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Title: An Intestine/Liver Microphysiological System for Drug Pharmacokinetic and Toxicological Assessment

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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 similar? **N**

2. **Software:** Does the part of your protocol being filmed demonstrate software usage? **N**

3. **Interview statements:** Considering the Covid-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

   ☒ Interviewees self-record interview statements outside of the filming date. JoVE can provide support for this option.

4. **Filming location:** Will the filming need to take place in multiple locations (greater than walking distance)? **N**

Protocol Length
Number of Shots: **41**

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Talita Miguel Marin**: MPS's have the ability to emulate pharmacokinetic and toxicological responses of the human body to specific treatments of interest **[1]**.

1.1.1. LAB MEDIA: To be provided by Authors: Named talent says the statement above in an interview-style shot, looking slightly off-camera NOTE: Talita's statements were recorded on the day of the shoot

REQUIRED:

1.2. **Talita Miguel Marin**: MPS's have the potential to replace animal testing, as their use can improve the predictability of in vitro methods and reduce the cost and time of pharmacokinetic and toxicological studies **[1]**.

1.2.1. LAB MEDIA: To be provided by Authors: Named talent says the statement above in an interview-style shot, looking slightly off-camera

OPTIONAL:

1.3. **Desiree Cigaran Schuck**: For example, cosmetic companies can use MPS's instead of animal models to guarantee the safety of new molecules in a fast, reliable, ethical manner **[1]**.

1.3.1. LAB MEDIA: To be provided by Authors: Named talent says the statement above in an interview-style shot, looking slightly off-camera NOTE: Desiree and Nathalia's statements should be self-recorded during the week of 10/12/2020 – 10/19/2020

OPTIONAL:

1.4. **Nathalia de Carvalho Indolfo**: The production of robust organoids and their proper integration into the device can be difficult. Be sure to check the quality of the organoids and to integrate them carefully into the chip **[1]**.

1.4.1. LAB MEDIA: To be provided by Authors: Named talent says the statement above in an interview-style shot, looking slightly off-camera

OPTIONAL:

1.5. **Nathalia de Carvalho Indolfo**: Visual demonstration is critical for showing important details, such as avoiding the formation of bubbles during organoid integration and correctly delivering the treatment **[1]**.

1.5.1. LAB MEDIA: To be provided by Authors: Named talent says the statement above in an interview-style shot, looking slightly off-camera

Introduction of Demonstrator on Camera

1.6. **Talita Miguel Marin**: Demonstrating the procedure with me and Nathalia will be Silvana Aparecida Rocco, and Murilo de Carvalho, researchers from my laboratory **[1][2]**.

1.6.1. LAB MEDIA: To be provided by Authors: Author saying the above
1.6.2. The named demonstrator(s) looks up from workbench or desk or microscope and acknowledges the camera

Protocol

2. Intestine and Liver Two-Organ Chip (2-OC) Microphysiological System (MPS) Assembly

2.1. Twenty-four hours before the test substance administration, split an-800 microliter aliquot of William E S medium between the larger and smaller compartments of the two-organ chip **[1-TXT]** and aspirate the basolateral and apical medium from each intestinal barrier equivalent in the prepared 24-well plates **[2-TXT]**.

2.1.1. WIDE: Talent adding medium to compartment(s), with medium container visible in frame **TEXT: See text for all medium and solution preparation details**

2.1.2. Talent aspirating medium from compartments in 24-well plate *Videographer: Important step* **TEXT: See text for full 2-OC setup details**

2.2. Using sterile forceps, integrate one insert per two-organ chip circuit into the larger compartment **[1]** and add 200 microliters of DMEM S (D-M-E-M S) to the apical side **[2]**.

2.2.1. Talent adding the intestine equivalent to apical side *Videographer: Important step*

2.2.2. Talent adding medium to apical side, with medium container visible in frame, with medium container visible in frame *Videographer: Important step*

2.3. Using wide-bore tips, integrate twenty liver equivalents per circuit into the smaller compartment of the two-organ chip **[1]** and connect the system to the control unit **[2]**.

2.3.1. Talent adding liver equivalent to smaller compartment *Videographer: Important step*

2.3.2. Talent connecting system to control unit *Videographer: Difficult step*

2.4. Then connect the control unit to a pressurized air supply **[1]** and set the pressure to approximately plus or minus 300 bars and a pumping frequency of 0.3 hertz **[2]**.

2.4.1. Talent setting pressure and pumping frequency

2.4.2. Added shot: Talent positioned the MPS connected to the controller unit, inside the cell incubator

3. "Oral" Acetaminophen (APAP) Administration

3.1. The next day, dilute the stock acetaminophen solution to a 12-micromolar concentration **[1]**.

3.1.1. WIDE: Talent adding APAP stock to medium, with stock and medium containers visible in frame **TEXT: Oral administration: 12 micromolar; Intravenous administration: 2 micromolar**

3.2. To replace the medium in the system, aspirate the basolateral and apical media from the intestinal barrier equivalent in the two-organ chip **[1]** and add 500 microliters of fresh appropriate culture medium into the large compartment at the organoid basolateral side and 300 microliters to the small compartment **[2]**.

