Medical Research Archives

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Published: July 10, 2023

Citation: Bianchi A, Francia G, Carletti V, Gigola F, et al., 2023. Ingestible capsules carrying 3D printed springs: a possible future prospective for Short Bowel Syndrome treatment? Medical Research Archives, [online] 11(7.1). https://doi.org/10.18103/mra.v11i7.1.3846

DOI: https://doi.org/10.18103/mra.v11i7.1.3846

ISSN: 2375-1924

RESEARCH ARTICLE

Ingestible capsules carrying 3D printed springs: a possible future prospective for Short Bowel Syndrome treatment?

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Abstract

Background: Short Bowel Syndrome (SBS) is a malabsorption syndrome characterised by a severe reduction of the absorbent surface of the intestinal mucosa. Treatment of this condition needs multi-professional teams and different therapies, which are not always enough to ensure enteral autonomy. New techniques are being explored, particularly distraction enterogenesis, which can allow the lengthening of the residual intestines of these patients. This study aims to demonstrate the possibility of using biodegradable materials to design ingestible capsules which carry 3D-printed springs capable of reaching the patient's intestine. These devices could be used as a slightly invasive distraction enterogenesis technique, stimulating cell proliferation and intestinal elongation without surgery.

Materials and methods: Capsules were realised with gelatin from pigskin type A from Sigma-Aldrich mixed with regenerated silk fibroin (RS) obtained by reverse engineering on Bombyx Mori cocoons. Springs are composed of a structure of regenerated silk (RS) modified with graphene nanoplatelets (GNP) externally covered with a biodegradable polyhydroxybutyrate-valerate (PHBV) shell. Springs were realised with 3D printing, through which, with an extruder, polyhydroxybutyrate-valerate and regenerated silk compounds are deposited simultaneously in a 3D structure. The springs' capsules were then analysed with solvents simulating the gastric and intestinal environment to verify their resistance to degradation. Phosphate Buffered Saline (PBS), composed of calcium chloride and magnesium chloride ($CaCl_2$ + $MgCl_2$), with a pH value of 7.4, was used as a degradative agent; for the gastric tract, we chose the acetic acid, CH_3COOH, at 12% with a pH value of 2.3.

Results: While the gelatin-only capsules showed poor resistance to degradation in Phosphate Buffered Saline, the new compound based on gelatin and regenerated silk showed excellent resistance in gastric and intestinal environments, allowing the pills to reach the intestine without dissolving. In addition, the results show variability in the release times of the springs as a function of the pH values and the elastic constants of the springs used: the latter determined that in acetic acid, the release time is increased at an increase of the elastic constant. In contrast, in Phosphate Buffered Saline, an opposite trend was observed.

Conclusions: Our results confirm the possibility of using gelatin, silk fibroin and polyhydroxybutyrate-valerate to design devices capable of transporting implantable endoluminal 3D structures, drugs, or growth factors, laying the foundations for a new approach to distraction enterogenesis in Short Bowel Syndrome (SBS) patients.

Keywords: Intestinal Failure, biodegradation, silk fibroin, pig gelatin

Introduction

Short bowel syndrome (SBS) is a rare disorder characterised by malabsorption of nutrients because of severe bowel loss. After losing an extensive small bowel length, the patient develops gastrointestinal (GI) symptoms such as diarrhoea, steatorrhea, weight loss, malnutrition, and dehydration. Short bowel syndrome is the leading cause of Intestinal Failure (IF), and it involves the need for long-term parenteral nutrition (PN) to ensure patients' nutritional needs[1–4].

Short bowel syndrome is a multi-systemic disease, and its treatment entails multidisciplinary teams involving surgeons, dieticians, gastroenterologists, specialised nurses, and psychologists. Nevertheless, overall survival has increased over the last decades, mainly due to improvements in medical and surgical treatment strategies and new PN formulas[5].

