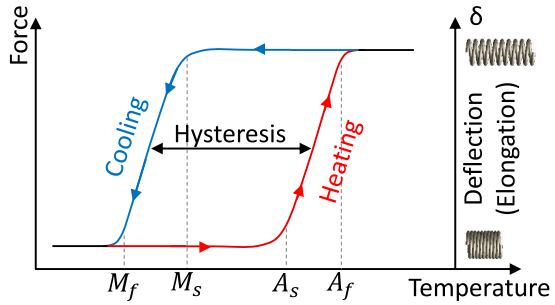

FIGURE 3. The relationship of force and deflection with different temperature states of SMA spring.

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

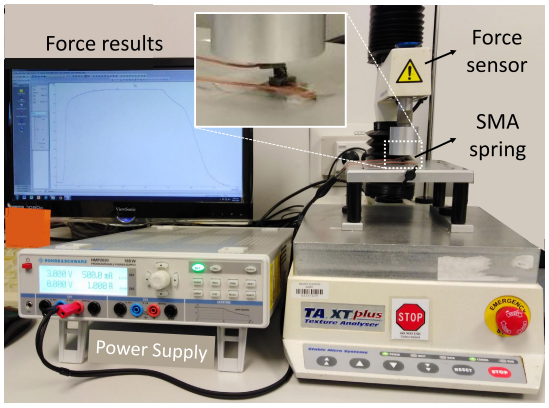

FIGURE 4. Experimental setup for force measurements of SMA spring.

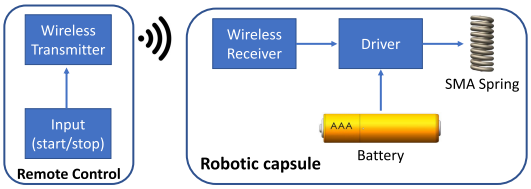

FIGURE 5. Electrical system design of the robotic capsule.

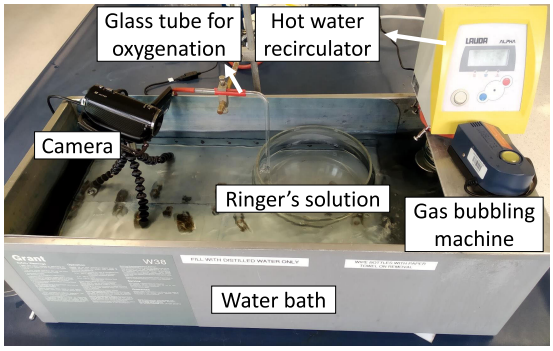

FIGURE 6. In-vitro experimental setup for postmortem tissue.

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

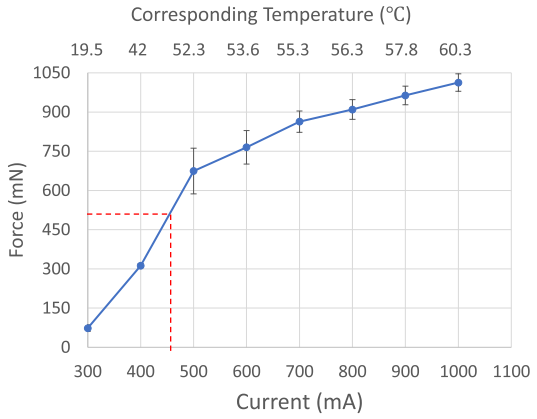

FIGURE 7. Force analysis of two-way SMA spring actuator.

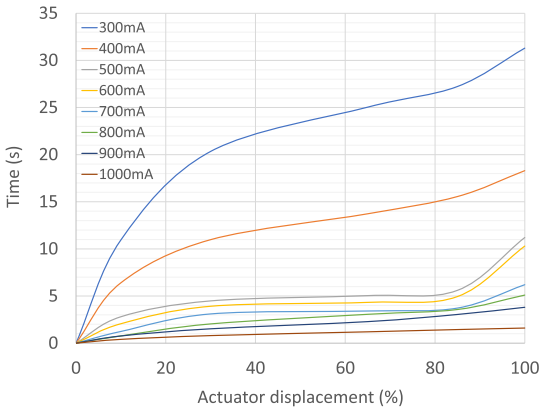

FIGURE 8. SMA spring actuator response time on varying currents.

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

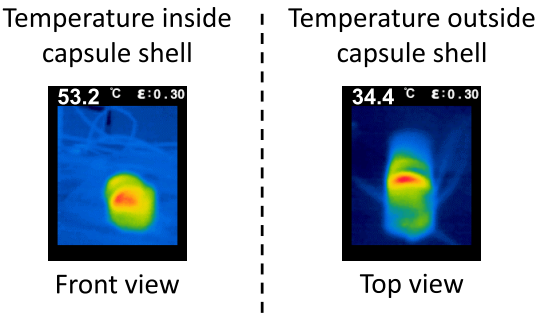

FIGURE 9. Temperature profile of robotic capsule at 500 mA current recorded by thermal imaging camera.

