

Submission ID #: 66778

Scriptwriter Name: Pallavi Sharma

Project Page Link: <https://review.jove.com/account/file-uploader?src=20367338>

Title: Isolation of Wharton's Jelly Mesenchymal Stem Cells and their Derived Exosomes/Small Extracellular Vesicles

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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

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



Interview Statements are read by JoVE's voiceover talent.

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: 21

Number of Shots: 38

Introduction

NOTE to VO: Please record the interview statements

INTRODUCTION:

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

- 1.1. This protocol describes the isolation and characterization of Wharton's jelly mesenchymal stem cells and their therapeutic exosomes from human umbilical cord Wharton's jelly tissue.

- 1.1.1. [2.5.1](#)

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

- 1.2. This protocol provides a foundation for researchers to establish stem cell and exosome research, applicable in regenerative therapy and exosome-based biomedical solutions.

- 1.2.1. [2.12.1](#)

CONCLUSION:

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

- 1.3. This protocol enables researchers to isolate, characterize, and study WJ-MSCs and their therapeutic extracellular vesicles.

- 1.3.1. [2.8.1](#)

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

- 1.4. Key considerations while performing this protocol are rapid umbilical cord processing, strict sterility, and careful exosome isolation to preserve integrity and efficacy.

- 1.4.1. [2.5.1](#)

Ethics Title Card

This research has been approved by the Ethical Committees of the Faculty of Pharmacy, Ain Shams University, and the Faculty of Pharmacy, The British University in Egypt

Protocol

NOTE: The protocol is scripted from the available footage

2. Isolation of Wharton's Jelly-Mesenchymal Stem Cells (MSCs) from Human Umbilical Cord

Demonstrator: Hend Atta

- 2.1. To begin, using sterilized forceps, transfer the collected umbilical cord from the sealed plastic bag into a Petri dish containing PBS to wash out the blood [1]. Repeat the washing until all visible blood is removed from the sample [2].
 - 2.1.1. LAB MEDIA: Shot 1- Horizontal first attempt.mp4: 01:33-01:40.
 - 2.1.2. LAB MEDIA: Shot 1- Horizontal first attempt.mp4: 03:16-03:26
- 2.2. Spray 70 percent ethanol onto a sterile swab [1] and wipe the umbilical cord thoroughly for 30 seconds [2].
 - 2.2.1. LAB MEDIA: Shot 1- Horizontal first attempt.mp4: 04:45-04:50.
 - 2.2.2. LAB MEDIA: Shot 1- Horizontal first attempt.mp4: 05:05-05:13
- 2.3. Then, transfer the umbilical cord to a new Petri dish filled with sterile PBS to remove any residual ethanol [1].
 - 2.3.1. LAB MEDIA: Shot 1- Horizontal first attempt.mp4: 05:25-05:40
- 2.4. Transfer the umbilical cord into another Petri dish prefilled with sterile PBS [1]. Using sterile scissors, cut the umbilical cord into pieces approximately 3 centimeters in length [2]. Transfer the cut pieces into another Petri dish containing fresh sterile PBS to avoid blood contamination [3].
 - 2.4.1. LAB MEDIA: Shot 1- Horizontal first attempt.mp4: 05:58-06:09
 - 2.4.2. LAB MEDIA: Shot 2- Cutting UC into pieces NEW 29.3.MP4: 00:16-00:42
 - 2.4.3. LAB MEDIA: Shot 2- Cutting UC into pieces NEW 29.3.MP4: 02:03-02:30
- 2.5. In a Petri dish with sterile PBS on ice, incise the clear side of the umbilical cord away from blood vessels and isolate the Wharton's jelly [1]. Clamp the blood vessels, cut around them, and pull them away from the tissue [2].
 - 2.5.1. LAB MEDIA: Shot 2- Cutting UC into pieces NEW 29.3.MP4: 04:00-04:05, 04:54-05:06,
 - 2.5.2. LAB MEDIA: Shot 2- Cutting UC into pieces NEW 29.3.MP4: 06:45-07:01, 07:40-07:55, 08:20-08:30
- 2.6. Next, place the clear Wharton's jelly pieces in another Petri dish filled with sterile PBS on ice [1]. Transfer the Wharton jelly pieces to a new Petri dish in the laminar flow [2].

