

Precision cut intestinal slices, a novel model of acute food allergic reactions

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Abstract

Background: Food allergy affects up to 8% of the pediatric population. Despite ongoing efforts, treatment options remain limited. Novel models of food allergy are needed to study response patterns downstream of IgE-crosslinking and evaluate drugs modifying acute events. Here, we report a novel human *ex vivo* model that displays acute, allergen-specific, IgE-mediated smooth muscle contractions using precision cut intestinal slices (PCIS). **Methods:** PCIS were generated using gut tissue samples from children who underwent clinically indicated surgery. Viability and metabolic activity were assessed from 0-24h. Distribution of relevant cell subsets was confirmed using single cell nuclear sequencing. PCIS were passively sensitized using plasma from peanut allergic donors or peanut-sensitized non-allergic donors, and exposed to various stimuli including serotonin, histamine, FcεRI-crosslinker and food allergens. Smooth muscle contractions and mediator release functioned as readouts. A novel program designed to measure contractions was developed to quantify responses. The ability to demonstrate the impact of antihistamines and immunomodulation from peanut oral immunotherapy (OIT) was assessed. **Results:** PCIS viability was maintained for 24h. Cellular distribution confirmed the presence of key cell subsets including mast cells. The video analysis tool reliably quantified responses to different stimulatory conditions. Smooth muscle contractions were allergen-specific and reflected the clinical phenotype of the plasma donor. Tryptase measurement confirmed IgE-dependent mast cell-derived mediator release. Antihistamines suppressed histamine-induced contraction and plasma from successful peanut OIT suppressed peanut-specific PCIS contraction. **Conclusion:** PCIS represent a novel human tissue-based model to study acute, IgE-mediated food allergy and pharmaceutical impacts on allergic responses in the gut.

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Abstract (249/250 words):

Background: Food allergy affects up to 8% of the pediatric population. Despite ongoing efforts, treatment options remain limited. Novel models of food allergy are needed to study response patterns downstream of IgE-crosslinking and evaluate drugs modifying acute events. Here, we report a novel human *ex vivo* model that displays acute, allergen-specific, IgE-mediated smooth muscle contractions using precision cut intestinal slices (PCIS).

Methods: PCIS were generated using gut tissue samples from children who underwent clinically indicated surgery. Viability and metabolic activity were assessed from 0-24h. Distribution of relevant cell subsets was confirmed using single cell nuclear sequencing. PCIS were passively sensitized using plasma from peanut allergic donors or peanut-sensitized non-allergic donors, and exposed to various stimuli including serotonin, histamine, FceRI-crosslinker and food allergens. Smooth muscle contractions and mediator release functioned as readouts. A novel program designed to measure contractions was developed to quantify responses. The ability to demonstrate the impact of antihistamines and immunomodulation from peanut oral immunotherapy (OIT) was assessed.

Results: PCIS viability was maintained for 24h. Cellular distribution confirmed the presence of key cell subsets including mast cells. The video analysis tool reliably quantified responses to different stimulatory conditions. Smooth muscle contractions were allergen-specific and reflected the clinical phenotype of the plasma donor. Tryptase measurement confirmed IgE-dependent mast cell-derived mediator release. Anti-histamines suppressed histamine-induced contraction and plasma from successful peanut OIT suppressed peanut-specific PCIS contraction.

Conclusion: PCIS represent a novel human tissue-based model to study acute, IgE-mediated food allergy and pharmaceutical impacts on allergic responses in the gut.

Key Words: Allergy model; food allergy; intestine; Precision Cut Intestinal Slices; PCIS

List of Abbreviations:

Area under the curve (AUC)

Food allergy (FA)

Gastrointestinal (GI)

Lactate dehydrogenase (LDH)

Oral food challenge (OFC)

Oral immunotherapy (OIT)

Ovalbumin (OVA)

Precision cut intestinal slices (PCIS)

Peanut extract (PE)

Single nuclear RNA sequencing (scNucSeq)

Water-soluble tetrazolium salt (WST-1)

Williams' medium E (WME)

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INTRODUCTION

Food allergy (FA) is a growing public health concern.¹ Current estimates report 7.6% of children and 10.8% of adults are affected by FA in the United States alone.^{2,3} While many children outgrow their allergy over time, some FAs persist into adulthood resulting in a chronic disorder. The symptoms of an allergic reaction can be unpredictable and may result in potentially fatal anaphylaxis.⁴ The current treatment approaches for FA are limited to avoidance of the allergen and emergency interventions upon accidental exposure, although allergen-specific immunotherapies (e.g., oral, sublingual, epicutaneous) are a promising potential treatment option for certain individuals.

