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Title: Cryosectioning of Synthetic Biocompatible Implants Using UV-Curable Resin for Enhanced Immunofluorescence Analysis

Authors and Affiliations: Amanda M. Richards^{*1}, Andrea Cheslea^{*1}, Cheryl E. Myers¹, Jonah D. Reichenbach¹, David G. Lott^{1,2}

¹Head and Neck Regenerative Medicine Laboratory, Mayo Clinic, Arizona

²Division of Laryngology, Department of Otolaryngology Head and Neck Surgery, Mayo Clinic, Arizona

* These authors contributed equally

Corresponding Authors:

David G. Lott lott.david@mayo.edu

Email Addresses for All Authors:

cheslea.andrea@mayo.edu
jdreichenbach06@gmail.com
lott.david@mayo.edu
myers.cheryl@mayo.edu
richards.amanda@mayo.edu

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**

Videographer: Please film the SCREEN shots

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

4. Testimonials (optional): Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **No**

Current Protocol Length

Number of Steps: 16

Number of Shots: 44 (3 Screen)

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**

INTRODUCTION:

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

- 1.1. **Amanda Richards:** This study develops a cryosectioning approach for synthetic implants and challenging tissues that maintain structural integrity and antibody accessibility.

~~Describe the research gap or limitation in existing methods and how this protocol addresses them.~~

- 1.2. **Amanda Richards:** Existing methods can damage implant–tissue structure and limit antibody access; this protocol preserves interfaces while maintaining immunofluorescence compatibility.

CONCLUSION:

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

- 1.3. **Amanda Richards:** This protocol lets researchers study tissue structure, immune cell localization, and protein expression directly at challenging implant–tissue interfaces.

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

- 1.4. **Amanda Richards:** The main challenge in this protocol is curing resin sufficiently to stabilize interfaces while avoiding over-curing that makes samples brittle.

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

- 1.5. **Amanda Richards:** Future studies may explore new biomaterials and tissues, develop low-autofluorescence resins, and design resin-based tapes for easier, more consistent application.

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

Ethics Title Card

This research has been approved by the Institutional Animal Care and Use Committee (IACUC) at Mayo Clinic

Protocol

***Videographer:** Please always record an audio slate while filming each shot mentioned in the shotlist.*

2. Cryostat Setup and Initial Sectioning

Demonstrator: Amanda Richards

2.1. To begin, set the temperature of the cryostat chamber to minus 15 degrees Celsius [1-**TXT**]. Verify the temperature using integrated digital monitoring [2] and equilibrate the chamber overnight before specimen introduction [3].

2.1.1. WIDE: Talent adjusting the cryostat chamber to -15 °C. **TXT: See the text protocol for tissue integrated implant processing and cryoprotection**

***Videographer:** Please obtain CU shot as well for this shot.*

2.1.2. Talent verifying the chamber temperature on the integrated digital monitor.

2.1.3. Shot of the cryostat chamber equilibrating.

2.2. Install new disposable blades in the cryostat [1]. Set the blade angle for sectioning of combined synthetic-biological material according to the manufacturer's specifications [2].

2.2.1. Talent installing new disposable blades in the cryostat.

2.2.2. Talent adjusting the blade angle for combined synthetic-biological material sectioning.

2.3. Program the desired section thickness of 50 to 100 micrometers into the cryostat by selecting **Thickness** on the control panel [1]. Adjust the setting with the arrow keys [2] and confirm the parameter by pressing **Set** [3].

***Videographer:** Please capture all shots labeled 'SCREEN'*

2.3.1. SCREEN: Show the cryostat control panel and select **Thickness**.

Authors: The shots labeled SCREEN show the cryostat control panel, which cannot be captured with screen-recording software, so the videographer will film the panel directly. Please confirm this is acceptable.

2.3.2. SCREEN: Show the thickness value being adjusted with the arrow keys. **NOTE:**

These shots could be in C9366 & C9367

2.3.3. SCREEN: Show the parameter being confirmed by pressing **Set**.

2.4. After mounting frozen specimens in the cryostat, section the embedded specimen to the predetermined depth at the implant–tissue interface [1]. Assess the advancing

section surface to identify the optimal plane for UV-resin application [2]. Confirm depth when subtle tissue opacity changes and initial implant-periphery exposure remain consistently visible across successive trimmed sections [3]. Stop sectioning immediately upon reaching this critical interface [4].