- 2.6.1. LAB MEDIA: Shot 2- Cutting UC into pieces NEW 29.3.MP4: 09:04-09:10
- 2.6.2. LAB MEDIA: Shot 5 – Transfer and speed and shorten.MP4: 0:04-0:17
- 2.7. Use a sterile, sharp scalpel and forceps to cut the collected Wharton's jelly tissues into small 10-millimeter-long pieces [1]. Transfer the collected pieces into a fresh 10-centimeter cell culture dish filled with sterile PBS to wash them [2].
 - 2.7.1. LAB MEDIA: Shot 6 cutting pieces.MP4: 00:01-00:23
 - 2.7.2. LAB MEDIA: Shot 7 - speed.MP4: 00:01-00:23
- 2.8. Next, transfer 5 to 6 Wharton's jelly pieces into each well of the previously prepared 6-well plates [1]. Incubate the plates in a humidified atmosphere with 5 percent carbon dioxide at 37 degrees Celsius for 3 days without disturbance [2].
 - 2.8.1. LAB MEDIA: Shot 7 - speed.MP4 00:05-00:23
 - 2.8.2. LAB MEDIA: Shot 9_V2_ Closing the incubator.MP4
- 2.9. On the fourth day, carefully add 1 milliliter of media to each well without disturbing the explants [1].
 - 2.9.1. Shot 10 Add media.MP4: 00:12-00:30
- 2.10. On the eighth day, change half of the media in each well every other day until day 12 to 14, removing about 2.5 milliliters from each well [1]. Then add 2.5 milliliters of fresh complete media at 37 degrees Celsius [3].
 - 2.10.1. LAB MEDIA: Shot 11 withdrawal half media.MP4 00:04-00:32
 - 2.10.2. LAB MEDIA: Shot 12 add media-speed.MP4: 00:00-00:20
- 2.11. Once the cells begin to proliferate and reach approximately 50–60% confluency, carefully remove the Wharton's jelly explants adhered to the bottom of the wells [1]. Hold the plates at a 45-degree angle and completely aspirate the medium [2].
 - 2.11.1. LAB MEDIA: Shot 13a Remove explant pieces-speed.MP4 00:06-00:25
 - 2.11.2. LAB MEDIA: Shot-14 Horizontal attempt.MP4: 00:05-00:20
- 2.12. Add 2 milliliters of PBS to each well [1] and gently swirl the plate [2]. Then, discard the PBS and repeat twice [3].
 - 2.12.1. LAB MEDIA: Shot 15 wash twice PBS shorten and speed.MP4 00:18-00:30
 - 2.12.2. LAB MEDIA: Shot 15 wash twice PBS shorten and speed.MP4: 00:32-00:37
 - 2.12.3. LAB MEDIA: Shot 15 wash twice PBS shorten and speed.MP4 00:49-00:57, 01:05-01:10
- 2.13. Next, add 2 milliliters of warm complete media to each well before incubating the plate in a humidified atmosphere with 5 percent carbon dioxide at 37 degrees Celsius [1].
 - 2.13.1. LAB MEDIA: Shot 15 wash twice PBS shorten and speed.MP4: 04:00-04:30

2.14. Change the medium every other day until the cells show fibroblast-like morphology, then proceed with cell characterization [1-TXT].

2.14.1. LAB MEDIA: Shot 16 - Image - WJMSCs isolation Explant. **TXT: Refer to the text protocol for MSCs characterization**

3. Isolation of Exosomes/sEVs from Wharton's Jelly-Mesenchymal Stem Cells Conditioned Media

Demonstrator: Dina Kassem

3.1. Once the cells attain 75-80 percent confluency, wash the cells twice with 1 milliliter of PBS per well [1] and add 1.5 milliliters of serum-free exosome collection media to each well [2].

