3D Printed Electrochemical Sensor for Simultaneous Dual Monitoring of Serotonin Overflow and Circular Muscle Contraction

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ABSTRACT

Serotonin (5-HT) is a key signalling molecule within the mucosal epithelium of the intestinal wall and has been shown to be an important modulator of motility. At present no single approach has been established for simultaneous dual measurement of 5-HT overflow and circular muscle contraction. We have developed a 3D printed carbon black / polylactic acid (PLA) electrochemical sensor, which had a geometry suitable for ex vivo measurement in the anorectum. The device was characterised for sensitivity and stability for 5-HT measurements as well as suitability for accurate tracking of anorectal contractions. The 3D printed electrochemical sensor had a linear range in physiological concentrations of 5-HT (1 - 10 µM) present within the intestinal tract and a limit of detection of 540 nM. The sensor was stable for 5-HT measurement following *ex vivo* tissue measurements. There was a signficant correlation in the amplitude and duration of individual contractions when comparing the measurements using an isometric force transducer and 3D printed electrochemical sensor. Finally, in the presence of 1 µM fluoxetine, the sensor was able to monitor a reduction in contractility as well as an increase in 5-HT overflow as predicted. Overall the 3D printed sensor has the ability to conduct dual simultaneous measurements of 5-HT overflow and contractility. This single device will have significant potential for clinical measurements of anorectum function and signalling, that can direct therapeutic management of patients with bowel disorders.

Keywords

3D printing, enterochromaffin cell, mucosa, ano-rectum, serotonin, muscle contraction, electrochemical sensor

INTRODUCTION

Serotonin (5-HT) is an important signalling molecule within the intestinal tract and is found in the enterochromaffin cells which are present in the mucosal lining of the intestinal wall[1,2]. There is much debate about the functional role of mucosal 5-HT, however more recently it has been shown to be a key modulator of intestinal motility and can drive the contraction of the smooth muscle [3-5]. Alterations in 5-HT have been observed in inflammatory bowel disorders, functional bowel disorders as well as in the ageing bowel, and all lead to intestinal dysmotility [6,7].

Measurement of 5-HT overflow from the intestinal tract mucosa of isolated *ex vivo* intestinal segments with carbon fibre and boron-doped diamond microelectrodes is an established technique that has led to a greater understanding on the regulation of 5-HT overflow [8-11]. However, this technique has provided limited insight into the functional activity that relates to changes in 5-HT overflow. Functional changes within the bowel are at present assessed through either traditional bioassays using isometric force transducer to track contractility or using imaging techniques to monitor the alterations in intestinal wall diameter due to contraction [12,13]. Very few approaches have aimed to monitor changes in 5-HT signalling and its relationship to function simultaneously. Studies have explored the relationship between the stretch of the bowel circular and longitudinal muscle and 5-HT release, however this required the use of a force transducer [4,9]. One study using a multi-wall carbon composite faecal pellet sensor was able to monitor 5-HT overflow whilst tracking motility through video imaging [5]. A similar approach was utilised to monitor norepinephrine in mesenteric arteries and veins using BDD microelectrodes, whilst once again simultaneously tracking the degree of vasoconstriction using video imaging [14,15]. In all these studies

two analytical measurement approaches are utilised to understand the relationship between signalling and function, which can have limitations in the precision of timing of these two events and limit the scope for translating the analytical measurement approaches towards clinical measurement.

Therefore, in this study we explored the potential to use an electrochemical sensor as a tool to simultaneously track both 5-HT overflow and circular muscle contraction. 3D printing was utilised to develop a sensor device that would have the appropriate geometry for ex vivo measurement. This approach allows for the robust manufacturing of conductive composite sensors into a single device platform. Measurements were conducted in the anorectum of the bowel. This region of the bowel was chosen as it has been prone to various pathophysiological changes. The device was fully characterised and assessed for the potential to track contractility. Studies were conducted to explore if the resultant device and methodology would respond to pharmacological manipulation of the anorectum.

EXPERIMENTAL SECTION

Fabrication of the 3D printed electrochemical sensor

The entire sensor was designed using SolidWorks 2017 (Dassault Systèmes) and a drawing highlighting the key dimensions of the mould as printed is shown in **Figure 1A**. The geometry of the device was constructed so it provided ease of insertion into the bowel and mimicked the observed natural diameter of a faecal pellet. For the conductive sensor, a hollow cone with a 4.5 mm diameter base and a length of 3 mm

and typical wall thickness of 1.25 mm was printed using a commercial polylactic acid (PLA) / carbon black filament (Proto-pasta, Vancouver, WA) using a Wanhao Duplicator 4 with a 0.4 mm nozzle at 220℃ and a bed temperature of 50℃. The layer height was set at 0.2 mm, with 2 shells (outer perimeter toolpaths) and 100 % infill density. Two skirt outlines were used to prime the extruder. To compensate for the filler content of the material, retractions were disabled, and the extrusion multiplier was set to 1.1. The electrode connection was achieved by attaching an insulated copper wire to the electrode using CircuitWorks Conductive (Silver) epoxy (**Figure 1B**). In order to seal the back end of the electrode, a 3D printed cap was printed using PLA filament (0.1 mm layer height, using a Raise 3D Pro printer with a 0.4 mm nozzle at 220℃ and a bed temperature of 50℃) and sealed to the conductive electrode and the insulated wire using epoxy resin. The final 3D printed electrochemical sensor can be seen in **Figure 1C**.