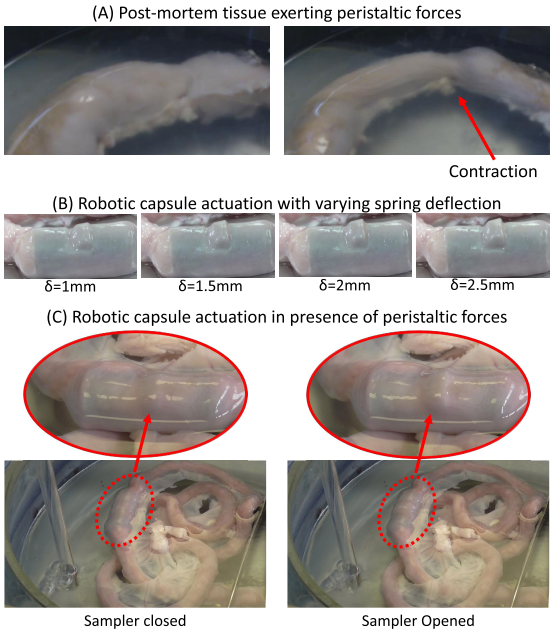

FIGURE 10. Sample collection process under in-vitro experimental setup.

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.

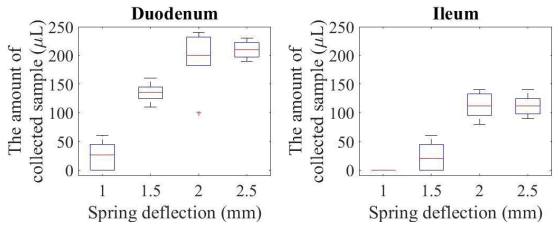

FIGURE 11. Variation in sample size collection by varying spring deflection on pig duodenal and ileal tissue.

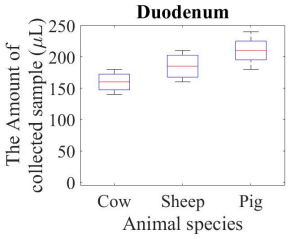

FIGURE 12. Variation in sample size collection on different animal species.

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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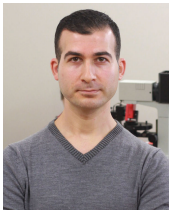

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5.5 Additional details

5.5.1 Figure 7: Additonal results

The Figure 7 in the published paper includes the variation of 3 springs with average values of each spring. In order to fully reflect the variation in the output forces of the individual springs, three separate graphs are added below. Each graph represents three repetitions of each spring.

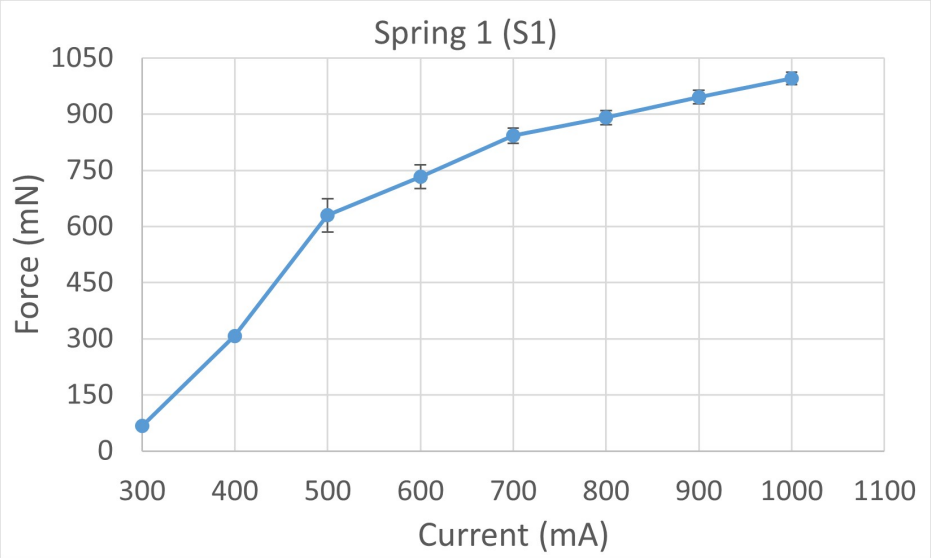

Figure 5.13: Force analysis of two-way SMA spring actuator - variation of forces for Spring 1 (S1)

