

Design and Validation of a Biosensor Implantation Capsule Robot

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Abstract

We have proposed a long-term, non-invasive, non-restrictive method of delivering and implanting a biosensor within the body via a swallowable implantation capsule robot (ICR). The design and preliminary validation of the ICR's primary subsystem—the sensor deployment system—is discussed and evidence is provided for major design choices. The purpose of the sensor deployment system is to adhere a small biosensor to the mucosa of the intestine long-term, and the modality was inspired by tapeworms and other organisms that employ a strategy of mechanical adhesion to soft tissue via the combined use of hooks or needles and suckers. Testing was performed to refine the design of the suction and needle attachment as well as the sensor ejection features of the ICR. An experiment was conducted in which needle sharpness, needle length, and vacuum volume were varied, and no statistically significant difference was observed. Finally, preliminary testing, coupled with prior work within a live porcine model, provided evidence that this is a promising approach for implanting a biosensor within the small intestine.

1 Introduction

In recent years, bio-sensing has seen increased demand in the medical field [1]. These sensors can be used to detect a wide variety of health-related biometrics such as body temperature or heart rate [2,3]. Many of these biosensors are worn externally and thus may interfere with the lifestyle or occupation of the wearer or lack the ability to continuously monitor the patient for long periods of time in all environmental conditions. A continuous, completely non-invasive *in vivo* system for gathering bio-indices could overcome these limitations and may also lead to quicker and more accurate diagnoses of complex chronic diseases [4]. There is a current need for a biosensor that is non-invasive, continuously monitors the desired metric over a long time period, and does not restrict movement or lifestyle. Researchers have recognized this need and are now developing “disappearables”—miniature sensors and actuators that are hidden on the body or in natural orifices [5–7]. In addition to solving the problem of sensor miniaturization, two additional questions associated with disappearables are: 1) how to noninvasively attach or implant the biosensor, and 2) how to make the biosensor attach to its target position long-term (at least one week) without causing serious trauma.

While research on biosensors is prevalent, development in the field of capsule robotics has been increasing as well and might offer insight into the problem of long-term, non-invasive biosensing. Capsule robots have been an intense area of research since the introduction of wireless capsule endoscopy in 2000 [8,9] and much has been done to further this technology, such as providing active locomotion to move the capsule against peristaltic forces of the intestine [10]. Recently, swallowable capsule technology has moved beyond simple passive traversal and diagnostics and has expanded to include controlled locomotion, long-term adhesion, drug delivery and biopsy [11,12].

Here we leverage the widely accepted, non-invasive swallowable capsule technology in order to implant and attach a micro-biosensor anchor to the inner lining of the small intestine for the purpose of creating a general platform for delivering many generic, long-term, disappearable biosensors. This work will lead to our ultimate goal of using the gastrointestinal (GI) tract as a location for long-term biosensing and control applications. Doing so may enable invisible, unobtrusive, long-term physiological monitoring to address the problem of infrequent, clinic-based measurements. The GI tract is an ideal location for miniature biosensing systems due to its large volume and surface area, proximity to vital organs and systems, and because it is a natural pathway into and out of the body.

Our approach is to create a general biosensor deployment platform that is capable of targeting any point along the small bowel. To do so, we are developing a swallowable implantation capsule robot (ICR) that performs the following sequence of events:

- 1) An ICR is swallowed and arrives at the desired location through the digestive tract.
- 2) The ICR deploys the sensor to the GI wall through a tissue attachment mechanism (TAM).
- 3) The ICR separates from the sensor and exits the body in a typical manner, leaving the sensor *in vivo*.

The previous work focused on the design, fabrication, and *in vitro* and *in vivo* testing of the TAM, a subsystem of the capsule and an adhesive device that anchors a bio-sensor to the mucosal and submucosal tissue long-term *in vivo* [13]. As described in that work, the TAM uses a combination of suction and needles to capture and grip mucosal tissue, a method that is inspired by some species of tapeworm which benignly adhere to the GI wall for long periods of time [14]. A limitation of our previous work, however, was that the pressure used by the TAM was generated by a large external vacuum pump with infinite vacuum volume, i.e. the TAM was able to generate near-perfect vacuum for an indefinite length of time. Therefore, the *in vivo* tests required a tethered vacuum line to the TAM. That strategy was sufficient to prove the TAM concept; however, it is not amenable to a swallowable, capsule-based system.

The purpose of this work was to design, build, and test a prototype capsule that accomplishes the sensor deployment capabilities of the ICR without the need for tethers to external components, such as a vacuum pump. Specifically: 1) An ICR sub system is designed that provides vacuum pressure for the TAM via a pressure vessel; 2) The required volume of the pressure vessel is investigated; 3) A custom microcontroller-based circuit board is designed to actuate the TAM vacuum pressure based on an external signal; 4) An onboard power system is developed, and 5) The performance of the integrated system and its ability to capture and maintain adhesion to the mucosal tissue is evaluated both *in vitro* and *in vivo*.

2 Methods

The approach of this work was to design, build, and test a prototype that accomplishes the sensor deployment capabilities of the ICR. The design was based partially on bio-inspiration and also on our knowledge of commercially available swallowable capsules used for diagnostic endoscopy. Two of the primary requirements of the ICR are transporting the biosensor to its ideal position in the intestine and attaching it to the intestine wall firmly for long-term monitoring. For the latter, tapeworms and other organisms that employ a strategy of mechanical adhesion to the soft tissue via the combined use of hooks and suckers inspired the design. Previous work provides details of these bioinspired features [13,15,16], and a component on the ICR called the tissue attachment mechanism (TAM) has been designed and optimized and consists of a sucker and a needle array around the sucker. During the attachment process, tissue is aspirated by the sucker and then fixed via needles. Once the TAM is integrated into the ICR, it can be used to benignly and noninvasively attach a variety of biosensors to the GI wall. Various tests were conducted during the design process to further optimize the performance of the TAM, the capsule body geometry, and the biosensor deployment function. The ICR was also functionally tested both *in vitro* and *in vivo*.

