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Title: A 3D Bone Culture Platform Using Human Osteocytes and Decellularized Extracellular Matrix for Modeling Musculoskeletal Diseases

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Author Questionnaire

1. We have marked your project as author-provided footage, meaning you film the video yourself and provide JoVE with the footage to edit. JoVE will not send the videographer. Please confirm that this is correct.

- ☐ Correct
- ☐ Incorrect

2. Interview statements: Which interview statement filming option is the most appropriate for your group? **Please select one.**

- ☐ Interviewees self-record interview statements. JoVE can provide support for this option.

3. Proposed interview filming date: Please indicate the proposed date that your group will self-film interviews: **MM/DD/YYYY**

When you are ready to submit your video files, please contact our Content Manager, [Utkarsh Khare](#).

Current Protocol Length

Number of Steps: 25

Number of Shots: 57

Introduction

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

Authors: Please note that **the questions will not appear on screen**. Please answer in **stand-alone full sentence with sufficient context**

Answers will be slightly edited in accordance with journal guidelines.

INTRODUCTION:

~~**REQUIRED:** Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Francisco Verdugo-Avello:** Our research develops accessible, biologically relevant 3D bone culture platforms for tissue engineering, disease modeling, regenerative medicine, and related translational applications.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~**Describe the research contexts or systems in which this protocol can be applied.**~~

- 1.2. **Francisco Verdugo-Avello:** This protocol can be applied to bone tissue engineering, biomaterial testing, musculoskeletal disease modeling, drug screening, and regenerative medicine research.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

CONCLUSION:

~~**What does this protocol allow researchers to study or measure?**~~

- 1.3. **Francisco Verdugo-Avello:** This protocol enables researchers to study cell viability, morphology, cell–matrix interactions, osteogenic behavior, and biomaterial biocompatibility within a 3D environment.
 - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.4. **Francisco Verdugo-Avello:** Key challenges include careful hydrogel handling, even cell distribution, and preventing premature GelMA gelation during construct preparation.
 - 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

- 1.5. **Francisco Verdugo-Avello:** Future studies can incorporate patient-derived cells, vascularized constructs, transcriptomics, and disease-specific models to study bone remodeling, metastasis, osteoporosis, and therapeutic responses.
 - 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 Scientific Ethics Committee of the Concepción Health Service

Protocol

Authors: During filming, please point out the organs or veins before dissecting them.

2. Decellularization and Lyophilization of Human Femoral Head Bone

2.1. **NOTE:** Step 2.1 was empty, an extra bullet was added by mistake, protocol starts from 2.2

2.2. To begin, thaw the human femoral head [1] obtained from a patient during hip replacement surgery gradually to 25 degrees Celsius [2]. Cut the femoral head in half and then cut each half into smaller pieces [3].

2.2.1. WIDE: Talent picking up the femoral head from a refrigerator.

2.2.2. Close-up of placing the femoral head in an incubator or on the work bench.

2.2.3. Talent cutting each femoral head in half and cutting the femoral head half into smaller pieces.

2.3. Immerse the bone pieces in sterile distilled water at 60 degrees Celsius [1] and sonicate for 15 minutes at 40 kilohertz continuously [2].

2.3.1. Talent immersing the bone pieces in sterile distilled water.

2.3.2. Talent placing the immersed bone pieces in the sonicator.

2.4. Then, wash the sample in 300 milliliters of distilled water preheated to 60 degrees Celsius while shaking at 200 revolutions per minute for 5 minutes [1].

2.4.1. Talent adding preheated distilled water to the sample and keeping it on a shaker.

2.5. Centrifuge the sample at 1850 g for 15 minutes at 25 degrees Celsius [1]. Remove the water and repeat the washing step 2 more times [2].

2.5.1. Talent placing the sample in the centrifuge.

2.5.2. Talent removing the water from the sample and adding fresh water.

2.6. Sterilize the tissue in a solution containing 3 percent hydrogen peroxide and 0.02 percent peroxyacetic acid [1]. Then, sonicate the sample at 40 kilohertz for 10 minutes

at 60 degrees Celsius [2].

2.6.1. Talent placing the tissue in the sterilization solution.

2.6.2. Talent placing the sample in a sonicator.

2.7. Transfer the tissue to 300 milliliters of 70 percent ethanol [1] and sonicate at 40 kilohertz for 10 minutes at 21 degrees Celsius [2].