3.2.1. Talent aspirating medium

3.2.2. Talent adding medium to basolateral side and small compartment and/or small compartment, with medium container visible in frame

3.3. After checking for bubbles, emulate oral administration with the addition of 200 microliters of a 12-micromolar acetaminophen solution on the apical side of the intestinal culture inserts **[1-TXT]** and connect the microphysiological system to the control unit **[2]**.

3.3.1. Talent adding solution to insert(s), with APAP solution container visible in frame *Videographer: Important step*

3.3.2. Talent connecting system to unit

3.4. Collect the total volume from both the intestinal the apical and basolateral sides as well as from the small compartment in triplicate at the indicated time points **[1-TXT]**.

3.4.1. Talent collecting sample **TEXT: *e.g.*, 0, 5, 15, 30, 60, 180, 360, 720, 1440 min**

3.5. When all of the samples have been collected **[1-TXT]**, set all of the relevant parameters for the HPLC (H-P-L-C) analysis as indicated in the Table **[2-TXT]** and filter the mobile phase through a 0.45-micron membrane filter under vacuum **[3]**.

3.5.1. Talent placing samples onto bench **TEXT: Perform all experiments in triplicate under static and dynamic conditions**

3.5.2. LAB MEDIA: Table 1 *Video Editor: please emphasize right column* **TEXT: HPLC: high pressure liquid chromatography**

3.5.3. Talent filtering sample

3.6. Then filter the samples through a 0.22-micron-pore size PVDF (P-V-D_F) syringe filter **[1-TXT]** and store the samples in a vial **[2]** before starting the HPLC UV measurement **[3]**.

3.6.1. Talent filtering sample through syringe **TEXT: PVDF: polyvinylidene fluoride**

3.6.2. Talent storing sample in vial

3.6.3. Talent starting measurement

4. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) Assay

4.1. To assess the organoid viability, transfer all 20 spheroids from each replicate to individual wells of a 96-well plate **[1]** and transfer the cell culture inserts to individual wells of 24-well plate **[2]**.

4.1.1. WIDE: Talent adding spheroids to well, with MPS visible in frame *Videographer: Important step*

4.1.2. Talent placing insert into well, with MPS visible in frame *Videographer: Important step*

4.2. Wash the tissue equivalents three times with fresh DPBS (D-P-B-S) per wash **[1-TXT]** and add 300 microliters of a 1-milligram/milliliter MTT (M-T-T) solution to each experimental well **[2]**.

4.2.1. Talent washing insert(s) **TEXT: DPBS: Dulbecco's phosphate buffered saline**

4.2.2. Talent adding MTT to well(s), with MTT container visible in frame

4.3. After a 3-hour incubation in the cell culture incubator, replace the MTT solution with 200 microliters of isopropanol per well for an overnight incubation at 4 degrees Celsius to extract the MTT formazan from the intestine and liver equivalents **[1]**.

4.3.1. Talent adding isopropanol to well(s), with isopropanol container visible in frame

4.4. The next morning, transfer 200 microliters of each supernatant to the appropriate well in the 96-well plate **[1-TXT]** and read the formazan on a plate reader at 570 nanometers **[2]** to allow calculation of the relative ability of the cells to reduce MTT using the average optical density of each time point compared to the negative control **[3]**.

4.4.1. Talent adding supernatant to well(s) **TEXT: Blank: isopropanol**

4.4.2. Talent loading plate onto plate reader

4.4.3. LAB MEDIA: Figure 2C

5. Cytochemical and Histological Analysis

5.1. For cytochemical and histological analysis of the samples, first fix the intestine and liver equivalents in 4% paraformaldehyde in 0.1-molar PBS for 25 minutes at room temperature **[1]**.

5.1.1. WIDE: Talent adding PFA to sample(s), with PFA container visible in frame

5.2. At the end of the incubation, wash the organoids five times in PBS for 10 minutes per wash **[1]** before staining the intestinal and the liver equivalents with tetramethylrhodamine isothiocyanate-phalloidin or Alexa Fluor 647 (six-four-seven) phalloidin for 1 hour at room temperature **[2]**.

5.2.1. Talent washing organoids, with PBS container visible in frame

5.2.2. Talent adding stain to sample(s), with stain containers visible in frame

5.3. At the end of the incubation, transfer the samples to freezing medium for a few minutes **[1]** before snap freezing the tissues in liquid nitrogen **[2]**.

5.3.1. Talent adding sample to OCT, with OCT container visible in frame

5.3.2. Talent adding sample to LN2

5.4. Next, use a cryostat to acquire 10-12-micron-thick liver spheroids cryosections **[1]** and mount the tissues sections in mounting medium with DAPI (DAP-ee) **[2]** for imaging by confocal fluorescence microscopy **[3]**.