2.1 Functional requirements

In order to deliver a long-term *in vivo* GI biosensor, three fundamental requirements of the ICR are: 1) The ICR must be able to deploy and firmly attach the sensor to the wall of the GI for at least one week; 2) The ICR must successfully separate from the TAM and sensor once it has attached; and 3) The ICR must exit the GI in the typical manner. Because the focus of this work is on the delivery and attachment systems of the ICR, a sham biosensor is used. Ongoing work is focused on the development of specific biosensors that will be carried by the ICR, such as those that measure pH, temperature, pressure, etc. Ongoing work is also focusing on a capsule subsystem and method that will be used to enable the ICR to utilize wireless communication and various position-enabling sensors to monitor the capsule's posture and location, and pressure of the tissue on the capsule. The keys to successful attachment are knowledge of capsule's location and intimate contact of the TAM with the tissue. The future sensor package will add these capabilities [17].

Besides these three basic functional requirements of the ICR, other important general requirements are that it must work in an *in vivo*, moist environment at body temperature and that the length and diameter must be less than or equal to 30 mm and 11 mm respectively, which are close to the dimensions of commercially available swallowable diagnostic capsules [18]. The ICR must also operate without tethering of any kind.

2.2 ICR System Architecture

The ICR consists of three different functional components: 1) The TAM which is connected via vacuum pressure to the ICR; 2) An ejection mechanism which deploys the TAM to the GI, and 3) A capsule body with batteries and circuit designed to control the whole deployment process.

2.2.1 ICR Sensor Deployment Architecture Overview

The first requirement for this ICR is to properly eject the TAM and sensor following activation by the user. To accomplish this design requirement, the ICR was designed to store energy in a compressed spring, a vacuum chamber, and batteries. These different potentials are stored such that they are in equilibrium while the ICR is in a resting state. Once the tissue capturing sequence is activated, a ‘domino effect’ is initiated, releasing these energies in sequence to accomplish the task of attaching the sensor to the wall of the intestine. For ease of testing, the current ICR prototype was designed to be reusable. This includes a battery compartment that can be opened so that a new set of batteries may be used on each test. The valve that releases the vacuum can also be reset externally.

The TAM deployment system consists of the TAM itself and a corresponding ejection mechanism which are shown in Fig. 1. The TAM is adhered to the ICR by pressure differential between the ambient pressure and the negative gauge pressure of the vacuum chamber. In the fabrication process, the control circuit and batteries are first installed onto the ICR body, then the ICR body, spring and TAM are moved to a custom vacuum assembly vessel that is equipped with an external pump to evacuate the chamber. The TAM and spring are further assembled onto the ICR body while within the vacuum environment to make sure the vacuum is introduced into the ICR vacuum chamber during the assembly process. This is done by means of a manipulator that traverses the wall of the vacuum assembly vessel via a sealed bearing. Once the assembly is complete, air is introduced into the assembly vessel and atmospheric pressure outside the ICR pushes the TAM against a spring. With a properly designed TAM surface area and spring stiffness, the atmospheric pressure will also hold the spring in a compressed state underneath the TAM. The ICR consists of three components labeled in Fig. 1: the TAM, the ejection mechanism, and the capsule body. These components are assembled and then placed inside of a casting mold. A mineral filled epoxy resin (EpoxAcast 650, Smooth-On) is then poured into the mold, encasing and sealing all electronics. Fig. 2 shows isometric renderings and a picture of the final manufactured ICR. The overall dimensions of the prototype ICR met all our design requirements except for the size. The prototype was 37.5 mm long and 15 mm diameter, which exceeds the requirement by 7.5 mm in length and 4 mm in diameter. Future work will reduce the size to meet this requirement.

2.2.2 Electronics

The ICR circuit consists of a PIC10F322 (Microchip Technology Inc.) microcontroller for logic with an operating voltage range from 1.8V to 5.5V, a transistor for activating the valve, an LED for providing user feedback in the form of blinks, and a reed switch for user input to activate the attachment sequence. Note that the LED in this prototype is for the convenience of direct observation and proof of concept testing. In future swallowable ICRs, a telemetry system will be embedded to provide feedback. All of the electronics are powered by three LR932 button cell alkaline batteries (1.5V). The circuit diagrams for the ICR circuit are shown in Fig. 3.

Battery life is important for ensuring sufficient time for the ICR to get to the correct position within the gastrointestinal tract. The PIC10F322 was chosen for its ability to go into a low-power “sleep mode” and wait for user input. While in this sleep mode, the microcontroller draws 25 μ A from the batteries. This gives the ICR a battery life of 67 days while in this state, providing ample time to get the ICR into position within the small intestine.

When power is connected to the circuit, the LED flashes two times to signal that it is functioning properly. The microcontroller then goes into sleep mode until the user passes a magnet over the reed switch, which begins another set of diagnostic blinks. Finally, the circuit is activated by passing the magnet over the reed switch a second time and the ICR begins its preset timer. Once the timer has expired, the circuit heats a resistive coil of nichrome wire,

setting off the TAM sequence described below, and then goes back into a sleep mode. The control flow for this process is shown in Fig. 4.

2.2.3 TAM ejection mechanism

The purpose of the TAM ejection mechanism is to separate the ICR from the TAM such that the ICR can exit the body and leave the TAM adhered to the wall of the intestine. The ejection mechanism consists of a wax valve, a vacuum chamber, and a spring, as shown in Fig. 1. The wax valve is a 4.5 mm length of copper tubing (2.4mm OD, 1.5mm ID) that is filled with paraffin wax (Gulfwax, 203-045-005). The melting point of the paraffin wax is altered using stearic acid, with a 9:1 ratio of wax to stearic acid, to keep its melting temperature sufficiently higher than body temperature while still melting easily given the limited energy supply. A nichrome wire (0.04 mm diameter) is wrapped eight times around the copper tube allowing it to be heated, melting the wax and opening the valve. The length of the nichrome wire was chosen such that the maximum amount of energy could be dissipated into the valve as quickly as possible. In order for this to happen, the resistance of the wire was found using (1).

$$P = \frac{V^2 R_L}{(R_{int} + R_L)^2} \quad (1)$$

where P = power dissipated into the wire (W), V = battery voltage (V), R_L = resistance of the wire (Ω), R_{int} = internal resistance of the batteries (Ω). V and R_{int} are both constant for a certain circuit system. Thus, for this single variable equation, the power is maximized when the resistance of the nichrome wire is equal to the internal resistance of the batteries.

Based on both theoretical analysis and testing, three LR932 batteries were chosen to provide enough power to melt the wax in a couple of seconds after the activation of the circuit.