The gastrointestinal (GI) tract plays a central role in FA as the site of exposure and immune response to food allergens. The gut is enriched with mast cells that are one of the primary effector cells of the allergic response.⁵ They express the high affinity IgE receptor (FcεRI) that binds allergen-specific IgE antibodies. Upon exposure to a relevant allergen, the receptor-bound IgE recognizes the allergen and crosslinks the receptors, activating the cell.⁶ This results in the release of pre-formed (e.g., histamine, tryptase, serotonin) and *de novo* synthesized (e.g., cytokines, leukotrienes) inflammatory mediators influencing both local and systemic allergic responses.⁷ The release of histamine causes increased intestinal smooth muscle contraction by binding to histamine receptors in the GI tract, which may manifest as abdominal pain during an allergic reaction.^{8–11} Similarly, other symptoms of an acute allergic reaction are often GI based (e.g., cramping, emesis, diarrhea) and reflect the direct and *in situ* immune response.¹²

Due to the inaccessibility of the gut, allergic exposure and the resulting immune cascade within human GI tissues remain understudied. Models of FA that reflect the complexity of human intestinal tissue, including the immune system and the functionality of the enteric nervous system, are rare.¹³

Precision cut intestinal slices (PCIS) are viable gut explants of a fixed thickness that maintain the structure and cellular diversity of intestinal tissue.¹⁴ They contain all relevant cell populations of interest and preserve the spatial distribution of the distinct cell subsets.¹⁵ PCIS can potentially represent any region of the intestine based on the surgical tissue harvested. To date, the PCIS system has been used to study the metabolism, toxicity, and interaction of pharmaceuticals as well as models for viral infection and intestinal fibrosis.^{16–21} However, the use of PCIS has not yet been reported in the context of FA.

Here we report that human PCIS can be used as a model of FA to study acute, IgE-mediated allergic reactions via measurement of smooth muscle contraction as a readout for allergic response.

MATERIALS AND METHODS

Source of human intestinal tissue

Human intestinal tissue was obtained from 16 children (median age: 4 months) undergoing clinically indicated resection of the small or large intestine (tissue donor information in Table S1). Patients with systemic inflammatory disorders, metabolic disorders, or systemic immunosuppression were excluded. The use of human tissue for research was approved by the SickKids Research Ethics Board (REB #1000059282). All experiments were carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving human subjects. Privacy rights of all human subjects were observed, and informed consent was obtained from the parents/guardians.

Preparation of PCIS

PCIS were prepared as previously published in detail by de Graaf et al with modifications.^{20,22} In short, a piece of full-thickness human intestinal tissue (approximately 1.0 x 0.5 x 0.2cm) was cut from the healthy region of a surgically resected specimen by a trained pathologist directly following surgical removal. The sample was immediately placed into oxygenated, chilled (4°C, 95% O₂, 5% CO₂) Krebs Henseleit Buffer (KHB) and transported to the laboratory within one hour. The sample was then dissected into strips approximately 0.5 x 0.2cm and embedded in low melting temperature agarose (37°C, 3% w/v, 0.9% NaCl; MilliporeSigma, Darmstadt, Germany). Once the agarose solidified, the tissue was cut into 400µm thick slices using a Krumdieck Tissue Slicer (Alabama Research and Development, Munford, AL, USA), or a VT1200S Vibratome (Leica Microsystems, Nussloch, Germany). PCIS were then incubated in 12-well culture plates in

1.2ml Williams' medium E (WME) containing L-glutamine and supplements (D-glucose 14mM, gentamycin 50µg/ml; Gibco, MA, USA) in an oxygenated incubator (37°C, 90% O₂, 5% CO₂; Thermo Fisher Scientific, MA, USA) with gentle shaking (Figure 1A).

Viability assays

The viability of PCIS were assessed using lactate dehydrogenase (LDH) release (cytotoxicity) assays and water-soluble tetrazolium salt (WST-1) (metabolic) assays (Roche, Mannheim, Germany) from 0-24h in culture. Treatment with the detergent Triton X-100 (MilliporeSigma, Darmstadt, Germany) was included to show maximum LDH release and as a dead-control.

Single cell nuclear sequencing of human colon tissue

Colon tissue samples (n=4), also used to generate PCIS, were snap-frozen and stored at -80°C prior to single nuclear RNA sequencing (scNucSeq). After library generation and quality control, the samples were deep sequenced using a NovaSeq (target 10 000 nuclei, 80 000 reads/nuclei). FASTQ files were aligned to a reference human transcriptome using 10X Genomics Cell Ranger (CA, USA). The resulting gene expression data were merged using the Seurat R package.^{23,24} Batch effects were removed by "regressing out" variables, and clustering was performed using the Leiden algorithm implemented in Seurat. Cell identities were assigned and confirmed using a list of pre-defined marker genes in reference to single cell gene expression databases and the top distinct genes expressed by each cluster.^{25,26}

Passive sensitization

PCIS were passively sensitized in a 1:10 dilution of human plasma from clinically confirmed peanut allergic donors or peanut-sensitized non-allergic donors (Table S2) in WME for 90-120min (37°C, 90% O₂, 5% CO₂) with gentle shaking. Human plasma was derived from ongoing and completed trials including the Markers of Nut Allergy Study (MONAS) cohort at the Hospital for Sick Children.²⁷ The use of human plasma for research was approved by the SickKids REB for MONAS (#1000053791) and the Low Dose Multi-OIT (LoMo) study (#1000060633).