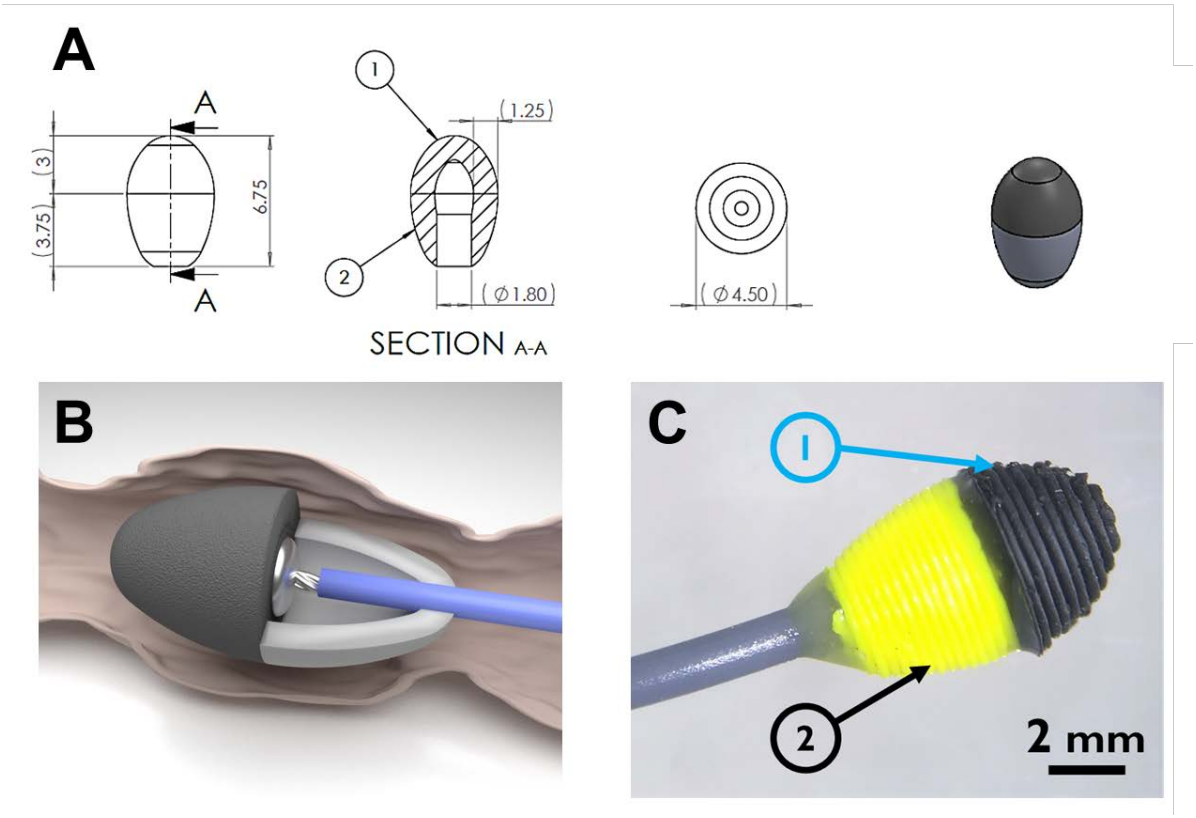

Figure 1: Fabrication of 3D printed electrochemical sensor. **(A)** key dimensions and 3D CAD model of the PLA sealing cap (2) and PLA/carbon black conductive electrode (1), where dimensions are in mm. **(B)** A schematic visualisation of the electrochemical sensor, showcasing how connection of the sensor was achieved. **(C)** The final 3D printed electrochemical sensor, with a geometry that mimics a faecal pellet.

Evaluation of sensor for monitoring of 5-HT overflow

All measurements were carried out with a 3-electrode system using a Ag|AgCl reference electrode and a Pt wire as the counter electrode. All measurements were conducted using CHI 760E multichannel potentiostat (CH Instruments).

To calibrate the 3D printed electrode, concentrations of 5-HT from 1 to 10 µM were added every 150 s in series to a modified Krebs' buffer solution, pH 7.4 (117 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgCl_2$, 1.2 mM NaH_2PO_4) and the current was recorded when the electrode was held at +0.6 V vs Ag|AgCl based on the oxidation peak potential obtained using differential pulse voltammetry (DPV). This concentration range of serotonin was utilised as it closely resembled the range observed in previous studies monitoring serotonin overflow from isolated bowel tissue. The current versus concentration response was obtained and the resultant sensitivity of the 3D printed electrode was obtained. In order to explore the potential for the electrode to foul, measurements were conducted in 1 and 10 µM 5-HT and the current response recorded every 2 minutes for a total of 20 minutes and the percent change in the current was obtained from the initial response.