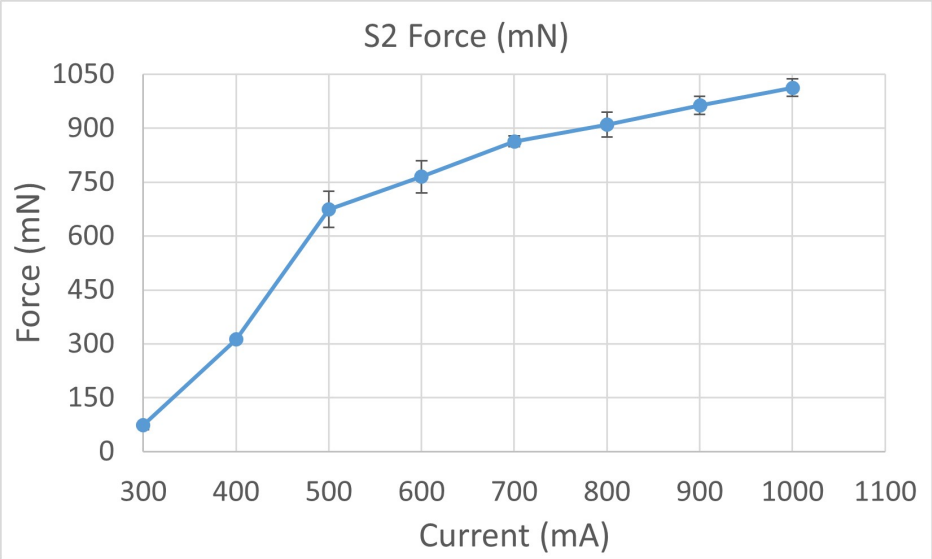

Figure 5.14: Force analysis of two-way SMA spring actuator - variation of forces for Spring 2 (S2)

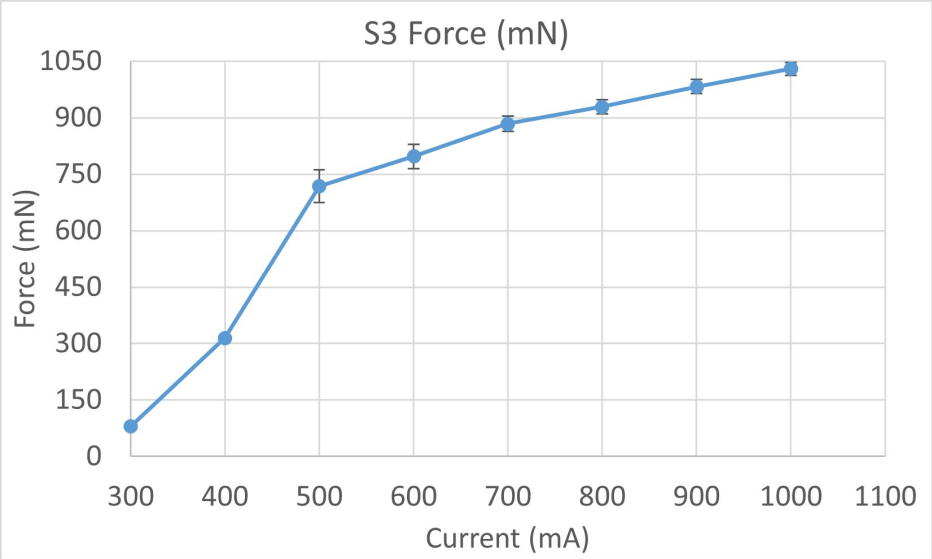

Figure 5.15: Force analysis of two-way SMA spring actuator - variation of forces for Spring 3 (S3)

Chapter 6

Integrated Design of Biocompatible Robotic Capsule with Sampling, Actuation and Sealing Mechanisms for *in-vivo* Sampling of Gut Microbiota

In this chapter the limitations of power source were resolved by developing an actuation system by tackling the high-drain current requirement of two-way SMA spring actuator. Another challenge of cross contamination for assuring effective sample collection was resolved by successfully encapsulating the collection chamber which was realised by testing 3 main sealing materials. Rigorous testing of the robotic capsule prototype is performed in a gut simulator that mimics *in-vivo* environment to ensure successful and safe travel of the capsule along the gastrointestinal tract. In addition, the capsule is also tested under in vitro experimental setup that keeps an small intestine alive to assure sample collection and its protection afterwards. The prototype presented in this chapter is the final prototype of this thesis that has a potential to become a vital apparatus for clinicians to sample human and animal gut in the future.

This chapter contains content from the following article.

- Rehan, M., Al-Bahadly, I., Thomas, D. G., & Avci, E. (2022). Development of a Robotic Capsule for *in-vivo* Sampling of Gut Microbiota. IEEE Robotics and Automation Letters, 7(4), 9517-9524. DOI: `https://doi.org/10.1109/LRA.2022.3191177`.

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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.)*

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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

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[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.

