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Investigation into the Acoustic Transparency of Reconstituted Mucus

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***Abstract*— Ultrasound (US) smart capsules are being developed with the potential to deliver medication via sonoporation in the intestine. However, a layer of mucus that can represent a barrier lines the luminal surface of the intestine. Consequently, it is important to study the propagation of US through mucus to the underlying mucosal cell layer. In order to promote sonoporation, it must be ensured that US propagates efficiently though the mucus, reaches sonoporation agents, such as microbubbles, and thus promotes cavitation. The objective of this work was to investigate the acoustic transparency of mucus. A 7 mm thick layer of mucus caused attenuation of 0.93 ± 0.16 dB/cm for a 4 MHz US signal. When controlled for the thickness of the mucus in the small intestine, mucus reduced the signal amplitude by 0.01 – 0.2%. Notably, the outcomes of this work are also relevant to US therapeutic devices acting in other cavities lined by mucus, such as the respiratory, excretory and female reproductive tracts.**

Keywords*— *barrier function, mucus, drug delivery, acoustic transparency

I. Introduction

In the human intestine, mucus lines the luminal surface [1]. Medical devices and drugs in the intestine thus encounter a mucus layer before the intestinal mucosa. This is important for the context of ultrasound (US) smart capsules being developed with the potential to deliver medication via sonoporation [2, 3]. It is thus important to study the propagation of US through mucus because contrast agents might have functionality in crossing the mucus layer and coming into contact with the cell layer. In order to promote *in situ* cavitation and sonoporation, it is necessary to ensure that US can propagate efficiently though the mucus, reach the cavitation agents and promote cavitation. Thus the aim of this paper is to describe the development of a system to determine the acoustic transparency of mucus.

The lining of the small intestine, which is a layer of epithelial cells, is protected by two layers of mucus, Fig. 1 [4]. The inner layer is firmly adherent; it penetrates the space between villi and crypts, attaches to the mucosa and cannot be removed easily. The outer layer, in contrast, is loose and can be scraped off.

Mucus thickness varies along the gastrointestinal tract and from species to species. Table 1 presents the differences in humans and pigs. Notably, the mucus in the small and large intestine varies in thickness between 9 – 218 µm in humans and 14 – 56 µm in pigs [5].

Table 1. Thickness of the mucus layer (in µm) in the gastrointestinal (GI) tract in humans and pigs [5]. In humans, the thickness ranges between 9 – 218 µm, whereas in pigs it ranges between 14 – 222 µm.

Species	GI Tract Region			
	Stomach	*Small Intestine*	*Large Intestine*	*Rectum*
Human	106 - 175	10 - 162	9 - 218	88 - 155
Pig	51 - 222	25 - 53	14 - 56	40 - 58

Mucus is produced by goblet cells and is composed mainly of water and mucin proteins (MUC) [6]. Mucins are linear, elongated, rod-like polymers [7]. Their C-terminals form dimers via disulphide (S-S) bridges, while their N-terminals form trimers via S-S linkages. Mucus is therefore a network cross-linked covalently via S-S bridges. Mucoadhesion is due to the many different interactions present in mucus. Besides covalent and S-S bonds, there are also electrostatic (van der Waals), hydrophobic and H-bonding interactions. Glycosylated regions of mucins are rich in sugars (oligosaccharides), which are suggested to be responsible for the water-holding capacity of the mucus and the resistance to proteolysis, which might help in maintaining the mucosal barrier.

Mucus has multiple roles: (1) it impedes pathogen interaction with the epithelium, (2) it lubricates the intestine during peristalsis, (3) it helps transport chyme from the gut to the colon, and (4) it protects the cell lining against the acidity in the lumen [8].

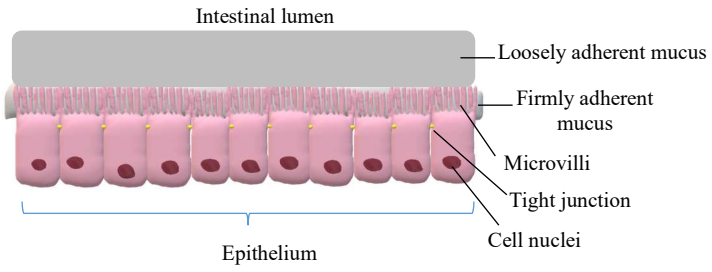

Fig. 1. Epithelium protected by two mucus layers. One layer is firmly adherent to the epithelium, whereas the other one is loosely adherent. The epithelium consists of simple columnar cells, held together by tight junctions. Microvilli increase the surface area of cell, maximising absorption ability.