- 2.4.1. Talent sectioning through the embedded specimen in the cryostat.
- 2.4.2. SCREEN: Show the advancing section surface as the optimal plane is approached. **NOTE: The author is positive that the videographer filmed this while she was applying the OCT compound**
- 2.4.3. SCREEN: Show subtle changes in tissue opacity and initial implant-periphery exposure remaining visible across successive trimmed sections.
- 2.4.4. Talent stopping sectioning at the critical interface.

UV-Curable Resin Application

NOTE: Treat this as a separate section

- 2.5. To prepare UV-curable resin, first cut parafilm to dimensions 2 to 3 millimeters beyond the implant perimeter [1]. Using sterile forceps, separate the parafilm from the protective paper backing [2].
 - 2.5.1. Talent cutting parafilm to dimensions 2 to 3 mm beyond the implant perimeter.
 - 2.5.2. Talent separating the parafilm from the protective paper backing with sterile forceps.
- 2.6. After optimizing the volume, dispense the desired volume of UV-curable resin onto the exposed parafilm surface [1]. Visually evaluate the spread to ensure uniform coverage of the specimen surface without overflow or excessive resin accumulation [2].
 - 2.6.1. Shot of the resin spread being visually assessed for uniform coverage without overflow or excessive accumulation.
 - 2.6.2. Talent dispensing the optimized volume of UV-curable resin onto the exposed parafilm surface.
- 2.7. Replace the protective paper backing immediately over the resin-loaded parafilm [1]. Apply a 2-inch polyurethane seam roller with consistent pressure in overlapping, parallel passes [2]. Continue with perpendicular passes to achieve a thin, uniform resin layer [3].
 - 2.7.1. Talent replacing the protective paper backing over the resin-loaded parafilm.
 - 2.7.2. Talent applying the 2-inch polyurethane seam roller in overlapping, parallel passes.

- 2.7.3. Talent continuing with perpendicular seam roller passes to form a thin, uniform resin layer.
- 2.8. Then, remove the protective paper layer carefully to reveal the uniformly distributed resin film [1]. Position the resin-parafilm directly over the exposed implant surface [2]. Ensure precise alignment with the implant-tissue interface [3].
 - 2.8.1. Talent removing the protective paper layer to reveal the resin film.
Videographer: Zoom in to show the resin film towards the end of the shot.
 - 2.8.2. Talent positioning the resin-parafilm over the exposed implant surface.
 - 2.8.3. CU: Shot of the resin-parafilm aligned with the implant-tissue interface.
- 2.9. Apply the polyurethane seam roller evenly from the implant center outward [1], while avoiding air bubbles [2]. Ensure intimate contact between the resin and both synthetic and biological surfaces [3].
 - 2.9.1. Talent rolling from the implant center outward with the polyurethane seam roller.
 - 2.9.2. Talent applying even pressure with a seam roller, while avoiding air bubbles.
 - 2.9.3. CU: Shot of the resin layer with air bubbles removed and full contact across the synthetic and biological surfaces.
- 2.10. Next, position the 405-nanometer UV LED light source at a distance of 2 to 3 centimeters from the implant surface [1]. Place the UV-protective cover over the cryostat chamber window before UV photopolymerization [2]. Expose the specimen for 90 seconds [3]. Verify complete polymerization by tactile assessment of resin hardness and non-tacky characteristics [4].
 - 2.10.1. Talent positioning the 405 nm wavelength UV LED light source 2 to 3 cm from the implant surface.
 - 2.10.2. Talent placing the UV-protective cover over the cryostat chamber window.
 - 2.10.3. Talent exposing the specimen to the UV LED light source for 90 seconds.
 - 2.10.4. Talent assessing the cured resin for hardness and non-tacky characteristics.

