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Title: An Intestinal Gut Organ Culture System for Analyzing Host-Microbiota Interactions

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Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **yes**

    If **Yes**, can you record movies/images using your own microscope camera?

    no

    If your protocol involves microscopy but you are not able to record movies/images with your microscope camera, JoVE will need to use our scope kit. If your microscope does not have a camera port, the scope kit will be attached to one of the eyepieces and **you will have to perform the procedure using one eye**.

    Please list the make and model of your microscope.

    Zeiss Stemi 305

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes**

4. **Filming location:** Will the filming need to take place in multiple locations? **No**

Current Protocol Length

Number of Steps: 11
Number of Shots: 25

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Nissan Yissachar:** The gut organ culture method combines the advantages of in vitro and in vivo assays, and thus serves as an intermediate experimental step between simple *in-vitro* assays to complex *in-vivo* experiments.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

1.2. **Hadar Bootz:** The main advantage of this technique is a combination of high controllability with the preservation of intestinal physiology.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.2.2 and 4.2.3*

OPTIONAL:

1.3. **Shalhevet Azriel:** This method can provide insight for analyzing intestinal responses to a specific stimulus with a high level of control over the host, microbial and environmental components.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.4.1 and 4.4.2*

Introduction of Demonstrator on Camera

1.4. **Nissan Yissachar:** Demonstrating the procedure will be Alon Shemesh, a M.Sc. student from my laboratory and Dr. Sivan Amidror, our lab manager.

1.4.1. INTERVIEW: Author saying the above.

1.4.2. The named demonstrator(s) looks up from workbench or desk or microscope and acknowledges the camera.

Ethics Title Card

1.5. This protocol follows the animal care guidelines approved by the ethics committee for animal welfare.

Protocol

2. Experiment Preparation

2.1. To begin, insert the blunt-end needles to the appropriate position within the device mold **[1-TXT]** and cast approximately 20 grams of polydimethylsiloxane, or PDMS, mix for one set of the device and lid **[2-TXT]**.

2.1.1. WIDE: Establish the shot of talent inserting the blunt end needles **TEXT: Needles: 22 G & 18 G**

2.1.2. Talent casting PDMS **TEXT: PDMS mix: 1:10 weight ratio, base to catalyst**

2.2. Place the molds in a vacuum chamber for 30 minutes to remove air bubbles from the PDMS mix **[1]**, then incubate the molds at 55 degrees Celsius overnight to complete PDMS polymerization **[2]**.

2.2.1. Talent placing the molds in vacuum chamber

2.2.2. Talent incubating the mold at 55 degrees

2.3. When the PDMS is set, pull out the needles from the mold and carefully release the culture device and lid from the plastic molds **[1]**. Remove PDMS residues from the well outline using a surgical blade **[2]**.

2.3.1. Talent pulling out the needles from mold and releasing culture device and lid

2.3.2. Talent removing PDMS from well outline using surgical blade

2.4. Attach the PDMS device and the device cover onto a cover glass of 75 by 50-millimeter micro slides using non-toxic silicon adhesive **[1]** and leave the parts to set overnight. Apply the glue to the smooth side of the device **[2]**.

2.4.1. Talent attaching PDMS device and cover onto microslides using adhesive
Videographer: This step is important!

2.4.2. Talent applying glue to the smooth side of the device

2.5. Insert twelve 22-gauge needles for the lumen **[1]** and twelve 18-gauge needles for the well **[2]**. Fix all the needles in place using silicone and let it set overnight **[3]**.

2.5.1. Talent inserting 22-gauge needles

2.5.2. Talent inserting 18-gauge needles

2.5.3. Talent fixing the needle using silicone

3. Experiment Setup Preparation

3.1. Purge the input syringes **[1]** and make sure that the well medium flows out from all tubes into a waste glass **[2]**.

3.1.1. Talent purging the well input syringes *Videographer: This step is important!*

3.1.2. Medium flowing out from tubes into waste glass *Videographer: This step is important!*

3.2. Then, purge the input syringes and make sure that the stimulations flow out of all the tubes into a waste glass, taking care to not contaminate the different stimulations **[1]**.