Under the effect of US, compounds can be immobilised in the mucus layer without penetrating the epithelium [9]. Consequently, it is important to study the propagation of US through mucus to the underlying mucosal cell layer. In order to promote sonoporation there, it must be ensured that US can propagate though the mucus, reach sonoporation agents such as microbubbles and thus promote cavitation. The objective of the present work was to investigate the acoustic transparency of mucus.

II. Methods

The mucus consisted of mucin (Mucin Type II, M2378-100G, Merck, UK) prepared in 1M NaOH by stirring on a magnetic stir plate for up to 50 h. The pH was adjusted to pH 6.5 with either HCl or NaOH and tested with a bench pH meter (HI-5221, Hanna Instruments, UK) [10].

A ring-shaped holder was prototyped with SolidWorks 2016 (Dassault Systemes SE, France). The holder had an inner diameter, ID = 23 mm, outer diameter, OD = 31.5 mm, height = 7 mm (Fig. 4.7.A). It was 3D printed in VeroGrey Gloss with an Objet Eden 350 printer. The holders were cleared of the coating material. Mylar membranes of varying thicknesses were tested to identify a suitable transparent acoustic window for the holder. The one chosen was 6 µm thick (410-993-06, Goodfellow, UK). It was cleaned with IPA and cut into appropriate sizes. UV epoxy (4UV80HV, Permabond, UK) was applied to the sides of the holder and the membrane was placed over its edges and weighted in place. The epoxy was cured using a UV lamp (UVGL-58, UVP LLC, Upland, CA, USA) to expose it for 5 min. Excess membrane was trimmed off and the holder was then filled with 3 ml reconstituted mucus using a syringe. As seen in Fig. 2 (a), the holder ring has two orifices of different diameters. Mucus was injected through the larger orifice and air bubbles exited through the smaller one. The orifices were then sealed with 3D printed plugs and epoxy. Fig. 2 (b) shows a holder filled with mucus.

Rigid supporting structures were constructed with optical components (Newport, UK) to hold and align the mucus holder, the transducer (3.1 MHz, element size = 2.5 cm, designation 7.5 cm in PTF, spherical focus, V380, Olympus, UK) and the fibre-optic hydrophone (FOH, Precision Acoustics and UCL, UK), **Error! Reference source not found.**.

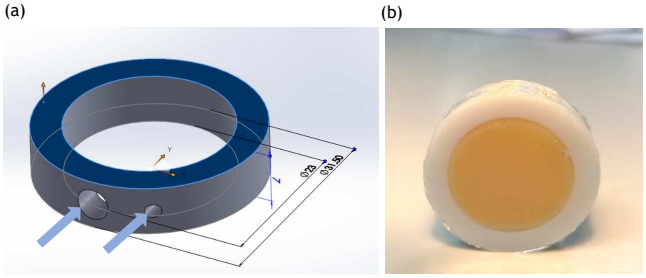

Fig. 2. Mucus holder. (a) Computer aided design prototype with dimensions in mm. Arrows point to two orifices, one for injecting mucus and one to enable air bubbles to exit the construct. (b) Holder with acoustically transparent membranes filled with reconstituted mucus. ID = 23 mm OD = 31.5 mm.

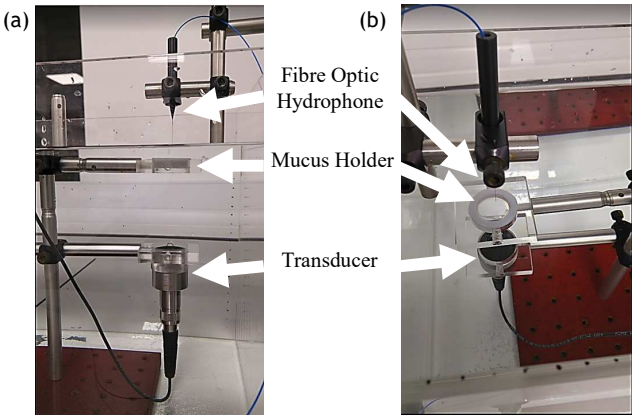

Fig. 3. Setup for investigating the acoustic transparency of mucus. (a) Side view. (b) Isometric view. The two supporting structures were constructed on two different breadboards: one immersed in the tank, holding the transducer and the mucus holder, and one external, holding the hydrophone. The structure with the hydrophone was attached to an xyz micropositioning stage for field mapping to ensure the maximum signal was recorded. The source transducer, mucus holder and hydrophone were fixed in optical holders using nylon screws.