3. Section Recovery and Slide Preparation

- 3.1. Remove the parafilm in a single continuous motion [1]. Inspect the exposed surface under cryostat lighting for uniform resin coverage [2], absence of lifting at the tissue edge [3], and lack of visible parafilm residue [4].
 - 3.1.1. Talent removing the parafilm in a single continuous motion.
 - 3.1.2. Shot of the exposed surface under cryostat lighting showing uniform resin coverage.
 - 3.1.3. CU: Shot of the tissue edge showing no lifting.

- 3.1.4. CU: Shot of the exposed surface showing no visible parafilm residue.
- 3.2. Use a calibrated microtome advance mechanism to section the specimens to a predetermined thickness of 8 micrometers [1]. Monitor each section for consistent thickness and absence of compression, tearing, and incomplete sectioning [2]. Position the anti-roll plate to prevent curling [3].
 - 3.2.1. Talent sectioning the specimen at 8 μm with the calibrated microtome advance mechanism.
 - 3.2.2. Shot of the section being monitored for consistent thickness and absence of compression, tearing, and incomplete sectioning.
 - 3.2.3. Talent positioning the anti-roll plate to prevent curling.
- 3.3. Then, place the slide adhesive surface so the initial contact occurs at the section edge [1] and continue progressive contact across the entire area [2]. Ensure that the UV-cured resin surface adheres to the slide while maintaining the tissue surface upward for immunofluorescence [3].
 - 3.3.1. Talent positioning the slide adhesive surface for initial contact at the section edge.
 - 3.3.2. Talent advancing the slide into progressive contact across the entire section area.
 - 3.3.3. CU: Shot of the UV-cured resin surface adhering to the slide with the tissue surface facing upward.
- 3.4. Press the section-slide assembly gently to eliminate air bubbles [1]. Verify the orientation under cryostat lighting [2] and confirm that the implant-tissue interface remains intact [3].
 - 3.4.1. Talent gently pressing the section-slide assembly to eliminate air bubbles.
 - 3.4.2. Shot of the section-slide assembly being checked under cryostat lighting.
 - 3.4.3. CU: Shot of the intact implant-tissue interface on the slide.
- 3.5. Place the completed slides in pre-labeled storage boxes at minus 20 degrees Celsius for applications requiring processing within 72 hours [1]. Store the slides at minus 80 degrees Celsius for periods longer than one week. Finally, after immunofluorescence staining, image the sections on an inverted fluorescence microscope [2].
 - 3.5.1. WIDE: Talent placing completed slides into pre-labeled storage boxes at $-20\text{ }^{\circ}\text{C}$.
 - 3.5.2. Talent storing the slides at $-80\text{ }^{\circ}\text{C}$

Results

4. Results

- 4.1. High-quality sections demonstrated intact morphology without significant cracks or fracturing artifacts, with continuous tissue architecture and a preserved implant-tissue interface [1].

- 4.1.1. LAB MEDIA: Figure 2A. *Video editor: Highlight the area around the arrows.*

- 4.2. Although the implant material exhibited autofluorescence, its uniform morphology and geometric structure allowed clear distinction from [1] specific immunofluorescence signals in the tissue-integrated constructs [2].

- 4.2.1. LAB MEDIA: Figure 3A. *Video editor: Emphasize the areas marked by asterisks.*

- 4.2.2. LAB MEDIA: Figure 3C. *Video editor: Highlight the areas marked by arrows at the tissue-implant border.*

- 4.3. Multi-parameter immunofluorescence imaging showed the whole specimen stained for laminin, wheat germ agglutinin, and a nuclear counterstain [1].

- 4.3.1. LAB MEDIA: Figure 7A.

- 4.4. Wheat germ agglutinin staining appropriately outlined membrane structures surrounding implant materials [1], while laminin and nuclear signals provided additional spatial definition [2].