Video microscopy

PCIS muscle contraction was filmed using a stereomicroscope with a camera attachment (Walter Products, ON, Canada; TCapture Software, Tucson Photonics, Fuzhou, China) for 10min without the addition of stimuli to establish baseline movement of the individual sample. The slices were then filmed for an additional 10min upon addition of either peanut extract (PE) (1µg/ml, ALK-Abelló, Hørsholm, Denmark), serotonin (10µg/ml, MilliporeSigma, Darmstadt, Germany), FceRI-crosslinker (10µl, Bühlmann, Amherst, NH, USA), histamine (10µg/ml, ALK-Abelló, Hørsholm, Denmark) or ovalbumin (OVA; 1µg/ml, InvivoGen, San Diego, CA, USA) as an irrelevant allergen. Any samples not passing quality control criteria (Table S3) were excluded from analysis.

Analysis of PCIS smooth muscle contractions

Statistical analysis was conducted using GraphPad Prism 6 (GraphPad Software, San Diego, CA, USA). Responses between different stimulations were compared using the Mann-Whitney U test with a *P* value <.05 considered statistically significant.

A novel video analysis software was developed internally (available at: <https://github.com/celalp/video.-parser>). This software measures muscle contraction using individual pixel movement per frame (Figure 2). Videos were analyzed using a semi-supervised approach with variables kept consistent between the unstimulated control and stimulated sample. Videos were processed using OpenCV (Open Source Computer Vision Library) and scikit-image Python packages.^{28,29} Pixels that changed from frame x-1 to frame x were calculated using a Gaussian mixture-based background/foreground segmentation algorithm.^{30,31} A background subtraction algorithm was chosen for this analysis as videos consisted of one large central object with a mostly static background, meaning that any observed movement was captured as foreground. The selection of the specific algorithm (MOG2 in OpenCV) was influenced by the large variability of exposure levels (e.g.,

glare) between different slices. A dynamic Gaussian Mixture Method allowed for the same background removal method for all experiments. Measurements were validated and confirmed by an independent assessor. Overall pixel movement per frame of the video was plotted for each stimulation and total Area Under the Curve (AUC) was calculated and compared for each contraction response using GraphPad Prism 6.

Tryptase quantification in cell free supernatant

Culture supernatant from PCIS passively sensitized and stimulated with PE (1 μ g/ml) or controls was collected 10min post-stimulation and stored at -80°C. Tryptase levels in supernatant were measured using a tryptase beta-2 ELISA (MilliporeSigma, Burlington MA, USA) after concentration using centrifugal filter devices (Amicon Ultra-0.5 10K device, MilliporeSigma, Burlington MA, USA).

Treatment with antihistamines

Passively sensitized PCIS were incubated with diphenhydramine hydrochloride (10 μ g/ml), cetirizine dihydrochloride (1 μ g/ml) and rupatadine (1 μ g/ml) (MilliporeSigma, Darmstadt, Germany) for 10min prior to stimulation with histamine (10 μ g/ml) and filmed for 10min post-stimulation. Concentrations were chosen based on published reports and dose-response experiments.^{32–35}

Effect of immunomodulation from successful OIT patients

PCIS were passively sensitized with plasma (1:10 in WME) and stimulated with PE and controls to assess differences in pre- (oral food challenge [OFC] positive) and post-successful peanut OIT (ingestion of 5x the baseline eliciting dose).

RESULTS

PCIS maintain viability for at least 24h

This model was generated to study acute, IgE-mediated allergic reactions, which are known to occur within seconds of exposure to a relevant allergen. To confirm that PCIS remained viable during the incubation process, they were monitored from 0-24h post-slicing. Cell death (LDH) and metabolism (WST-1) were assessed in each sample and demonstrated consistent viability of PCIS during the investigated 24h in culture (Figure 1B, C).

scNucSeq of gut tissue confirms the presence of mast cells and other cell types

The cellular composition of PCIS was analyzed using scNucSeq. Single cell gene expression profiles from colon tissue donors (n=4) were compiled to reveal the cellular composition of PCIS (Figure 1D). Expression profiles aligned with the cellular identities of each cluster and were confirmed using single cell gene expression databases.^{25,26} All expected cell types in colon tissue were observed (Figure 1D, Table S4) including epithelial cells (enterocytes, goblet cells, enteroendocrine cells, endothelial cells), stromal cells (fibroblasts, smooth muscle cells) and immune cells (T cells, $\gamma\delta$ T cells, B cells, mast cells, dendritic cells, macrophages).