The transducer was connected to a waveform generator (gated sine wave, 20 cycles, 10 ms bursts, 33250A, Agilent, USA) and a power amplifier (A075, E&I RF, USA) and the hydrophone was connected to an oscilloscope (DSO-X 3014, Agilent, USA) and a PC for data acquisition. Transducer calibration and field mapping were conducted (data not shown) to ensure the devices were working correctly and to identify the maximum signal which could be recorded. The water in the tank was degassed and allowed to reach RT. The oscilloscope displayed voltage after averaging 512 pulses. The voltage recorded was transformed into pressure using the manufacturer's software for the FOH.

III. Results

The acoustic pressure recorded with the FOH ranged between 1.65 – 2 MPa when there was no holder between the transducer and the FOH, Fig. 44 (a). When a holder filled with water from the tank was placed between the FOH and the transducer, the acoustic pressure recorded was lower, ranging between 1.65 – 1.9 MPa. The values decreased further when the holder was filled with reconstituted mucus, ranging between 1.45 – 1.6 MPa.

According to Fig. 4 (b), the holder with water from the tank decreased the acoustic pressure recorded by 7%, whereas the holder with mucus decreased it by 14%. In order to account for the holder and isolate the effect of the mucus, only the difference between the two instances was considered further, which is 7%.

The mucus in the human intestine ranges in thickness from approximately 10 to 200 µm, see Table 1. If 7 mm of mucus reduced the signal by 7%, Fig.4 (b), and the thickness and US attenuation are considered directly proportional, then it is expected that 10 – 200 µm will reduce the signal by 0.01 – 0.2%. If this difference is deemed important, it can be accounted for by increasing the MI. The reconstituted mucus attenuated 0.93 ± 0.16 dB/cm of a 4 MHz US signal.

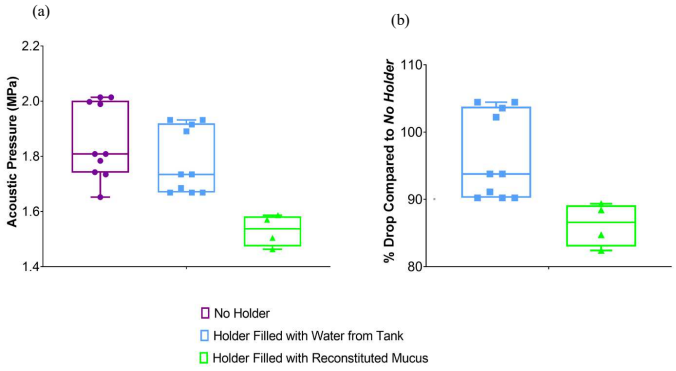

Fig. 4. Acoustic transparency of reconstituted porcine mucus. (a) The holder and the mucus caused attenuation of the US pressure; (b) When the mucus holder was filled with water from the tank, US pressure was reduced by ~7%. When the mucus holder was filled with reconstituted mucus, the US pressure was reduced by ~14%. $n \geq 4$.

IV. Discussion and Conclusions

The work presented in this paper describes a system for measuring the acoustic transparency of mucus and recorded the attenuation of an US signal through a reconstituted mucus layer. The reconstituted mucus attenuated 0.93 ± 0.16 dB/cm of a 4 MHz US signal.

Potential strategies to ensure the passage of US through the mucus layer with sufficient pressure for use at the interface between the mucus and the cell layer include decreasing the frequency to decrease the attenuation, increasing the MI [11], increasing the quantity of MBs applied simultaneously to promote more cavitation that might disrupt the mucus layer, and using mucolytics. The latter make mucus less thick and sticky, hence potentially easier to pass.

The current experiment measured only the effect of a 4 MHz beam, since that frequency had proved effective at delivering compounds to the porcine small intestine *in vivo* and thorough epithelial cell layers *in vitro* [9,12]. A broadband transducer could have been used to investigate simultaneously attenuation at a large range of frequencies. However, the use of broadband pulse could have increased the probability of error at each frequency, affecting the accuracy of the results.

The reconstituted mucus recipe was chosen in order to replace the use of fresh mucus. The present experiment would have required 12 ml of fresh mucus harvested from substantial amounts of small intestine from freshly sacrificed pigs that had been fasted prior to culling. As this approach was not possible, reconstituted mucus was used instead.

In order to harvest fresh mucus from the human small intestine, the patient needs to give consent and the researcher must collect it quickly post mortem. A patient whose time of death is reasonably predictable is likely to be ill and illness may affect food intake and the health of the GI tract. Therefore, the mucus may be dehydrated or affected in different ways. Such mucus samples will not be representative of the healthy human intestine, hence of little value. Although mucus could be scraped from resected tissue from the small intestine, this will often originate from diseased tissue and hence will be unrepresentative.

There is a paucity of available literature on the interaction between mucus and US. Although focused on the small intestine, this work is also relevant to US therapeutic devices acting in other cavities lined by mucus, such as the respiratory, excretory and female reproductive tracts.

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