Submission ID #: 68405
Scriptwriter Name: Sulakshana Karkala
Project Page Link: https://review.jove.com/account/file-uploader?src=20863573

Title: Reproducible Manufacturing of SPOT as a High-Throughput Scaffold-Based Culture Platform

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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? **No**

2. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

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

Current Protocol Length
Number of Steps: 16
Number of Shots: 44

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

1.1. **Ruonan Cao:** Our research focuses on developing high-throughput scaffold-supported platforms for robust, reproducible patient-derived organoid cultures to improve drug screening assay workflow and help advance disease modelling.
   1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll: 3.3*

What technologies are currently used to advance research in your field?

1.2. **Chantelle Shing:** Automated liquid handlers, scaffold-supported hydrogel platforms, organ-on-a-chip technologies, and single-cell assays like flow cytometry and CyTOF are some of the currently available technologies.
   1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:3.1*

What are the current experimental challenges?

1.3. **Ruonan Cao:** Current challenges include preserving hydrogel integrity, overcoming gel meniscus for real-time imaging, and ensuring uniform cell distribution in long-term organoid-based assays.
   1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

What advantage does your protocol offer compared to other techniques?

1.4. **Chantelle Shing:** Our protocol enables consistent scaffold-supported hydrogel loading, minimizes meniscus issues, supports long-term organoid culture, and integrates smoothly with automated liquid handlers and high-content imaging systems.
   1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

What research questions will your laboratory focus on in the future?

1.5. **Alison McGuigan:** We are most excited about using our disease modelling platforms to explore disease progression mechanisms in disease such as cancer and the development of obesity.
   1.5.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

Videographer: Obtain headshots for all authors available at the filming location.

Ethics Title Card

This research has been approved by the Ethics Board at the University of Toronto

Protocol

2. Fabrication and Assembly of 96- and 384-SPOT Platforms for Organoid Cultures
Demonstrator: Ruonan Cao

2.1. To begin, take a PMMA *(P-M-M-A)*-patterned scaffold for either 96- or 384-SPOT *(Spot)* **[1-TXT].** Trim the excess paper around the blue region of the scaffold, leaving a 5-millimeter white margin around the perimeter **[2]**. Ensure the resulting sheet measures approximately 760 millimeters by 1300 millimeters **[3]**.

2.1.1. WIDE: Talent holding a PMMA patterned scaffold for SPOT.
TXT: PMMA: Polymethyl Methacrylate;
SPOT: Scaffold-supported Platform for Organoid-based Tissues

2.1.2. Shot of excess paper being cut off from a blue-centered scaffold while leaving a white margin.
Videographer's Note: 2.1.2 is combined with 2.1.3

2.1.3. Shot of the final sheet.

2.2. To prepare a 96-SPOT scaffold for assembly, lay a piece of polyethylene film over a rigid acrylic support **[1]**. Place this film-covered support onto the work surface **[2]** and lay the scaffold on top of the polyethylene film **[3]**.

2.2.1. Talent spreading polyethylene film over a large acrylic board.
Videographer's Note: 2.2.1 is combined with 2.2.2

2.2.2. Shot of the film covered support being placed on the work surface.

2.2.3. Talent positioning the scaffold centrally on top of the polyethylene film.

2.3. Align the edge of a ruler between the first two rows of the scaffold to avoid tracing into the wells **[1]**. Place the tracing wheel at the bottom of the scaffold **[2]** and roll it upward to the top, guided by the ruler **[3-TXT]**.

2.3.1. Talent carefully positioning a ruler between scaffold rows.
Videographer's Note: 2.3.1 is combined with 2.3.2-3

2.3.2. Shot of the tracing wheel being placed at the bottom of the scaffold.

2.3.3. Talent rolling a tracing wheel along the scaffold's inter-row space using the ruler as a guide. **TXT: Repeat for all 7 inter-row and inter-column spaces**

2.4. For a 384-SPOT scaffold, do not perform perforation **[1].**

2.4.1. Shot of the unperforated 384 SPOT scaffold.

2.5. Next, lay the scaffold with the rough side facing up on a PDMS *(P-D-M-S)* slab, ensuring rows run horizontally **[1-TXT]**. Use the priming line to determine which side is rough **[2].**

2.5.1. Talent orienting the scaffold correctly and placing it onto the polydimethylsiloxane slab. **TXT: PDMS: Polydimethylsiloxane**
Videographer's Note: 2.5.1 is combined with 2.5.2

2.5.2. Talent pointing to the priming line.

2.6. Then smoothen the scaffold onto the slab to remove wrinkles or bubbles **[1]**. Remove the protective layer from the non-engraved side of the double-sided tape **[2]**. Slightly buckle the tape in the middle **[3]** and align the middle two columns of the tape with the scaffold **[4]**.

