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# Microbiota Sampling Capsule: Design, Prototyping and Assessment of a Sealing Solution Based on a Bistable Mechanism

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*The objective of the presented work is to allow non-invasive sampling of microbiota, while today surgery is still needed to collect intestinal liquid samples. Given the recent developments of endoscopic capsules and their added value for the diagnosis and the treatment of gastrointestinal pathologies, the paper is focused on the design of a microbiota sampling capsule. The device has to offer an efficient collection of intestinal liquid and at the same time to protect it from any contamination as the device navigates out of the human body. To ensure this and safety at the same time, a passive capsule is developed based on a bistable mechanical structure actuated by an expanding foam that itself collects the intestinal fluid. The device is developed using additive manufacturing. It is shown the process is compatible with the production of the complete capsule with biocompatible material, while ensuring the presence of bistability for capsule closing. In-vitro trials validate the operating mode of the capsule. This work represents a significant step towards the creation of simple and yet efficient tools to better understand microbiota impact and future pathology detection.*

## 1 Introduction

The microbiota consists of a collection of microorganisms such as micro-fungi, bacteria and viruses that live in the microbiome, which is a specific environment hosted by a plant or an animal host [1]. There are several types of microbiota in the human body, including the skin microbiota which is the collection of microorganisms living on the skin, the vaginal microbiota which is found in the vagina and the intestinal microbiota which is located in the gastrointestinal (GI) tract. In this paper, we are interested in the intestinal microbiota of humans and more specifically that located in the small intestine which is the host of the largest number of bacterial species in the human body. The intestinal microbiota is dominated by a composition of five bacterial species. The concentration of these bacteria varies depending on its location in the small intestine which is composed of three sections illustrated in Fig. 1 [2]. In the duodenum and jejunum, the concentration of bacteria varies between  $10^3$  and  $10^4$  per mL. In the ileum, the number of bacteria is around  $10^8$  per mL [1].

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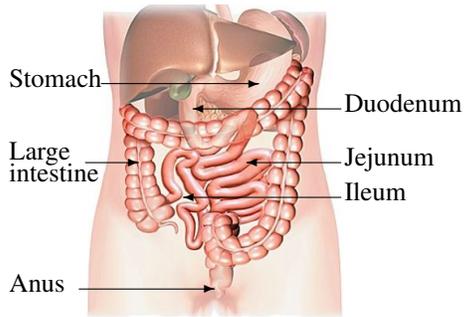


FIGURE 1: Representation of the small intestine's sections and its neighboring organs [3].

### 1.1 Relation Between the Microbiota and Pathologies

Described as the second brain in the human body, the intestinal microbiota play an important role in maintaining health as its balance helps prevent and treat several diseases. Recent research has proven that there is a fundamental relationship between the imbalance of the intestinal microbiota, also known as dysbiosis, and the detection of several pathologies such as obesity [4], type 2 diabetes [5], chronic inflammatory bowel disease [6] and colorectal cancer [7]. Dysbiosis can also affect human mood and behavior and can even lead to stress and depression [8].

Therefore, it is important to collect the intestinal microbiota to advance basic clinical research, understand the clinical relevance and, in the future, help identify the pathologies described above.

### 1.2 Conventional Microbiota Sampling Method

Two methods have been developed for microbiota sampling. The first one consists of collecting the human stool in order to remove bacteria, culture them and determine DNA sequences which will be used to identify the dysbiosis [9,10]. Although this method is non-invasive, it represents two major limitations. Only 50% of the intestinal bacteria can be cultured due to precise oxygen requirements which means that several species of intestinal bacteria cannot be analyzed. In addition, the intestinal environment is not homogeneous, which means that some bacteria species cannot be found in the stool. Therefore, this method does not allow the collection of all the information needed to discover and identify the intestinal environment.

The second method of microbiota sampling consists of making an incision in the small intestine in order to access it and take a sample [6]. It is often performed simultaneously with other surgical operations on the abdomen [10]. Although this method is precise, it remains very invasive and uncomfortable for the patient.

### 1.3 Capsules Used for Microbiota Sampling

In order to have an accurate and non-invasive sampling, the bacteria must be collected directly from the small intestine with a tool that gains access without creating any complications for the human body or contamination of the sam-

pled bacteria. Swallowable devices also known as "capsules" have been considered for microbiota sampling.

A capsule is a swallowable device originally developed to inspect the digestive tract. It has a cylindrical shape and dimensions adapted to ingestion: a maximum of 11 mm for the diameter and 26 mm for the length [11]. It facilitates the access to different sections of the gastrointestinal tract in a non-invasive way which makes it an interesting concept that was used by many researchers to perform different functions in the digestive tract such as locomotion, localisation, diagnostic and treatment [12].

One of the first capsules for microbiota sampling was developed in 1999 by Gu et al. [13]. It has an oval shape with a diameter of approximately 10 mm. The operating mode of this capsule is based on the biological interactions, mainly the change in pH in the gastrointestinal tract, of the latter and the intestinal environment. Although this capsule is non-invasive, the sealing of its orifice remains weak because it has no resistance to the reopening of the device. Therefore, the absence of contamination cannot be guaranteed.

In 2014, Amoako-Tuffour et al. introduced two concepts of intestinal microbiota samplers composed of sample storage chambers, a motor that actuates the opening and the closing of the latter and a power unit based on the use of batteries [14]. Although the integration of several power supply and communication modules allows for precise sampling, these devices do not have the capacity to potentially store more liquid. The presence of electronic components is, in addition, a concern in terms of risk analysis for the device.