Studies to evaluate the ability of the sensor to track intestinal contraction

To understand the ability for the sensor device to be able to track contraction, a silicon tube with internal diameter of 9 mm was utilised to mimic the guinea pig anorectum. This tube was placed in a solution of modified Krebs buffer and the 3D printed electrochemical sensor was inserted into the tube, so that it was completely submerged into Krebs buffer solution. To simulate the behaviour of a circular muscle contraction the tube was compressed in the vicinity of the 3D printed electrochemical sensor using a pair of tweezers to exert a range of forces. The force applied was designed to reduce the distance between the wall of tube of the silicon tube to the 3D printed sensor by ~25, 50 and 100 % and mimic a typical contraction of the rectum proximal to a faecal pellet (see **Figure 4A**). The compression was applied for 5 s and

due to the potential variability between each measurement, a video recording was used to provide a precise % reduction in the silicon tube during compression. The recorded current during each compression measurement was obtained through amperometric detection at +0.6 V vs Ag|AgCl. This experiment was carried out in Krebs buffer and in Krebs buffer plus 1 µM 5-HT (where the 5-HT was introduced as a bolus in the silicon tube). The current amplitude obtained during each simulated contraction was compared to the percent change in the diameter of the silicon tubing during each compression.

Sensor measurements in the ano-rectum

All procedures were carried out according to U.K. Home Office regulations and were approved by the University of Brighton Ethics Committee. Male Duncan Hartley guinea pigs (200 g) were obtained from Harlan UK and housed under barrier-reared conditions until required. Animals were maintained at 19.0 ± 1 °C, 55% humidity and fed on a maintenance diet and had free access to irradiated water. Animals were euthanized in CO_2, followed by cervical dislocation and the distal colon to anus was harvested and placed in ice cold oxygenated (95% O_2 and 5% CO_2) Krebs' buffer solution, pH 7.4 (117 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgCl_2$, 1.2 mM NaH_2PO_4, 25 mM $NaHCO_3$ and 11 mM glucose) prior to experiments.

The tissue was placed in a Sylgard®- (Dow Corning, UK) lined Teflon recording chamber and superfused with warm (37 °C) Krebs solution at a flow rate of 2 mL/min. The anorectum region was defined as the region within 10-30 mm of the anus of the bowel. Fine suture silk was tied through the muscle layers of the anorectum and connected to the isometric force transducers. The muscle was placed initially under a

low level of tension 0.2 g. The signal from the force transducer then passed to a preamplifier and ADI Powerlab (ADI Instruments, Oxford, UK) before being stored on computer using LabChart 7 software (ADI Instruments, Oxford, UK). The 3D electrochemical sensor was fixed 5 mm into the bowel from the anus using a pin to hold down the sensor in the anorectum region for monitoring of 5-HT and circular contraction. Tissues were perfused for 20 minutes prior to commencing a series of measurements. For measurements of 5-HT overflow and circular contraction, amperometric recordings were carried out on all electrodes for a duration of 30 s at 0.6 V vs. Ag|AgCl reference electrode. Measurements were carried out to compare 5-HT overflow and circular muscle contraction in the presence and absence of serotonin transporter (SERT) blocker 1 µM fluoxetine. Pre- and post-tissue measurements, of the 3D printed device in 5 µM 5-HT were conducted to explore the stability of sensor. The change in the current baseline was monitored following application of 1 µM fluoxetine to understand how 5-HT overflow levels were altered. The standard deviation of the current amplitude was monitored for 1-minute duration pre and post application of fluoxetine as a marker of circular muscle contractions recorded on the 3D printed electrodes. For the same 1-minute duration, the integral of the contraction response was recorded from the force transducer measurements and the two measurements of contractile strength compared.

Data Analysis

Data on the fouling study were statistically analysed using a one-way ANOVA, followed by a Tukey's multiple comparison test. For all biological studies, measurements were analysed using either a Students t-test or two-way ANOVA

followed by a Bonferroni's post-hoc test. Data was presented as mean ± standard deviation and P<0.05 was taken as being significant.

RESULTS AND DISCUSSION

Measurement of 5-HT using the 3D printed electrochemical sensor

The response of the 3D printed electrochemical sensor to 5 µM 5-HT by DPV is shown in **Figure 2A**. There is the presence of an oxidation peak at +0.45 V vs Ag|AgCl. The response of the electrode to varying concentrations of 5-HT is shown in **Figure 2B**. The calibration response of the electrode is shown in **Figure 2C**, where the sensitivity of the electrode to 5-HT was 135 nA μM^{-1} and the limit of detection (LOD) was 540 nM (n=4). Although the LOD of detection may be perceived to be high, the value is fit for purpose in the context of intestinal monitoring, where the physiological range is between 1 and 10 µM. The value of the LOD is slightly higher than observed for carbon fibre sensors used for intestinal tissue measurements [8], but comparable to carbon nanotube based composite sensors for intestinal monitoring [5,16].