3.1.1. LAB MEDIA: Shot 17a_Start EVs isolation_withdraw media (6 well plate).MP4 : 00:08-00:20, Shot 17b - PBS wash twice (6 well plate).MP4 : 00:23-00:35

3.1.2. LAB MEDIA: Shot 18- Add serum-free media (6-well plate).MP4: 00:13-end

3.2. After incubating the plate for 48 hours under standard conditions, collect the conditioned media from the plates into 15 milliliter centrifugation tubes [1]. Centrifuge the collected conditioned media at 300 *g* for 10 minutes at 4 degrees Celsius to remove suspended cells [2].

3.2.1. LAB MEDIA: Shot 19- Collect Exo media after 48 hr (6-well plate).MP4: 00:09-00:38

3.2.2. LAB MEDIA: Shot 20 Centrifuge 300 Xg 10 min.MP4: 00:00-00:24

3.3. Then, collect the conditioned media [1] and centrifuge again at 3000 *g* for 15 minutes at 4 degrees Celsius to get rid of necrotic cells as well as cell debris [2].

3.3.1. LAB MEDIA Shot 21- After 300xg media collect.MP4: 01:07-01:36 Talent placing the tubes into a centrifuge.

3.3.2. LAB MEDIA: Shot 22 Centrifuge 3000Xg 15 min .MP4: 00:00-00:31

3.4. Collect the supernatant [1] and pass it through a 0.22 micrometer filter into a new 50 milliliter polypropylene tube to remove residual debris and large extracellular vesicles [1].

3.4.1. LAB MEDIA: Shot 23 - After centrifuge 3000xg media collect.MP4 00:25-0:43

3.4.2. LAB MEDIA: Shot 24- Filtration 0.2 micron.MP4 0:00-0:01 *Video editor: Use as a still image since the rest of the video is moving in an unprofessional manner*

3.5. Transfer 7.5 milliliters of the cleared conditioned media into a 15-milliliter centrifuge tube and add 1.5 milliliters of precipitating reagent and mix thoroughly [1].

3.5.1. LAB MEDIA: Shot 25 Adding Exoquick 5 to 1 ratio. MP4: 00:00-00:02, 00:30-00:50, 01:25-01:34

3.6. Refrigerate the tube upright at 4 degrees Celsius for approximately 16 hours [1].

3.6.1. LAB MEDIA: Shot 27 place upright in fridge.MP4: 0:01-0:08

3.7. The following day, centrifuge the mixture at 1500 *g* for 30 minutes at 4 degrees Celsius [1]. Carefully aspirate the supernatant without disturbing the pellet [2]. Suspend the exosome pellet in 100 to 300 microliters of sterile PBS, depending upon the pellet size, and proceed with exosome characterization [4].

3.7.1. LAB MEDIA: Shot 28 Centrifuge 1500Xg 30 min.MP4: 0:01-0:19

3.7.2. LAB MEDIA: Shot 29 remove media exoquick after centrifugation. MOV: 00:00-00:29

3.7.3. LAB MEDIA: Shot 30 suspend exosomes in PBS.MOV: 00:00-00:45 **TXT: Refer to the text protocol for exosome characterization**