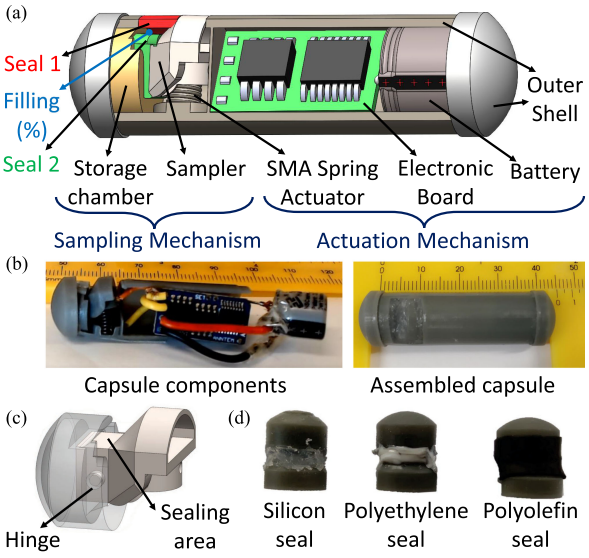

Fig. 1. Standalone robotic capsule for gut sampling. (a) CAD model. (b) Robotic capsule prototype. (c) Hinge mechanism between sampler and storage chamber. (d) Tested sealing materials at the hinge mechanism.

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

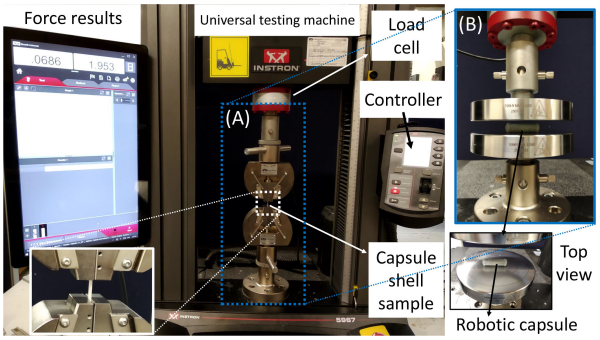

Fig. 2. Universal Testing Machine. (a) Tensile strength measurement of bio-compatible materials for capsule fabrication. (b) Actuator force output measurement of robotic capsule under various sealing mechanisms.

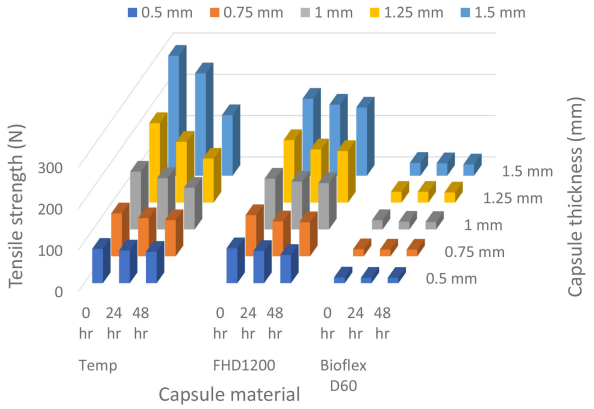

Fig. 3. Material strength testing.

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

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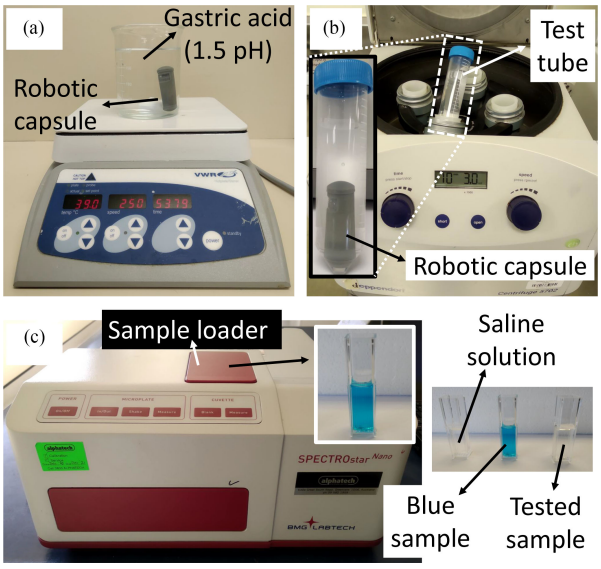

Fig. 4. (a) A magnetic stirrer machine used to replicate the stomach model and test the pre-sampling sealing mechanism. (b) A centrifuge machine to test the post-sampling sealing mechanism under agitation. (c) A spectrophotometer to analyse any potential leakage from the sealing mechanism.

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

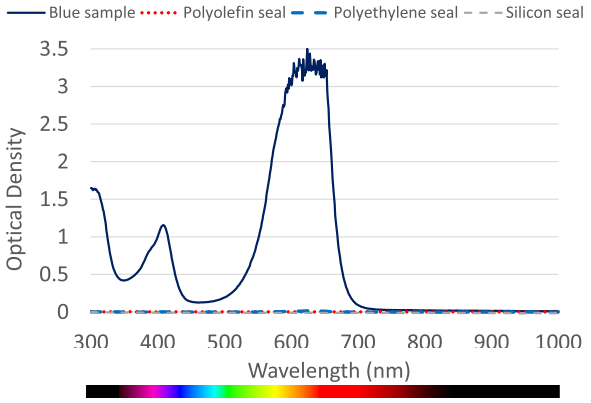

Fig. 5. Spectrophotometer results for the post-sampling sealing test under agitation conditions. The blue sample shows OD above 3 while all the three tested samples returned zero OD showing there was no leakage from the storage chamber.