The most frequent occurrences that bring to the subtotal loss of the small intestine in early life are necrotising enterocolitis (NEC), small intestinal volvulus, gastroschisis and "apple peel" intestinal atresia. Other rare conditions, such as incarcerated congenital diaphragmatic hernia, have also been reported in[6]. Early surgical intervention should focus on preserving bowel length in all these cases.

Short bowel syndrome is a devastating disorder that profoundly affects children's and their families lives. Complications of IF are severe and still common among SBS patients despite the establishment of innovative Intestinal Rehabilitation Programs. Sepsis from central line infections and intestinal failure-associated liver disease (IFALD) are the most frequent conditions influencing patient outcomes. Furthermore, autologous gastro-intestinal reconstruction (AGIR) techniques have high mortality and morbidity, and managing their complications is difficult and expensive[7,8].

New techniques have been studied to improve intestinal length in SBS and IF patients since the end of the past century[9–12]. It is well known that biological systems and tissues such as skin, bone and lungs react to mechanical strengths. Therefore, mechanical distraction is routinely used in different clinical contexts, e.g., bone lengthening by distraction osteogenesis or skin growth by tissue expansion.

Distraction enterogenesis (DE) is based on applying linear longitudinal traction in different isolated bowel loops to stimulate the tissue's growth and proliferation and promote intestinal lengthening[13,14].

Several devices and materials have been proposed as a means of intestinal distraction; Okawada and colleagues[8,15] created a murine model of DE using an infusion of high molecular weight polyethene glycol (PEG) in isolated intestinal loops. Polyethene glycol is an osmotic laxative that induces bowel dilation by drawing liquids in the intestinal lumen [7,15]. In addition, Fisher *et al.* designed a shape-memory polymer cylinder positioned extraluminally that self-expands causing bowel distraction[12].

Later, further studies introduced wholly implantable intraluminal devices made of different materials such as nitinol and

polycaprolactone (PCL)[11,16,17]. The implantation of these biocompatible springs inside the lumen assumes a surgical intervention or an endoscopic procedure. In addition, if the material used is not biodegradable, a second intervention will be necessary to remove it after the distraction process is considered satisfactory.

Distraction enterogenesis has also been shown to induce adaptive histologic changes e.g., thickening of the muscular layer and deepening of the crypts; mechanisms at the base of those events still need to be well known[18,19].

This study discusses the possibility of using biodegradable materials (e.g., silk fibroin and gelatine) to convey devices that facilitate intestinal growth. These devices could be used in new mini-invasive DE techniques. As proof of this concept, we consider using gelatine capsules modified with silk fibroin as carriers for 3D-printed springs.

Materials and methods

The study aimed to create edible capsules that can be used as a vehicle for drugs and/or devices that can enhance intestinal growth in specific intestine tracts. To reach their target, these capsules should be biodegradable, soluble in water, resistant to degradation in acidic conditions and simple to create.

The choice of materials used to produce the capsules was based on their biocompatibility, biodegradability, and solubility[20–22]. After creation, the capsules were tested to check their resistance to degradation in acidic solutions using Phosphate Buffered Saline (PBS), composed of Calcium chloride and Magnesium chloride ($CaCl_2$+$MgCl_2$) with a pH value of 7.4 and acetic acid (CH_3COOH) with a pH value of 2.3, both at 35° to recreate the conditions found in the gastrointestinal tract. The capsule resistance to degradation was studied using the Fourier-transform infrared spectroscopy (FTIR) and analysing their weight before and after immersion. The analysis of resistance to degradation required 1-minute immersions, a drying process and weight assessment for gelatine-only and gelatine and fibroin capsules. Weight variation was calculated using the formula: [(Final weight - Initial weight)/Initial weight] *100.

In this study, the capsules were used as vehicles for 3D-printed springs, which are used for the distraction enterogenesis process in SBS patients. The springs' expansion process is a function of the degradation of the capsules in which the springs are contained. Therefore, our tests were focused on the timing of spring release both in gastric and intestinal environments; obtained results were then compared with the elastic constant of the encapsulated spring.