The vacuum chamber is formed by two 3D-printed parts. Two different volumes of 0.25 cm³ and 0.35 cm³ were produced for testing. The vacuum volumes were determined through a method that will be discussed later in this paper.

As mentioned, the spring is compressed under the TAM and held in place by the vacuum contained within the vacuum chamber. Once the ICR has been activated, the attachment sequence (Fig. 5) is initiated. This sequence consists of three major steps. First, the wax in the valve melts and the vacuum pressure is then directly exposed to the tissue through the valve. Next, the ICR captures the tissue by aspirating it and fixing it onto the needles. After adhesion, the vacuum in the chamber decreases and the internal and external pressures reach equilibrium. Once equilibrium is reached, the spring overcomes static friction and the TAM is ejected from the ICR. Finally, the ICR is pushed by peristaltic force out of the intestine, leaving the TAM attached to the side wall.

The opposing vacuum and spring forces are balanced in the device so that the TAM remains firmly attached to the ICR until the wax valve opens. When the ICR is assembled, the force in the compressed spring must be less than the vacuum force to maintain an adequate seal, and the length of the spring must be long enough to push the TAM away from the ICR. Thus, the precise spring length and its stiffness are crucial. With too short of a spring, not enough separation occurs between the capsule and the sensor plate; too long of a spring can cause the sensor plate to pop off despite a good vacuum seal. The force of the spring and the vacuum can be calculated according to (2).

$$F_s = Kx \quad F_v = P \times A_{tot} \quad (2)$$

where K is the spring constant, x is the spring deflection, P is the gauge vacuum pressure, and A_{tot} is the combined area of the sensor plate seals. Naturally, F_v needs to be greater than F_s for the sensor plate to remain sealed to the capsule. Therefore, the spring deflection can be represented in (3).

$$x < \frac{P \times A_{tot}}{K} \quad (3)$$

Vacuum pressure of 94.87 kPa can be consistently achieved in the current vacuum system and the diameter of the seal location in the capsule is measured to be 5.5 mm. The applied spring was cut from a commercial compression spring (McMaster-Carr 94125K521) with original stiffness value 1.82 N / mm and 11.5 mm length. The stiffness, K , of the cut spring is inversely proportional to its original length as shown in (4)

$$K = 1.82 \times \left(\frac{11.5}{L} \right) \quad (4)$$

where L is the length of the cut spring. Since the well in which the spring sits is 4.35 mm deep, the deflection x in (3) is therefore equal to $L - 4.35$. Thus, (3) is fully expressed as (5).

$$(L - 4.35) < \frac{P \times A_{tot}}{1.82 \times \left(\frac{11.5}{L} \right)} \quad (5)$$

According to the calculation, the total length of the spring theoretically should not exceed 5.54 mm. The calculation provided a general estimation of the spring length. This calculated length was fine-tuned by iteratively cutting down the spring until the capsule sealed repeatedly for a long period of time. The capsule was able to seal reliably at a spring length of 7.5 mm, and it remains sealed with this spring length for longer than 5 days. Although F_s slightly exceeds F_v , this excess is balanced by the friction of the O-ring in the seal sites and the spring release length of 3.15 mm (7.5 mm - 4.35 mm) is able to push the TAM such that it clears the ICR. The tensile normal force needed to prematurely remove the TAM from a sealed ICR (with a compressed spring installed) was measured to be 5.59 ± 0.74 (mean $\pm$ SD) on the tensile test machine. Compared to the 8.79 N spring force in its compressed condition, the safety factor for premature separation due to spring compression force variability is therefore 1.64. This indicates that an unexpected tensile normal force generated by handling the capsule during implantation or from peristalsis must exceed approximately 5.59 N to cause premature separation. Forces on the capsule TAM are expected to be shear or normal compression, which have no effect on premature separation.

From the *in vivo* experiments, we found that the ejection motion of the TAM is occasionally snagged by the spring resulting in a deficient release, especially when the irregularities of the ejection mechanism are introduced in the fabrication and assembly process. In addition, although the TAM ejection spring has posed no complications or problems during *in vivo* testing, future versions of the device may incorporate an alternate strategy for TAM ejection in order to reduce the risk of ICR retention and increase the deployment efficiency.

2.2.4 Tissue attachment mechanism (TAM)

Since the aim of this research was to explore the ICR, a TAM with a sham biosensor was used for all tests described above. Exact geometry, functionality, and tissue adhesion features of the TAM were optimized and tested in a live porcine model in previous work [13]. Briefly, three parameters that determine TAM geometry were varied in the previous study: hole diameter, needle length and needle angle. The adhesion strength of the TAM to the tissue was tested using the tensile testing machine. In the tests it was determined that a hole diameter of 5 mm and needle angle of 45° were the optimum needle protrusion geometry. These parameters were replicated for this study. Future work will include studying materials that are optimally suited to long-term contact with the mucosa and submucosa (316L stainless steel needle, biocompatible 3D printing material). Regardless of the materials chosen, because the mechanism of the TAM attachment is purely mechanical, we anticipate that attachment performance will be similar if the future biocompatible materials or coatings have similar mechanical and geometric properties. Also in future work, the bio-sensor will be integrated on the TAM and deployed as a single component.

2.3 Testing

Testing of the current iteration of the ICR was performed with the overall purpose of assessing the repeatability of the ICR's ability to attach a TAM to the wall of the small intestine and ensure that the ICR separates from the TAM following tissue attachment. Note that current development is targeting the small intestine only and that all the *in vitro* tests were performed on freshly excised porcine intestine tissue.

2.3.1 Vacuum Volume Test

As mentioned, vacuum pressure is used to hold the TAM onto the ICR as well as to aspirate tissue into the capture mechanism once the tissue capture sequence is initiated. The vacuum volume, which is the volume of the cavity in the ICR from which air is evacuated, is considered as a crucial parameter because changing the size of the vacuum volume affects the tissue capture dynamics by enabling more or less tissue to be pulled into the ICR.

Previous versions of the ICR sought to maximize this parameter, resulting in a vacuum volume of 2.5 cm³ [16]. The entire volume of the ICR is approximately 5.5 cm³, so this is a relatively large portion of the ICR's total available volume that is dedicated to vacuum. Furthermore, with such a large vacuum volume, the residual vacuum after tissue capture generates too large a force for the spring to push the TAM off, leaving the entire ICR attached to the intestinal wall, which creates an undesirable retention issue. Ideally, the minimum vacuum volume that still successfully captures tissue should be used as this leaves the maximum amount of volume for ICR components. Thus, an experiment was designed to determine the optimal vacuum volume.

