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Imaging small intestinal motility at macro and micro scales

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1. Introduction

The motility of small intestine (SI) has a fundamental role on the absorption of nutrients (Kisielinski et al. 2002) and the spatio-temporal organisation of gut microbiota (Cremer et al. 2016). Furthermore, mixing and propulsion of the chyme at macroscopic scales is achieved thanks to standing or propagating contractions of circular and/or longitudinal smooth muscles (Lentle and de Loubens 2015).

At sub-millimetric scales, small intestinal mucosa is covered by finger-like or leaf-like structures, the so-called villi. Further experiments have indicated that the villi are moved by the transfer of mechanical stress from the smooth muscles layers to the mucosa layer (Lentle et al. 2013) and that their structure is rigid (Lim et al. 2014). Furthermore, recent simulations show that their motility could enhance mixing and mass transfers (Lim et al. 2015). However, simulations with realistic boundary conditions extracted from experiments remain challenging.

At the organ level, various techniques have been used to characterize in-vivo the contractions of the smooth muscles using MRI (Ailiani et al. 2009). Ex-vivo experiments has also been done using portions of the SI submerged on Krebs while the movement was recorded (Melville et al. 1975). However, it is not well understood how this complex movements may affect the movement of the villi and thus the resulting flow and mixing conditions.

The present work main objective is to develop an image technique that permit the quantification of the small intestine motility at the scale of the organ and at the scale of the villi in order to understand the interplay between smooth muscle motility and villi movement.

2. Methods

2.1 Preparation of the duodenal segments

Adult male Wistar rats (300-400 g) were euthanised by heart excision following an injection of Pentobarbital (180 mg/kg). An incision was then made on the abdomen to access the intestine. The initial 15 mm of the duodenum was resected from the

pylorus and immediately immersed in A Tyrode buffer at room temperature.

The mesenteric part was then removed from the excised segment and the duodenal digesta contain was cleaned. Graphite marks were done on the duodenal surface with a 25G needle to improve the contrast for image analysis. The segment was then attached to the set up and immersed in 80mL of oxygenated Tyrode buffer at 36°C without pre-load during 20 minutes for stabilization.

2.2 Experimental Set-up

A custom setup was developed for simultaneous deformation measurements and imaging, Figure 1. One end of the duodenum was connected to the sensor that had a weight to produce a tension closer to the physiological state, while the other end was fixed. A macroscope was fixed over it to simultaneously record the motion at the micro scale.

A pre-load of 0.25g was added every 5 min until 1.5g was reached. Once this was achieved, another 5 min were waited before finishing the experiment. The camera was started 15 minutes after the stabilization period started. This permitted to have videos under different constraints for the later analysis.

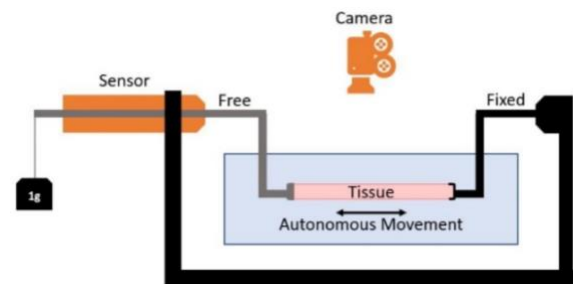


Figure 1: Diagram of the set-up used to make the experiments.

2.3 Image analysis

The image analysis was performed following a previous publication (Lentle et al., 2007). Briefly, we tracked 20 sections (20x20px) of the frames to obtain the displacement versus time data. The velocity of each portion was obtained with central difference scheme and interpolated using spline over the positions for which displacement were calculated. The derivative of this interpolation gave us the strain-rate.

3. Results and discussion

At macroscopic scale, strain-rate maps were done at different pre-loads, an example is seen on Figure 2-a. Two patterns were observed on the strain-rate maps. The first one is red or blue lines, which are related to propagating contraction (red) or relaxation (blue) along the length of the duodenum (e.g. between 45

and 60 s). The second one is localized contraction at different points of the organ at the same time (e.g. between 0 and 15 s).

Propagating contractions were correlated to higher amplitude of the strain rate than localized contractions. The occurrence of localized contractions was more evident when the applied load was small (<0.5 g). The frequency (0.45 ± 0.03 Hz) was independent on the apply load. The strain rate amplitude decreased when the load was larger than 1 g.

To assess the movement of the villi, a strip of small intestine tissue was used instead of the closed duodenum. The movement of each villi could be resolved and tracked thanks to image analysis, Figure 2-b.

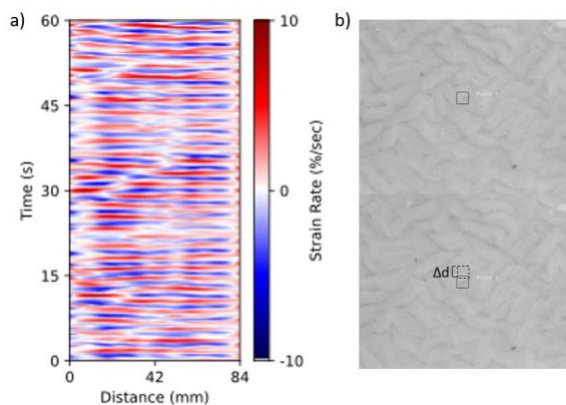


Figure 2: a) Strain-rate map obtained after an image analyses done during 60 seconds at 1g. The tissue length analysed was less than 1cm, where 0 is the closest point to the sensor. b) Villi displacement, Δd , after 1s.

4. Conclusions

The set-up allowed to characterize the motility of the duodenum in iso-tonic conditions. The spatio-temporal organization was dependent on the applied load. The set-up made possible also to track the movements of the villi. Future experiments will focus on the tracking of villi in ex-vivo situations and correlated it to smooth muscle motility.

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