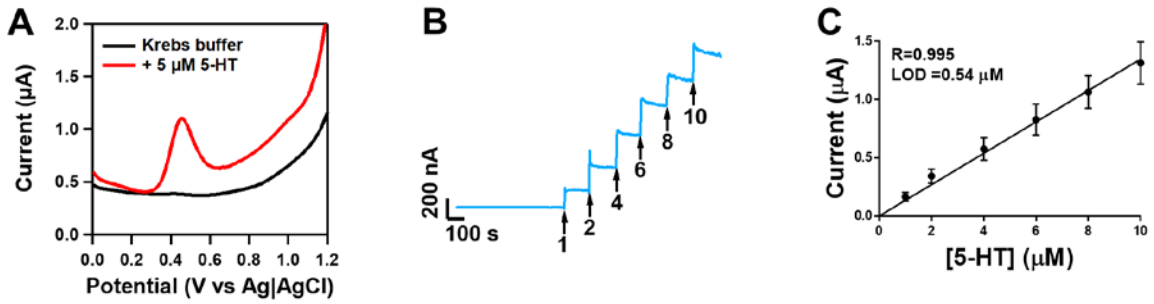

Figure 2: Measurement of 5-HT on the 3D printed electrochemical sensor. **(A)** DPV responses of modified Krebs buffer and 5 μM serotonin. **(B)** An experimental response showing the addition of varying concentrations of 5-HT (μM) to the electrochemical sensor, when held at 0.6 V vs Ag|AgCl. **(C)** Calibration response of 5-HT conducted in modified Krebs buffer. Data shown as mean ± S.D., n=4.

Investigation of electrode fouling

Electroanalytical measurements conducted from gastrointestinal tissue pose significant problems from biofouling due to the release of mucins from the mucosa, and from oxidative-by products of 5-HT [17,18]. **Figure 3A** shows a representative trace, where 1 μM 5-HT was added to modified Krebs buffer to explore the change in the current over time for a duration of 20 minutes. The overall responses from multiple sensors when exposed to 1 and 10 μM 5-HT is shown in **Figure 3B**. The results are shown as the percentage loss in the concentration of 5-HT from the initial response for a duration of 20 minutes. For the duration of 20 minutes, in 1 μM 5-HT, there was no change in the concentration of 5-HT (n=4). In the presence of 10 μM 5-HT, the response of 5-HT was stable until 15 minutes. From 15 to 20 minutes there was a signficant loss in the concentration of 5-HT ($p<0.05$, n=4, Figure 3B). Therefore, under the physiological range of concentrations of 5-HT observed in the intestinal tract, the 3D printed electrode was shown to be stable for a duration of up to 15 minutes. This is slightly improved that other sp^2 carbon materials such as carbon fibre and graphite, which have been shown to be prone to fouling from oxidative by-products of 5-HT [17,19]. The resistance to fouling may be due to the PLA hydrophobicity, which exerts a strong influence on the strength of molecular adsorption by polar molecules on the

presumably nanometer-sized clusters of carbon black, therefore leading to no significant loss in the signal for the first 15 minutes.

In order to explore if the electrochemical sensor was stable for the *ex vivo* measurements, DPV recordings of 5 μM 5-HT were compared before and after measurements (**Figure 3C**). *Ex vivo* recordings were conducted for a maximum of 10 minutes for each protocol. No significant differences in the responses were observed ($p=0.24$, **Figure 3D**). This may have been due to the nature of the measurement environment as contractions within the bowel provided a natural convection that may have prevented oxidative by-products from causing extensive fouling of the sensor.