To determine the ideal range for the vacuum volume, a replica of the tissue capture mechanism was fabricated with an attached tube that could be pinched off, yielding varying vacuum volumes as shown in Fig. 6. A piece of fresh tissue (approximately 30 mm × 30 mm) was then placed over the TAM and captured at varying volumes. Each volume was tested three times and the pressure reading was averaged. A pressure transducer was attached to the vacuum chamber so that the pressure could be measured before and after capturing. The successful vacuum volume was determined by observing the tissue during each test and determining the smallest vacuum volume for successful and consistent tissue capture.

2.3.2 *In vitro* Functional Validation Experiment

Our previous work has shown that the geometry of the TAM, specifically the needle length, needle angle, and sucker hole diameter, has a significant influence on the attachment performance, and those variables have been tested and finalized. However, these TAM parameters were determined by deploying the TAM manually using an infinite vacuum volume. Thus, an experiment was designed to investigate TAM functionality with other vacuum volumes. Furthermore, in previous work, the TAM was tested prior to the invention of the ICR ejection mechanism, so the integrated system was also tested. This experiment was designed to investigate the efficiency of TAM and ICR function in a simulated *in vitro* environment as a step towards *in vivo* testing. The ICR was tested intraluminally using intact, freshly excised porcine intestine. The geometry of the TAM with a needle length of 2 mm, a 5 mm sucker hole diameter, and 45° needle angle was chosen due to those parameters having been tested previously [13]. Two different vacuum volumes were used, 0.25 cm³ and 0.35 cm³. These volumes were chosen from the results of the vacuum volume test which will be discussed later. Furthermore, mucus in the intestine may be sucked into the vacuum chamber during the attachment process and inhibit the working vacuum. Thus, the functionality of the TAM and the ICR with (and without) mucus was also investigated by scraping mucus from inside the intestine downstream from the anticipated attachment location and inserting a liberal amount of it into the ICR through the suction hole. The four different combinations of ICRs (0.25 cm³ / 0.35 cm³ vacuum volumes, with or without mucus in the ICR) were each tested three times. Each configuration was tested with the full ICR assembly including the electronics, valve, and timing sequence. This is the first time the completed assembly has been tested.

Each ICR configuration was inserted such that its long axis was parallel to the longitudinal axis of the small bowel. It was inserted midway into an approximately 15-20 cm long section of porcine small intestine and tested at room temperature. The only external force applied to the capsule during this test was to push it past the incision point and into place. Once in place, no external force was applied to the tissue or the capsule to aid in adhesion (or for any other purpose). The intestine was stored in saline (NERL blood bank saline, Thermo Inc.) and used within 30 hours of excision to avoid deterioration of tissue properties [19]. After insertion, the ICR was activated by sweeping a magnet across an integrated reed switch and after a five-second delay it captured the tissue. One end of the tissue was then attached to a tensile testing machine (ADMET eXpert 5601, ADMET Inc.) and the other end was free. Two strings were attached to both ends of the ICR and the TAM as shown in Fig. 7. The tensile testing machine then

pulled on the string attached to the ICR after the separation of the TAM and ICR was observed. The maximum forces were recorded as separation forces and were compared to the maximum expected peristaltic traction force of a human intestine [20]. In each experiment, the force of the ICR weight pulling on the load cell was subtracted from the reading. This force indicated the TAM ejection efficiency which is critical to ensure the ICR is not retained in the small intestine. Similar to the separation force test, the maximum pull force required to separate the TAM from the tissue was also recorded in an independent test run. This attachment force indicated the attachment strength of the TAM deployed by the ICR. The attachment forces were also recorded with intestinal mucus being placed in the ICR suction hole prior to the test.

The reliability of the ICR was also investigated independently. Sixteen ICRs (8 ICRs for each vacuum volume) were placed in the intestinal tissue one by one and were activated to perform the entire aspiration-ejection-separation sequence, and the aspiration and separation rates were recorded. The reliability of the ICR was analyzed based on the results.

The vacuum volume of the ICR for the following *in vivo* animal testing was determined from the results.

2.3.3 *In vivo* Animal Experiment

To investigate the TAM deployment function and attachment duration *in vivo*, a 70 kg crossbred, neutered, domestic pig was selected for this study. The animal received humane care according to the Guide for the Care and Use of Laboratory Animals prepared by the U.S. Department of Health and Human Services and published by the National Institutes of Health (NIH). The study was approved by the University of Nebraska-Lincoln's (UNL's) Institutional Animal Care and Use Committee (IACUC, Protocol No. 887). An ICR with a vacuum volume of 0.35 cm³ was used to deploy the TAM. The self-localization features of the capsule are beyond the scope of this project, so to ensure the TAM deployed in the proper location, the ICR was implanted via enterotomy instead of via swallowing. According to a previous study, the difference in intestinal contact pressure on a solid bolus between a closed and an open abdomen is 0.28 kPa [17]. Thus, the ICR ejection performance under surgical implantation conditions (with an open abdomen) is likely similar to the performance of a capsule that is swallowed and activates in a closed abdominal pressure environment. Prior to surgery, the pig fasted for 24 hours. An abdominal laparotomy was performed to expose a section of the jejunum, and the ICR was inserted along its long axis direction through an approximately 15 mm jejunal incision and then pushed 10 cm aboral to the incision. The ICR was then activated remotely via a magnetic field. No external force is applied between the ICR and the tissue to improve attachment during the capture process. After observing that the tissue was partially aspirated by the ICR, the enterotomy was sutured closed and a radiopaque marker was sutured on the intestinal mesentery close to the ICR position. This marker enabled tracking the migration of the ICR and TAM from the implant site. Radiographs were taken prior to recovery from anesthesia to record the initial locations of the TAM. Ventral dorsal (VD) and lateral abdominal X-ray images were taken to establish the initial position. The pig was recovered and returned to the stall, following which radiographic images were acquired four hours after recovery, then every twelve hours until the TAM appeared to detach. The pig had free access to food and water for the duration of the experiment and veterinary staff monitored the behavior and body temperature of the pig to detect signs of discomfort or sepsis. After the detachment of the TAM was confirmed by X-ray, the time was recorded as the attachment duration and end-point of the study. The pig was euthanized right after and the tissue from the TAM attachment position was collected for a histological survey. In addition to this test, the same *in vivo* experiment was performed a second and third time using four and two ICRs in the second and third experiments, respectively. The purpose of repeating the *in vivo* studies was to provide a repeatable measure of attachment success and duration.