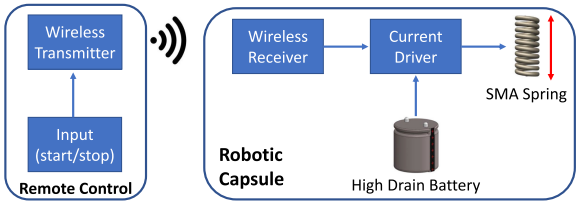

Fig. 6. Actuation mechanism of robotic capsule.

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)

$$C(mAh) = I(mA) * t(h) \quad (1)$$

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

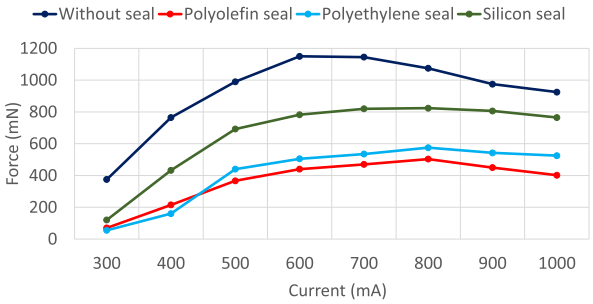

Fig. 7. Force analysis before and after sealing mechanism.

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

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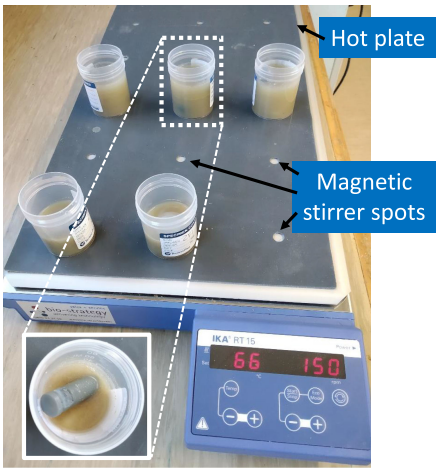

Fig. 8. In-vitro gut simulator.

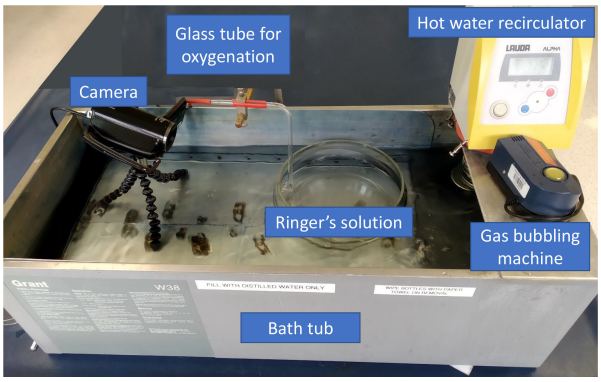

Fig. 9. In-vitro experimental setup to maintain peristaltic movements of ex-vivo intestine.

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

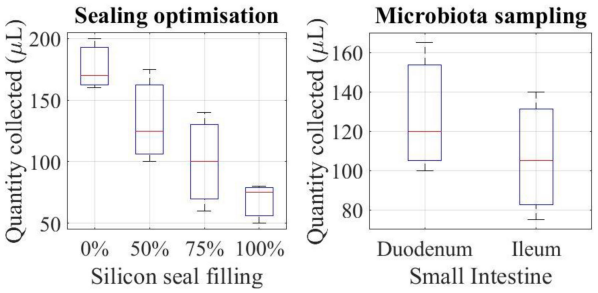

Fig. 10. (Left) Optimisation of filling percentage for the silicon seal of robotic capsule under intestinal trials. (Right) Sample collection from duodenal and ileal tissue after optimisation.

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.

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Chapter 7

Conclusion

A robotic capsule prototype has been developed in this thesis that has a potential to collect gut microbiota both from humans and animals. This sampling robot can serve as a clinical tool in future to assist in better understanding of the human health. In addition, the developed device can also be used to sample an animal's gut for understanding digestion and nutrient absorption and for optimising diets.

7.1 Conclusions

The human gastrointestinal tract is a mystery and researchers know very little about it. This is mainly due to the lack of minimally invasive tools and the complexity of accessing the full length of the gut. Initially, this thesis covers the importance of gut microbiota, the role microbiota play in food digestion and nutrients absorption, highlighting its relationship with human health and disease, and showing its linkage with mental health. Furthermore, it was established that the location from where the microbiota is collected and the mechanism or tool by which it is collected is important to assure the complete assessment of the microbial community. Despite the increasing importance of microbiota and its role in host health, it is not fully explored yet due to the limitations of traditional tools and methods. Therefore, it is crucial to develop a tool that has a potential to collect samples from gut lining (radial precision) throughout the length of the gut (longitudinal precision) to assure full microbiota assessment.

