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Title: Bioprinting of Hydrogel Tumor Slices as a 3D Model for Mantle Cell Lymphoma

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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? **Yes, all done**

3. **Filming location:** Will the filming need to take place in multiple locations? **Yes.**

Current Protocol Length
Number of Steps: 15
Number of Shots: 38 (4 SC)

Introduction

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

1.1. **Meng Dong:** We developed a hydrogel-based, bioprinted model to replicate the conditions of mantle cell lymphoma in patients, so we can better study how it survives and responds to treatments.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.4.1*

What are the current experimental challenges?

1.2. **Jan Schlegel:** The challenge is to figure out the best way to bioprint, culture and analyse 3D models specifically designed for lymphomas.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.3.1*

What research gap are you addressing with your protocol?

1.3. **Julia Thiel:** Due to drug resistance and relapse, mantle cell lymphomas are still incurable. Our model can help to understand how tumor heterogeneity and the microenvironment influence drug responses.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.2.1*

What advantage does your protocol offer compared to other techniques?

1.4. **Jan Schlegel:** Compared to other 3D models of mantle cell lymphoma, our model mimics the network of extracellular matrix fibers within a lymph node by using a bioink containing collagen and Matrigel.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 5.1.1*

How will your findings advance research in your field?

1.5. **Meng Dong:** The presented model enhances physiological relevance compared to traditional 2D culture systems. It has the potential to advance both biological and therapeutic studies of mantle cell lymphomas.

1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.1.3*

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

Ethics Title Card

This research has been approved by the Ethics Committee of the Eberhard-Karls-University and University Hospital Tuebingen

Protocol

NOTE: LAB MEDIA/SCREEN/SCOPE timestamps for protocol were added at the postshoot stage. Please contact the postshoot note integrator (Sulakshana) for queries regarding lab media.

2. Preparation of Cell-Laden Bioink

Demonstrator: Julia Thiel

2.1. To begin, gather materials to prepare the bioink adapted for the cells of interest **[1]**. For Mantle cell lymphoma or MCL *(M-C-L)* Jeko-1 cells mix alginate and collagen in a suitable buffer to obtain 1 milliliter of bioink **[2]**.

2.1.1. WIDE: Talent gathering all components needed to prepare the bioink.

2.1.2. Talent pipetting and combining each component into a microcentrifuge tube.

And
TEXT ON **2.1.2's** BACKGROUND:

Bioink composition:

0.5% (w/v) Alginate

20% (v/v) Type-I collagen

1x RPMI and 2 mM HEPES in PBS

2.2. Transfer the MCL cell suspension from the culture flask into a 50-milliliter conical tube **[1]**.

2.2.1. Talent pouring cell suspension from a flask into a 50 milliliter conical tube.

2.3. Mix the cells with 0.4 percent trypan blue solution in a 1 to 1 ratio **[1]**. Using a cell counter or Neubauer chamber, count the viable unstained cells **[2]** and calculate the volume of cell suspension corresponding to 14 million cells for 1 milliliter of bioink **[3]**.

2.3.1. Talent mixing equal volumes of trypan blue and cell suspension in a microcentrifuge tube.

2.3.2. Talent placing the stained cell suspension on a counting chamber or inserting it into a cell counter.

2.3.3. Talent writing down in a notebook.

2.4. Transfer the calculated volume into a fresh 50 milliliter conical tube **[1]** and centrifuge the tube at 340 *g* for 5 minutes **[2]**. After centrifugation, remove the supernatant **[3]** and resuspend the cell pellet in the prepared bioink to generate the final cell-laden bioink **[4]**.

2.4.1. Talent transferring the calculated volume of cell suspension to a new 50 milliliter tube.

2.4.2. Talent placing the tube in a centrifuge and closing the lid.

2.4.3. Talent removing supernatant from the tube.
Videographer's note: file number is DSCF8914.

2.4.4. Talent gently resuspending the cell pellet in the bioink by pipetting up and down.

3. In-Bath Bioprinting Using Gelatin Support

Demonstrator: Jan Schlegel

3.1. Switch on the bioprinter and connect it to the 3D-printer software **[1]**. Open the **.stl** *(S-T-L)* file containing the hydrogel tumor slice blueprint using the software **[2]**. Ensure each hydrogel slice has a diameter of 8 millimeters and a height of 1.5 millimeters **[3]**. Then, set the fill density to 85 percent and home the printer head **[4]**.