Capsules were first realised with a solution of 10% gelatin (pigskin type A from Merck) mixed in 20 ml of distilled water. To create a homogeneous solution, magnetic stirrers were used at 60°; after 45 minutes, we obtained a complete dissolution of the gelatin, which was then poured into the mould (Figure 1a). Moulds were designed with 3 pairs of elliptical holes, 0.6 cm deep, with dimensions of 1.1 cm x 0.5 cm, 1.1 cm x 0.4 cm and 0.9 cm x 0.4 cm, respectively.

Capsules are obtained by pouring the solutions into the moulds (Figure 1B) and will subsequently be analysed and characterised.

To obtain more excellent resistance to degradation in acidic solutions, regenerated silk fibroin was added to the solution - the presence of gelatin grants further protection from light and oxygen[23]. The gelatin and regenerated silk fibroin mixture can change the hydrophilic interactions between silk and water, thus creating a more stable and homogeneous system by creating porous structures.

Bombyx mori silk cocoons (10g) were boiled for 30 minutes in 200ml of water containing 5g $NaHCO_3$. The extract fibres were washed two times with water and dried at room temperature under a chemical hood in laminar flow. Subsequently, the fibroin fibres were dissolved in 5ml of FA containing an amount of $CaCl_2$ equal to 60:40 concerning the weight of the dried fibroin fibres (*i.e.* 0.65g) at 30°C for 1 hour (*e.g.* 0.43g).

The compound thus obtained is dissolved distilled water to get a 20 ml solution to which 10% gelatin is added. The solution is placed on a 5 cm Petri dish to let the solvent overnight and then put at 40° for 4 hours. Finally, the solution is homogenised using magnetic agitators at 60° for 30 minutes and then poured into a mould and left to solidify for 6 days (Figure 1).

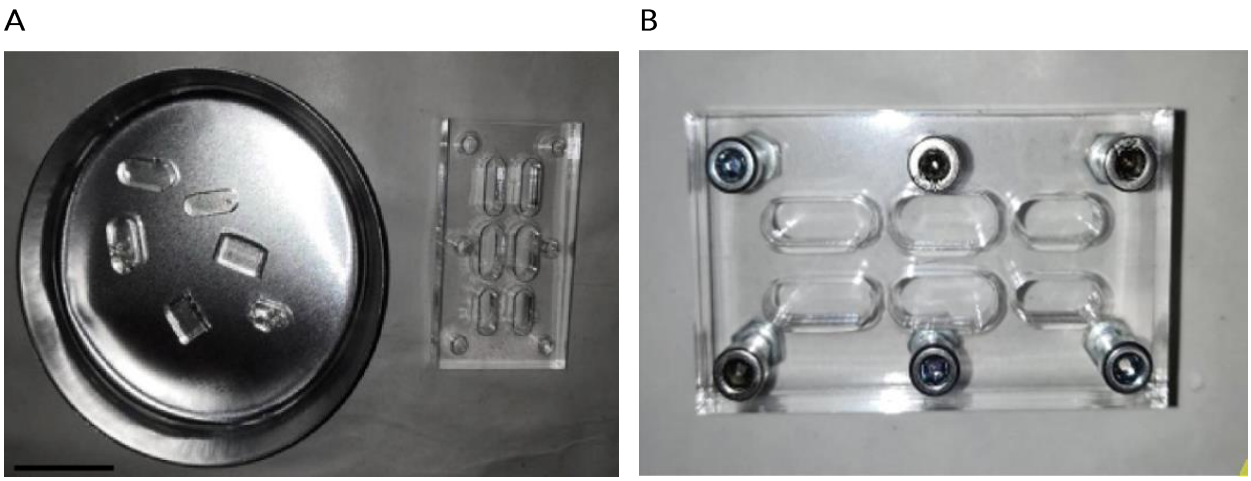

Fig. 1. (A) Picture of the mould used to realise the capsules and (B) picture of the capsules of different shapes and dimensions after the extraction from the mould. The scale bar indicates 1 cm.