PCIS contract in response to antigen-independent stimuli

To validate the model and determine the sensitivity of PCIS to non-specific antigen stimulations, FceRI-crosslinker, histamine and serotonin were selected as positive controls. All conditions elicited a strong, reproducible smooth muscle contraction response, elevated in comparison to the unstimulated control (Figure 3A; Video S1-6).

Based on raw video data, a novel software was designed to quantify smooth muscle contractions using object and movement detection of irregular shapes. This was achieved by tracking the overall movement of individual pixels within each frame (Figure 2) which were then quantified and graphed resulting in a curve of the contraction response. Total AUCs of each response per stimuli were compared, showing a significant increase in movement between the unstimulated slices and PCIS stimulated with the positive controls (Figure 3B).

Allergen-specific induction of PCIS contractions reflects allergic status of the donor

In order to establish PCIS as a model of FA, responses must be reproducible, allergen-specific, and clinically relevant. PCIS passively sensitized with plasma from clinically confirmed peanut allergic children displayed a strong contractile response after stimulation with PE (Figure 4A; Video S7,8). This response was strictly allergen-specific, as stimulation with a clinically irrelevant food allergen (OVA) did not result in an increased response (Figure 4A; Video S9,10).

To evaluate whether response patterns were linked to allergen-specific IgE or match clinical reactivity at the individual level, PCIS were passively sensitized with plasma from peanut-sensitized non-allergic donors. These donors had measurable peanut-specific IgE but were asymptomatic upon peanut exposure. PCIS sensitized with this plasma did not show significant muscle contraction responses following stimulation with PE, while maintaining reactivity to positive controls (Figure 4A; Video S11, 12). The total AUCs of each contraction response revealed a significantly greater response following stimulation with a relevant allergen (PE), in comparison to an irrelevant allergen (OVA) in slices sensitized with peanut allergic plasma, as well as to PE stimulation in sensitized non-allergic tissue (Figure 4B).

Tryptase was measured in the culture supernatant following stimulation to confirm the release of mast cell derived mediators (Figure 4C). Tryptase concentrations in the supernatant of PCIS were significantly greater following exposure to PE and elevated in response to FcεRI-crosslinker in tissue sensitized with peanut allergic plasma. Unstimulated tissue or stimulation with an irrelevant allergen (OVA) did not result in similar tryptase levels, demonstrating the specificity of the response and linkage to degranulation (Figure 4C). PE stimulation in slices sensitized with peanut sensitized non-allergic plasma did not result in a significant change in tryptase concentration, reflecting their lack of reactivity to PE exposure (Figure 4C).

PCIS can be used to study anti-allergic drugs

Antihistamines were used to demonstrate the suitability of PCIS as a model of FA to assess specific mechanisms of action. Pre-treatment of the tissue with the antihistamines diphenhydramine hydrochloride, cetirizine dihydrochloride and rupatadine resulted in significant suppression of contraction responses to histamine stimulation (Figure 4D).

Peanut OIT suppresses allergen-specific responses

As a promising treatment option for FA, the suppression of allergic responses following OIT was also investigated using the PCIS model. PCIS passively sensitized with plasma from a peanut allergic individual pre-peanut OIT, stimulated with PE resulted in a strong smooth muscle contraction response (Figure 5). Following completion of the OIT protocol, plasma from the same individual resulted in a diminished response after stimulation with PE, which is reflective of the clinical phenotype of the plasma donor. Co-incubation of pre- and post-OIT plasma also resulted in a reduced contraction response in comparison to the pre-OIT condition, reflecting the sensitivity of the model (Figure 5).

DISCUSSION

As the incidence and awareness of FA increases, there is a demand for appropriate models of FA for mechanistic studies and the development of therapeutics. This study aims to generate a human gut tissue-based model of acute, IgE-mediated FA. Using the PCIS system, allergic responses that occur within GI tissues may be characterized.

Cellular composition is key for accurate inferences on the nature of an investigated response. To ensure the representation of distinct cell subsets, scNucSeq was employed to define the cellular composition of the PCIS. The resulting map of cellular distribution confirmed that all relevant cell types were present in the samples, including stromal and epithelial cells as well as key immune cell populations that are commonly described in human colon tissue.^{36,37} Not only is this dataset useful in this context, but forms the framework for future functional assessments and adds to single cell libraries for healthy infant colon tissue, a relatively understudied group.