2.3.4 Histopathology trauma identification

To identify the possible damage caused by the TAM attachment, tissue from the attachment location was collected from the pigs immediately after humane euthanasia and fixed in 10% neutral buffered formalin. Two adjacent pieces of tissue 100 mm orally and aborally of the ICR were analyzed as a control. Cross sections of the intestines at the sites marked with radiopaque markers were collected and processed (Sakura VIP 5 Tissue Processor) for histological

evaluation the next day after fixation. Paraffinized sections were cut at 4 μm thickness and were stained with hematoxylin and eosin (Ventana Symphony Stainer). The samples were evaluated by a board certified veterinary anatomic pathologist to describe the presence or absence of inflammatory changes in the sections.

3 Results

Following are the results of validation of the ICR to meet the functional requirements.

3.1 Vacuum Volume Test

Fig. 8 shows the average residual vacuum pressure for each vacuum volume tested. Ideally, the residual vacuum pressure should be as small as possible to allow the spring to successfully eject the TAM. As shown in the figure, the residual vacuum pressure decreases as the vacuum volume decreases (as expected), until the vacuum volume is decreased below approximately 0.23 cm^3 . After this point, the TAM no longer successfully captured tissue. The residual vacuum pressure rises after the TAM no longer captures tissue due to the vacuum no longer aspirating the tissue because it cannot overcome the elasticity and viscous friction between the tissue and the ICR. This causes the final volume to be larger, increasing the final vacuum pressure magnitude.

Using this test, the two volumes to be used in the *in vitro* experiment were chosen as 0.25 cm^3 and 0.35 cm^3 . The 0.25 cm^3 volume was chosen since, during the manufacturing process of the ICR, less than 0.15 cm^3 of the internal vacuum space gets displaced by adhesive. Since it was determined that a volume below 0.23 cm^3 does not capture tissue, this gives a margin of error for the manufacturing of the ICR's internal volume. The 0.35 cm^3 volume was chosen to discover if an increase in vacuum volume provides any significant improvement in performance.

3.2 *In vitro* Experiment

As can be seen in Fig. 9, an ICR with 0.35 cm^3 vacuum volume performed slightly better than the 0.25 cm^3 vacuum volume configuration. The attachment force of the TAM applied by the 0.35 cm^3 ICR increased 17% and 43% in no-mucus and mucus cases compare to the 0.25 cm^3 ICR. Meanwhile the separation force needed decreased 27% which means the separation process would be easier.

However, using a one way ANOVA table for the attachment/separation force, it was determined that none of the variables (vacuum volume and mucus) had a statistically significant effect on the ICR performance. This means that the ICR is not highly sensitive to changing parameters and allows for some variability in the design.

The presence of mucus in the suction chamber enhanced the attachment performance of the TAM in both vacuum volume configurations. One possible explanation might be that mucus works as lubrication and allows the needles to penetrate the tissue more easily and deeply, hence achieving firmer attachment.

The mean separation forces of the both ICRs (0.96 N-1.31 N) are still higher than the average human peristaltic traction force (0.6N) [20], but 50% (3 out of 6) of the ICRs separated with a force less than 0.6N.

Due to the better performance of the larger vacuum volume, the 0.35 cm^3 ICR will be used in the *in vivo* test. Considering that only 0.35 cm^3 out of the 2.5 cm^3 volume is used as the vacuum chamber in the ICR body, there is still a large amount of available space in the ICR body that can be eliminated to shrink the overall size. This size optimization process will be conducted in the future.

For the reliability test, of the 16 total trials run with the ICRs, 2 were excluded due to electrical circuit failure. Out of the 14 remaining ICRs, all aspirated tissue as expected for an aspiration success rate of 100%. The success rate of separating the TAM from the ICR after aspiration was 78.57% (11 out of 14 capsules).

3.3 *In vivo* Animal Experiment

Body temperature measurements by the veterinary staff were in the normal range of a healthy pig during the test. The results from the first animal study are shown by inverted contrast lateral abdominal radiographs of the pig in Fig. 10. In the subfigures, the small black rod is the radiopaque marker on the intestinal mesentery. The TAM is indicated by a circular pattern of needles, which is the only radiopaque portion of the TAM (Fig. 10a). Another capsule unrelated to this study is also visible in these images, but it was located in a different position of the intestine so it would not affect the result of this study. From the X-ray images, it was clear that the TAM separated from the ICR successfully after 16 hours (Fig. 10c). The ICR kept moving downwards along the intestine while the TAM stayed fixed relative to the stationary mesenteric marker. The dashed circles in Fig. 10d-f show the location of the TAM with respect to the marker with a zoomed-in view in Fig. 10c). Based on the results from the radiographs, the attachment lasted at least 40 hours and detachment was observed by 52 hours (Fig. 10f).

For the second and third *in vivo* experiments, no attachment duration improvement of the TAM occurred, and only 3 out of 7 ICRs separated from the TAM successfully according to the X-ray images. Compared to the separation rate acquired during the *in vitro* study, the reliability of the ICR *in vivo* decreased from 78.57% to 42.85%. A possible reason for the decrease in success rate is the different biomechanical properties of the intestinal tissue *in vitro* versus *in vivo* or imperfections of the ejection mechanism. A full investigation of the tissue and ICR behavior *in vivo* will be conducted in future work.