The primary challenge in designing such a tool is the lack of knowledge about gut mechanics in terms of amount and frequency of peristaltic forces applied on a robotic capsule. This information is critical as the robotic capsule needs to overcome the peristaltic forces to collect microbiota from the gut lining and without the knowledge of these forces it is not possible to design efficient small scale actuators and related components. Therefore, in this thesis, the quantification of peristaltic forces was performed

systematically by developing an analytical model of small intestine initially, then simulating this model in COMSOL Multiphysics and lastly performing experiments with living small intestines to measure the peristaltic forces applied on a robotic capsule by small intestinal tissue. This is covered in Chapter 3 of the thesis and allowed me to set the precedence for the amount of peristaltic forces that robotic capsule needed to overcome for collecting a sample from the gut lining. This further allowed the development of actuators dedicated for a robotic capsule or a similar small scale device, in the next chapters.

The key limitation of existing laboratory prototypes (sampling devices), as identified in the Chapter 2 of this thesis, is that they only capture the surrounding fluid which is mainly a digesta sample and cannot guarantee full microbiota assessment. The Chapter 4 of the thesis focuses on the design and fabrication of a robotic capsule that collects the sample from both luminal digesta (surrounding the capsule) and gut lining (intestinal wall). This unique design ejects a collection bucket from the capsule shell using a hinge-based mechanism that collected more than 100 μL of mucus/digesta content during *ex-vivo* intestinal trials. Lab analysis of the collected sample indicated that the proposed design of the sampling mechanism shows promise for sampling microbiota from the GI tract. The sample collected with this design of robotic capsule should allow a full microbiota assessment as it clearly captures a sample of both the mucosa layer and digesta ensuring the collection of all major microbial communities.

The knowledge of peristaltic forces, covered in Chapter 3, allowed me to develop two separate designs of SMA spring actuators consecutively in Chapters 4 and 5. In Chapter 4, a concentric configuration of two SMA springs was organised to perform push and pull movements that were utilised by the sampling mechanism to eject the sampler outside of the capsule (for sample collection) and then back inside (for sample storage). This unique design of two SMA springs, work in antagonistic configuration that allow to and fro motion when the relevant spring is activated. However, this design was complex and required high amounts of power which was a challenge in a robotic capsule with limited space to accommodate batteries. Therefore, the design was improved in Chapter 5 by introducing a two-way SMA spring that provided both push and pull movements at different temperatures. This unique two-way effect was tested for the first time in any robotic capsule application and allowed space and power requirement savings. To assure that the new design of actuator (Chapter 5) was compatible with the previously designed sampling mechanism (Chapter 4), the robotic capsule was tested in living small intestines of three animal species (cow, sheep and pig). The average sample collection across all the species were more than 100 μL which is an adequate quantity for performing microbiota assessment through next generation sequencing techniques.

However, the design of robotic capsule presented in Chapter 5, is lacking in two

aspects. First, it was powered by a AAA battery that is 45 mm x ϕ 10.5 mm in size which restricted its testing under *in-vivo* conditions (in animals or humans). Second, the sample contamination issue was not addressed and it was possible for the collected sample to get mixed with content from non-targeted sites. Therefore, these limitations were addressed in Chapter 6, by analysing small size high-drain current batteries which were necessary for SMA actuators. Furthermore, three sealing materials were tested for the proposed capsule to secure the sample from contamination and the selected material (silicon) was tested to ensure optimum sample collection at the target-site. The challenges of safety, both for the host and the capsule itself were resolved by fabricating the capsule with bio-compatible materials that neither deteriorate nor harm the host. Lastly, to ensure that the developed capsule prototype was fit for *in-vivo* trials, consideration was given to rigorous testing under various gut conditions. A special gut simulator was designed to replicate the different parts of the *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 a proxy *in-vivo* environment (living small intestine), which provides enough confidence to perform *in-vivo* studies in future.

The stage-wise development of robotic capsule in this thesis has significantly reduced the limitations as laid down in Chapter 1. These findings will help researchers to develop more tools for sampling the gut that would eventually increase our knowledge of microbial communities and their relationship with human health. The design detailed in this thesis is a proof of concept for *in-vivo* testing after miniaturisation and it will allow to perform full microbiota assessment in future.

7.2 Future research directions

The robotic capsule prototype developed in this thesis has a potential to collect digesta and gut microbiota from big animals like a cow or horse. In future, the size of the capsule can be miniaturised to test it in smaller animals and humans. Following research directions will improve the gut health diagnosis.