In addition to confirmation of cellular diversity, establishing tissue viability and metabolic activity in the

tissue is essential to ensure accurate readouts. Using routine LDH and WST-1 assays, PCIS maintained consistent viability for at least 24h in culture.³⁸ This model was specifically designed for acute allergic responses, which did not warrant investigations beyond 24h. Previous reports have shown that the viability and morphology of human PCIS can be maintained up to 48h²⁰ and even 72h.^{19,39}

Smooth muscle contraction was chosen as the readout for allergic responses in the PCIS model, as allergic reactions stemming from food exposure often result in symptoms centralized to the GI tract.⁴⁰ Moreover, food allergen-induced muscle contraction in sensitized individuals has been reproducibly demonstrated in both animal and human models.^{41–43} The inflammatory mediators stored in mast cell granules, which includes histamine, serotonin and tryptase, have all been shown to directly cause smooth muscle contraction in the gut.^{5,44,45} Similar studies using precision cut lung slices have also utilized bronchoconstriction to model allergic responses to aeroallergens.^{46–48} To measure the contraction response in PCIS, a novel analysis program was developed internally, as other commercial motion tracking software were unable to reflect the patterns observed in the videos. This program, designed to quantify the muscle contraction responses in PCIS, also has the potential to be applied beyond this system. Any model in which movement is a central aspect of response could utilize this program to quantify results. The configurations can also be set based on controls and adjusted to accommodate changes in lighting during filming.

PCIS generated from non-allergic donor tissue underwent passive sensitization to induce sensitivity to the allergen of the allergic plasma donor. Passive sensitization is a common experimental procedure used to induce sensitivity in non-allergic cells or tissue.^{47,49–51} Use of this process not only reduces the need for relatively rare allergic tissue donors, but also extricates the humoral component of the allergic response. PCIS passively sensitized with clinically confirmed peanut allergic plasma stimulated with PE or positive controls displayed a strong contraction response that was significantly greater than the baseline movement due to enteric reflexes.⁵² This response was allergen-specific, as demonstrated by the lack of response to a clinically irrelevant allergen (OVA). In contrast, PCIS passively sensitized with plasma from a peanut-sensitized non-allergic donor did not display a strong response to PE stimulation, indicating that the PCIS model reflects the clinical phenotype of the donor. Although the overall patterns of response were clear between stimuli, the kinetics and magnitude of the muscle contraction responses may vary between individual PCIS. This was expected as the gut slices differ in terms of smooth muscle composition and mast cell distribution due to variability between tissue donors and along an individual GI tract.

Tryptase release from intestinal mast cells occurs immediately upon activation.⁵³ Measurements of tryptase in the PCIS culture supernatant confirmed inflammatory mast cell-derived mediators were released following stimulation with a relevant allergen (peanut) or FcεRI-crosslinker but not an irrelevant allergen (OVA), further highlighting the specificity of responses.

As observed in the PCIS model, histamine is a key mediator of the allergic response that affects smooth muscle contraction. Thus, antihistamines were the drug of choice to demonstrate the utility of this *ex vivo* model for testing anti-allergic therapeutics in development, as illustrated by the suppression of contraction responses. While anti-allergic drugs are commonly tested in animal models,^{41,54,55} the use of a human tissue-based model either in place of or in addition to animal testing would allow for more translatable and clinically relevant results. Of note, the PCIS model has often been used to study pharmacokinetics in the context of human gut tissue.^{14–16,20,21}

Passive sensitization of PCIS with plasma from a peanut allergic donor pre-OIT resulted in a robust contraction response following stimulation with PE, while a comparatively diminished response was observed post-peanut OIT as well as in combination. OIT has become a standard treatment option for FA and has been broadly effective in treating children with established FA. During OIT, levels of allergen-specific IgG, IgG4 and IgA increase, and are considered to act as protective antibodies, whereas levels of allergen-specific IgE decline over time.^{56,57} These results demonstrate the use of PCIS as a model of FA in potentially assessing responses to therapy and immunomodulation. Moreover, at the start and end of OIT, an OFC is completed to determine the threshold of response and the development of desensitization respectively; however, severe reactions may occur. As PCIS reflects allergic status, this model may also be used as a proxy for OFCs to

prevent higher risk challenges.

In summary, a novel human gut tissue-based FA model has been developed. PCIS generated from non-allergic tissue, passively sensitized with plasma from allergic donors, displayed visible and quantifiable allergen-specific smooth muscle contractions upon allergen stimulation. This model has great potential as a valuable experimental tool in FA research as it can be used to differentiate sensitized allergic versus sensitized non-allergic individuals, test anti-allergic drugs within a relevant environment and observe the progression of allergen-specific immunotherapy. As mast cells are difficult to isolate in peripheral blood, and the complex interactions between immune cells and structural cells in the gut are not easily observed, the utility of this FA model addresses a relevant research need.