2.6.1. Talent smoothing the scaffold flat.

2.6.2. Talent peeling off the tape's protective layer.

2.6.3. Shot of the tape being buckled in the middle.
Videographer's Note: 2.6.3 is combined with 2.6.4

2.6.4. Talent aligning the tape to the centre of the scaffold.

2.7. Now, press the middle of the tape onto the scaffold to secure alignment **[1]**. Smooth the tape outward from the centre to affix it evenly **[2]**.

2.7.1. Talent pressing down on the centre of the tape to make first contact.

2.7.2. Talent pressing along the tape from the centre outwards to secure it.
Videographer's Note: 2.7.1 is combined with 2.7.2

2.8. Using tweezers, gently lift the scaffold from the PDMS slab **[1].** Store the slab for future use **[2]**.

2.8.1. Talent using tweezers to lift the scaffold.

2.8.2. Talent placing the slab into storage.

2.9. To attach the scaffold to the second well tape, flip the scaffold so the smooth, non-taped side faces up on the workbench **[1]**. Remove the middle protective layer from the engraved side of the second tape to expose two central columns **[2-TXT]**.

2.9.1. Talent rotating the scaffold carefully to expose the smooth side.

2.9.2. Talent peeling back the centre section of the engraved tape. **TXT: If using a non-engraved design, remove the full protective layer from one side**

2.10. Slightly buckle the middle of the second tape and align it to the middle columns of the scaffold **[1]**. Then press the tape's centre to fix it in place **[2]**. If engraved tape is used, remove the remaining protective layers adjacent to the exposed centre **[3]**. Smoothen the tape starting from the middle and moving outward **[4]**.

2.10.1. Talent gently bending the tape and aligning it over the scaffold.

2.10.2. Talent pressing down the tape's centre section.
Videographer's Note: 2.10.1 is combined with 2.10.2

2.10.3. Talent peeling off adjacent protective layers.
Videographer's Note: 2.10.3 is combined with 2.10.4

2.10.4. Talent smoothing out the tape with a firm gloved hand.

2.11. To attach the scaffold to the bottomless plate, remove the protective layer **[1]**.

2.11.1. Talent removing the middle section for engraved version.

2.12. Align the scaffold under the bottomless plate with a lid **[1].** Once aligned, press down its middle onto the plate **[1]**. If using engraved tape, remove adjacent layers and smooth the scaffold onto the plate outward from the centre **[3]**.

2.12.1. Talent aligning scaffold to the underside of the bottomless plate.
Videographer's Note: 2.12.1 is combined with 2.12.2

2.12.2. Talent pressing the scaffold onto the plate underside.

2.12.3. Talent peeling additional tape layers and smoothing scaffold.

2.13. To attach the scaffold to the polycarbonate film, use tweezers to carefully loosen the protective layer from one side of the polycarbonate film **[1]**. Then slowly peel off the protective layer, without causing any indentations in the film **[2].** Repeat the process for the second side **[3].** Then set the film aside on the workbench **[4]**.

2.13.1. Shot of the protective layer being loosened from one side of the polycarbonate film, with tweezers.
Videographer's Note: 2.13.1 is combined with 2.13.2-4

2.13.2. Talent peeling off the protective film layer.

2.13.3. Talent peeling off second side.

2.13.4. Talent laying the film aside on the workbench.

2.14. Peel the protective tape layer from the plate bottom and press the polycarbonate film onto the scaffold from the center outwards **[1]**. Then firmly press the polycarbonate film onto the scaffold to ensure each well has a defined border **[2]**. Using a precision knife , carefully cut off any excess material from the bottom of the plate **[3]**.

2.14.1. Talent removing tape and pressing the polycarbonate film into position.
Videographer's Note: 2.14.1 is combined with 2.14.2

2.14.2. Talent using hands to seal the film over the plate.

2.14.3. Talent trimming edges neatly using a knife .

2.15. After full assembly, place the 96-SPOT plate into a clear resealable plastic bag **[1]** and take it outside the biosafety cabinet **[2]**.

2.15.1. Talent placing the plate in a resealable bag.

2.15.2. Talent moving it outside the biosafety cabinet.

2.16. Place a rigid acrylic support underneath the plate **[1]** and clamp both at opposite corners **[2-TXT]**. Store the plate in a clean, dry space until further use **[3]**.

2.16.1. Talent positioning the acrylic support .
Videographer's Note: 2.16.1 is combined with 2.16.2

2.16.2. Talent clamping the corners. **TXT: Keep clamped for at least 30 min on each corner before use**

2.16.3. Talent placing the assembled plate on a clean shelf or storage drawer.
Videographer's Note: shot involves unclamping before storing, and then showing 384 spot variation

Results

3. Results

3.1. No media leakage or exchange was observed when alternating wells in the 384-SPOT plate were filled with fluorescein and PBS during a 30-day test period, confirming the efficacy of the PMMA barrier in maintaining well-to-well isolation **[1]**.