3.2.1. Stimulation flowing out of the tubes into waste glass

4. Organ Cultures

4.1. After sacrificing the mouse, use sharp scissors and forceps to dissect it **[1]** and take out the digestive tract from the stomach to the anus by cutting all the fat and connective tissues **[2]**. Cut the colon and place it on a new plate. Minimize contact while holding the tissue gently and only at the edges **[3]**.

4.1.1. WIDE: Talent performing dissection

4.1.2. Talent taking out the digestive tract of mouse

4.1.3. Talent cutting the colon and placing it on a new plate

4.2. Perform the colon flush under a dissection microscope **[1]**. Gently flush the colon content with sterile IMDM with the prepared 10-milliliter syringe as described in the text **[2]**. After removing the feces from the intestinal tissue ~~**[3],**~~ place the colon in a new 6-well plate filled with 0.5 milliliters of sterile IMDM **[3]** ~~**[4].**~~

4.2.1. WIDE: Talent looking through dissection microscope

4.2.2. SCOPE: Talent flushing the colon content *Videographer: This step is important!*

4.2.3 ~~SCOPE: Talent removing the fecal content *Videographer: This step is important!*~~ NOTE: This shot was not filmed

4.2.3. Colon placed in a new well plate filled with sterile IMDM

4.3. Next, take the colon tissue and carefully connect it to the 22-gauge needle, making a tight tie with the two threads **[1]**. Maintain the correct orientation of the colon to the lumen flow such that proximal and distal is equal to input and output, respectively. Repeat colon flushing and needle-tying for all the tissues **[2]**.

4.3.1. SCOPE: Talent connecting the tissue onto the needle and tying it with the two threads *Videographer: This step is important!*

4.3.2. The device full with 6 colons connected

4.4. Connect the input and output tubes to the device **[1]**, then begin the experiment by starting the pumps at the desired rates **[2-TXT].**

4.4.1. Talent connecting input and output tubes to the device

4.4.2. Talent starting the pump **TEXT: Flow rate: lumen: input– 30 µL/h, output– 35 µL/h, External medium: input– 1000 µL/h, output– 950 µL/h**

Results

5. Results: Assessment of Intestinal Structure and Host-Microbiota Interactions using Gut Organ Culture

5.1. Mucus-filled goblet cells in the colonic epithelium and mucus secretion within the lumen were detected as well as proliferating IEC in the colonic crypts, as indicated by Ki-67 staining. These results show that the gut culture system maintains intestinal function and structure ex vivo **[1]**.

5.1.1. LAB MEDIA: Figure 2

5.2. After 2 hours of segmented filamentous bacteria, or SFB, introduction, typical SFB filaments were detected in close association with small intestine villi using fluorescence in situ hybridization **[1]**. Additionally, a transmission electron microscopy presented SFB within a few microns of the small intestine epithelium brush border **[2]**.

5.2.1. LAB MEDIA: Figure 3a

5.2.2. LAB MEDIA: Figure 3b

5.3. Gene expression profiles of whole-tissue samples were produced, in triplicate, 2 hours after infusion with SFB. Control cultures were infused with fecal suspensions of germ-free or Bacteroides fragilis-monocolonized mice. The changes persuaded by SFB were mainly of small amplitude, compared to the germ-free control **[1]**.

5.3.1. LAB MEDIA: Figure 3c

Conclusion

6. Conclusion Interview Statements

6.1. **Alon Shemesh:** The orientation of the colon is highly important. Take extra care when tying the colon on the needle, proper tie will prevent the contamination of the well with the lumen content.

6.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.3.1 and 4.3.2*

6.2. **Sivan Amidror:** A wide range of readout techniques can follow this protocol, such as, next-generation sequencing, imaging, cell sorting and many more. These readouts will provide a novel insight into host-microbiome interactions in health and disease.

6.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.