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Title: A Pre-Clinical Model of Synovitis Using Ex Vivo Human Synovial Tissue with Preserved Function and Architecture

Authors and Affiliations:

Karen I. Cyndari^{1,2}, Jacob M. Elkins³, Mitchell C. Coleman³, KariAn E. Mulford^{1,2}, Rachel M. Johnson⁴, Katherine L. Butler^{1,2}, Corey P. Parlet^{5,6}, Hanna C. Huston^{1,2}, Tyler Draayer¹, Preethika Naveen⁷, Christine A. Petersen⁸

¹Department of Emergency Medicine, University of Iowa Health Care

²Interdisciplinary Graduate Program in Immunology, University of Iowa

³Department of Orthopedics and Rehabilitation, University of Iowa Health Care

⁴University of Iowa, Carver College of Medicine

⁵Office of Research and Development, Iowa City Veterans Affairs Health Care System

⁶Department of Internal Medicine, University of Iowa Health Care

⁷Iowa State University

⁸Department of Veterinary Biosciences, Ohio State University

Corresponding Authors:

Karen I. Cyndari Karen-cyndari@uiowa.edu

Email Addresses for All Authors:

Jacob M. Elkins	Jacob-elkins@uiowa.edu
Mitchell C. Coleman	mittchell-coleman@uiowa.edu
KariAn E. Mulford	Karian-mulford@uiowa.edu
Rachel Johnson	rachel-johnson-2@uiowa.edu
Katherine L. Butler	Katherine-l-butler@uiowa.edu
Corey P. Parlet	Corey-parlet@uiowa.edu
Hanna C. Huston	Hanna-huston@uiowa.edu
Tyler Draayer	Tyler-draayer@uiowa.edu
Preethika Naveen	pnaveen@iastate.edu
Christine A. Petersen	petersen.307@osu.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, all done**
- 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. **Yes**

Current Protocol Length

Number of Steps: 14

Number of Shots: 34 (4 SC)

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 are slightly edited in accordance with journal guidelines to fit the length limit. 5 best answers are chosen.

INTRODUCTION:

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

- 1.1. **Karen Cyndari:** The main questions we want to answer are: what is the native synovial immune response to infectious and inflammatory stimuli, and how is that reflected in alterations to synovium structure?
 - 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. **Karen Cyndari:** This protocol can be utilized in the context of infectious stimuli, such as Lyme Arthritis or *S. aureus*, as well as with inflammatory stimuli, such as LPS.
 - 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. **Karen Cyndari:** This protocol allows researchers to study the alterations in synovial intimal and sublining layers including depth, cellularity, and intactness, which is all indicative of inflammation.
 - 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. **Karen Cyndari:** The most important considerations while performing this protocol are viable tissue acquisition from appropriate donors, sterility, and intimal lining identification for biopsy selection.

- 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. **Karen Cyndari:** Future studies will evaluate improving the length of viable tissue culture to evaluate regeneration of the synovial intimal lining.

- 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.

Testimonial Questions (OPTIONAL):

Videographer: Please capture all testimonial shots in a wide-angle format with sufficient headspace, as the final videos will be rendered in a 1:1 aspect ratio. Testimonial statements will be presented live by the authors, sharing their spontaneous perspectives.

- Testimonial statements will **not appear in the video** but may be featured in the journal's promotional materials.
- **Provide the full name and position** (e.g., Director of [Institute Name], Senior Researcher [University Name], etc.) of the author delivering the testimonial.
- Please **answer the testimonial question live during the shoot**, speaking naturally and in your own words in **complete sentences**.

What problem in your field of research does your JoVE article address?

- 1.6. **Karen Cyndari, Assistant Professor**: (authors will present their testimonial statements live): This work addresses the complex cross-talk present in joint spaces that can cause arthritis by isolating synovium-initiated signals in various clinically relevant context.
- 1.6.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

What value do you expect this video article to have for other researchers working in your field?

