Developing a Precision-cut Tissue Sectioning Protocol for Fresh Porcine Colonic Tissue for Downstream Mechanical Analysis

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Introduction

Cancer is among the leading obstacles to improving life expectancy worldwide. Specifically, colorectal cancer accounts for 1 in 10 cancer cases and deaths, making it the third most common cancer with the second highest mortality rate [1]. There is increasing evidence to suggest that mechanical changes to microenvironment can elicit an oncogenic response. However, this pathway has yet to be investigated in detail [2] and there has been no robust mechanical analysis of the colon at a microscale.

Atomic force microscopy (AFM) is a popular method for measuring stiffness at this microscale level in an environment that mimics the physiological environment [3]. The Optics 11 Chiaro Nanoindenter (**Fig. 1 C)** is a type of AFM that is suitable for soft biological tissue. However, the tissue must first be sectioned to be compatible for AFM to perform a microscale mechanical analysis (**Fig 1 B**). The gold standard for tissue sectioning is the cryosectioning, but this procedure requires the tissue to be fixed and snap frozen beforehand, leading to the loss of some of the native mechanical properties. Precision-cut tissue slices (PCTS) are a popular method of preparing tissue for studying the native microenvironment. However, again these protocols often require fixing or snap freezing of the tissue to achieve relatively thin sections. This is due to the difficulty in achieving fresh tissue sections that are small enough to be analysed on a microscale but large enough to retain the native properties of the tissue. Currently, no standardised protocol exists for PTCS of fresh soft biological tissue without fixing the tissue prior to sectioning. This study aims to develop a protocol for sectioning fresh biological tissue, specifically the colon, with the aim of using these sections for downstream microscale mechanical characterisation of the colon layers using the Optics 11 Chiaro Nanoindenter. Ultimately, this protocol can lead to the creation of a microscale map of fresh tissue to better understand the mechanics of tissue on this scale.

Materials & Methods

Tissue Sectioning Protocol This study set out to develop and optimise a protocol for sectioning fresh porcine colonic tissue using a Compresstome® VF-210-0Z (**Fig 1 A**), which can be used for downstream mechanical analysis with the Optics 11 Chiaro Nanoindenter. Porcine colonic tissue is sourced from a local abattoir, cut into 1 cm^2 sections, and placed onto a petri dish on ice submerged in 1X Phosphate Buffered Saline (PBS). The concentration at which agarose is used varies across studies, and is important to ensure precise tissue section cuts. For example, concentrations range from 0.5% to 4% for the preparation of precision cut lung slices [4]. Agarose combined with gelatin allows the tissue to remain flat once sectioned [5] this is useful for downstream mechanical analysis (**Fig. 1 B**).The variable chosen to optimize for the study is reagent concentration. Three different agarose concentrations (2%, 2.5% and 3%) and 3 gelatin concentrations (1%, 1.5% and 2%) will be tested (n=3).

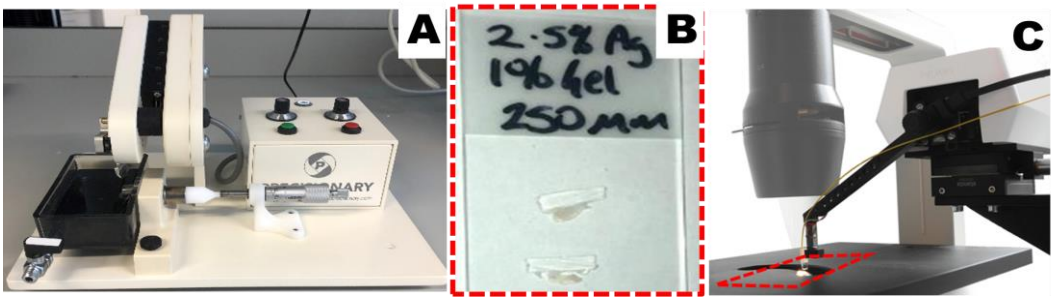

Fig. 1 Setup of the Compresstome® VF-210-0Z (a); PCTS 250µm thickness (b); set up of Optics 11 Chiaro Nanoindenter (c).

Certain conditions remain constant across the majority of studies. The embedding reagents used are cooled to 37°C before embedding to prevent heat shock [6]. Once this step is complete, the buffer temperature is maintained at between 0-4°C to promote tissue viability. It was found that intestinal sections will only remain viable for up to 24h [2]. Begin sectioning at a bigger cutting thickness e.g. 1 mm and work in reducing increments to the required thickness in approximately 3 cuts. Section thickness is another inconsistent variable across studies. One study achieved tissue section thickness of between 300-400 µm on fresh rat intestinal tissue and it is not recommended to cut tissue sections less than 100 µm as this damages the cells [6]. Gently remove sections with a paintbrush and place on coverslip with double-sided tape to secure in place for indentation [7].