3.1.1. Talent pressing the power button on the bioprinter.

3.1.2. SCREEN: 68417_screenshot_1.mp4. 00:03-00:15

3.1.3. SCREEN: 68417_screenshot_1.mp4. 00:16-00:21

3.1.4. SCREEN: 68417_screenshot_1.mp4. 00:22-00:32

3.2. Now, transfer the gelatin slurry into a 35 millimeter Petri dish until it is half full **[1]**. Use disposable precision wipes to remove excess water and eliminate any air gaps in the gelatin **[2]**, forming a stable support bath **[3]**.

3.2.1. Talent filling the Petri dish halfway with gelatin slurry.

3.2.2. Talent dabbing the slurry with disposable precision wipes to remove water.

3.2.3. Shot of the stable bath.

3.3. Load the cell-laden bioink into a 2.5 milliliter glass syringe and attach a 0.8 millimeter blunt nozzle **[1]**. Invert the syringe and slowly eject air bubbles **[2]** and then insert the prepared syringe into the bioprinter **[3]**. Using the 3D-printer software, extrude a small amount of bioink to check the flow **[4]**. Wipe off the extruded drop with a disposable precision wipe to avoid nozzle clogging **[5]**.

3.3.1. Talent filling a 2.5 milliliter glass syringe with cell-laden bioink and connecting the blunt nozzle.

3.3.2. Talent inverting the syringe and gently pushing out visible air bubbles.

3.3.3. Talent inserting the syringe into the bioprinter's syringe holder.

3.3.4. Shot of the drop being extruded.

3.3.5. Talent using a wipe to clean the nozzle tip after extrusion.

3.4. Next, place the gelatin support bath beneath the syringe nozzle **[1]** and lower the printer head until the nozzle is 2 millimeters above the bottom of the gelatin support bath **[2]**.
Videographer's Note: 3.4.1 and 3.4.2 are separate. (I say we do them together and that's false)

3.4.1. Talent positioning the Petri dish under the syringe nozzle on the printer platform.

3.4.2. Shot of the printer head being lowered.

3.5. Start the printing process using the 3D-printer software **[1]**.

3.5.1. SCREEN: 68417_screenshot_2 00:02-00:10

Added shot3.5.2 : 3D printer printing.

4. Bath Processing and Hydrogel Tumor Slice Culture

4.1. Once printing is complete, remove any excess gelatin slurry from the nozzle using a disposable precision wipe to prevent clogging **[1]**. Take out the Petri dish containing the gelatin support bath with the printed hydrogel tumor slices **[2]** and cover it with a sterile lid **[3]**.
Videographer's Note: 4.1.1-4.1.3 were shot in one clip

4.1.1. Talent wiping the nozzle with a precision wipe to remove residual gelatin.

4.1.2. Talent removing the Petri dish from the bioprinter.

4.1.3. Talent placing a lid over the dish.

4.2. Place the covered support bath in an incubator set to 37 degrees Celsius to allow the gelatin to melt and release the printed hydrogel tumor slices **[1]**.

4.2.1. Talent placing the covered Petri dish into an incubator.

4.3. Then, warm the wash buffer and cell culture medium to 37 degrees Celsius **[1]**. While the gelatin is melting, prepare a 6-well plate by filling two wells with 10 millimolar HEPES and 14.4 millimolar calcium chloride wash buffer **[2]**, and two wells with pre-warmed cell culture medium **[3]**.