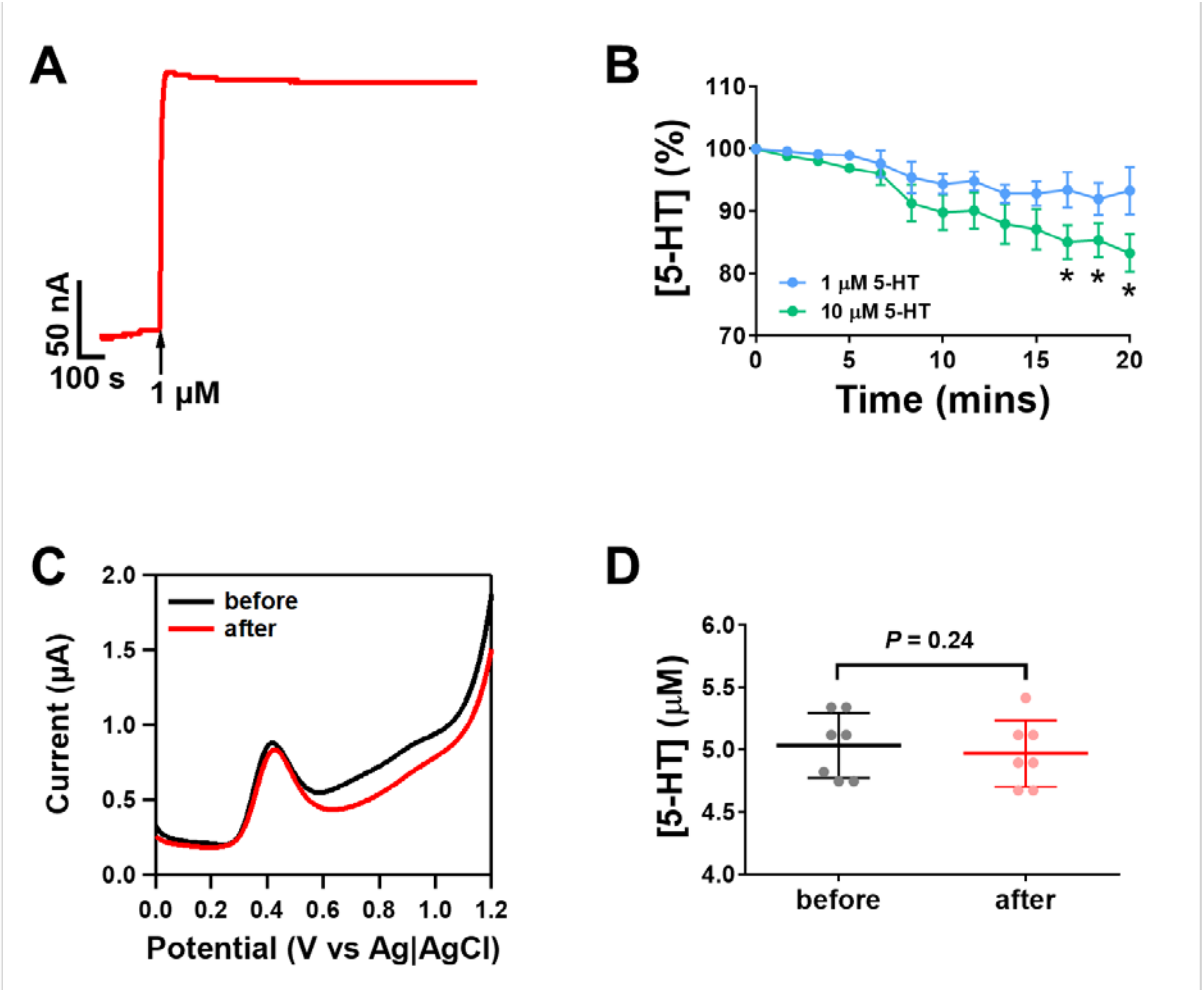

Figure 3: Evaluating the extent of electrode fouling from oxidative by products of 5-HT on the 3D printed electrochemical sensor. **(A)** Amperometric response at 0.6 V vs Ag|AgCl, in the presence of 1 µM 5-HT. **(B)** The % change in the recorded concentration of 5-HT following incubatin in 1 and 10 µM 5-HT standards over a 20 minute period. Data shown as mean ± S.D., n=4, *p<0.05. (**C**) DPV response of 5 µM 5-HT before and after *ex vivo* tissue measurements. **(D)** Current responses of 5 µM 5-HT before and after ex vivo tissue measurements. Data shown as mean ± S.D., n=7

Assessment of the electrochemical sensors ability to track reductions in the internal diameter of a simulated anorectum

In order to simultaneously monitor the overflow of 5-HT and muscle contraction, the 3D printed electrochemical sensor must respond to change in the internal diameter of the muscle. Therefore, to simulate this effect, a silicon tube was used to mimic the intestinal wall and compressed to varying degrees of force in the presence of the electrochemical sensor. **Figure 4A** shows a schematic diagram of the experiment, where the change in the distance in the outer layer of the silicone tubing was tracked following compression was recorded as a % reduction in the silicone tubing. **Figure 4B** shows photographs of the experimental setup, where varying degrees of compression were applied to the silicone tubing. The resultant changes in the current traces following squeezing by 25, 50 and 100 % reduction of the internal diameter of the tube are shown in **Figure 4C**. A transient increase in the current is observed each time the tube is squeezed. The same experiment was conducted in the presence of 1 µM 5-HT as shown in **Figure 4D**, where the 5-HT was introduced into the silicon tube. There is a rise in the steady state current following addition of 5-HT, with clear presence of transient increases in the current where the tube has been squeezed. The current increases following squeezing in 5-HT were approximately 3 times greater than observed in the modified Krebs buffer. Using the current to track both means that the responses are additive at a given timepoint, and thus are difficult to tell apart. However due to the nature of how the changes occur on different timescales, monitoring dynamic (seconds) differences in the current provides insight into contractions, whilst monitoring more cumulative changes (minutes) provides an ability to see alterations in 5-HT overflow. The measurement from multiple trials are shown in **Figure 4E**. Due to the imprecise nature of the squeezing, the accurate percent reduction in diameter

of the silicone tubing was obtained from the video recordings and plotted against the current amplitude. There is a good linear correlation between the % compression of the tubing and the recorded current in both the modified Krebs buffer and 5-HT (both $R^2 = 0.97$).