- 1.7. **Karen Cyndari, Assistant Professor**: (authors will present their testimonial statements live): This video will clarify nuances of tissue identification and processing as well as provide a tutorial on the tools used for masks and measurements.
- 1.7.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

Ethics Title Card

This research has been approved by the Institutional Review Board (IRB) at the University of Iowa

Protocol

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

2. Biopsy Coring of Human Synovial Tissue

Demonstrator: Karen Cyndari

- 2.1. To begin, place a specimen container filled with cold Dulbecco's PBS or DPBS on ice [1] and transfer the human anterior synovial tissue acquired during total knee arthroplasty or tibial tubercle osteotomy into the container [2].
 - 2.1.1. WIDE: Talent placing the specimen container with DPBS on ice.
 - 2.1.2. Talent placing the synovium into a 50-milliliter specimen container containing cold Dulbecco's PBS on ice.
- 2.2. Wash the tissue two times in cold DPBS for 10 minutes on a plate rocker [2].
 - 2.2.1. Talent transferring the synovium into another container with cold DPBS and placing it on a rocker.
- 2.3. Next, transfer the synovium to a 10-centimeter or larger sterile petri dish under a biosafety level 2 cabinet [1]. Add cold, sterile, low-glucose DMEM (*D-M-E-M*) containing sodium pyruvate and L-glutamine to fill the dish halfway [2]. Identify the synovial lining by the pearlescent intimal layer, without peeling it away [3].
 - 2.3.1. Talent transferring the synovium to a sterile petri dish in a biosafety level 2 certified biosafety cabinet.
 - 2.3.2. Talent adding cold, sterile, low glucose DMEM containing sodium pyruvate and L-glutamine until the dish is halfway filled.
 - 2.3.3. Talent pointing to the pearlescent intimal layer.
- 2.4. Arrange the required tools to obtain 3-millimeter biopsy cores for a minimum of 2 technical replicates [1]. Using iris scissors or a scalpel, dissect away the extraneous adipose and stromal tissue from the cauterized side to isolate the synovium, maintaining a tissue depth of approximately 4 to 5 millimeters throughout [2]. Change the petri dish media as necessary to maintain the field of view [2].

- 2.5.1,. Talent arranging tools to pick biopsy cores such as forceps, hemostats, punch, etc. **Author's NOTE: Move this shot before 2.4.1**
- 2.4.1. Talent dissecting away extraneous adipose and stromal tissue on the cauterized side while preserving approximately 4 to 5 millimeters of tissue depth.
- 2.4.2. Talent changing the media in the petri dish to maintain the field of view.
- 2.5. ~~Arrange the required tools to obtain 3 millimeter biopsy cores for a minimum of 2 technical replicates per tested condition [1].~~ Position the tissue with the synovial lining facing the coring tool [2]. Stabilize the tissue with the non-dominant hand using forceps or hemostats [3], and core the tissue by applying a firm, perpendicular pressure with gentle twisting [4]. Replace the biopsy punch after approximately 10 to 15 uses, or when the tissue twists during coring, to avoid damaging the intimal lining structure [5].
 - 2.5.1. ~~Talent arranging tools to pick biopsy cores such as forceps, hemostats, punch, etc~~ **Author's NOTE: Move this shot before 2.4.1**
 - 2.5.2. Talent positioning the synovium with the synovial lining facing the coring tool.
 - 2.5.3. Talent stabilizing the tissue with forceps or hemostats using the non-dominant hand.
 - 2.5.4. Talent coring the tissue with a forceful perpendicular motion and gentle twisting.
 - 2.5.5. Talent placing the biopsy punch in use aside and picking up a new one.
- 2.6. Open a second petri dish and fill it with 5 to 10 milliliters of media containing 10 percent FBS tempered at 37 degrees Celsius [1]. With flat or non-toothed forceps, transfer the cores to the dish [2] and gently agitate for 1 to 2 seconds to wash away debris without pinching them tightly [3].
 - 2.6.1. Talent opening a second petri dish and filling it with warmed media containing 10 percent FBS.
 - 2.6.2. Talent transferring the biopsy cores with flat or non-toothed forceps into the second dish.
 - 2.6.3. Talent gently agitating the cores in the media to remove debris without pinching them tightly.