Springs are composed of a structure of regenerated silk (RS) modified with graphene nanoplatelets (GNP) externally covered with a biodegradable polyhydroxybutyrate-valerate (PHBV) shell[24]. Briefly, springs were realised with 3D printing, through which, with an extruder, PHBV and RS compounds are deposited simultaneously in a 3D structure with the addition of graphene nanoplatelets (GNPs) to the RS[25]. Polyhydroxybutyrate-valerate was chosen because it is a non-cytotoxic biocompatible polymer suitable for *in vivo* use. Table 1 shows the details of the solution of RS used for 3D printing.

	Composition		
Solution	$CaCl_2$ (wt.%)	KNO_3 (wt.%)	GNP (wt.%)
RS 60:40	40	3.5	/
RS/GNP_s 60:40	40	3.5	1
RS 80:20	20	3.5	/
RS/GNP_s 80:20	20	3.5	1

Table 1. RS solutions are used for 3D printing.

Springs were inserted into the moulds before pouring the RS and gelatine solution to encapsulate them with the solidification phase, the length of which was defined through trial and error (Figures 2 A and 2 B).

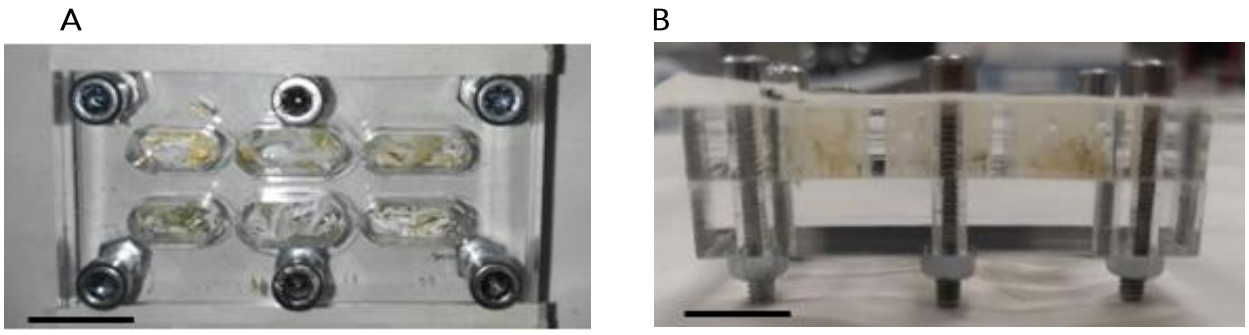

Fig. 2. (A) Springs inserted in the mould and (B) lateral view of the springs inside the solution of gelatine and silk fibroin. The scale bar indicates 1 cm.

If the process lasts longer than 3 days, the solution alters, making it useless. Figure 3 shows a spring inside a capsule after the solidification phase.

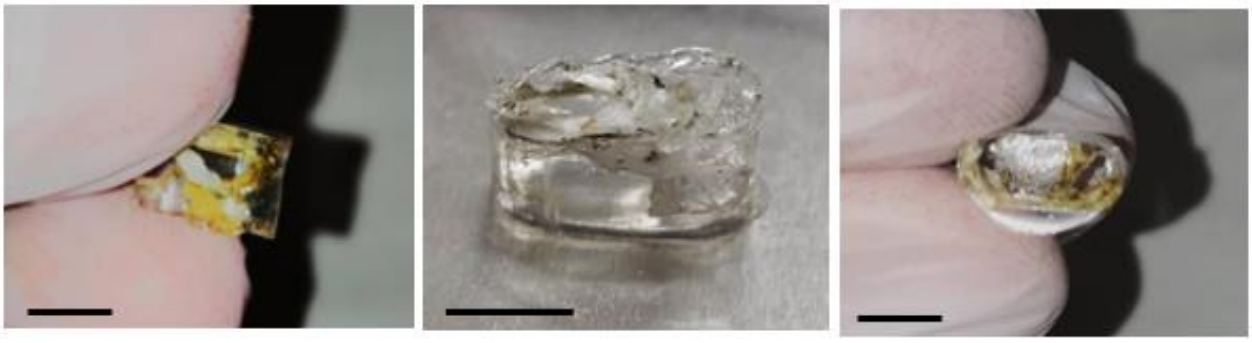

Fig. 3. Pictures of the capsules containing the 3D-printed springs. The scale bar indicates 0.5 cm.