In both the modified Krebs buffer and in the presence of 1 μM 5-HT, there is a clear transient positive deflection in the current response following squeezing. This was unexpected as we assumed that compression of the silicone tube would decrease the current by perturbing the electrode surface region, particularly when 100 % squeezed. However, the net effect was an increase in current, which is assumed to be through enhanced mass transfer following the compression of the silicone tube which directed the flow of either electrolytes in the modified Krebs buffer or 5-HT to the electrode surface to enhancement the current response.

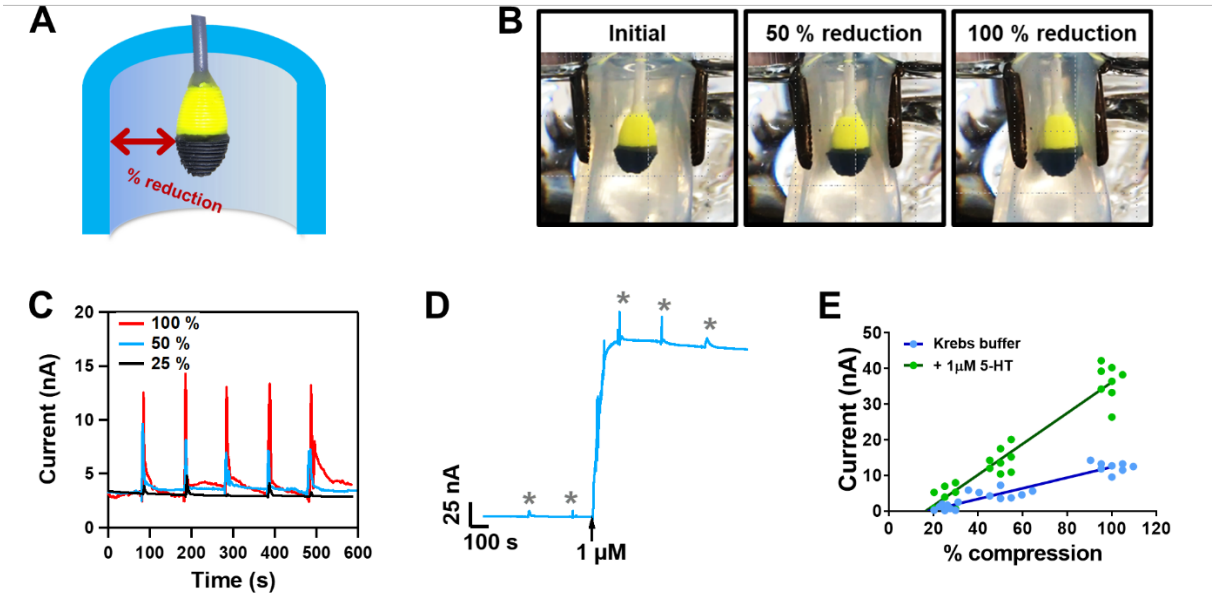

Figure 4: Can the 3D printed electrochemical sensor to monitor a simulated contraction? **(A)** Schematic showcasing how the % reduction in the silicone tube was measured as a marker of compression. **(B)** Photographs of the experimental set-up showing the changes in the internal diameter of the silicone tube following 50 and 100 % reduction in the diameter. **(C)**

Changes in the current of the modified Krebs buffer as a result of reducing the internal diameter of the silicone tube. **(D)** Experimental response showing the presence of 5 consecutive squeezes of the silicone tube before and after the application of 1 µM 5-HT. **(E)** Cumulative responses from multiple measurements, where the current amplitude is plotted against the percentage reduction in the internal diameter of the silicone tubing following squeezing. Data shown as Mean ± S.D., n=8 different sensors.

Comparison of the electrochemical sensor with force transducer for the determination of anorectal contractions

Observations using the simulated anorectum demonstrated that the 3D printed sensor had potential for tracking circular muscle contractions. Therefore, measurements were made in *ex vivo* tissue and a comparison made between responses recorded using the electrochemical sensor and those recorded using an isometric force transducer, the gold standard for monitoring muscle contraction of smooth muscle. **Figure 5A** shows a clear correlation between the responses on both the electrochemical sensor and force transducer for a duration of 40 seconds. The shape time that the responses on both traces are not completely aligned, as this is due to location in which the recording conducted are not identical and that each technique has varied sensitivity. **Figure 5B** shows that there is a signficant correlation between the amplitudes of individual contractions measured from both the electrochemical sensor and force transducer (n=7 animals, $p<0.001$). A signficant correlation was also seen when measuring the duration of contraction using both techniques ($p<0.001$; **Figure 5C**). This demonstrates for the first time that electrochemical measurement can be used as a viable tool for the tracking of circular muscle contraction in the anorectum. This approach should also be suitable for measurement of contractions from other tubular

regions that undergo smooth muscle contractions such as other regions of the intestinal tract and the cardiovascular system.