3. Setting up the Joint Space Analysis System (JSAS)

- 3.1. To prepare the bottom well of the joint space analysis system [1], add 600 microliters of low glucose DMEM containing 10 percent FBS tempered at 37 degrees Celsius [2].
 - 3.1.1. Talent showing the bottom well of the joint synovial assay system.
 - 3.1.2. Talent pipetting 600 microliters of warmed low glucose DMEM containing 10 percent FBS into the bottom well.
- 3.2. Add the vehicle control or treatment reagent of choice, such as LPS or MCP-1, to the bottom well [1-TXT].
 - 3.2.1. Talent adding the treatment solution to the bottom well. **TXT: LPS: Lipopolysaccharide; MCP-1: Monocyte Chemoattractant Protein 1**
- 3.3. Select the transwell pore size based on the desired experimental outcome [1] and add 300 microliters of low glucose DMEM containing 10 percent FBS to the transwell [2].
 - 3.3.1. Talent picking up and showing an appropriate transwell insert.
 - 3.3.2. Talent pipetting 300 microliters of media into the transwell insert.
- 3.4. Using sterile forceps, gently transfer individual tissue cores into the media of the transwell, avoiding frayed and floating cores [1].
 - 3.4.1. Talent transferring individual tissue cores into the transwell using sterile forceps.
- 3.5. Next, place the assembly in an incubator at 37 degrees Celsius with 5 percent carbon dioxide for 8 to 72 hours, depending on the downstream assays to be performed [1].
 - 3.5.1. Talent placing the joint synovial assay system into the incubator.

Histologic and Quantitative Analysis of Synovial Tissue

NOTE: Treat the following as a separate section (4) and add the above section title

Authors: The following are only representative shots for bridging the results

- 3.6. Fix the synovial tissue in 10% neutral buffered formalin, then embed and section it for histologic analysis [1].
 - 3.6.1. Talent placing synovial tissue into a tube/jar labeled 'Fixative' .

NOTE: The authors were very reluctant to film any representative shots for downstream analyses, and they agreed to film only a couple of shots after requesting multiple times

3.7. After staining with hematoxylin and eosin, image the tissue sections at 10x and 40x using brightfield microscopy [1]. Use Photoshop to outline the intimal lining [2] and sublining regions in the 40x TIFF (*tiff*) image [3], masking non-target areas, erythrocytes, and large vasculature for subsequent quantitative analysis [4].

3.7.1. Talent placing a slide under a microscope and adjusting magnification to visualize tissue regions.

3.7.2. SCREEN: 3.4-5.mp4 00:38-00:44

3.7.3. SCREEN: 3.6.mp4 00:42-00:46

3.7.4. SCREEN: 3.6.1.mp4 00:37-00:42

3.8. Analyze the final masks to quantify intimal lining and sublining thickness, integrity, and cellularity [1]. Finally, assess the migratory cells by flow cytometry and the soluble factors in the collected media by enzyme-linked immunosorbent assay [2].

3.8.1. LAB MEDIA: Figure 4E and F.

3.8.2. Talent placing the sample in a FACS machine or loading a plate into an ELISA plate reader.

Results

4. Results

4.1. The synovial tissue cultured with increasing doses of MCP-1 or LPS showed distinct intimal lining morphology changes [1], including friable intimal lining [2] and a thickened intimal lining with decreased cellular density [3] when compared to the control [4].

4.1.1. LAB MEDIA: Figure 8A. *Video editor: Highlight the images for MCP-1 and LPS*

4.1.2. LAB MEDIA: Figure 8A. *Video editor: Highlight the black arrows marking the friable lining in the MCP-1 100 ng/ml panel.*

4.1.3. LAB MEDIA: Figure 8A. *Video editor: Highlight the black brackets in, MCP-1 200 ng/ml and LPS 250 ng/ml images.*

4.1.4. LAB MEDIA: Figure 8A. *Video editor: Highlight the black bracket in control image.*

4.2. MCP-1 and LPS treatment altered the synovial cytokine response [1]. MCP1 induced pathogenic fibroblast marker Podoplanin expression in the sublining [2], whereas LPS increased podoplanin in both the intimal lining and the sublining [3].