Results

As mentioned above, the stability of gelatine-only and gelatine and fibroin capsules was tested by submerging them in 10 ml of PBS. Gelatine-only capsules showed a rapid degradation time with total weight loss after five minutes.

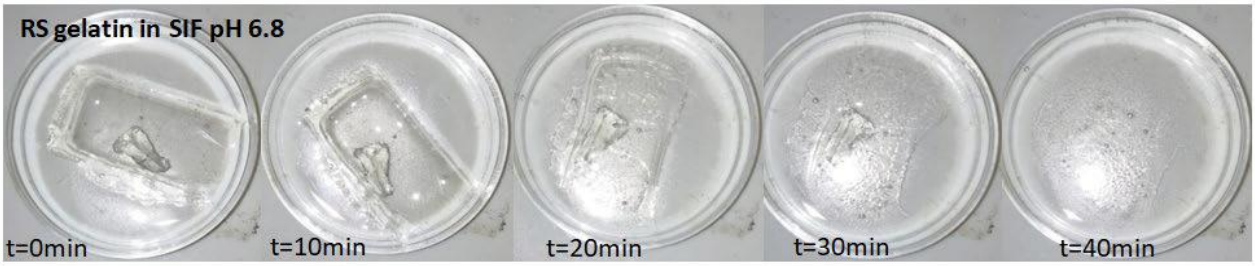

Figure 4. Degradation progress sequence of gelatine-only capsules in PBS [22].

On the other hand, gelatine and fibroin capsules proved to be more resistant, with a degradation time of 45 minutes (Figure 4). We also analysed this compound in acetic acid (pH 2.3), simulating the gastric environment with a resistance of over 90 minutes. These results should be considered satisfying considering the medium gastric and intestinal transit time[26].

In both experiments, we observed an initial growth in the weight and size of the capsules; a starting PBS absorption can explain this by the capsules themselves.

Once we established that the gelatine and fibroin capsules were the most stable choice, we analysed the releasing times of the springs in pH-controlled environments. The dissolution tests were conducted at 35°C, submerging the capsules in 10 ml of PBS or acetic acid to simulate intestinal and gastric environments.

Capsules obtained in 1.1 x 0.5 cm moulds containing type 1 e 2 springs were studied in acetic acid and PBS, respectively; capsules obtained in 1.1 x 0.4 cm and 0.9 x 0.4 cm moulds containing type 3 and 4 springs were tested in both fluids instead.

For example, we report in Figure 7 the degradation process of the capsule containing type 1 springs with a release time of around 120 min.

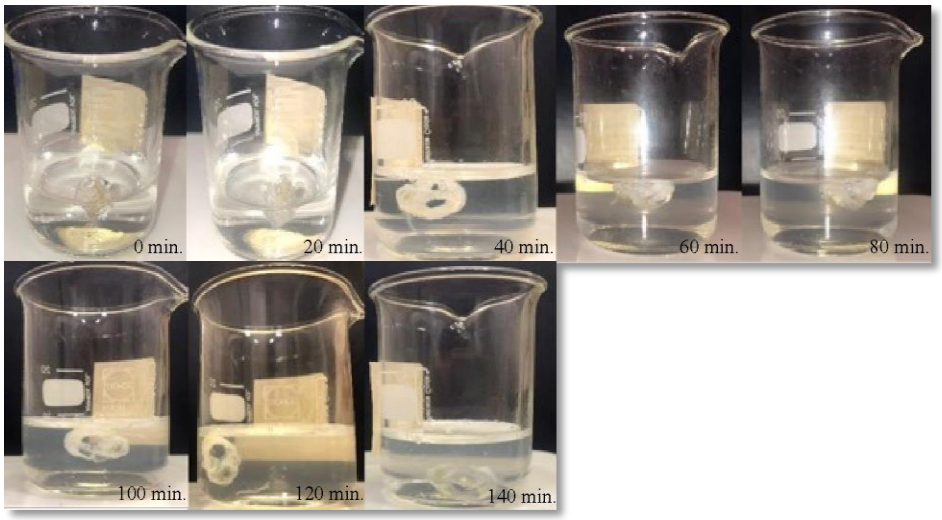

Figure 5. Photographic sequence of dissolution test of the capsule containing type 1 spring in acetic acid.