3.4 Histology

Tissue from the attachment and control locations of the first animal test (only one ICR tested in this procedure) were collected and processed for the histology study. The results were shown below (Fig.11). At the attachment area, there is a 0.85-1.35 mm thick crust of neutrophils, fibrin, eosinophils, and cell debris with small amounts of hemorrhage along the surface of the mucosa. Some moderate inflammatory changes at the site of attachment and changes associated with the incision are observed. These are normal phenomena after surgery and suturing. No serious trauma or inflammation was observed from this site. Mild proliferation of slightly plump fibroblasts and collagen focally along the serosa due to the surgical stimulation were also found in both controls, which, with the occurrence of the crust, may provide an explanation for the intestine thickening phenomenon and natural detachment we observed from our previous TAM test [13]. This natural detachment caused either by sloughing of the mucosa, tissue thickening, or some other unknown phenomenon is the current mechanism we relied on to detach the TAM after a long-period dwell (one week). These phenomena will need to be addressed if the very long-term attachment is pursued (weeks to months). Also, a more device-controlled detachment mechanism is planned if the tissue thickening is proven problematic or dangerous to the host. In the third *in vivo* experiment, a single inflammatory polyp was found on one of the TAM attachment sites. In general, the inflammatory polyp was found most often in animals with an inflammatory bowel disease and it is a typical reaction to chronic inflammation in the GI. This phenomenon may have been caused by unsterilized needles, a non-biocompatible material in the attachment mechanism, or that the attachment process itself causes undesirable stimulations to the intestinal tissue (infection, bleeding and tearing). A comprehensive animal experiment using more than 20 ICR samples will be performed in our following study and the pathogenesis of the polyp and the damage will be investigated both biologically and statistically with image analysis processing. Furthermore, the device used in future work will use biocompatible and sterile components.

4 Summary and Conclusions

The attachment forces based on *in vitro* testing are nearly equivalent to the results achieved by manually deploying the TAM with infinite vacuum in our previous work. Although 50% of the ICRs required more force to separate the ICR from the TAM than is typically generated by the small intestine, the results were different for *in vivo* testing. As can be seen in the X-ray images, the TAM separated successfully, possibly because *in vivo* the ICR is subjected to

cyclic peristaltic waves over a long period of time which will be advantageous to separating the ICR from the TAM. Furthermore, it is likely that the vacuum pressure gradually dissipates over longer time scales than were used in the *in vitro* tests. There is also the possibility that the fluidic drag of chyme on the ICR helped it to separate from the TAM. These theories have not yet been tested and are the subject of future work. The *in vivo* test verified the proper functioning of the ICR system, and histology indicated this attachment method does not cause unacceptable damage to the host. Compared to our previous single *in vivo* TAM test, the attachment duration of the integrated TAM with ICR decreased from six days to less than 52 hours. This may indicate that the process of separation by pulling the ICR from the TAM under periodic intestinal peristalsis may cause a weakening of the TAM attachment strength and duration. Thus, a separation mechanism that functions with less force will be investigated in future work. The final goal is to develop a system with the most flexibility that can achieve very long-term attachment (weeks to months), but that can be modulated for shorter durations based on the application. Future work will also include additional animal tests and corresponding histology in order to acquire statistically significant results of the whole ICR implantation process. As mentioned, a very important component of the ICR technology is self-localization, which will enable the TAM to activate at the correct point in the small intestine. Ongoing work is pursuing this goal. The ICR will also need to be miniaturized to within acceptable swallowable size limits of 11 mm by 30 mm.

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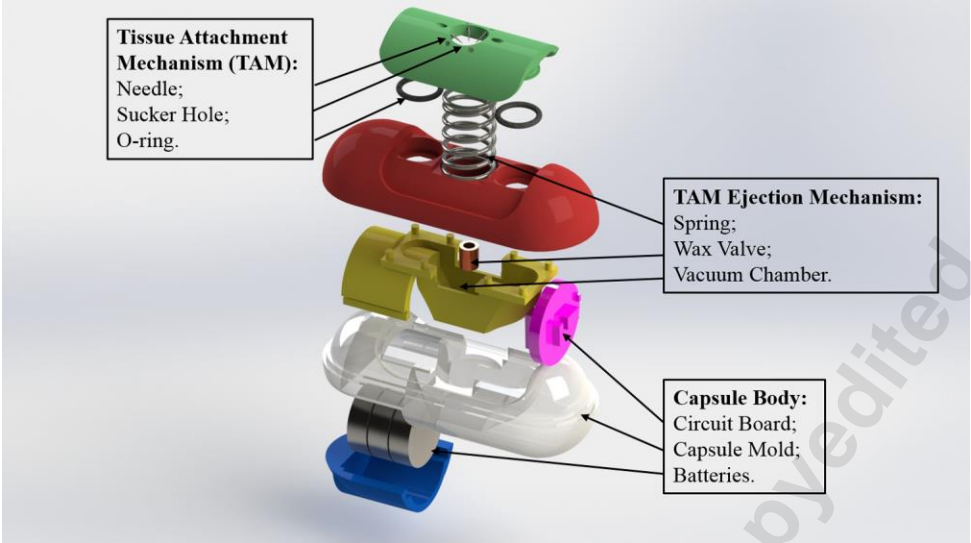


Fig. 1 Exploded view of the ICR showing major components

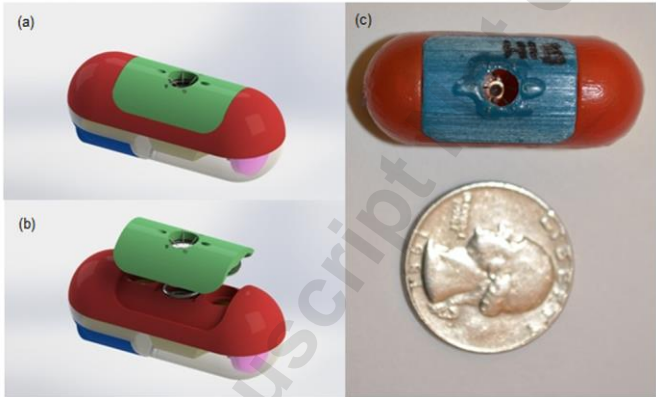


Fig. 2 Integrated ICR working schematic and the real product. (a) ICR with TAM attached; (b) ICR after TAM has been ejected; (c) manufactured ICR.