7.2.1 Miniaturisation of the developed capsule

The capsule developed in this thesis can be miniaturised to test it in humans. A relatively quick way to reduce the prototype dimension to swallowable size is by changing the shape and reducing the size of the wireless receiver. Further miniaturisation can be achieved by using modern manufacturing tools like a micro-milling machine to fabricate the capsule shell. The miniaturisation to ingestible size may allow us to better diagnose human health conditions that will in turn improve the treatment methods. A

sampling device of ingestible size may enable better treatment of gut related problems like inflammatory bowel diseases, ulceration, coeliac disease, Crohn's disease, and irritable bowel syndrome. Furthermore, early diagnosis of diseases like cancer, obesity and diabetes might be realised which would help to treat these deadly diseases effectively.

However, the miniatursation process will have its own limitations. Therefore, it is critical to determine the optimal size of the capsule that can pass through the gut of animals and humans. A dummy capsule study should be conducted to determine the maximum size of the capsule that will allow the safe passage of the capsule while utilising the possible maximum space to incorporate the mechanical and electronic components for robotic capsule.

7.2.2 Determination of optimal size to pass through the gut

A study with a patency capsule (dummy capsule shell without any internal components) should determine the maximum size of the capsule that can pass through different animals. This patency capsule study will allow testing of the capsule developed in this thesis in smaller animals like pigs and dogs. This development will extend to range of species able to be studied using the technology and will ultimately contribute to studying nutrient absorption and digestion which will improve our understanding of nutritional requirements of animals and allow to develop better foods for animals.

The patency capsule with smaller size, e.g., 25 mm, in length and 12 mm in diameter should be tested initially to pass through the gut of small animal, e.g., dog. Once the capsule has proven to pass through the gut, 30 mm length with similar diameter (12 mm) can be tested next. Furthermore, 35 mm and 40 mm lengths can be tested to determine the maximal size of the capsule that can pass through the gut without posing any danger to the animal. Similarly, other species can be tested to determine the maximal size of a capsule for their gut. The patency capsule can be developed with a biodegradable material to pose less harm to the animal, and be designed to dissolve in the gut over the time, in case the capsule gets stuck. Therefore, this will not endanger the animal while allowing determination of the maximum capsule size. This study will benefit the researchers working in the gut robotics field to develop better tools with the size that can pass through the animal's gut. Additionally, this study can further help to test the maximal size of a capsule to be used in humans for gut diagnosis.

7.2.3 Localisation methods to track the capsule

Onboard actuation

The identification of the target-site is critical to trigger the sampling process in order to collect gut microbiota from specific locations. This can be achieved by placing a small

pH sensor inside the capsule that can determine the transition from the stomach to the small intestine in the gut. Furthermore, a microcontroller may be able to trigger the sample collection process using transit profiles. An efficient mechanism that is small enough to fit inside the capsule and draws little power is desired to solve the localisation problem. Considering that the space is a major factor in the design of robotic capsule, external devices can be used to track the capsule that will not consume any power and space from the capsule.

External tracking

Another method to track the capsule movement is through the imaging devices, e.g., x-ray or ultrasound. Imaging devices can be used but the precise tracking of the capsule inside the gut needs to be improved. Long term x-ray exposure may pose challenges and are not considered as efficient way to localise the capsule. Therefore, tracking via ultrasound might be a good option but the current literature provides a handful possibilities around the use of ultrasound machines. Considering that the ultrasound machines do not harm the host even with long term exposure and the latest machines are portable so performing these tests will be relatively easier as opposed to x-ray. A diversified team of engineers, animal scientists, ultrasound technicials and clinicians may able to develop some solution that will help to expand the field of robotic capsule to perform a range of activities (therapeutics, diagnosis and treatment) inside the gut.

7.2.4 Gadgets for gut

In the future, the robotic capsule may be made less dependent on any external system like tracking via imaging devices or triggering through external activation. This may simplify the process and allow capsule testing at home for personalised treatments which will reduce the burden on the healthcare system. Software on a mobile device may interpret the results in a similar way to portable blood-sugar testing machines which are operated by the users at home currently. The mobile software may also update the diagnoses regularly to make and prepare weekly diet plans for optimum results. The smart sampling devices could also specify optimal diets for each individual based on their microbiota composition. The rapid pace in development could determine that this happens in near future and the next generation may keep a log of their gut health from childhood to assess any drastic changes in their health to treat themselves with the aid of prescriptions provided by machine learning algorithms and artificial intelligence, without even visiting a doctor. This will not remove the doctors and physicians from this treatment process, rather their role will be changed from looking the patients physically to checking their health status online and they can intervene if needed. Specially, with the COVID restrictions observed recently, this will allow the

doctors to perform diagnosis and treatment from their offices or personal space.