4.2.1. LAB MEDIA: Figure 8B. *Video editor: Highlight the images for MCP-1 and LPS*

4.2.2. LAB MEDIA: Figure 8B. *Video editor: In the MCP-1 200 ng/ml row, highlight the areas pointed by the black arrows*

4.2.3. LAB MEDIA: Figure 8B. *Video editor: Highlight the red signals in LPS 250 ng/ml row*

4.3. Migratory immune cells in the bottom well included neutrophils, NK cells, NKT cells, T cells, B cells or dendritic cells, monocytes or macrophages, and M2 macrophages [1]. T cells were the most abundant migratory immune cells [2].

4.3.1. LAB MEDIA: Figure 9A. *Video editor: Show panel A and highlight the x-axis labels*

4.3.2. LAB MEDIA: Figure 9A. *Video editor: Highlight the group labelled CD3 in panel A*

4.4. Statistically, there was no difference in cell migration based on bottom well stimulus [1].

4.4.1. LAB MEDIA: Figure 9A.

- 4.5. Osteoprotegerin, an arthritis-relevant protein, was detected at 24, 48, and 72 hours in untreated cultured arthritic synovium [1], and was significantly higher at 72 hours than at 24 hours [2].

- 4.5.1. LAB MEDIA: Figure 9B.

- 4.5.2. LAB MEDIA: Figure 9B. *Video editor: Highlight the 72h bar*

- 4.6. Inducible nitric oxide synthase expression in the intimal lining was high in controls at 0 hours [1], and was nearly absent after 24 hours [2]. The expression of nitric oxide synthase was maintained when treated with N-acetyl cysteine [3].

- 4.6.1. LAB MEDIA: Figure 10. *Video editor: Show the left column (set labelled iNOS), highlight the red signal along the surface layer in the '0 hr Control' panel*

- 4.6.2. LAB MEDIA: Figure 10. *Video editor: Show the left column set labelled iNOS, highlight the 24 hour Control panel*

- 4.6.3. LAB MEDIA: Figure 10. *Video editor: Show the left column set labelled iNOS, highlight the red surface signal in the left border in the +NAC panel*

- 4.7. Conversely, N-acetyl cysteine did not alter Matrix metalloproteinase 9 expression, a marker of both synovitis and potential cartilage damage [1].

- 4.7.1. LAB MEDIA: Figure 10. *Video editor: Show the right column set labelled MMP9, highlight the red dots*