2.7.1. Talent transferring the tissue to 300 milliliters of 70 percent ethanol.

2.7.2. Talent placing the tissue in ethanol in the sonicator.

2.8. Next, wash the sample twice with 300 milliliters of distilled water [1]. Incubate the sample at 60 degrees Celsius for 10 minutes on a shaker set to 200 revolutions per minute [2] and centrifuge at 1850 *g* for 15 minutes at 25 degrees Celsius [3].

2.8.1. Talent washing the sample with distilled water.

2.8.2. Talent placing the sample for shaking in an incubator.

2.8.3. Talent placing the sample in the centrifuge.

2.9. Pre-freeze the bone at minus 80 degrees Celsius for 4 hours [1]. Then, place the decellularized bone in a wide-base container [2] and lyophilize the sample at 0.05 millibars and minus 40 to minus 60 degrees Celsius for 24 hours [3].

2.9.1. Talent placing the bone for pre-freezing in a freezer.

2.9.2. Talent placing the decellularized bone in a wide-base container.

2.9.3. Talent placing the sample in lyophilizer.

3. Preparation of Decellularized Bone Microparticles

3.1. Place the decellularized bone fragments in a 250-milliliter flask containing the sterilizing solution [1] and homogenize until a uniform white solution is obtained [2].

3.1.1. Talent placing decellularized bone fragments into 250 milliliter flask with sterilizing solution.

3.1.2. Talent homogenizing the bone fragments until a uniform white solution forms.

- 3.2. Centrifuge the homogenate at 1850 *g* for 15 minutes at 25 degrees Celsius [1]. After discarding the supernatant, wash the pellet twice with 300 milliliters of distilled water at 25 degrees Celsius, agitating at 200 revolutions per minute for 10 minutes [2]. Then, perform a final centrifugation at 1850 *g* for 15 minutes at 25 degrees Celsius [3].
 - 3.2.1. Talent placing the sample in the centrifuge.
 - 3.2.2. Talent adding water to the pellet and placing the sample under agitation.
 - 3.2.3. Talent placing the sample back into the centrifuge for final centrifugation.
- 3.3. Lyophilize the resulting material at 0.05 millibar and minus 40 to minus 60 degrees Celsius for 48 hours [1]. Next, mechanically mill the lyophilized cortical or medullary bone using a sterilized ceramic mortar until a fine powder is obtained [2].
 - 3.3.1. Talent placing the material in the lyophilizer.
 - 3.3.2. Talent milling the lyophilized bone in a sterilized ceramic mortar.
- 3.4. Pass the dry powder through a 200-micrometer mesh or sieve [1] and weigh the final material before storage at minus 80 degrees Celsius [2].
 - 3.4.1. Talent adding the dry powder onto a 200 micrometer mesh or sieve.
 - 3.4.2. Talent weighing the final powdered material on a weighing balance.

4. Preparation of Methacrylated Gelatin-Bone Composite Hydrogel

- 4.1. Dissolving 1 gram of methacrylated gelatin in 9.5 milliliters of deionized water tempered at 50 degrees Celsius to prepare a 10% solution [1]. Cool the solution to 37 degrees Celsius [2] and add Irgacure 2959 photo-initiator solution [3].
 - 4.1.1. Talent adding GelMA to deionized water and placing the mixture.
 - 4.1.2. Talent placing the solution aside on the work surface to cool.
 - 4.1.3. Talent adding Irgacure 2959 photoinitiator solution to the mixture.
- 4.2. Filter-sterilize the solution using a 0.22 micrometer filter, maintaining the solution at 37 degrees Celsius [1]. Then, add decellularized bone microparticles to achieve a final concentration of 1% weight by volume [2].

- 4.2.1. Talent adding the solution onto a 0.22 micrometer filter.
- 4.2.2. Talent adding decellularized bone microparticles to the solution.

4.3. Mix the components by pipetting up and down carefully while avoiding bubble formation until a homogeneous suspension is obtained [1].

- 4.3.1. Talent mixing the solution by pipetting the mixture up and down carefully and then showing the homogeneous suspension without bubbles.

5. Isolation and Culture of Bone Marrow-Derived Cells

5.1. Extract and mince the bone marrow tissue from the interior of the femoral head using sterile surgical instruments [1] and transfer the minced tissue to a sterile Petri dish [2]. Wash the tissue three times with alpha minimum essential culture medium supplemented with antibiotic-antimycotic solution [3-TXT].

- 5.1.1. Talent extracting and mincing bone marrow tissue from the femoral head using sterile surgical instruments.
- 5.1.2. Talent transferring the minced tissue into a sterile Petri dish.
- 5.1.3. Talent washing the tissue with supplemented culture medium. **TXT: 100 µg/mL Penicillin; 100 µg/mL Streptomycin; 0.25 µg/mL Amphotericin**

5.2. After rinsing the sample with Hank's Balanced Salt Solution or HBSS, incubate it [1] with collagenase and EDTA with intervening HBSS washes to thoroughly dissociate the tissue [2].