- 4.4.1. LAB MEDIA: Figure 7D. *Video editor: Highlight the green regions*

- 4.4.2. LAB MEDIA: Figure 7 C and B. *Video editor: Highlight the red regions and blue-stained nuclei*

1. Cryostat

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

IPA: /'kraɪəˌstæt/

Phonetic Spelling: kry·uh·stat

2. Cryoprotection

Pronunciation link: No confirmed link found

IPA: /ˌkraɪoʊprəˈtɛkʃən/

Phonetic Spelling: kry·oh·pruh·tek·shuhn

3. Micrometers

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

- IPA: /maɪˈkraːmətər/
Phonetic Spelling: my·krah·muh·ter
4. Implant
Pronunciation link: <https://www.merriam-webster.com/dictionary/implant>
IPA: /ˈɪmˌplænt/, /ɪmˈplænt/
Phonetic Spelling: im·plant
 5. Opacity
Pronunciation link: <https://www.merriam-webster.com/dictionary/opacity>
IPA: /oʊˈpæsəti/
Phonetic Spelling: oh·pas·uh·tee
 6. Periphery
Pronunciation link: <https://www.merriam-webster.com/dictionary/periphery>
IPA: /pəˈrɪfəri/
Phonetic Spelling: puh·rif·uh·ree
 7. UV-curable
Pronunciation link: No confirmed link found
IPA: /ˌjuːˈviːˈkjʊrəbəl/
Phonetic Spelling: yoo·vee kyur·uh·buhl
 8. Parafilm
Pronunciation link: No confirmed link found
IPA: /ˈpærəˌfɪlm/
Phonetic Spelling: pair·uh·film
 9. Polyurethane
Pronunciation link: <https://www.merriam-webster.com/dictionary/polyurethane>
IPA: /ˌpɑːliˈjʊrəˌθeɪn/
Phonetic Spelling: paa·lee·yur·uh·thayn
 10. Photopolymerization
Pronunciation link: No confirmed link found
IPA: /ˌfoʊtoʊpəˌlɪməˈzeɪʃən/
Phonetic Spelling: foh·toh·puh·lim·uh·ruh·zay·shuhn
 11. Polymerization
Pronunciation link: <https://www.merriam-webster.com/dictionary/polymerization>
IPA: /pəˌlɪməˈzeɪʃən/
Phonetic Spelling: puh·lim·uh·ruh·zay·shuhn
 12. Tactile
Pronunciation link: <https://www.merriam-webster.com/dictionary/tactile>
IPA: /ˈtækˌtaɪl/, /ˈtæk.təl/
Phonetic Spelling: tak·tyle; tak·tuhl
 13. Microtome
Pronunciation link: <https://www.merriam-webster.com/dictionary/microtome>
IPA: /ˈmaɪkrəˌtoʊm/
Phonetic Spelling: my·kruh·tohm
 14. Immunofluorescence
Pronunciation link: No confirmed link found

- IPA: /ɪˌmjuːnoʊflʊəˈresəns/
Phonetic Spelling: ih·myoo·noh·floor·es·uhns
15. Fluorescence
Pronunciation link: <https://www.merriam-webster.com/dictionary/fluorescence>
IPA: /flʊˈresəns/
Phonetic Spelling: floor·es·uhns
16. Microscope
Pronunciation link: <https://www.merriam-webster.com/dictionary/microscope>
IPA: /ˈmaɪkrəˌskoʊp/
Phonetic Spelling: my·kruh·skohp
17. Morphology
Pronunciation link: <https://www.merriam-webster.com/dictionary/morphology>
IPA: /mɔːrˈfɒlədʒi/
Phonetic Spelling: mor·faa·luh·jee
18. Autofluorescence
Pronunciation link: No confirmed link found
IPA: /ˌɔːtoʊflʊəˈresəns/
Phonetic Spelling: aw·toh·floor·es·uhns
19. Multi-parameter
Pronunciation link: No confirmed link found
IPA: /ˌmʌlti pəˈræmətər/
Phonetic Spelling: mul·tee puh·ram·uh·ter
20. Laminin
Pronunciation link: <https://www.merriam-webster.com/dictionary/laminin>
IPA: /ˈlæməniːn/
Phonetic Spelling: lam·uh·nin
21. Agglutinin
Pronunciation link: <https://www.merriam-webster.com/dictionary/agglutinin>
IPA: /əˈɡluːtəniːn/
Phonetic Spelling: uh·gloo·tuh·nin
22. Counterstain
Pronunciation link: No confirmed link found
IPA: /ˈkaʊntərˌsteɪn/
Phonetic Spelling: kown·ter·stayn