Results

4. Results

- 4.1. Flow cytometric analyses showed that most isolated cells expressed CD90 (*C-D-Ninety*) [1], CD73 (*C-D-Seventy-Three*) [2], CD105 (*C-D-One-Zero-Five*) [3], and CD44 (*C-D-Forty-Four*) [4].
 - 4.1.1. LAB MEDIA: Figure 4A. *Video editor: Highlight the plot labeled “CD90” with 93.72%.*
 - 4.1.2. LAB MEDIA: Figure 4A. *Video editor: Highlight the plot labeled “CD73” with 93.77%.*
 - 4.1.3. LAB MEDIA: Figure 4A. *Video editor: Highlight the plot labeled “CD105” with 92.53%.*
 - 4.1.4. LAB MEDIA: Figure 4A. *Video editor: Highlight the plot labeled “CD44” with 98.55%.*
- 4.2. The isolated cells were almost null for CD34 (*C-D-Thirty-Four*) [1], CD14 [2], and human leukocyte antigen-DR [3].
 - 4.2.1. LAB MEDIA: Figure 4A. *Video editor: Highlight the plot labeled “CD34” with 3.96%.*
 - 4.2.2. LAB MEDIA: Figure 4A. *Video editor: Highlight the plot labeled “CD14” with 3.15%.*
 - 4.2.3. LAB MEDIA: Figure 4A. *Video editor: Highlight the plot labeled “HLA-DR” with 0.57%.*
- 4.3. Differentiated adipocytes showed positive oil-red staining of lipid vacuoles compared to control uninduced cells [1]. Differentiated osteocytes showed typical alizarin red staining [2].
 - 4.3.1. LAB MEDIA: Figure 4B. *Video editor: Highlight the left panel labeled “Adipocytes,”.*
 - 4.3.2. LAB MEDIA: Figure 4B. *Video editor: Highlight the middle panel labeled “Osteocytes” in the differentiated row.*
- 4.4. Chondrogenically induced cells showed positive blue staining of the extracellular matrix with Alcian blue compared to control uninduced cells [1].
 - 4.4.1. LAB MEDIA: Figure 4B. *Video editor: Highlight the right panel labeled “Chondrocytes,” comparing the control row with the differentiated row.*
- 4.5. Wharton’s jelly mesenchymal stromal cells maintained a healthy, homogenous

appearance after 48 hours in serum-free media [1].

4.5.1. LAB MEDIA: Figure 5A. *Video editor: Highlight the image labeled “WJ-MSCs 48 h after adding SF media.”*

4.6. Transmission electron microscopy showed freshly isolated exosomes and small extracellular vesicles [1]. Particle size analysis revealed a single peak with an average diameter of about 87.69 nanometers [2].

4.6.1. LAB MEDIA: Figure 5C. *Video editor: Show the gray image with many small round particles.*

4.6.2. LAB MEDIA: Figure 5D. *Video editor: Highlight the tall peak near 87.69 nanometers and the table row labeled “Peak 1.”*

1. Extracellular
Pronunciation link: <https://www.merriam-webster.com/dictionary/extracellular>
IPA: /ˌɛkstrəˈsɛljələr/
Phonetic Spelling: ek·struh·SEL·yuh·ler
2. Adipocytes
Pronunciation link: <https://www.merriam-webster.com/dictionary/adipocyte>
IPA: /ˈædəpəˌsaɪts/
Phonetic Spelling: AD·uh·puh·sites
3. Co-culture
Pronunciation link: No confirmed link found
IPA: /ˌkoʊˈkʌltʃər/
Phonetic Spelling: koh·KUL·cher
4. Differentiated
Pronunciation link: <https://www.merriam-webster.com/dictionary/differentiate>
IPA: /ˌdɪfəˈrenʃiˌeɪtɪd/
Phonetic Spelling: dif·uh·REN·shee·ay·tid
5. Adipocyte
Pronunciation link: <https://www.merriam-webster.com/dictionary/adipocyte>
IPA: /ˈædəpəˌsaɪt/
Phonetic Spelling: AD·uh·puh·site