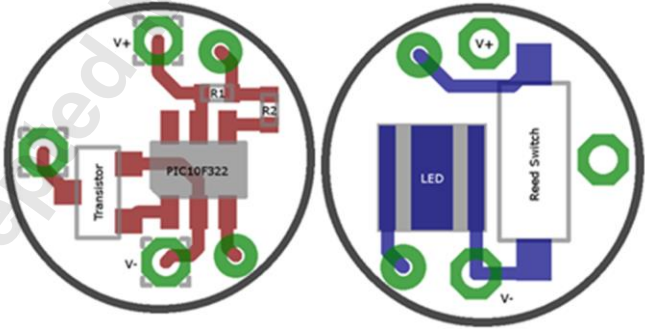


Fig. 3 Front and back circuit diagrams of the ICR's internal circuit

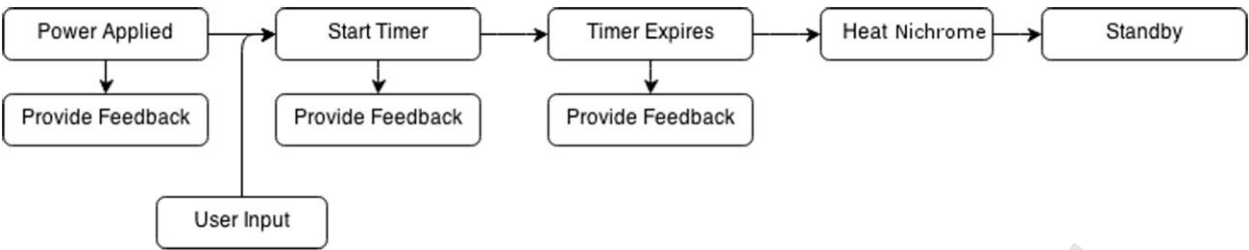


Fig. 4 Control flow diagram for the ICR circuit

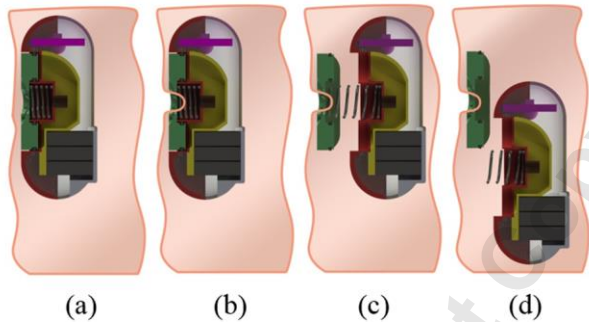


Fig. 5 (a) The ICR enters the small intestine. (b) The wax valve is opened allowing the vacuum to aspirate the tissue through the TAM. (c) After the vacuum has dissipated the spring ejects the TAM off of the ICR. (d) The ICR exits the small bowel, leaving the TAM attached to the intestinal wall.

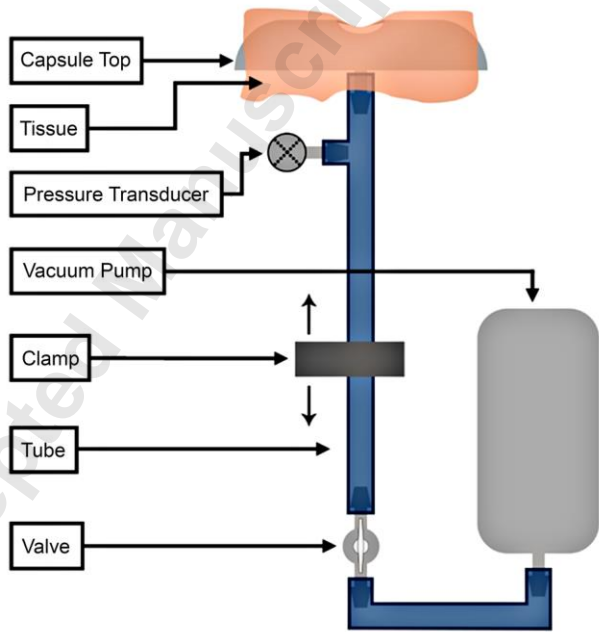


Fig. 6 Vacuum volume test setup. ICRs with different vacuum volume chamber were connected to a vacuum pump; when the vacuum was created by the pump, success rate of tissue capture and residual vacuum pressure were recorded.

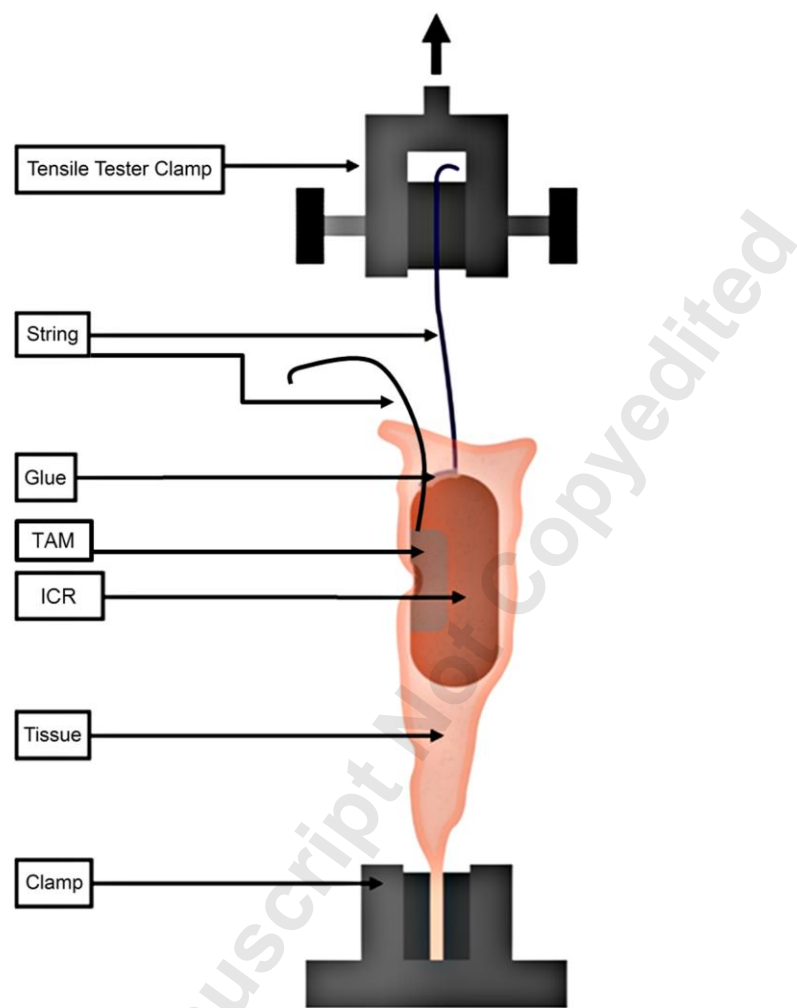


Fig. 7 *In vitro* experiment test setup. Integrated ICRs were inserted into a fixed fresh intestine tissue, then the ICR was activated and pulled by a tensile test machine, and the force which separated the ICR from the TAM was recorded. Similar to the separation test, the string on the TAM was pulled and the force which detached the TAM from the tissue was recorded.