Results shown in Table 2 and Figure 6 suggest that the pH plays a vital role in the correlation between the elastic constant of the springs and the releasing time. The releasing time grows with the increase of the elastic constant when the capsules are tested in acetic acid (e.g., simulated gastric fluid (SGF)). At the same time, the opposite happens in PBS (e.g., simulated intestine fluid (SIF)). These results must be confirmed with *in vivo* experiments.

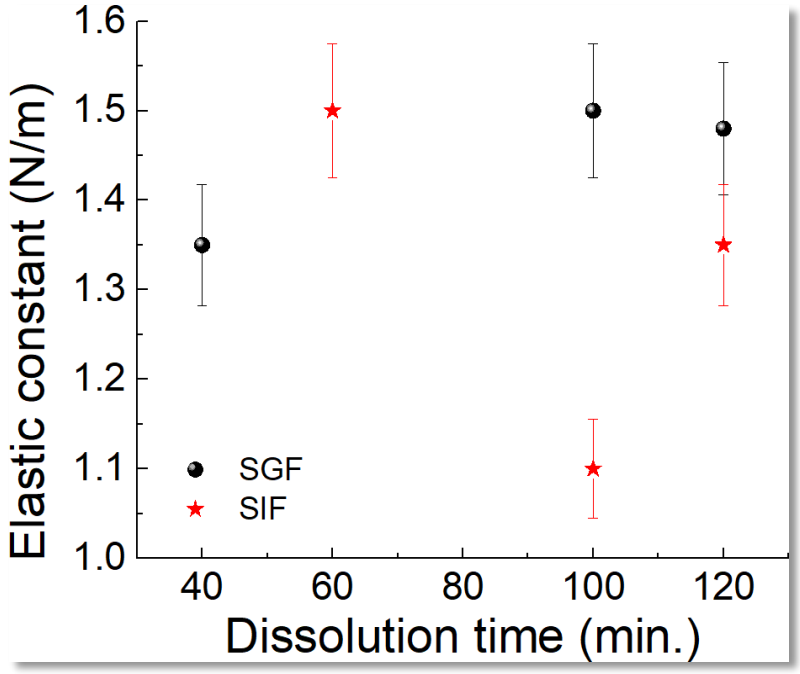

Figure 6. Values of the elastic constant of the springs according to the dissolution time in RS gelatine capsules.

Spring type	Capsule weight (g)	Degradation environment	Releasing time (min)	Elastic constant (N/m)
1	0.052±0.001	Acetic Acid	120±5	1.48±0.01
2	0.056±0.001	PBS	100±5	1.10±0.01
3	0.035±0.001	PBS	60±5	1.50±0.01
3	0.042±0.001	Acetic Acid	100±5	1.50±0.01
4	0.046±0.001	PBS	120±5	1.35±0.01
4	0.032±0.001	Acetic Acid	40±5	1.35±0.01

Table 2. Degradation times of RS gelatine capsules as regards the elastic constant of the 3D coated springs.

Data from Figure 8 and Table 2 show that while carrying the springs, gelatine and fibroin capsules' releasing times are compatible with gastric and intestinal transit time in PBS and acetic acid.