Nanoindenter Protocol Once the above protocol has been optimised these sections of fresh porcine colon will be used to carry out a microscale mechanical characterisation using the Optics 11 Chiaro Nanoindenter. The piezo-motor displacement rate is set at 1 µm/s, which is standard across most studies [3, 7, 8]. The probe selection depends on the type of tissue used, for example probe 0.45 N/m cantilever stiffness and 48.5 µm radius spherical tip was carried out on abdominal aortic aneurysms at 700 µm in thickness [7]. For this study, the probe used to perform this analysis was probe 0.32 N/m cantilever stiffness and 52.0 µm radius spherical tip. The temperature used for carrying out AFM on tissue samples varies with some experiments being performed at room temperature and other studies being conducted at 37°C [3, 8]. Another study demonstrates no difference between samples tested at room temperature and 37°C [7]. Therefore, the analysis is performed at room temperature. To preserve the fresh tissue and cells Dulbecco's Modified Eagle Medium is used for this study [3, 8]. A 20x20 matrix scan is then performed on the tissue moving in 50 µm increments and covering an area of 1 mm^2. This provides a surface area map of an Effective Young's Modulus (E.eff) of the tissue section, beginning at the serosa layer and moving towards the mucosa layer (**Fig. 2**).

Results & Discussion

The purpose of this study is to develop a protocol to facilitate the mechanical characterisation of fresh biological tissue at the microscale. The optimised protocol achieved 250 µm thickness sections, which are thin enough to be used with the Optics 11 Chiaro Nanoindenter but also retain the native tissue properties.

Preliminary data was obtained from frozen porcine colon samples, which were sectioned using a cryotome to 45µm in thickness (**Fig. 2**)**.** Three technical sample of tissue from the same pig were prepared (n=3). A 20x20 matrix scan was then performed on four 1 mm^2 sections of these samples (n=4 per technical sample). The data from these sections will be used as a baseline for comparison with the fresh colon sections from the optimised PCTS protocol. The initial data show a unique profile for each layer. The serosa layer ranges from 90-480 kPa; the muscularis propria layer ranges from 80-710 kPa; and the mucosa layer ranges from 12-710 kPa. An interesting observation from the mucosa layer is that the tissue appears to get softer along the length of the crypt. More in-depth analysis is required to support this observation. The fresh tissue analysis should lend itself to a more realistic profile of the native mechanical microscale properties of the tissue.

The study highlights that fresh tissue can be sliced into PCTS that allow for accurate mechanical testing of the native tissue environment on a microscale. The optimised PCTS protocol for soft biological tissue is the first step in this microscale mechanical characterisation This will allow for more representative properties of human tissues to help develop our understanding of the effects of disease on tissue structure.

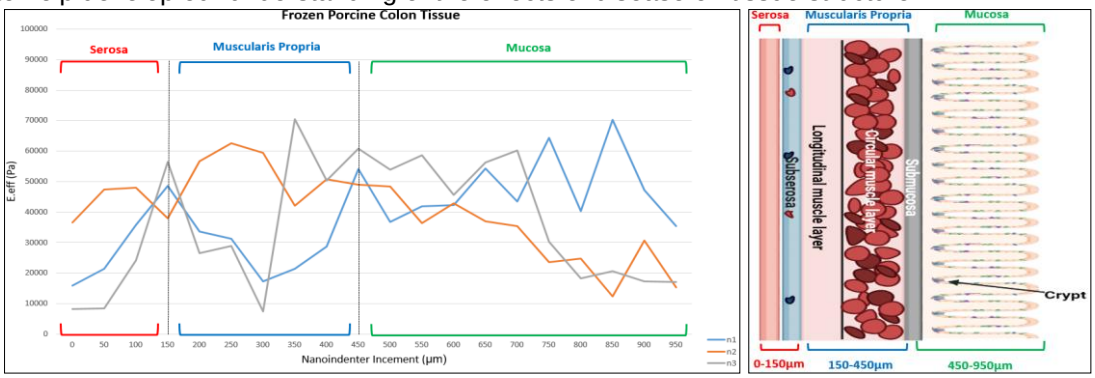

Fig. 2 Preliminary data from frozen porcine samples showing the change in E.eff across the different layers of the colon (left). A cross-section of the colon layers are displayed on the right.

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