1. Dulbecco's

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

IPA: /du:l'bɛkoʊ/

Phonetic Spelling: dool·BEK·oh

2. Synovial

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

IPA: /sə'nouviəl/

Phonetic Spelling: suh·NOH·vee·uhl

3. Arthroplasty

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

IPA: /'ɑrθroʊ plæsti/

Phonetic Spelling: AAR·throh·plas·tee

4. Tibial

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

IPA: /'tɪbiəl/

Phonetic Spelling: TIH·bee·uhl

5. Tubercle
Pronunciation link: <https://www.merriam-webster.com/dictionary/tubercle>
IPA: /'tu:bərkəl/
Phonetic Spelling: TOO·ber·kuhl
6. Osteotomy
Pronunciation link: <https://www.merriam-webster.com/dictionary/osteotomy>
IPA: /,əsti'atəmi/
Phonetic Spelling: ah·stee·AAT·uh·mee
7. Synovium
Pronunciation link: <https://www.merriam-webster.com/dictionary/synovium>
IPA: /sə'noʊviəm/
Phonetic Spelling: suh·NOH·vee·uhm
8. Biosafety
Pronunciation link: <https://www.merriam-webster.com/dictionary/biosafety>
IPA: /,baɪoʊ'seɪfti/
Phonetic Spelling: bye·oh·SAYF·tee
9. Sterile
Pronunciation link: <https://www.merriam-webster.com/dictionary/sterile>
IPA: /'sterail/
Phonetic Spelling: STER·uhl
10. Pyruvate
Pronunciation link: <https://www.merriam-webster.com/dictionary/pyruvate>
IPA: /paɪ'ruː,veɪt/
Phonetic Spelling: pie·ROO·vayt
11. Glutamine
Pronunciation link: <https://www.merriam-webster.com/dictionary/glutamine>
IPA: /'glu:tə,mi:n/
Phonetic Spelling: GLOO·tuh·meen
12. Pearlescent
Pronunciation link: <https://www.merriam-webster.com/dictionary/pearlescent>
IPA: /pər'lesənt/
Phonetic Spelling: per·LESS·uhnt
13. Intimal
Pronunciation link: <https://www.merriam-webster.com/dictionary/intimal>
IPA: /'ɪntəməl/
Phonetic Spelling: IN·tuh·muhl
14. Biopsy
Pronunciation link: <https://www.merriam-webster.com/dictionary/biopsy>
IPA: /'baɪ,əpsi/
Phonetic Spelling: bye·OP·see
15. Replicates
Pronunciation link: <https://www.merriam-webster.com/dictionary/replicate>
IPA: /'rɛplə,ket/
Phonetic Spelling: REP·luh·kayts

16. Scalpel
 Pronunciation link: <https://www.merriam-webster.com/dictionary/scalpel>
 IPA: /'skælpəl/
 Phonetic Spelling: SKAL·puhl
17. Adipose
 Pronunciation link: <https://www.merriam-webster.com/dictionary/adipose>
 IPA: /'ædəˌpəʊs/
 Phonetic Spelling: AD·uh·pohs
18. Stromal
 Pronunciation link: <https://www.merriam-webster.com/dictionary/stromal>
 IPA: /'stroʊməl/
 Phonetic Spelling: STROH·muhl
19. Cauterized
 Pronunciation link: <https://www.merriam-webster.com/dictionary/cauterize>
 IPA: /'kəʊtərˌaɪzd/
 Phonetic Spelling: KAW·tuh·ryzed
20. Hemostats
 Pronunciation link: <https://www.merriam-webster.com/dictionary/hemostat>
 IPA: /'hi:məˌstæt/
 Phonetic Spelling: HEE·muh·stat
21. Centrifuge
 Pronunciation link: <https://www.merriam-webster.com/dictionary/centrifuge>
 IPA: /'sentrəˌfjuːdʒ/
 Phonetic Spelling: SEN·truh·fyooj
22. Enzymatic
 Pronunciation link: <https://www.merriam-webster.com/dictionary/enzymatic>
 IPA: /ˌenzəˈmætɪk/
 Phonetic Spelling: en·zye·MAT·ik
23. Collagenase
 Pronunciation link: <https://www.merriam-webster.com/dictionary/collagenase>
 IPA: /kəˈlædʒəˌneɪs/
 Phonetic Spelling: kuh·LAJ·uh·nays
24. Incubation
 Pronunciation link: <https://www.merriam-webster.com/dictionary/incubation>
 IPA: /ˌɪnkjəˈbeɪʃən/
 Phonetic Spelling: in·kyuh·BAY·shuhn
25. Cytokines
 Pronunciation link: <https://www.merriam-webster.com/dictionary/cytokine>
 IPA: /'saɪtəˌkaɪn/
 Phonetic Spelling: SIGH·toh·kyne
26. Histological
 Pronunciation link: <https://www.merriam-webster.com/dictionary/histological>
 IPA: /ˌhɪstəˈlɒdʒɪkəl/
 Phonetic Spelling: hiss·tuh·LOJ·ih·kuhl

27. Immunofluorescence

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

IPA: /ɪˌmjuːnoʊˈflɔːˈresəns/

Phonetic Spelling: ih·MYOO·noh·flor·ESS·uhns

28. Fibroblasts

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

IPA: /ˈfaɪbrəˌblæst/

Phonetic Spelling: FYE·broh·blast

29. Macrophages

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

IPA: /ˈmækroʊˌfeɪdʒ/

Phonetic Spelling: MAK·roh·fayj

30. Rheumatoid

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

IPA: /ˈruːməˌtɔɪd/

Phonetic Spelling: ROO·muh·toyɪd