Recent researchers have opted for the development of passive devices. Shape memory spring [15,16], foam or even hydrogel [17] have been considered to actuate and target the sampling task in the small intestine. To optimize the precision and localization of the sample in such passive capsules, magnetic actuation systems have been developed in [18,19]. In 2017, a research team from the laboratory TIMC-IMAG (Grenoble, France) developed a microbiota sampling capsule [20]. The device is composed of an elastic membrane and a foam for intestinal liquid absorption. The membrane has an entry orifice which is closed after sampling by a locking system as shown in the example in Fig. 2. The foam used to collect the intestinal liquid is also being used as an actuator that triggers the locking system. The use of foam is of great interest because it is biocompatible and can rapidly absorb the microbiota. However, the closing force will be completely constrained by the choice and the volume of the foam.

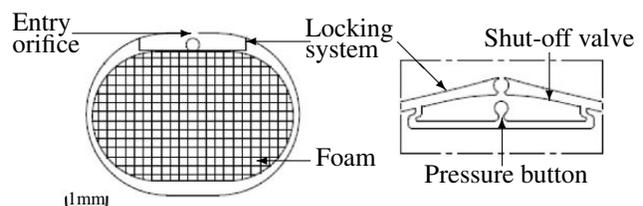


FIGURE 2: Concept of the capsule proposed by [20] for microbiota sampling.

Given the capsules presented in this state of the art summary, the capsule that we propose must ensure the sampling of microbiota while guaranteeing the sealing of its closing and its resistance to any force applied by the gastrointestinal tract after sampling. To eliminate electrochemical risk and reduce sources of risks, we avoid the integration of electronic components and batteries in the device. In addition, the risk of capsule blocking is limited by respecting maximum standard sizing and the risk of toxicity is reduced by considering biocompatible materials. Our goal is to collect the microbiota from a part of the small intestine with a non-invasive, non-contaminating and less expensive device.

Following preliminary work in [21], we present a microbiota sampling capsule that operates in the human small intestine and is used for diagnostic purposes. We develop an approach based on the use of a foam to both store the intestinal liquid and actuate the closing of the capsule. To do so, we need a structure which can be passively triggered by the foam and can resist reopening. A bistable mechanism where the closing energy is a mechanical energy stored in it is the most suitable structure to be used [22, 23]. In section 2, we elaborate on the design requirements that the capsule must meet, followed by its components the most important of which are the foam used as an actuator and the bistable mechanism used to close the capsule. In addition, the operating mode of the capsule in the gastrointestinal tract is described.

Section 3 is dedicated to the characterization of the actuator in order to determine the force applied by the foam and its force-displacement characteristic. We focus, in section 4, on the modeling of the bistable mechanism by an analytical model, the manufacturing process as well as the experimental characterization. Finally, section 5 is dedicated to the prototyping of the capsule in its final version and the validation of its operating mode by in-vitro trials.

## 2 Microbiota Sampling Capsule

### 2.1 Key Requirements

The design of the microbiota sampling capsule must respect certain constraints imposed by the gastrointestinal environment, the safety of the patient and the protection of the sample. The capsule will be admitted into the human body through the mouth. Therefore, it must be able to transit through the gastrointestinal tract without creating any complication for the patient. Its dimensions must not exceed the maximum dimensions which are those of the largest swallowable pharmaceutical capsule 000 according to the capsule size classification standard [24, 25], i.e. 9.5 mm for the diameter and 26.1 mm for the length. Here our capsule has a diameter of 9 mm and a length of 26 mm.

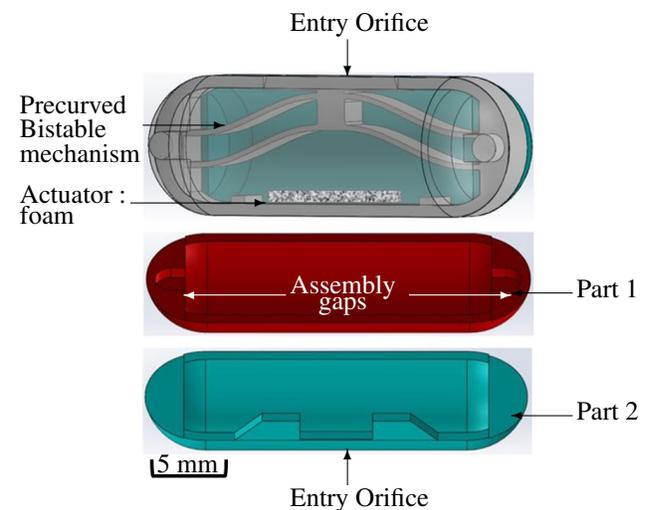
While passing through the stomach, the capsule must withstand the acidity of the gastric environment which has a pH close to 2 [26]. Once in the small intestine, where the pH level is higher and close to 7 [27], the capsule must open to collect the sample of intestinal microbiota. It is necessary to ensure that the intestinal liquid can penetrate into the capsule considering that it has a viscosity of approximately 0.085 Pa·s [28, 29]. The sampled volume must be sufficient

to analyze the bacteria species located in the small intestine. For this, a volume of 0.20 mL is adequate because it represents approximately  $2.0E+7$  of bacteria [30].

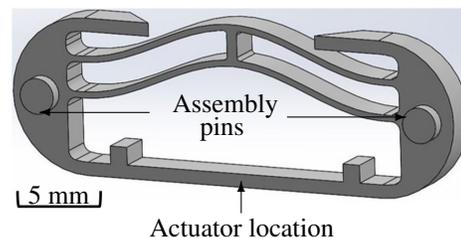
Afterwards, the capsule transits through the rest of the gastrointestinal tract to exit the human body. At this stage, it is important to guarantee its sealing to protect the sample from contamination. In total, the transit of the capsule takes approx. 5 hours to arrive at the sampling location [31]. When passing through the anus, the capsule must resist reopening considering that the anal sphincter can apply a pressure that can go up to 0.02 MPa [32].

