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Determining the Biomechanics of Layer-Separated Mouse Colorectum Using Biaxial Tensile Stretch

[1]Saeed Siri, [2]Franz Maier, [1,2]David M. Pierce, [1]Bin Feng,
[1]Department of Biomedical Engineering, University of Connecticut, Storrs, CT 06279, USA
[2]Department of Mechanical Engineering, University of Connecticut, Storrs, CT 06279, USA

Background: Visceral pain is the cardinal symptom of irritable bowel syndrome (IBS), afflicting 15% of the US population and costing healthcare ~$30bn annually. Current drug treatments for visceral pain have significant side effects that often outweigh the analgesic benefit. Unlike other types of pain, visceral pain has a unique biomechanical component: it is mechanical distension/spasm of hollow visceral organs – not heat, cutting or pinching – that reliably evokes pain from these organs. Encoding of mechanical stimuli (i.e. mechanosensation), relies first on a biomechanical process, namely transmission of bulk mechanical deformation in visceral organs to local stress/strain distribution in microns-thick sensory nerve endings (i.e., afferent endings) in the organ. However, this biomechanical process of mechanosensation has never been mechanistically understood. In this study, we systematically studied the biomechanical properties of mouse distal colon and rectum (colorectum) using combined biaxial tissue testing and nonlinear imaging method on different layers of the colorectum to reveal the bulk and micro-mechanical environment of sensory nerve endings.

Materials and Methods: The ~30mm distal colon and rectum were harvested from mice, placed in phosphate buffered saline containing L-type Ca^{2+} blocker (nifedipine), protease inhibitors and antibiotics (penicillin and streptomycin), and stored at 4^{o}C overnight to completely relax smooth muscle activities, consistent with our previous studies [1,2]. The cylindrical colorectum is then cut open, pinned flat, and separated into mucosal and muscular layers by fine blunt dissection at the interstitial space below the submucosa. Tissue squares of 7x7 mm were harvested at three different longitudinal locations of the colorectum either from mucosal or muscular layers, which were subjected to bi-axial tissue testing via two servo-controlled force actuators (Model 300D, Aurora Scientific) that records both bulk tissue displacement and force. Immediately after biaxial testing, some tissue samples were subjected to non-linear imaging (second harmonic generation, [SHG]) to reveal collagen fiber orientations throughout the thickness of the tissue via two-photon fluorescent microscopy (Zeiss LSM 780).

Results and Discussion: Our novel layer-separated biaxial stretch tests reveal significant tissue anisotropy between the axial and circumferential directions in both mucosal and muscular layers at all three locations of the colorectum (colonic, intermediate and rectal). Colorectal tissue has consistently higher stiffness in axial direction than circumferential direction. Mucosal stiffness in the axial direction is almost twice as in circumferential direction, whereas muscular stiffness is only slightly higher in axial direction than in circumferential direction. Mucosal layer is stiffer than muscular layer in axial direction, indicating the load-bearing roles of the mucosal layer in contrast to what is predicted from their folded villi-crypt structures. The SHG imaging data reveal a significant network of collagen fibers at the basal layer of the mucosa and submucosa, which likely subserves the unexpected load-bearing feature of the mucosal layer.

Conclusions and significance: This is the first study to characterize the layer-separated mechanical properties at three different regions of the colorectum, revealing the unexpected mechanical strength of the submucosal layer. Further studies on the biomechanics of distal colorectum will nicely complement the neurophysiological approach to synergistically advance our mechanistic understanding of colorectal afferent neural encoding and sensitization in disease conditions of IBS. In addition, studying the biomechanics of colorectum will likely guide the next-generation drug development that targets biomechanical factors to treat visceral pain.

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