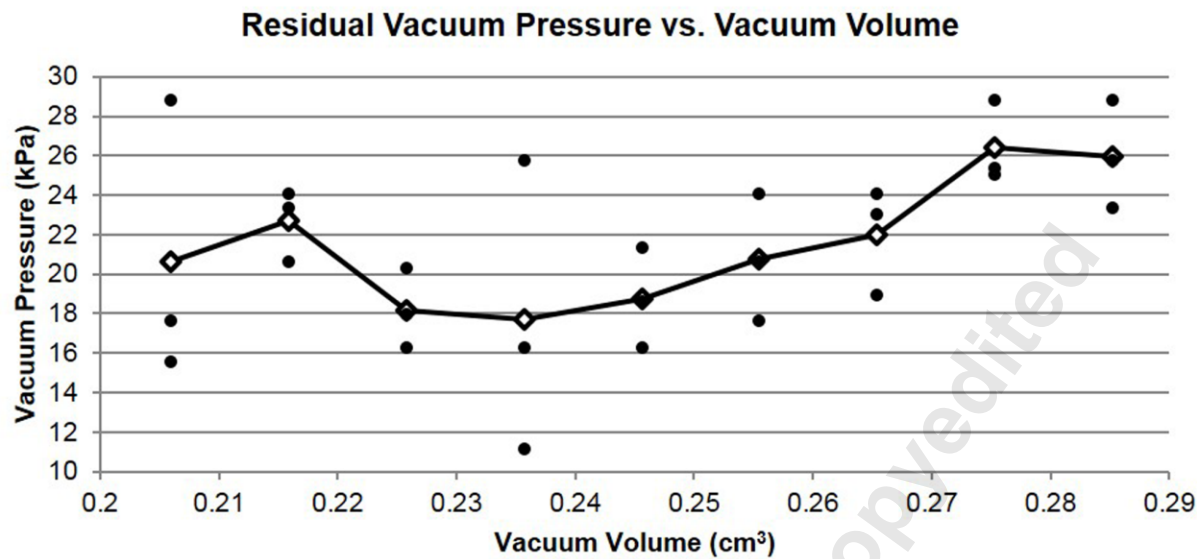


Fig. 8 Residual gauge vacuum pressure after capturing tissue. All measurements are shown; the line is the mean.

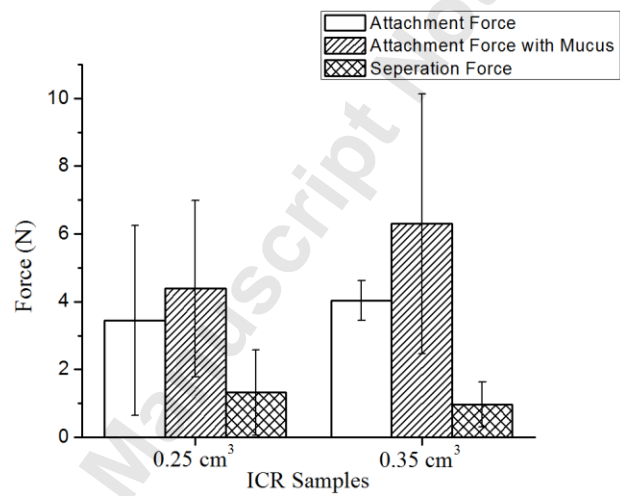


Fig. 9 Attachment/separation forces for ICR samples with different vacuum volumes

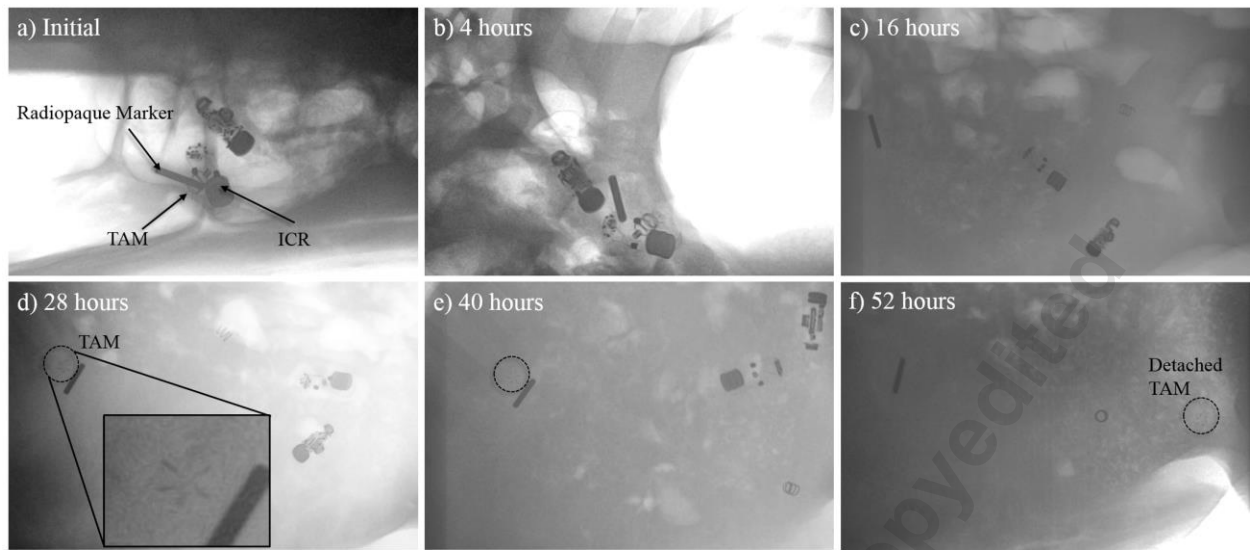


Fig. 10 TAM deployment process and its duration in a live porcine model. (a) X-ray image taken before the recovery of pig. (b) X-ray image taken 4 hours after recovery. (c) X-ray image taken 16 hours after recovery. (d) X-ray image taken 28 hours after recovery. (e) X-ray image taken 40 hours after recovery. (f) X-ray image taken 52 hours after recovery; the TAM was detached at this point.



Fig. 11 Histology microscope images. (a) Microscope image of the intestinal tissue at the attached position. (b) Control tissue collected 10 cm orally from the attached position. (c) Control tissue collected 10 cm aborally from the attached position.