### 2.2 Capsule Components

In order to satisfy the key requirements described above, we have chosen to develop a capsule controlled and closed in a passive manner. It measures 9 mm in diameter and 26 mm in length and is composed of a shell that has an entry orifice. The shell is composed of two parts as shown in Fig. 3a.



(a) Main components of the microbiota sampling capsule.



(b) CAD of the bistable mechanism as manufactured in its first stable position.

FIGURE 3: Components of the microbiota sampling capsule.

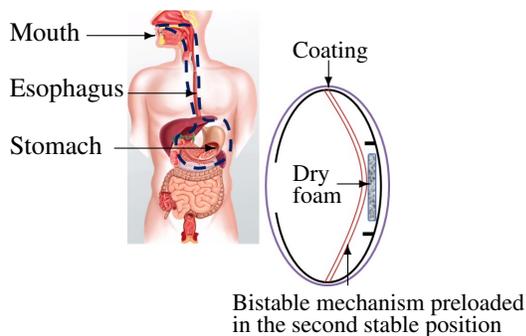
A PVA foam (Polyvinyl Alcohol) is used to both store the intestinal liquid and actuate the bistable mechanism to close the capsule [20]. As the name suggests, the bistable structure (shown in Fig. 3b) has two stable positions: one for opening the capsule and the other one for sealing it once the sampling is done. This structure meets our needs as it has a configuration that stores the mechanical energy used to ensure closing in an instantaneous way rather than a progres-

sive way. At the same time, it offers a resistance to reopening which is used to guarantee the protection of the sample from contamination and/or loss. In addition, the structure is manufactured precurved in its first stable position. Therefore, there is no need to buckle the beams during assembly which is important to get a repeatable behavior.

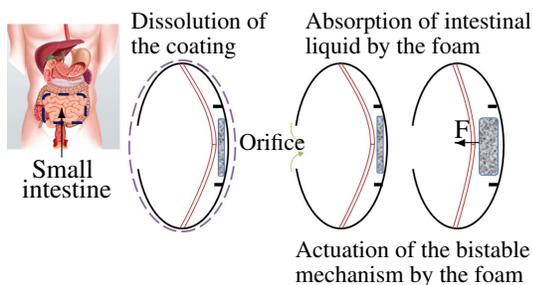
The capsule is made in three parts and assembled by the pins and gaps that are located respectively in the bistable mechanism and the part 2 of the capsule shell, as shown in Fig. 3a and Fig. 3b.

### 2.3 Operating Mode

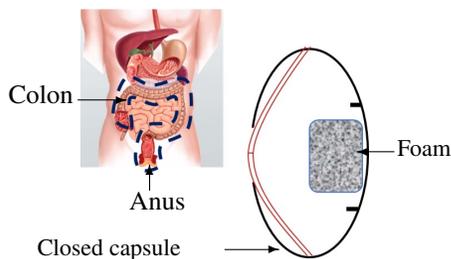
When transiting through the gastrointestinal tract, the capsule follows the scenario described in this section. In its initial state, the capsule shell is assembled with the bistable mechanism. The latter is preloaded in the second stable position for which the capsule remains open as shown in Fig 4a.



(a) Initial state of the capsule after assembly.



(b) Operating mode of the capsule in the small intestine.



(c) Final state of the capsule after sampling.

FIGURE 4: Scenario followed by the capsule in the gastrointestinal tract.

The capsule is initially surrounded by a gastro-resistant

gelatin coating. It protects the capsule during its transit through the stomach and it dissolves at a pH close to 7 which is that of the small intestine. Although the coating does not allow to precisely control the time or place of capsule opening, the variation in pH is sufficient to sample in a relevant zone in terms of analysis of intestinal bacteria. In addition, it is possible to choose a coating thickness to ensure opening in the small intestine or to ingest several capsules with different thicknesses of the soluble coating allowing samples to be taken from various places.

Thus the capsule reaches the small intestine, the pH level gradually increases which causes the coating to dissolve until it totally disappears. The capsule is then open, allowing intestinal liquid to enter through the entry orifice. Once in contact with the liquid, the foam absorbs it and swells to provide a sufficient force to trigger the bistable mechanism and close the capsule. These steps are shown in Fig. 4b and Fig. 4c.

## 3 Actuator Selection

### 3.1 Actuator Description

Here the actuator used in the microbiota sampling capsule is a PVA foam which is a high absorbency material used in surgeries [33]. It is also used to store the sampled intestinal liquid. It is biocompatible and can quickly absorb a sufficient volume of microbiota while tripling its volume [20].

In this section, we will focus on the characterization of the foam to be integrated into the capsule using an experimental method in order to obtain the generated force according to its expansion. The intestinal environment considered, in this experiment, consists of water. Later, when the validation of the capsule sealing is carried out, a liquid of the same characteristic as that of the human intestinal fluid will be used.

### 3.2 Experimental Characterization of the foam

The experimental characterization of our actuator consists of determining the force applied by the foam considering its dimensions which are constrained by its integration into the capsule. The identification of these dimensions are constrained by those of the capsule and the bistable mechanism. The foam must be positioned below the bistable mechanism to guarantee its actuation as shown in Fig. 3a. Therefore, its dimensions must not exceed a maximum length of 13 mm, a width of 5 mm and a height of 2 mm. The initial height of the foam is set at 10 mm before compression with a compression ratio equal to 5 which facilitates its integration into the capsule.

An experimental setup is made to obtain the force provided by the foam. It is composed of a laser-based displacement sensor (LK-H152, Keyence) that has a measuring range of 80 mm with a resolution of 0.25  $\mu\text{m}$ , a bending beam for which the stiffness has been initially determined and a mounting system for both the beam and the foam. The experimental setup is illustrated in Fig 5.