6. Monolayer
Pronunciation link: <https://www.merriam-webster.com/dictionary/monolayer>
IPA: /'mɑːnoʊˌleɪər/
Phonetic Spelling: MON·oh·lay·er
7. Hemocytometer
Pronunciation link: <https://www.merriam-webster.com/dictionary/hemocytometer>
IPA: /ˌhiːmoʊsaɪˈtɑːmətər/
Phonetic Spelling: hee·moh·sy·TOM·uh·ter
8. Mammary
Pronunciation link: <https://www.merriam-webster.com/dictionary/mammary>
IPA: /'mæməri/
Phonetic Spelling: MAM·uh·ree
9. Polymerized
Pronunciation link: <https://www.merriam-webster.com/dictionary/polymerize>
IPA: /pəˈlɪməˌraɪzd/
Phonetic Spelling: puh·LIM·uh·ryzed
10. Conditioned media
Pronunciation link: No confirmed link found
IPA: /kənˈdɪʃənd ˈmiːdiə/
Phonetic Spelling: kuhn·DISH·uhnd MEE·dee·uh
11. Immunofluorescence
Pronunciation link: No confirmed link found
IPA: /ˌɪmjənoʊflʊˈresəns/
Phonetic Spelling: im·yuh·noh·floor·ESS·uhns
12. Paraformaldehyde
Pronunciation link: No confirmed link found
IPA: /ˌpærəfɔːrˈmældəˌhaɪd/
Phonetic Spelling: pair·uh·for·MAL·duh·hyde
13. Glycine
Pronunciation link: <https://www.merriam-webster.com/dictionary/glycine>
IPA: /ˈɡlaɪˌsiːn/
Phonetic Spelling: GLY·seen
14. Permeabilization
Pronunciation link: No confirmed link found
IPA: /ˌpɜːmiəbələˈzeɪʃən/
Phonetic Spelling: per·mee·uh·buh·luh·ZAY·shuhn
15. Antibodies
Pronunciation link: <https://www.merriam-webster.com/dictionary/antibody>
IPA: /ˈæntɪˌbɑːdɪz/
Phonetic Spelling: AN·tee·bod·eez
16. DAPI
Pronunciation link: No confirmed link found
IPA: /ˈdæpi/
Phonetic Spelling: DAP·ee

17. Micromolar
 Pronunciation link: No confirmed link found
 IPA: /ˌmaɪkroʊˈmoʊlər/
 Phonetic Spelling: my·kroh·MOH·ler
18. Diamidino
 Pronunciation link: No confirmed link found
 IPA: /daɪˌæmɪˈdiːnoʊ/
 Phonetic Spelling: dy·am·ih·DEE·noh
19. Phenylindole
 Pronunciation link: No confirmed link found
 IPA: /ˌfenəlˈɪndool/
 Phonetic Spelling: fen·uhl·IN·dohl
20. Silicone
 Pronunciation link: <https://www.merriam-webster.com/dictionary/silicone>
 IPA: /ˈsɪlɪˌkoon/
 Phonetic Spelling: SIL·ih·kohn
21. Sealant
 Pronunciation link: <https://www.merriam-webster.com/dictionary/sealant>
 IPA: /ˈsiːlənt/
 Phonetic Spelling: SEE·luhnt
22. Antifade
 Pronunciation link: No confirmed link found
 IPA: /ˌæntiˈfeɪd/
 Phonetic Spelling: an·tee·FAYD
23. Coverslip
 Pronunciation link: <https://www.merriam-webster.com/dictionary/coverslip>
 IPA: /ˈkʌvərˌslɪp/
 Phonetic Spelling: KUV·er·slip
24. Confocal
 Pronunciation link: <https://www.merriam-webster.com/dictionary/confocal>
 IPA: /kɑːnˈfoʊkəl/
 Phonetic Spelling: kon·FOH·kuhl
25. Fluorescence
 Pronunciation link: <https://www.merriam-webster.com/dictionary/fluorescence>
 IPA: /flʊˈresəns/
 Phonetic Spelling: floor·ESS·uhns
26. Stellate
 Pronunciation link: <https://www.merriam-webster.com/dictionary/stellate>
 IPA: /ˈstelət/, /ˈsteˌleɪt/
 Phonetic Spelling: STEL·uht / STEL·ayt
27. Viability
 Pronunciation link: <https://www.merriam-webster.com/dictionary/viability>
 IPA: /ˌvaɪəˈbɪləti/
 Phonetic Spelling: vy·uh·BIL·uh·tee