As with all models, there are several constraints. A significant limitation is the lack of tissue availability from appropriate sources. Additionally, gut tissue is sensitive to culture conditions and has a limited viability. While this is appropriate for acute, short-term outcomes, this model cannot be used without modifications for long-term studies or for the study of delayed allergic responses. Additional considerations include the isolated nature of the PCIS model, which cannot replicate circulation or migration of cells from other tissue, as well as equal exposure to stimulants on both surfaces (apical/basal) which does not reflect the human system. Importantly, as the tissue is from non-atopic donors, it cannot reflect the pre-existing inflammation observed within the GI tract of allergic patients.

Despite these limitations, this human tissue-based model is directly translatable in contrast to individual cell culture models or animal models of disease which cannot replicate the human system. It contains all resident cell types interacting in a physiologically accurate environment. This allows for observation of cellular mechanisms in tissues that are not represented by peripheral blood samples and may also be adapted to investigate other GI-based diseases. The promising potential of PCIS as a model of FA will also expand to future studies including early changes in gene expression following allergen exposure.

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FIGURE LEGENDS

Figure 1. Precision Cut Intestinal Slice (PCIS) preparation, viability, and cellular distribution

(A) After surgical excision of a portion of intestinal tissue as part of a clinically indicated procedure, a sample (approximately 1.0 x 0.5 x 0.2cm) was taken from the proximal site of the excision and placed in oxygenated, chilled, buffer (I) . The tissue was dissected into smaller strips (approximately 0.5 x 0.2cm), embedded in low melting temperature agarose (II) and 400µm thick slices(III) were generated. PCIS were kept in an oxygenated incubator(IV) (37°C, 90% O₂, 5% CO₂). (B) Cell death in PCIS was determined using the lactate dehydrogenase (LDH) assay. Absorbance was measured as OD 490nm (n=16). Detergent Triton X-100 (TX-100) used as a positive control (C) Metabolic activity in PCIS was measured using the water-soluble tetrazolium salt (WST-1) assay. Absorbance was measured as OD 450nm (n=16). (D) Single nuclear RNA sequencing gene expression data of human colon tissue samples (n=4) merged to display approximate cellular distribution of PCIS using Uniform Manifold Approximation and Projection. *P <.05, ****P<.0001, bars indicate mean, error bars represent ± SEM.

Figure 2. Example muscle contraction measurement using novel video analysis software

Peanut sensitized, allergic PCIS show visible and quantifiable smooth muscle contraction responses following peanut extract stimulation, illustrated by the appearance of yellow pixels in the program output at 5min. Sensitized non-allergic PCIS stimulated with peanut extract did not show comparable movement as indicated by the reduced yellow pixel appearance.

Figure 3. FceRI-crosslinker, serotonin and histamine induce smooth muscle contractions in PCIS

(A) Example graphs showing PCIS filmed for 10min without stimulation to establish baseline movements, then an additional 10min following stimulation. FceRI-crosslinker, serotonin and histamine were used as positive controls. PCIS smooth muscle contraction was measured and quantified using movement per frame. X-axis indicates each frame of the video; y-axis indicates overall movement of individual pixels (n=1). (B) Total area under the curve (AUC) of muscle contraction responses to different stimulations (n=16). Unstimulated (MC); FceRI-crosslinker stimulation (α -FceRI); Serotonin stimulation (5-HT); Histamine stimulation (Hist). * $P < .05$, ** $P < .01$, **** $P < .0001$, bars indicate mean, error bars represent \pm SEM.

Figure 4. Passively sensitized PCIS show allergen-specific muscle contraction responses, mediator release and are affected by antihistamines

(A) Example graphs showing PCIS filmed for 10min without stimulation to establish baseline movements, then an additional 10min following stimulation. PCIS passively sensitized with peanut allergic plasma (PA) stimulated with peanut extract (PE, 1 μ g/ml) or irrelevant allergen (ovalbumin [OVA], 1 μ g/ml) (n=1) and PCIS passively sensitized with peanut sensitized non-allergic plasma (PS NA) stimulated with PE (1 μ g/ml) (n=1). (B) Total area under the curve (AUC) of muscle contraction responses to PE or irrelevant allergen (OVA) (n=16). (C) Tryptase concentration in culture supernatant from PCIS passively sensitized with PA stimulated with PE, FceRI-crosslinker (α -FceRI), irrelevant allergen (OVA) or unstimulated (MC), and PCIS passively sensitized with PS NA stimulated with PE (n=9). (D) Muscle contraction responses to histamine stimulation (10 μ g/ml) by passively sensitized PCIS untreated or pre-treated with antihistamines diphenhydramine hydrochloride (10 μ g/ml), cetirizine dihydrochloride (1 μ g/ml) or rupatadine (1 μ g/ml). Y-axis compares total AUC following histamine stimulation to the respective unstimulated (baseline) movements for each slice, represented by 100% on the y-axis and the horizontal dotted line (n=3). * $P < .05$, ** $P < .01$, **** $P < .0001$, ns is not significant, bars indicate mean, error bars represent \pm SEM.