Initially the foam is compressed and placed, with zero contact, below the beam as shown in Fig. 5. A volume of

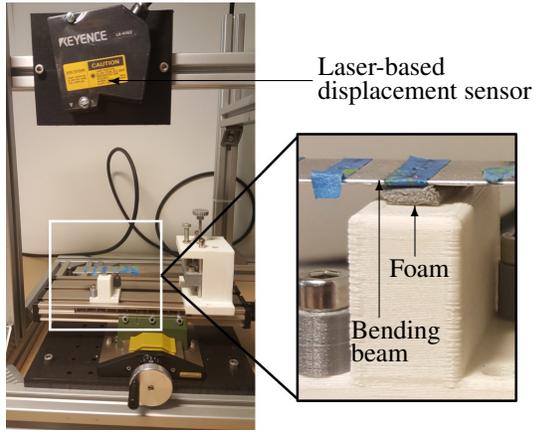


FIGURE 5: Experimental setup for the actuator characterization.

0.20 mL of water is absorbed. Afterwards, the foam swells, generates a force and pushes the beam which displacement is obtained by the laser sensor. As the stiffness of the beam is initially determined, the force applied by the foam can be easily calculated from the beam deflection.

### 3.3 Results

The foam can generate a maximum force of 1.5 N. Figure 6 shows the curve of the force provided by the foam in relation to its expansion from the compressed height. The foam height can grow up to an additional 3 mm. When the foam expands, the force decreases to a minimum value of 0.45 N. The force obtained will be used in the following section to determine the dimensions and characteristics of the bistable mechanism.

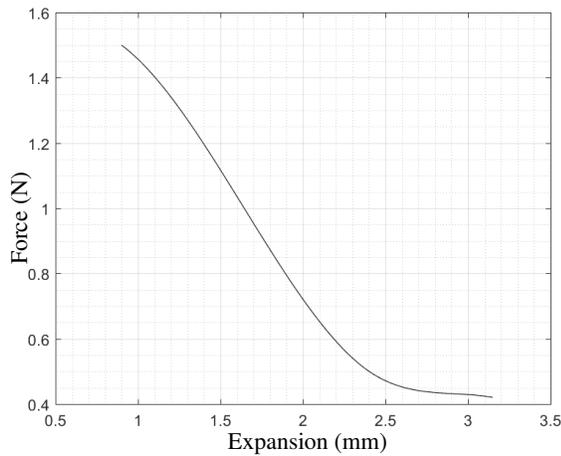


FIGURE 6: Curve of the force provided by the foam as it expands from its compressed form.

## 4 Design of the Sealing Mechanism

### 4.1 Definition and Geometry

A bistable mechanism is a structure which has two stable positions. The one used in the microbiota sampling capsule is composed of the superposition of two beams initially manufactured preshaped curved and connected together with a shuttle as shown in Fig. 7. This prevents the shuttle from rotating when the mechanism is triggered and also ensures its bistability [34].

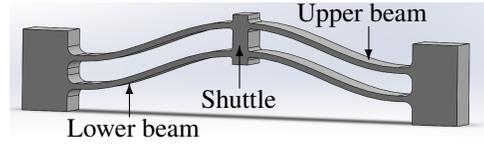


FIGURE 7: CAD of a bistable mechanism composed of the superposition of two preshaped curved beams.

The neutral fiber of the preshaped curved beams follows an initial shape which is that of the first buckling mode of a straight beam as illustrated in Fig. 8, and given by the following expression:

$$\bar{w}(x) = \frac{h}{2} [1 - \cos(2\pi \frac{x}{L})] \quad (1)$$

where  $\bar{w}(x)$  the vertical position of the beam's neutral fiber in the first buckling mode,  $L$  the length of the preshaped curved beam,  $t$  its thickness,  $b$  its width and  $h$  its initial height.

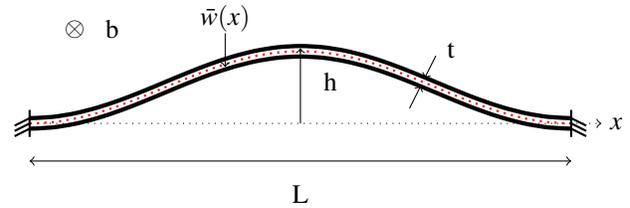


FIGURE 8: Preshaped curved beam at the initial position.

The theoretical behavior of this structure can be described by the force-displacement curve shown in Fig. 9. It shows two stable equilibrium positions represented in black (Position 1 and Position 2) and one unstable position represented in white. A maximum force  $f_{top}$  is necessary to transition from the first stable position to the second one. A lower force  $f_{bot}$  is needed to go from the second position to the first one.

As shown in Fig. 9, the force-displacement curve is asymmetrical due to the fact that the mechanism is initially manufactured in its first stable position. This asymmetry is advantageous because the mechanism can be triggered by a

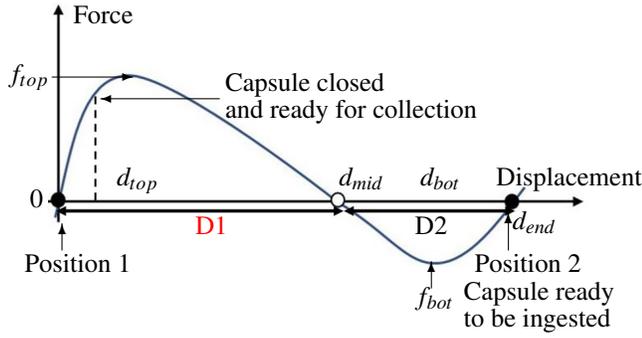


FIGURE 9: Force-displacement curve of a bistable mechanism, measured at its center.

low force  $f_{bot}$  which is supplied by the foam. On the contrary, the large value of  $f_{top}$  helps to seal the capsule.