The increase in the current observed during a muscle contraction may be due to enhanced mass transfer as observed in the simulated environment, however, it may also be due to the variation in the distance between the tissue and sensor during measurements. Previous studies from tissue segments have highlighted that the 5-HT current can be significantly affected by the distance between the tissue and sensor [20-22]. Therefore, the higher currents observed may be a marker of higher 5-HT concentrations as the sensor was directly positioned above the release sites of 5-HT on the anorectum mucosa.

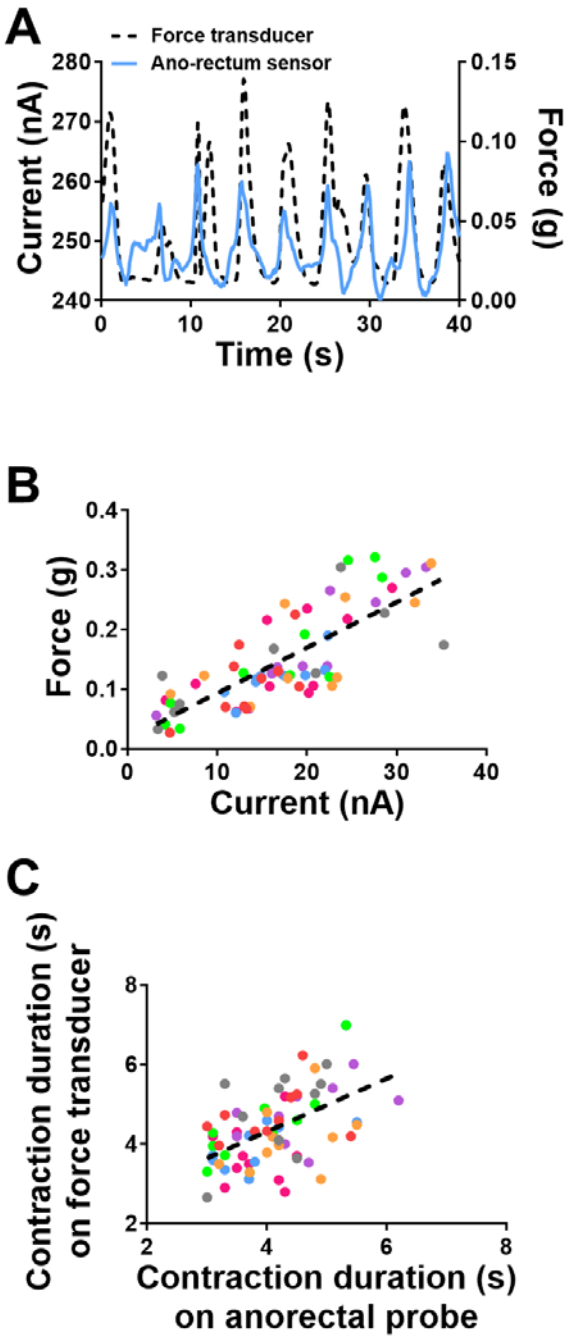

Figure 4: Simultaneous detection of anorectum contraction using electrochemical sensor and isomeric force transducer. **(A)** Recordings of the force transducer and electrochemical sensor for a duration of 40 s, which was analysed for the amplitude and duration of each contraction. **(B)** Correlation of current amplitude recorded at the electrochemical sensor and force recorded using the isometric transducer ($p<0.001$). **(C)** Correlation of the contraction duration recorded

using the two techniques ($p<0.001$). Each set of coloured dots represents the responses obtained from an individual animal. n=7.

Simultaneous monitoring of 5-HT overflow and circular muscle contraction

The 3D printed electrochemical sensors presented in this study have been shown to be equitable with the gold standard (force transducer). The sensor was then further challenged to investigate if changes in 5-HT overflow could also be monitored during *ex vivo* tissue measurements. Recordings were carried out in the presence of 1 µM fluoxetine, a 5-HT reuptake inhibitor, which is known to block the 5-HT transporter and increase the 5-HT overflow. **Figure 6A** shows a representative response from the electrochemical sensor (blue) and force transducer (red) following the addition of 1 µM fluoxetine. There is a clear reduction in the amplitude of the phasic current changes on the electrochemical sensor post the addition of fluoxetine. However, there was an increase in the basal current response following 1 minutes after addition of fluoxetine. A reduction in the amplitude of phasic contractions was recording using the force transducer post addition of 1 µM fluoxetine. Analysis of a series of experiments showed there was a significant increase in the basal current due to increased 5-HT overflow after the addition of 1 µM fluoxetine ($p<0.001$, n=7, **Figure 6B**). There was also a significant reduction in the amplitude of the transient currents recorded in a 40 second period after the addition of 1 µM fluoxetine compared to an equivalent control period ($p<0.001$, n=7; **Figure 6C**) suggesting a decrease in the force of anorectal contractions. Similar reductions in amplitude were also observed using the force transducer, as the integral of contractile response for the same duration was significantly reduced in the presence of 1 µM fluoxetine ($p<0.001$, n=7; **Figure 6D**).