5.4.1. Talent at cryostat, making section

5.4.2. Talent adding mounting medium to section, with mounting medium container visible in frame

5.4.3. Talent at microscope, imaging slide

6. Mitochondrial and Nuclear Staining

6.1. For mitochondrial and nuclear staining, completely cover the samples with mitochondrial staining solution for a 15-45-minute incubation at 37 degrees Celsius in a humidified atmosphere with 5% carbon dioxide **[1]**.

6.1.1. WIDE: Talent adding solution to sample

6.2. At the end of the incubation, carefully replace the staining solution with 2-4% paraformaldehyde in PBS for 15 minutes at room temperature **[1]**.

6.2.1. Talent adding PFA to sample

6.3. After fixing, gently rinse the cells two times with fresh PBS for 5 minutes per wash **[1]** before labeling the samples with nucleic acid staining working solution for 10 minutes at room temperature **[2]**.

6.3.1. Talent washing cells, with PBS container visible in frame

6.3.2. Talent adding nucleic acid stain to sample, with stain container visible in frame

6.4. At the end of the incubation, wash the samples with three, 5-minute washes in PBS protected from light **[1]** and use the nucleic acid stain to facilitate quantification of the number of mitochondrial stain-expressing cells on a fluorescence microscope with the appropriate filter sets **[2]**.

6.4.1. Talent washing cells protected from light, with PBS container visible in frame
Videographer: Important step

6.4.2. Talent at microscope, looking at slide

Protocol Script Questions

A. Which steps from the protocol are the most important for viewers to see?
2.1.-2.3., 3.3., 4.1., 6.4.

B. What is the single most difficult aspect of this procedure and what do you do to ensure success?
2.4. is the proper integration of the organoids without the presence of bubbles or leaks from the culture medium. success is guaranteed by the technique of integrating organoids and adding the medium.

Results

7. Results: Representative Intestine/Liver Microphysiological System Recapitulation of Pharmakokinetic and Toxicological Responses Induced by Acetaminophen Exposure

7.1. Integrating the human intestinal barrier **[1]** and a liver equivalent into a two-organ chip microfluidic device **[2]** allows assessment of the pharmacokinetic and toxicological properties of treatments of interest **[3]**.

7.1.1. LAB MEDIA: Figure 1A *Video Editor: please emphasize Intestine equivalent text*
7.1.2. LAB MEDIA: Figure 1A *Video Editor: please emphasize Liver equivalent text*
7.1.3. LAB MEDIA: Figure 1A

7.2. After treatment, the media samples can be analyzed by HPLC **[1]**.

7.2.1. LAB MEDIA: Figures 5A and 5B

7.3. The organoids can be analyzed **[1]** for their gene expression **[2]**, transepithelial electrical resistance values **[3]**, and protein expression and activity **[4]** as well as for their morphological characteristics **[5]**.

7.3.1. LAB MEDIA: Figures 5D-5I
~~7.3.2.~~ LAB MEDIA: Figures 5D-5I Author NOTE: Use Figure 2 F and 4 A – D. ~~*Video Editor: please emphasize Figures 5F, 5G, and 5H*~~
~~7.3.3.~~ LAB MEDIA: Figures 5D-5I Author NOTE: Use Figure 2 D and 2E. ~~*Video Editor: please emphasize Figures 5D and 5E*~~
7.3.4. LAB MEDIA: Figures 3E-3I Author NOTE: Use Figure 4 E - J.
7.3.5. Author NOTE: Use Figures 2A-B, Figures 3B, 3C and 3J.

7.4. MTT analysis can be performed to assess organoid viability **[1]** as well as to detect very early toxic events in response to acetaminophen treatment **[2]**.

7.4.1. LAB MEDIA: Figure 2C *Video Editor: please emphasize grey and black data bars from 0 to 24 h*
7.4.2. LAB MEDIA: Figure 2C *Video Editor: please emphasize 100 mM APA grey and black data bars*

7.5. The media flow does not significantly affect acetaminophen absorption **[1]**, whereas it significantly improves liver equivalent functionality **[2]**, indicating that human

intestinal acetaminophen absorption and hepatic metabolism can be emulated in this microphysiological system **[3]**.

7.5.1. LAB MEDIA: Figure 5 *Video Editor: please emphasize Figures 5A and 5B*
7.5.2. LAB MEDIA: Figure 5 *Video Editor: please emphasize Figure 5C*
7.5.3. LAB MEDIA: Figures *Video Editor: please emphasize Figures 5D and 5E*

Conclusion

8. Conclusion Interview Statements

8.1. **Talita Miguel Marin**: MPS's have the potential to replace animal models in drug development and discovery, as they more accurately mimic human physiology and can be used to promote drug development at higher speeds and lower costs, risks, and complexity **[1]**.

8.1.1. LAB MEDIA: To be provided by Authors: Named talent says the statement above in an interview-style shot, looking slightly off-camera