- 5.2.1. Talent adding Hank's Balanced Salt Solution to the tissue.

5.2.2. TEXT ON PLAIN BACKGROUND:

- Incubate the tissue in collagenase Type I (25 min, 37 °C, gentle agitation)
- Wash with HBSS
- Repeat collagenase digestion + HBSS wash (2x)
- Incubate the sample with 0.5 mM EDTA (25 min, 37 °C)
- Wash with HBSS
- Repeat incubation with EDTA + HBSS (2x)
- Perform final collagenase digestion (25 min, 37 °C) + HBSS wash

- 5.3. Collect the collagenase solution [1] and the final wash solution into a 50-milliliter centrifuge tube [2].
 - 5.3.1. Talent collecting the collagenase solution into a tube.
 - 5.3.2. Talent transferring the wash solution into the same tube.
- 5.4. Centrifuge the solution at 1000 *g* for 5 minutes at room temperature [1]. Discard the supernatant [2] and resuspend the pellet in 2 milliliters of complete alpha minimum essential medium [3].
 - 5.4.1. Talent placing the tube in the centrifuge.
 - 5.4.2. Talent discarding the supernatant from the tube.
 - 5.4.3. Talent resuspending the pellet in complete culture medium.
- 5.5. Then, seed the cell suspension into two T75 culture flasks [1] and incubate at 37 degrees Celsius with 5 percent carbon dioxide [2]. Replace the culture medium every 2 to 3 days until adequate confluency is achieved [3].
 - 5.5.1. Talent seeding the cell suspension into two T75 culture flasks.
 - 5.5.2. Talent placing the flasks into the incubator.
 - 5.5.3. Talent removing the culture medium in the flask and adding fresh medium.

6. Bioink Preparation and 3D Cell Encapsulation

- 6.1. Prewarm the required amount of bone gel hydrogel at 37 degrees Celsius [1]. Detach the cultured osteocytes using a standard trypsinization protocol [2]. Centrifuge the sample in a 15-milliliter tube at 500 *g* for 5 minutes [3].
 - 6.1.1. Talent placing the bone gel hydrogel for prewarming at 37 degrees Celsius.
 - 6.1.2. Talent detaching cultured osteocytes.
 - 6.1.3. Talent placing the 15 milliliter tube in the centrifuge.
- 6.2. Resuspend the pellet in 200 microliters of culture medium tempered at 37 degrees Celsius [1]. Mix the cell suspension with the bone gel to obtain a final cell density of at least 1,000 cells per microliter, forming the bioink [2]. Using a P-1000 pipette, add 50

microliters of the bioink per construct to each well of the culture plate and allow the bioink to physically gel for 5 minutes at room temperature [3].

6.2.1. Talent resuspending the pellet in culture medium.

6.2.2. Talent mixing the cell suspension with bone gel hydrogel.

6.2.3. Talent pipetting bioink into wells of the culture plate and keeping the plate aside.

6.3. Expose each well to 365-nanometer ultraviolet light for 1 minute to achieve irreversible crosslinking at 0.3 joules per square centimeter [1]. Then, add culture medium to each well [2] and incubate the constructs at 37 degrees Celsius with 5 percent carbon dioxide for the required time points using a separate plate for each time point [3].

6.3.1. Talent placing the culture plate under ultraviolet light for crosslinking.

6.3.2. Talent adding culture medium to the wells.

6.3.3. Talent placing the culture plates into the incubator.

6.4. Finally, assess scaffold biocompatibility by measuring cell viability using live-dead fluorescence staining and metabolic activity using the alamarBlue assay [1].

6.4.1. Talent adding the stain to the sample wells.

Results

7. Results

7.1. Primary osteocytes were detectable in culture approximately 5 days after extraction and displayed a stellate morphology forming interconnected networks characteristic of osteocytes [1].

7.1.1. LAB MEDIA: Figure 3.

7.2. Metabolic activity in osteocyte-laden constructs at each time interval showed significant differences [1] relative to the control [2].

7.2.1. LAB MEDIA: Figure 5. *Video editor: Highlight the bars for TE1, TE2, and TE3*

7.2.2. LAB MEDIA: Figure 5. *Video editor: Highlight the bars for Control*

7.3. After 7 days of culture, strong calcein acetoxymethyl fluorescence indicated widespread viable cells throughout the hydrogel matrix [1].