To allow the bistable mechanism to be preloaded into its second stable position, it is important to keep a sufficient distance between the first stable position and the bottom of the frame as shown in Fig. 10 so that the bistable mechanism can overcome the unstable equilibrium position.

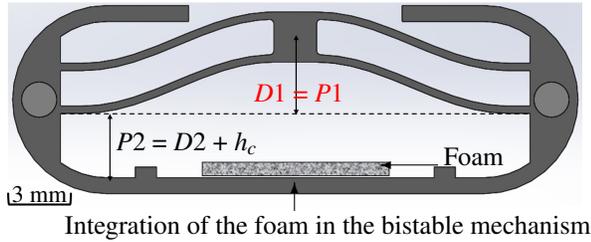


FIGURE 10: Positioning of the bistable mechanism represented in its first stable position.

According to Fig. 9, the distance between the first stable position and the unstable equilibrium position is  $D1$  and the one between the unstable equilibrium position and the second stable position is  $D2$ . It is also important to take into consideration the compressed height of the foam  $h_c$ . Therefore, the height of the foam is added to the distance  $D2$  to determine the position of the bistable mechanism between the unstable equilibrium position and the second stable position.

The two positions ( $P1$ ) and ( $P2$ ) corresponding to the distances ( $D1$ ) and ( $D2 + h_c$ ) are respected in the positioning of the bistable mechanism as shown in Fig. 10.

#### 4.2 Modeling

In order to determine the dimensions of the bistable mechanism to be integrated into the capsule, sizing constraints must be respected as well as the actuation forces. The dimensions shown in Fig. 8 can be calculated using an analytical model developed by Hussein in [35] and based on the buckling phenomenon [36].

To guarantee that the structure has a bistable behavior, the ratio  $Q$  between its initial height  $h$  and its thickness  $t$

must exceed 2.31. Once this condition is met, the mechanism can be characterized by the force-displacement curve of its shuttle as shown in Fig. 9.  $f_{top}$  and  $f_{bot}$  are the forces needed to trigger the structure and switch between the two stable positions.  $d_{top}$  and  $d_{bot}$  are the displacements of the shuttle when maximum forces are applied.  $d_{mid}$  and  $d_{end}$  are the displacements for a zero force.

The value of these forces and displacements can be calculated using the following expressions [35]:

$$d_{top} = h \left( \frac{28}{27} - \frac{2\pi}{3} \sqrt{\frac{1}{6} + \frac{16}{81\pi^2} - \frac{1}{Q^2}} \right) \quad (2)$$

$$d_{bot} = h \left( \frac{28}{27} + \frac{2\pi}{3} \sqrt{\frac{1}{6} + \frac{16}{81\pi^2} - \frac{1}{Q^2}} \right) \quad (3)$$

$$d_{end} = h \left( \frac{3}{2} + \sqrt{\frac{1}{4} - \frac{4}{3Q^2}} \right), \quad d_{mid} = 1,33h \quad (4)$$

$$f_{top} = 64\pi^2 \frac{EIh}{L^3} \left( \frac{4}{3} - \frac{d_{top}}{h} \right) \quad (5)$$

$$f_{bot} = 64\pi^2 \frac{EIh}{L^3} \left( \frac{4}{3} - \frac{d_{bot}}{h} \right) \quad (6)$$

with  $E$  the Young's modulus of the material,  $I$  the quadratic moment and  $Q = \frac{h}{t}$  the bistability ratio.

Although the  $f_{top}$  and  $f_{bot}$  forces play a very important role in determining the dimensions of the bistable mechanism, other dimensional constraints also have an impact on this choice. The initial height  $h$  is determined by taking into consideration the bistability ratio  $Q$  and the integration of the mechanism in the capsule. As for the value of the length  $L$ , it is impacted by the dimensions of the capsule as well as its assembly. Finally, the thickness  $t$  is determined by the manufacturing parameters.

Taking into consideration these dimensional constraints and the force provided by the foam to trigger the mechanism and close the capsule, a set of parameters for the bistable mechanism is determined and given in Table 1.

The force-displacement characteristic of this mechanism is determined using the analytical model. The force value of  $f_{top}$  is 8.2 N and that of  $f_{bot}$  is 4 N.

TABLE 1: Geometric parameters of the bistable mechanism.

Parameter	t	b	L	h
Value	0.35 mm	2 mm	18 mm	2.4 mm

### 4.3 Additive Manufacturing with Fused Deposition Modeling

Additive manufacturing using Fused Deposition Modeling (FDM) is chosen to manufacture the prototype of the capsule and the bistable mechanism due to its several advantages. FDM deposits a fused filament in successive layers through a nozzle. Therefore, the final part consists of a stack of thin layers [37]. It is of great interest because it offers the possibility of using biocompatible materials (e.g. ABS-M30i, Stratasys, Ltd) which is very important considering the use of the capsule in the human body [38, 39]. In addition, it enables rapid manufacturing of the prototype, which accelerates the design cycle at a relatively low cost.

The capsule parts are manufactured on a centimeter scale with the Fortus 400mc industrial printer (Stratasys, Ltd) using the T12 nozzle (diameter = 0.178 mm). It is manufactured by depositing a contour that has a thickness of 0.35 mm. Therefore, it is necessary to understand and master the process as well as the behavior of the parts after the manufacturing.