These findings clearly indicated the ability to track changes in 5-HT overflow and circular contraction simultaneously for the first time. These were evident following he addition of fluoxetine, where an increase in the 5-HT overflow and a simultaneous decrease in the amplitude of muscle contraction were observed. This finding supports previous observations, where high concentrations of fluoxetine were shown to elevate 5-HT overflow and lead to the desensitisation of 5-HT receptors on intrinsic primary afferent neurons (IPANs), leading to a reduction in contractility [23,24]. Our findings also suggest that a doubling of the baseline concentration of 5-HT is sufficient to desensitise 5-HT_3 receptors in the guinea pig colon [24]. This finding will therefore allow important insights into the regulation of 5-HT overflow and its effect on motility.

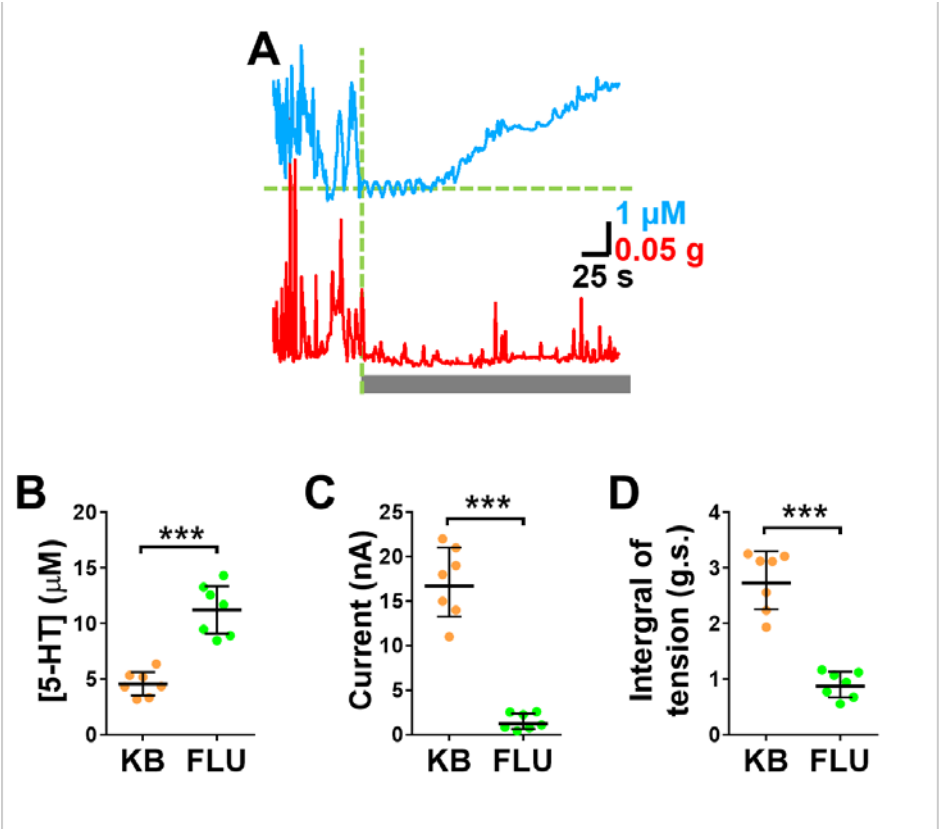

Figure 6: Simultaneous monitoring of 5-HT overflow and circular contraction from the anorectum. **(A)** Recordings of the force transducer (red) and electrochemical sensor (blue) for measurements in the anorectum following addition of 1 µM fluoxetine. The grey bar indicates

the duration that 1 µM fluoxetine was added. **(B)** Change in basal 5-HT overflow following addition of 1 µM fluoxetine. **(C)** Change in the current amplitude of phasic contractions on the electrochemical sensor in the presence of 1 µM fluoxetine. **(D)** Changes in the integral of tension monitored following addition of fluoxetine. For measurement of contractility by both electrochemical and force transducer, recordings were analysed 40 s before and after the addition of 1 µM fluoxetine. Data shown as mean ± S.D., n=7, ***p<0.001.

CONCLUSION

Our 3D printed electrochemical sensor has the ability to detect physiological concentrations of 5-HT present within the intestinal tract for a duration of 15 minutes without significant fouling. There was a clear correlation in the ability of the electrochemical sensor to track the amplitude and duration of individual contractions when compared to the widely used isometric force transducer. In the presence of fluoxetine, the electrochemical sensor was able to track the predicted increase in 5-HT overflow and decrease in contractility. Overall this is the first device able to conduct dual measurement of signalling and contractility, which offers signficant scope towards clinical measurement of bowel function and can be a useful tool to direct therapeutic management.

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TOC Figure