3.1.1. LAB MEDIA: Figure 7A and B. *Video Editor: Please show A first and then B*

3.2. GFP-expressing pancreatic tumor organoids seeded into 96-SPOT plates remained viable and expanded progressively from day 0 through day 12 of culture **[1]**.

3.2.1. LAB MEDIA: Figure 7C. *Video editor: Highlight the top row images corresponding to "OT2" from day 0 to Day 12*

3.3. On day 12, SPOT-grown organoids displayed strong cytokeratin 19 **[1]** and zonula occludens-1 expression, along with the presence of internal lumen structures **[2]**.

3.3.1. LAB MEDIA: Figure 7D**.** *Video editor: Please highlight the top row corresponding to "CK19"*

3.3.2. LAB MEDIA: Figure 7D. *Video editor: Please highlight the bottom row corresponding to "ZO-1". Also emphasise the area pointed at by the white arrows in the left most image in the row*

3.4. Suboptimal and optimal tissue seeding outcomes in 384-SPOT plates were visually distinguishable by edge definition and cell distribution uniformity **[1]**.

3.4.1. LAB MEDIA: Figure 8A. *Video editor: Highlight the image labelled "Not optimal"*

3.5. Consistently uniform seeding was achieved across the entire 96-SPOT and 384-SPOT plates using the automated liquid handler **[1].**

3.5.1. LAB MEDIA: Figure 8B and C**.**

Pronunciation Guide:

Plymethyl Methacrylate (PMMA)

Pronunciation link: https://www.howtopronounce.com/pmma justpronounce.com+10How To Pronounce+10How To Pronounce+10

IPA: /ˌpɑliˌmɛθəl mɛθəˈkrɪleɪt/

Phonetic spelling: PAH-lee-meth-uhl meth-uh-KRIL-ayt

Polydimethylsiloxane (PDMS)

Pronunciation link: https://www.howtopronounce.com/polydimethylsiloxane Forvo.com+8How To Pronounce+8YouTube+8

IPA: /ˌpɑliˌdaɪˌmɛθəlˌsɪlɒkˌseɪn/

Phonetic spelling: PAH-lee-dye-MEH-thul-sil-OX-ayn

Hydrogel

Pronunciation link: https://www.merriam-webster.com/dictionary/hydrogel YouGlish+14YouGlish+14YouTube+14Merriam-Webster+13Merriam-Webster+13WordReference+13

IPA: /ˈhaɪdroʊˌdʒɛl/

Phonetic spelling: HY-droh-jel

Organoid

Pronunciation link: https://www.merriam-webster.com/medical/organoid Definitions+15Merriam-Webster+15Merriam-Webster+15

IPA: /ˈɔrgəˌnɔɪd/

Phonetic spelling: OR-guh-noid

Meniscus

Pronunciation link: https://www.merriam-webster.com/dictionary/meniscus Merriam-Webster+12How To Pronounce+12YouTube+12Merriam-Webster+15Merriam-Webster+15OpenMD+15

IPA: /məˈnɪskəs/

Phonetic spelling: muh-NIS-kuhs

Fluorescein

Pronunciation link: https://www.merriam-webster.com/dictionary/fluorescein Pronounce Kiwi+2How To Pronounce+2Cambridge Dictionary+2Merriam-Webster+5Merriam-Webster+5Merriam-Webster+5

IPA: /flu̇ˈɒr-əˌsin/ or /flu̇ˈrɛs-in/

Phonetic spelling: floo-OR-uh-seen or floo-ress-een

Cytokeratin

Pronunciation link: https://www.merriam-webster.com/medical/cytokeratin Merriam-Webster+1Merriam-Webster+1Merriam-Webster+13Merriam-Webster+13Oxford English Dictionary+13

IPA: /ˌsaɪtoʊˈkɛrətɪn/

Phonetic spelling: SIGH-toh-KEHR-uh-tin

Epithelium

Pronunciation link: https://www.merriam-webster.com/dictionary/epithelium Merriam-Webster

IPA: /ˌɛpəˈθiliəm/

Phonetic spelling: EH-puh-THEE-lee-um

Fluorescence

Pronunciation link: https://www.merriam-webster.com/dictionary/fluorescence How To Pronounce+15YouTube+15Merriam-Webster+15Merriam-Webster+6Merriam-Webster+6Merriam-Webster+6

IPA: /ˌfluːəˈrɛsəns/

Phonetic spelling: floo-uh-RESS-ens

CyTOF ***(time-of-flight cytometry)***

Pronunciation link: No confirmed link found

IPA: /ˈsaɪtoʊf/

Phonetic spelling: SIGH-tohf