The first manufacturing iterations revealed problems with the shuttle and the surfaces between the latter and the beams which present several gaps [40]. In order to master the filament deposit used to manufacture the parts, certain parameters must be identified and optimized, i.e.  $e_{cc}$  the gap between the deposited contours,  $e_{cr}$  the gap between the contour and the filling and  $e_{rr}$  the gap between the filling deposited. Finally, experimental trials allowed the identification of adequate values of each parameter for successful parts manufacturing as shown in Fig. 11. We used  $e_{cc} = -0.05$  mm,  $e_{cr} = -0.005$  mm and  $e_{rr} = -0.05$  mm which guarantees optimal filling of the printed parts.

Dimensional measurements for the length  $L$  and the initial height  $h$  were carried out to determine the dimensional accuracy of the manufactured mechanisms. The total length of the structure is equal to an average of 21.4 mm with a maximum deviation of 0.40 mm and the initial height is equal to an average of 2.37 mm with a maximum deviation of 0.13 mm. Following these results, the bistability ratio  $Q$  and the forces  $f_{top}$  and  $f_{bot}$  of the mechanisms manufactured with FDM are verified. The average value of the ratio  $Q$  is equal to 6.7 which guarantees the bistable behavior.  $f_{top}$  is equal to 6.5 N and  $f_{bot}$  is equal to 3.1 N.

### 4.4 Experimental Characterization

Manufacturing the bistable mechanism has been proved achievable at the centimeter scale by FDM. However, this technology can have an impact on the force-displacement characteristic of the mechanism. In this section, we carry

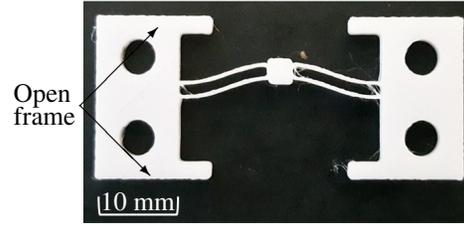


FIGURE 11: Bistable mechanism manufactured with Fused Deposition Modeling with  $e_{cc} = -0.05$  mm,  $e_{cr} = -0.005$  mm and  $e_{rr} = -0.05$  mm.

out an experimental characterization of mechanisms made by FDM to study its influence on the bistability and the force-displacement curve.

In order to conduct the experimental characterization of the bistable mechanism, a dedicated setup was built as shown in Fig. 12. It is composed of a laser-based displacement sensor (LK-H152, Keyence) that has a measuring range of 80 mm with a resolution of  $0.25 \mu\text{m}$ , a force sensor (FK50, Sauter) with a maximum force of 50 N and an accuracy of 0.02 N and a mounting bracket to fix the bistable mechanism.

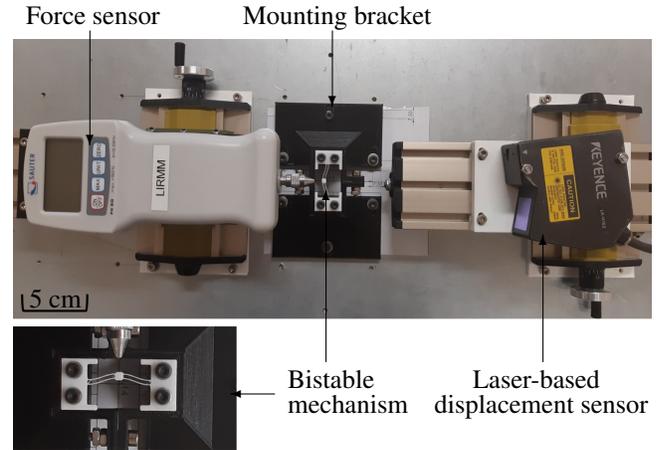
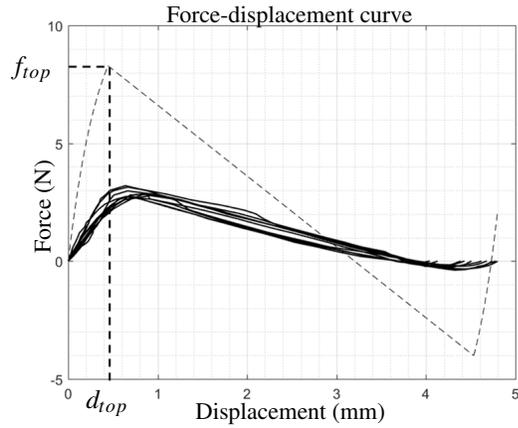


FIGURE 12: Experimental setup for the characterization of the bistable mechanisms

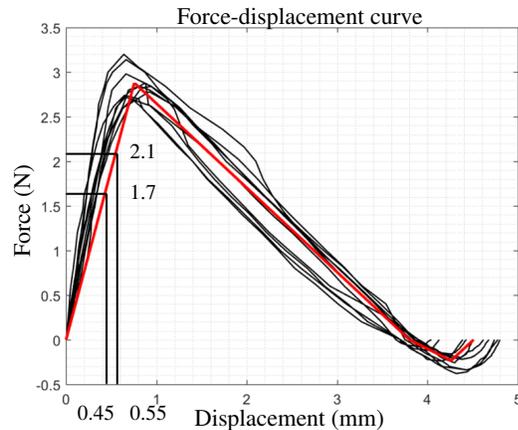
For this characterization phase, ten bistable mechanisms are manufactured with the manufacturing parameters determined above. They are made with an open frame, as shown in Fig. 11, to allow both measurement and actuation. A force is applied to the bistable mechanism by moving the linear table on which the force sensor is mounted and the corresponding displacement is measured using the laser sensor. Once the positive forces are determined, the bistable mechanism is reversed to determine the negative forces.

The bistable behavior of each mechanism is characterized by determining the force-displacement curve. These results are compared to the force-displacement curve obtained by the analytical model and presented in Fig. 13a.