Figure 5. PCIS passively sensitized with peanut allergic donor plasma before and after peanut oral immunotherapy (OIT) shows change in contraction response

Contraction responses of PCIS passively sensitized with peanut allergic plasma (PA) from a donor prior to peanut OIT, unstimulated, stimulated with peanut extract or an irrelevant allergen (ovalbumin); PCIS passively sensitized with plasma from the same donor after successful peanut OIT (OIT) stimulated with peanut extract or histamine; PCIS passively sensitized with a mix of plasma from before and after successful peanut OIT (PA+OIT mix) at a ratio of 1:3 respectively, stimulated with peanut extract.

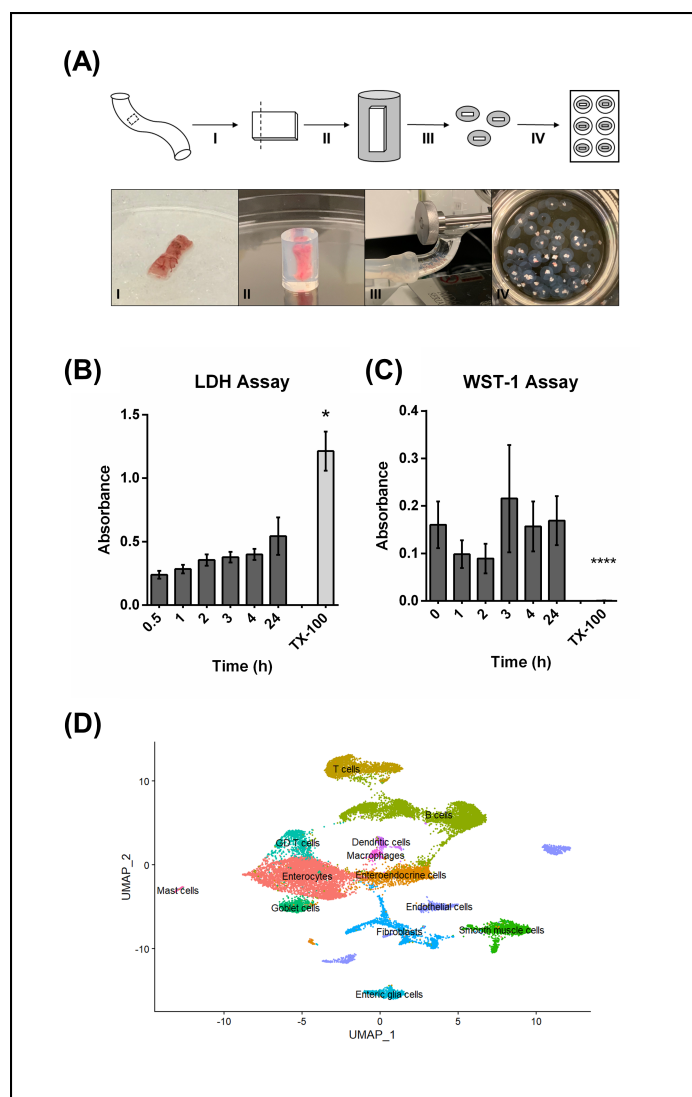


Figure 1_Hung et al.