Discussion

Surgical treatment of Short bowel syndrome focuses on lengthening and remodelling procedures to restore intestinal function, peristalsis, calibre, and length. Non-transplant surgery in SBS focuses on the principle of Autologous Gastrointestinal Reconstruction procedures (AGIR): controlled bowel expansion[27,28], lengthening and tailoring procedures such as Longitudinal Intestinal Lenghtening and Tailoring (LILT), Serial Transverse Enteroplasty (STEP) and Spiral Intestinal Lenghtening and Tailoring (SILT)[29–31], slowing transit time using reverse segments or colonic interposition[32]. Sometimes more than one surgical procedure is required to achieve enteral autonomy, together with medical and nutritional management[33]. Unfortunately, often these techniques are not enough to obtain weaning from PN. New frontiers are continuously researched in medical management, such as GLP-2 treatments, new PN formulas and surgical side, such as organoids[34,35] and distraction enterogenesis. Distraction enterogenesis is a promising method for less invasive treatment of SBS of increasing intestinal length.

A few studies have been conducted so far to demonstrate the effectiveness of this method on pre-clinical and *in vivo* animal models[36–39]. Our study focused on the possibility of creating edible capsules that could reach the human intestine and release 3D-printed springs. The first step required a careful choice of materials. Initially, capsules were realised only using gelatine from pigskin type A from Sigma-Aldrich, which is already broadly used in the pharmaceutical industry to produce edible capsules. The low resistance

to degradation in PBS and subsequently in the intestinal tract at a pH of 7.4 brought us to combine gelatine and RS to realise our capsules. We conducted different experiments with various compositions and concentration levels to define the perfect mixture. This new compound showed an optimal resistance to degradation both in gastric and intestinal environments, with a substantial increase in timings of dissolution compared to gelatine alone. Analysis in acidic and basic conditions confirmed the stability of the solution with a transit time that could allow the capsule to reach the intestine without dissolving (Table 1). After testing the capsules' resistance, we combined them with 3D-printed springs, confirming the potential ability of the device to reach the intestine and release the 3D springs. During this phase, we noted variability in release timings as a function of the pH value to which the capsules were exposed, and the elastic constants of the spring contained. When immersed in acetic acid, the release time grew following the elastic constant of the spring, while in PBS, the opposite trend was observed.

Our work is a preliminary study for the realisation of biocompatible devices that could carry endoluminal implantable 3D structures and drugs or growth factors in the human intestine, setting the foundation for a new approach to distraction enterogenesis for the treatment of SBS patients. With this new approach, we could deliver scaffolds enriched with growth factors into the intestine to enhance cellular proliferation, treat ulcers or anastomosis leaks, or even delay surgical treatment in selected patients. The capsules must be tested *in vivo* in animals to study the springs' feasibility and correct positioning to reach these goals. Springs need specific characteristics of diameter, length, and elastic constants that need to be correlated with intestinal dimensions [11,18,39]. The goal is to have enough elastic strength to determine intestinal lengthening without causing damage. From previous studies, it has been shown that the relationship between the elastic strength and the weight of the animal should be better at 0.83 and 0.117 [11,39,40]. In our study, the elastic constants range from 1.10 ± 0.01 N/m to 1.60 ± 0.01 N/m, similar to what was achieved in rats by Sullins *et al.* [11]. With these capsules, the springs would reach the intestine without surgery. However, subsequently, they will not have a secure attachment to the intestinal wall, which could lower their ability to exert the traction necessary for distraction enterogenesis. Previous studies have tried using high-friction materials to overcome this problem and diminish dislodgement risk. Recent studies solved this problem by altering the 3D structure of the spring and adding anchoring devices to the external surface, which allows a safer attachment of the springs to the intestinal wall [19]. In further experiments, our 3D-printed springs could be modified in the design phase to add these anchoring structures for more stability.

Conclusions

This preliminary study showed that capsules realised from gelatine and RS are resistant to degradation in the intestinal environment,

possibly carrying 3D springs, drugs or other devices in specific intestine tracts without the need for surgery. These devices could greatly assist the distraction enterogenesis processes for treating SBS patients. However, further animal *in vivo* studies are needed to better understand the springs' feasibility and correct positioning.

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Conflicts of Interests:
None.

Funding Statement:
None

Acknowledgement
None

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