The experimental characterization shows a difference of



(a) Force-displacement curves of the bistable mechanisms: the continuous curves are obtained by the experimental characterization in comparison with the dashed curves, which was obtained by the analytical model.



(b) Force-displacement curves: the black curves represent the experimental results for each mechanism and the red curve is the average of the experimental data.

FIGURE 13: Results of the experimental characterization of the bistable mechanisms.

5.29 N for  $f_{top}$  and 3.6 N for  $f_{bot}$ . Two mechanisms broke at the shuttle of the structures after 6 triggering cycles. The values of the actuating force  $f_{bot}$  are remarkably low for mechanisms manufactured by FDM as they do not exceed 0.38 N. The difference between these results may be related to the impact of FDM such as the variation of the Young's modulus after manufacturing as well as the geometric proportions of the bistable mechanism [40]. However, this does not affect the operating mode of the capsule because  $f_{bot}$  is smaller than the force provided by the actuator which can reach 1 N. As for  $f_{top}$ , we must experimentally verify that this force is sufficient to guarantee the sealing of the capsule which will be conducted in the next section.

## 5 Validation of the Microbiota Sampling Capsule

The experimental characterization of the bistable mechanism obtained previously is used in this section to up-

date the design of the capsule according to these results and to propose a closing cap that guarantees its sealing. The improved version of the capsule is then manufactured and used to validate the operating mode using an artificial intestinal liquid. At this stage, it is important to use such a liquid in order to validate the ability to seal the capsule when the surrounding fluid is high viscosity intestinal fluid.

### 5.1 Design Update

In order to improve the design of the capsule, first we refine that of the bistable mechanism based on the results of the experimental characterization. This step is very important because it allows the definition of the shape and the dimensions of the closing cap that will be added to the bistable mechanism.

Therefore, the positioning of the bistable mechanism in the capsule is modified while respecting two important aspects to its proper functioning which are ensuring the preloading of the bistable mechanism in its second stable position and the integration of the foam below it as described in section 4. According to the experimental result, the distances  $D1$  and  $D2$ , illustrated in Fig. 10, must be equal to respectively 3.7 mm and 1.2 mm.

The second step consists in incorporating a closing cap to the bistable mechanism as shown in Fig. 14. It will not only ensure the sealing of the orifice but it will also apply a sufficient pressure to prevent the capsule from reopening. The cap is of rectangular shape and of dimensions 2 mm x 5 mm and is used to close an orifice of dimensions 1 mm x 3 mm.

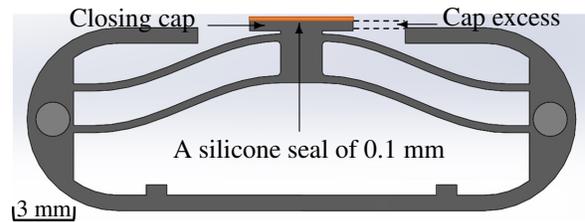


FIGURE 14: Design of the closing cap added to the bistable mechanism to ensure the sealing of the capsule.

As for the pressure applied by the cap, its value is determined from the force-displacement curve of the bistable mechanism. Theoretically, the point  $(d_{top}, f_{top})$ , illustrated in Fig. 13a, corresponds to the maximum and ideal force to ensure the sealing of the capsule and its resistance to reopening. However, due to dimensional constraints, we do not implement the cap at this maximum position but rather in its vicinity which nevertheless corresponds to an important force. To do so, the cap is designed such that when the capsule is sealed, the bistable mechanism exerts a maximal locking force of 1.7 N according to Fig. 13b. With this force, the mechanism is able to apply a pressure of 0.17 MPa to a surface of 5 mm x 2 mm. The closing cap is combined with a 0.1 mm thick silicone seal (composed of methyltriacetox-

silane and triacetoxethylsilane) to prevent liquid from entering. With the additional seal and according to Fig. 13b, the bistable mechanism can exert a pressure which varies from 0.17 MPa to 0.21 MPa, i.e. 10 times the pressure encountered in the sphincter.

## 5.2 Prototype Manufacturing

For the capsule for in-vitro validation, the same parameters detailed in section 4 were used. A polycarbonate filament (diameter = 1.78 mm) supplied by Stratasys, Ltd is the material used for prototyping. Before assembling the capsule, a layer of silicone is deposited on the cap of the bistable mechanism as shown in Fig. 15a.

Afterwards, the first part of the capsule body is assembled to the bistable mechanism. Then, the mechanism is preloaded in its second stable position and the actuator i.e. a standard PVA foam, used for medical purposes, is inserted below without having any contact with it as shown in the first image of Fig. 15b. Finally, the capsule assembly is completed by inserting it in a 000 gelatin coating which is an empty pharmaceutical capsule.

The intestinal liquid used for the in-vitro trial is a special liquid that makes it possible to reproduce as closely as possible the conditions encountered by the capsule in the intestinal environment. Fasted State Simulated Intestinal Fluid (FaSSIF) provided by Biorelevant.com Ltd, a fluid considered for solubility studies and drug testing [41], is used.