7.3.1. LAB MEDIA: Figure 6A. *Video editor: Show only day 7 images and highlight the green fluorescent cells in panels A3*

7.4. Minimal ethidium homodimer-1 signal indicated low cytotoxicity in the constructs [1].

7.4.1. LAB MEDIA: Figure 6. *Video editor: Show only day 7 images and highlight the image B3*

1. Femoral

Pronunciation link: <https://www.merriam-webster.com/dictionary/femoral>

IPA: /'femərəl/

Phonetic Spelling: fem·uh·ruhl

2. Sonicate

Pronunciation link: <https://www.merriam-webster.com/dictionary/sonicate>

IPA: /'sɑ:nəˌkeɪt/

Phonetic Spelling: saa·nuh·kayt

3. Centrifuge

Pronunciation link: <https://www.merriam-webster.com/dictionary/centrifuge>

IPA: /'sentrəˌfjuːdʒ/

Phonetic Spelling: sen·truh·fyooj

4. Peroxyacetic
Pronunciation link: <https://www.howtopronounce.com/ peroxyacetic>
IPA: /pəˌrɑːksɪəˈsiːtɪk/
Phonetic Spelling: puh·rok·see·uh·see·tik
5. Decellularized
Pronunciation link: <https://www.howtopronounce.com/decellularized>
IPA: /diːˈsɛljələraɪzd/
Phonetic Spelling: dee·sel·yuh·luh·ryzd
6. Lyophilize
Pronunciation link: <https://www.merriam-webster.com/dictionary/lyophilize>
IPA: /laɪˈɑːfəˌlaɪz/
Phonetic Spelling: ly·aa·fuh·lyz
7. Homogenize
Pronunciation link: <https://www.merriam-webster.com/dictionary/homogenize>
IPA: /həˈmɑːdʒəˌnaɪz/
Phonetic Spelling: huh·maa·juh·nyz
8. Supernatant
Pronunciation link: <https://www.merriam-webster.com/dictionary/supernatant>
IPA: /ˈsuːpərˌneɪtənt/
Phonetic Spelling: soo·per·nay·tuht
9. Methacrylated
Pronunciation link: <https://www.howtopronounce.com/methacrylated>
IPA: /məˈθækɹɪleɪtɪd/
Phonetic Spelling: muh·thak·ruh·lay·tid
10. Gelatin
Pronunciation link: <https://www.merriam-webster.com/dictionary/gelatin>
IPA: /ˈdʒələtɪn/
Phonetic Spelling: jel·uh·tin
11. Deionized
Pronunciation link: <https://www.merriam-webster.com/dictionary/deionize>
IPA: /diˈaɪəˌnaɪzd/
Phonetic Spelling: dee·eye·uh·nyzd
12. Photoinitiator
Pronunciation link: <https://www.howtopronounce.com/photoinitiator>
IPA: /ˌfoʊtoʊˈɪnɪʃeɪtər/
Phonetic Spelling: foe·toe·ih·nish·ee·ay·ter
13. Osteocytes
Pronunciation link: <https://www.merriam-webster.com/dictionary/osteocyte>
IPA: /ˈɑːstɪəˌsaɪt/, /ˈɑːsteɪəˌsaɪt/
Phonetic Spelling: ahs·tee·uh·syts
14. Collagenase
Pronunciation link: <https://www.merriam-webster.com/medical/collagenase>
IPA: /kəˈlædʒəˌneɪs/
Phonetic Spelling: kuh·la·juh·nays

15. Amphotericin
Pronunciation link: <https://www.merriam-webster.com/medical/amphotericin>
IPA: /,æmfou'terəsɪn/
Phonetic Spelling: am·foh·ter·uh·sin
16. Biocompatibility
Pronunciation link: <https://www.merriam-webster.com/dictionary/biocompatibility>
IPA: /,baɪoʊkəm,pætə'bɪləti/
Phonetic Spelling: by·oh·kuhm·pat·uh·bil·uh·tee
17. Cytotoxicity
Pronunciation link: <https://www.merriam-webster.com/medical/cytotoxicity>
IPA: /,saɪtoʊtɪ:k'sɪsɪti/
Phonetic Spelling: sy·toe·tok·sis·uh·tee
18. Irgacure 2959

Pronunciation link: <https://www.howtopronounce.com/irgacure>
IPA: /'ɜːrɡəkjʊr tuː 'naɪn faɪv naɪn/
Phonetic Spelling: ur·guh·kyur too·nine·five·nine