4.3.1. Talent placing wash buffer and medium tubes in a 37 degrees Celsius water bath.

4.3.2. Talent pipetting wash buffer into two wells of a 6-well plate.
Videographer's Note: 4.3.2-4.3.3 were shot together

4.3.3. Talent filling the remaining two wells with pre-warmed cell culture medium.

4.4. Once the gelatin has melted, use a sterile spatula to carefully transfer the hydrogel tumor slices into the two wash buffer wells **[1-TXT]**. Then, transfer them into the two medium-containing wells and wash for 1 minute in each **[2]**.

4.4.1. Talent lifting hydrogel tumor slices with a sterile spatula and placing them into the wash buffer wells. **TXT: Wash for 1 minute in each well.**

4.4.2. Talent transferring the slices into the medium-containing wells.

4.5. To culture the hydrogel tumor slices, place them onto a 0.4-micrometer pore-size filter support positioned inside a 6-well plate **[1]**. Add 1.5 milliliters of cell culture medium below the filter support **[2]**, followed by a small drop of medium directly on top of each hydrogel tumor slice to prevent drying **[3]**. For drug treatment, add the desired amount of drug to the culture medium **[4]**.

4.5.1. Talent placing up to five tumor slices on a filter support inside a well.

4.5.2. Talent pipetting medium below the filter support into the well.

4.5.3. Close-up of a pipette adding a drop of medium on top of each hydrogel slice.

4.5.4. Talent adding a measured dose of drug to the medium using a micropipette.

4.6. Final, place the 6-well plate into a cell culture incubator set to 37 degrees Celsius and 5

percent carbon dioxide for the desired cultivation period **[1-TXT]**.

4.6.1. Talent opening the incubator and placing the 6-well plate inside. **TXT: After incubation, perform four-color live 3D fluorescence imaging**

Results

5. Results

5.1. The hydrogel tumor slices retained their structure over 3 days of culture **[1]**, with air bubbles present after printing **[2]** that disappeared during cultivation **[3]**.

5.1.1. LAB MEDIA: Figure 2A

5.1.2. LAB MEDIA: Figure 2A. *Video editor: Show the slice on d0*.

5.1.3. LAB MEDIA: Figure 2A. *Video editor: Show the image d3*.

5.2. The viability of Jeko-1 cells cultured in hydrogel slices for 3 days was 80% **[1]**, which was not significantly different from cells cultured in 2D suspension at 88% **[2]**.

5.2.1. LAB MEDIA: Figure 2B. *Video editor: Highlight the 3D bar* .

5.2.2. LAB MEDIA: Figure 2B. *Video editor: Highlight the 2D bar*.

5.3. Immediately after printing, Jeko-1 cells were evenly distributed in the hydrogel slice and were predominantly TMRM *(T-M-R-M)*-positive, indicating viability **[1]**. Only a few cells were positive for Caspase-3 and PicoGreen *(Pie-Co-Green)* , markers of apoptosis and death respectively **[2]**.

5.3.1. LAB MEDIA: Figure 2D. *Video editor: Zoom in on the red-stained "TMRM" cells on d0 panel*.

5.3.2. LAB MEDIA: Figure 2D. *Video editor: Highlight the few yellow "Caspase-3" and "pico-green" dots in the d0 panel*.

5.4. After 3 days of culture, Jeko-1 cells formed clusters and remained mostly TMRM-positive **[1]**.

5.4.1. LAB MEDIA: Figure 2D. *Video editor: Highlight clustered red-stained cells for TMRM* .

5.5. The hydrogel tumor slices maintained their structural integrity after 3 days of Doxorubicin treatment **[1]**.

5.5.1. LAB MEDIA: Figure 3A.

5.6. Jeko-1 cells cultured in hydrogel slices showed a dose-dependent decrease in viability in response to Doxorubicin treatment **[1]**.

5.6.1. LAB MEDIA: Figure 3B. *Video editor: Highlight the red 3D line*.

5.7. The half-maximal inhibitory concentration of Doxorubicin was higher for Jeko-1 cells in hydrogel slices at around 5.8 micromolar **[1]** compared to suspension culture at 2 micromolar, indicating reduced sensitivity in 3D culture **[2]**.