## 5.3 Validation of the Operating Mode of the Capsule

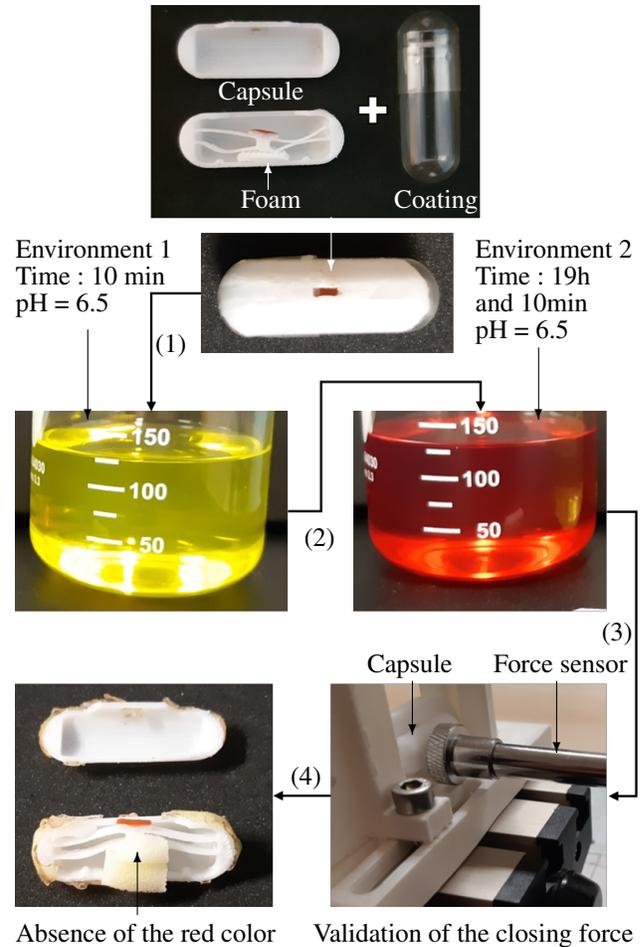
As shown in Fig. 15b, the setup dedicated to the in-vitro validation is composed of two recipients which represent the most important stages of the operating mode of the capsule in the gastrointestinal tract: a first intestinal environment for the sampling process and a second one after the sampling process. In the first intestinal environment which is colored in yellow and has a pH of 6.5, the capsule must be open after the coating has dissolved to take a sample from the intestinal liquid and then close by means of the bistable mechanism triggered by the swollen foam. Once in the second intestinal environment, which is colored in red and has also a pH of 6.5, the sealing of the capsule is verified. During its transit through environments 1 and 2, the capsule did not undergo pressure that imitates the intraluminal pressures of the gastrointestinal tract because the latter are smaller than the anus pressure [42, 43].

Finally, to mimic the force applied by the anus and validate the resistance of the bistable mechanism to the reopening, a maximum force of 0.2 N [32] is applied to the entry orifice. The anus pressure is obtained using a force sensor mounted on an XY stage. Initially, the sensor is placed in front of the orifice with zero contact. Then, a force of 0.2 N is successively applied to the orifice by advancing the sensor.

Concerning the timing, once immersed in the first intestinal environment (step (1) in Fig. 15b), the coating of the capsule immediately begins to dissolve. It takes seven minutes for the coating to completely disappear and uncover the entry orifice. When in contact with the intestinal liquid and



(a) Final version of the bistable mechanism.



(b) The in-vitro validation of the microbiota sampling capsule.

FIGURE 15: Capsule assembly and in-vitro validation of its operating mode.

after exactly three minutes, the foam swells to trigger the bistable mechanism and close the capsule. Afterwards, the capsule is put into the second intestinal environment with a transition time of 30 seconds (step (2) in Fig. 15b). It remains in this environment for 19 hours and 10 minutes. This time is determined according to the standard ISO 10993-1:2018 [44] and the transition time of the capsule between the mouth and the small intestine [31]. Finally, pressure from the anus is simulated on the capsule before checking for contamination (step (3) in Fig. 15b). The capsule is then opened for inspection (step (4) in Fig. 15b). As shown in the last image of the same figure, the absence of the red color in the capsule validates sealing performance. The quantity of the liquid absorbed by the foam during this experiment is 0.4 mL, which

corresponds to  $4.0E+7$  bacteria and is sufficient for the analysis of intestinal bacteria.

## 6 Conclusion

In this paper, we proposed a non-invasive microbiota sampling capsule that combines the use of a bistable mechanism and additive manufacturing. Using the interaction between a gelatin coating and the intestinal environment, the capsule collects a liquid sample from the small intestine. It does not integrate electronic components or batteries, which makes it affordable, eco-friendly and disposable. First, the capsule components were presented. Then, the operating mode performed by the capsule in the gastrointestinal tract is detailed. To guarantee its proper functioning, we carried out an experimental characterization of the actuator that measured its force-displacement curve.

Afterwards, we focused on the bistable mechanism used to close the capsule. A model is used in order to size the structure. Since we use FDM to manufacture the capsule and the bistable mechanism, we studied the impact of this technology on the bistable behavior with an experimental method. FDM has a significant impact on the value of the triggering forces  $f_{top}$  and  $f_{bot}$  compared to the analytical ones. However, the results show that the bistable behavior is guaranteed. Some future work could consist of developing modified models to take into account additive manufacturing impact on the bistable behavior.

Finally, the design of the capsule is updated according to the experimental results. A final prototype is then manufactured and used to validate its operating mode with an in-vitro trial using an artificial intestinal liquid proving the success of the capsule operating mode and validating the quantity of the sampled liquid.

We also validated in section 5 the sealing of the capsule in order to protect the sample from contamination. It is of course now important to ensure that the bacteria collected are properly stored in order to be analyzed and to avoid their degradation by using a storage substance. In addition, it is possible to consider different coating thicknesses, so the opening of the capsule can happen at different locations, i.e. at different positions in the intestine. Therefore, a study on the relationship between the thickness of the coating and the time of its dissolution is also part of future work. Perspective of this work will thus concern this validation, before production with biocompatible material to go toward in vivo trials. These latter will be of great importance to build statistics on the transit kinematics of the capsule in the GI tract and therefore define more precisely the sampling location and effective achievable sampling volume. In addition, in-depth risk analysis will be carried out.

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