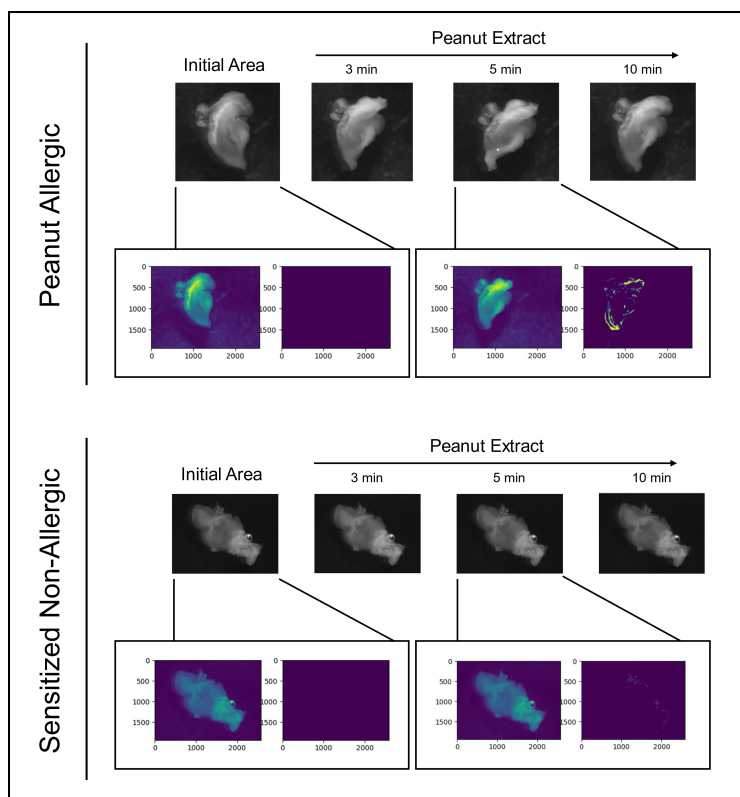


Figure 2_Hung et al.

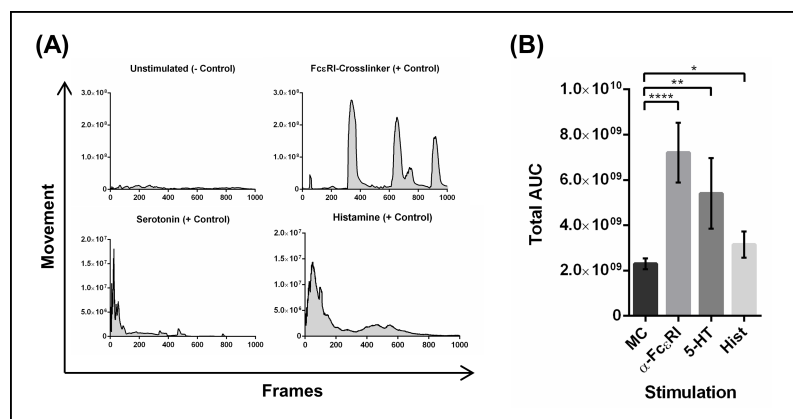


Figure 3_Hung et al.

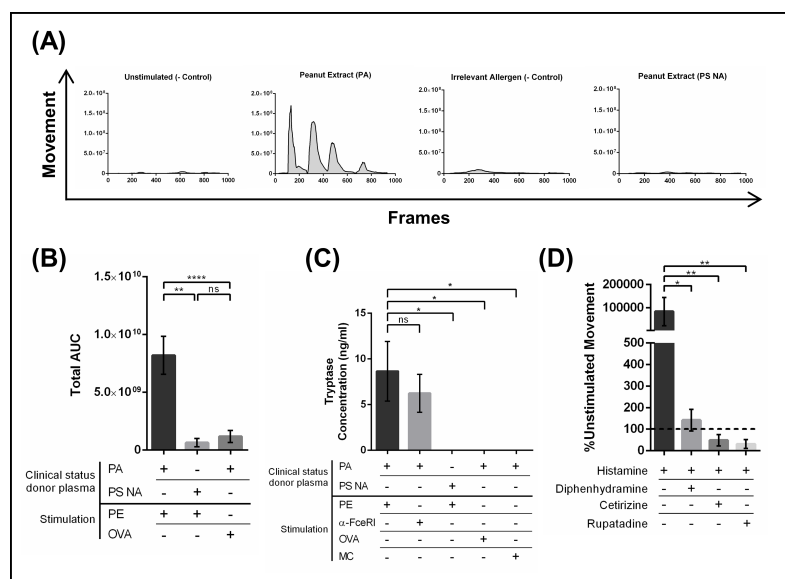


Figure 4_Hung et al.

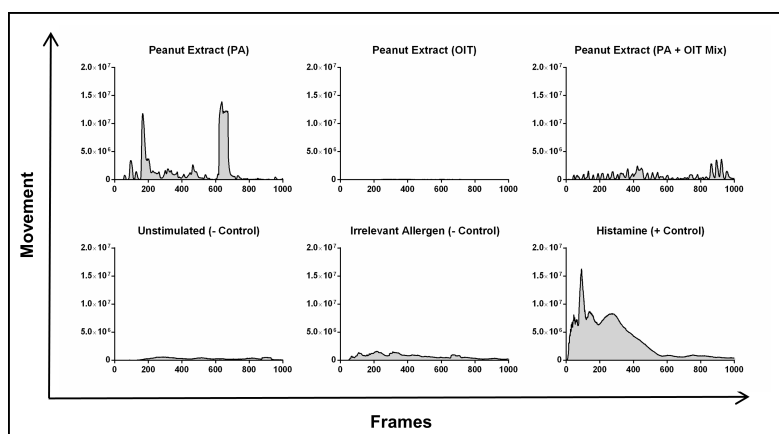


Figure 5_Hung et al.