5.7.1. LAB MEDIA: Figure 3B. *Video editor: Highlight the point on the red 3D curve where it intersects the IC50 horizontal line*.

5.7.2. LAB MEDIA: Figure 3B. *Video editor: Highlight the black 2D curve where it intersects the IC50 horizontal line*.

5.8. Live fluorescence imaging of hydrogel slices treated with Doxorubicin showed reduced TMRM signal, indicating loss of mitochondrial membrane potential **[1]**, and increased PicoGreen signal, indicating enhanced cell death **[2]**.

5.8.1. LAB MEDIA: Figure 3C. *Video editor: Zoom in on the TMRM cells in DOXO panel*.

5.8.2. LAB MEDIA: Figure 3C. *Video editor: Highlight the PicoGreen image in cells in DOXO panel*.

5.9. After 4 days of culture, primary MCL cells in hydrogel slices maintained a viability of 43.6% **[1]** which was significantly higher than the 19.6% observed in suspension culture **[2]**. Fluorescence imaging indicated that cells in hydrogel were majorly TMRM-positive **[3]**.

5.9.1. LAB MEDIA: Figure 4A. *Video editor: Highlight the 3D bar at d4* .

5.9.2. LAB MEDIA: Figure 4A. *Video editor: Highlight the lower 2D bar at d4*.

5.9.3. LAB MEDIA: Figure 4B. *Video editor: Zoom in on the red-stained TMRM-positive cells*.

Pronunciation Guide:

1. **Extracellular**
    - Pronunciation link: https://dictionary.cambridge.org/us/pronunciation/english/extracellular forvo.com+10dictionary.cambridge.org+10collinsdictionary.com+10
    - IPA: *US* /ˌek.strəˈsel.jə.lɚ/
    - Phonetic Spelling: ek-struh-SEL-yu-ler
2. **Matrix**
    - Pronunciation link: No confirmed link found
    - IPA: /ˈmeɪ.trɪks/
    - Phonetic Spelling: MAY-triks
3. **Hydrogel**
    - Pronunciation link: No confirmed link found
    - IPA: /ˈhaɪ.drə.dʒɛl/
    - Phonetic Spelling: HY-droh-jel
4. **Bioprinting**
    - Pronunciation link: No confirmed link found
    - IPA: /ˌbaɪ.oʊˈprɪn.tɪŋ/
    - Phonetic Spelling: BY-oh-PRIN-ting
5. **Alginate**
    - Pronunciation link: No confirmed link found
    - IPA: /ˈæl.dʒɪ.neɪt/
    - Phonetic Spelling: AL-juh-nayt
6. **Collagen**
    - Pronunciation link: No confirmed link found
    - IPA: /ˈkɒl.ə.dʒən/ (AmE tends toward /ˈkɑː.lə.dʒən/)
    - Phonetic Spelling: KOL-uh-jen
7. **Matrigel**
    - Pronunciation link: No confirmed link found
    - IPA: /ˈmæ.tri.dʒɛl/
    - Phonetic Spelling: MAT-ri-jel

8. **MCL (Mantle Cell Lymphoma)**
    - Pronunciation link: No confirmed link found
    - IPA (acronym): /ɛm.si.ˈɛl/
    - Phonetic Spelling: em-see-ELL
9. **Jeko-1**
    - Pronunciation link: No confirmed link found
    - IPA: /ˈdʒɛ.koʊ-wʌn/
    - Phonetic Spelling: JEH-koh-one
10. **Trypan Blue**
    - Pronunciation link: No confirmed link found
    - IPA: /ˈtraɪ.pæn blu/
    - Phonetic Spelling: TRY-pan BLUE
11. **Centrifuge**
    - Pronunciation link: No confirmed link found
    - IPA: /ˈsɛn.trɪ.fjuːdʒ/
    - Phonetic Spelling: SEN-trih-fyooj
12. **Doxorubicin**
    - Pronunciation link: No confirmed link found
    - IPA: /ˌdɒk.səˈruː.bə.sɪn/ (AmE)
    - Phonetic Spelling: dok-suh-ROO-buh-sin