Appendix A

Appendix: DRC 16 Forms

This Appendix contains DRC 16 forms for each publication that is included in this thesis. The order-wise list of all publications that has contributed to this thesis is below,

1. Chapter 2 - Will be submitted to a journal.
2. Chapter 3 - Published in IEEE/ASME Transactions on Mechatronics.
3. Chapter 4 - Published in International Journal of Medical Robotics and Computer Assisted Surgery.
4. Chapter 5 - Published in IEEE Access.
5. Chapter 6 - Published in IEEE Robotics and Automation Letters.

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Appendix B

Appendix: Research recognition and awards

The research in this thesis has obtained following research recognition and awards,

1. Runner-up best presentation award in the IEEE Postgraduate Symposium at Massey University in August 2019. The Symposium was attended by 33 Postgraduate presenters from Massey University and Victoria University of Wellington.

2. Winner and People's choice award in 3MT competition at Massey University in September 2020. In this competition, participants are required to explain their PhD research in 3 minutes.

    - Semi-finalist in Asia-Pacific 3MT competition organised by The University of Queensland, Australia in October 2020. Represented Massey University along side a total of 54 competitors from Australia, New Zealand, Oceania, Northeast Asia and Southeast Asia.

3. Research work published in VetScript that is a flagship magazine of the New Zealand Veterinary Association in December 2020.

    - Rehan, M., (2020). Massey University research update: Technology that transforms. VetScript, 33 (11), pp. 20-22.

4. Finalist (top 11 out of 90 applicants) in Grand Ideas competition organised by Massey ecentre in June 2021.

5. Finalist for the best paper award (top 6 out of 56 papers) in ICAM conference at Aichi, Japan in July 2021.

- Paper title: Mechanical Characterisation of Robotic Capsule Anchoring Mechanism for Gastrointestinal Tract
- Conference: 7^{th} International Conference on Advanced Mechatronics (ICAM 2021)

6. Winner in Pitch.ME competition organised by Massey ecentre in September 2021.

7. Winner and People's choice award in Falling Walls Lab New Zealand organised by Royal Society Te Apārangi in September 2021. This is a national level (New Zealand) competition where participants present their research ideas (that can transform the world in future) along with their contributions.

   - Finalist in Falling Walls Lab – Emerging Talent category in November 2021. Represented New Zealand along side 75 national winners from different countries and shared the research work on robotic capsule.

8. Winner in 180 Seconds of Research Studies organized by Universiti Teknologi PETRONAS (UTP), Malaysia in October 2021. A total of 70 presenters from UK, France, New Zealand, Italy, Poland, Malaysia, Pakistan and India participated in this competition to present their postgraduate research in 180 seconds.

9. Work on robotic capsule featured in "Afternoons with Jesse Mulligan" broadcasted at Radio New Zealand (RNZ) on 9^{th} November 2021.

10. Successful in obtaining a research grant of $10k from Palmerston North Medical Research Foundation under general grant 2021-2022 in November 2021.

11. Work on robotic capsule featured on Education New Zealand (ENZ) website in December 2021.

12. Work on robotic capsule featured on Massey University's website under research impact stories in March 2022.

13. Invited talk delivered on robotic capsule at University of the Third Age (U3A), Christchurch Central chapter, New Zealand in April 2022.

14. Won a research grant of $5k from Massey ecentre under grand ideas competition (3^{rd} prize) in April 2022.

15. Profile featured on the website of Royal Society Te Apārangi in April 2022.

16. Royal Society Te Apārangi published online Highlights Te Tau 2021, featuring the work on robotic capsule in June 2022.

17. Runner-up best presentation award in the IEEE Postgraduate Symposium at Victoria University of Wellington in August 2022. The Symposium was attended by 27 Postgraduate presenters from Massey University and Victoria University of Wellington.

18. Finalist (top 3) for the 2022 KiwiNet Momentum Student Entrepreneur of the year award for “Robotic capsule for gut health” in October 2022.

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		End Page	70
		Issue	1
Author/Editor	Royal Society of Chemistry (Great Britain)	Volume	22
Date	01/01/2001		
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Title	Robotic Capsule for Sampling Gut Microbiota: Design, Development and Evaluation	Institution name	Massey University
		Expected presentation date	2022-10-18

Instructor name	Muhammad Rehan

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Title, description or numeric reference of the portion(s)	Fig. 1 (a and b) and Fig. 2 (a)	Title of the article/chapter the portion is from	Small intestinal sampling capsule for inflammatory bowel disease type detection and management
Editor of portion(s)	Nejati, Sina; Wang, Jiangshan; Heredia-Rivera, Ulisses; Sedaghat, Sotoudeh; Woodhouse, Ian; Johnson, Jay S.; Verma, Mohit; Rahimi, Rahim	Author of portion(s)	Nejati, Sina; Wang, Jiangshan; Heredia-Rivera, Ulisses; Sedaghat, Sotoudeh; Woodhouse, Ian; Johnson, Jay S.; Verma, Mohit; Rahimi, Rahim
Volume of serial or monograph	22		
Page or page range of portion	57-70	Issue, if republishing an article from a serial	1
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