

Submission ID #: 71420

Scriptwriter Name: Poornima G

Project Page Link: <https://review.jove.com/account/file-uploader?src=21783148>

Title: Large-Scale Production of Extracellular Vesicles from High-Density 3D Cell Cultures in Hollow Fiber Perfusion Bioreactors

Authors and Affiliations:

John J.S. Cadwell

FiberCell Systems Inc.

Corresponding Author:

jcadwell@fibercellsystems.com

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? **No**
- 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: 28

Number of Shots: 61

Interviews

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**

The answers and questions are edited for clarity

INTRODUCTION:

Videographer's NOTES: For the interview portion we did the two testimonials first and then did the interview questions. Did each response at least twice

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

- 1.1. **John Cadwell:** This protocol describes the collection of concentrated secreted exosomes while maintaining optimal cell mass and 3D perfusion culture conditions over several weeks or months.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~Describe any limitations in the existing methods and how this protocol overcomes them?~~

- 1.2. **John Cadwell:** Progress in extracellular vesicle research is hampered by the low levels of secretion, non-physiologic conditions, and inefficiencies of 2-D flask culture. This protocol allows any cell culture lab to scale up their EV production to clinically relevant amounts.
 - 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. **John Cadwell:** This protocol supports the production of extracellular vesicles in a 3-D perfusion, continuous production hollow fiber bioreactor at a clinically relevant scale.
 - 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. **John Cadwell:** The key challenge is maintaining sterile bioreactor handling and monitoring glucose uptake to control cell growth for optimizing extracellular vesicle harvest.

- 1.4.1. 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.~~

- 1.5. **John Cadwell**: This protocol can be applied to any mammalian cells that secrete extracellular vesicles and can be scaled up using hollow-fiber bioreactor systems.
 - 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. **John J.S. Cadwell, President and CEO of FiberCell Systems Inc.**: (authors will present their testimonial statements live)

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. **John J.S. Cadwell, President and CEO of FiberCell Systems Inc.**: (authors will present their testimonial statements live)

1.7.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

Protocol

Videographer's NOTE: Talent wanted to do large sections continuously rather than breaking up the steps.

There was one step where talent's hand was blocking the action, so did an additional close up labeled CU on the take.

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

2. Reservoir Cap Preparation and Cartridge Connection

Demonstrator: John Cadwell

2.1. To begin, remove the two short pieces of tubing from the double-bagged cartridge [1] and attach them to the hose barbs on the reservoir cap's stainless-steel tubing [2].

2.1.1. WIDE: Talent removing the two short pieces of tubing from the double-bagged cartridge. ***Videographer:** Please obtain CU shot as well for this shot.*

Videographer's NOTE: Did a closeup for the first shot 2.1.1 as well as wide

2.1.2. Talent attaching the tubing to the hose barbs on the stainless-steel tubing of the reservoir cap.

2.2. Wrap the short tubing and the exposed ends of the stainless-steel tubing with aluminum foil to maintain sterility after autoclaving [1]. Autoclave using a dry cycle at 121 degrees Celsius for 15 to 30 minutes. Allow the cap to cool to room temperature before use [2].

2.2.1. Talent wrapping the short tubing and the exposed ends of the stainless-steel tubing with aluminum foil.

2.2.2. Talent placing the autoclave bag into the autoclave.

2.3. Prepare the PBS reservoir by transferring 50 milliliters of PBS from a 1-liter bottle into a 50-milliliter conical tube for later use [1].

2.3.1. Talent transferring PBS from a 1-liter bottle into a 50-milliliter conical tube.

2.4. Replace the cap of the PBS bottle with the reservoir cap [1]. Then, remove the cartridge from the inner sterile bag [2].

- 2.4.1. Talent screwing the reservoir cap onto the PBS bottle.
- 2.4.2. Talent removing the cartridge from the inner sterile bag.

2.5. Remove the Luer cap from one long piece of cartridge tubing and attach it to the reservoir tubing on the reservoir cap [1]. Use a little backspin to prevent kinking [2-TXT]. Gently squeeze the brown pump tubing to drive PBS through the system until all air bubbles are removed [3].

- 2.5.1. Talent removing the Luer cap from one long piece of cartridge tubing and attaching it to the reservoir tubing on the reservoir cap.
- 2.5.2. Talent using a little backspin to prevent kinking. **TXT: Repeat the process for the second tubing connection**
- 2.5.3. Talent gently squeezing the brown pump tubing to drive PBS through the system.

3. Filling the Extra Capillary Space (ECS)

3.1. Draw 20 milliliters of PBS into a sterile syringe with a large-gauge blunt needle at least 1.5 inches in length [1]. Disinfect both side-port Luer fittings with alcohol and allow them to dry [2].

- 3.1.1. Talent drawing PBS into a sterile syringe using a large-gauge needle.
- 3.1.2. Talent wiping both side-port Luer fittings with alcohol.

3.2. Attach the PBS-filled syringe to one side port and attach an empty sterile syringe to the opposite port [1]. Close both end ports and open both side ports [2].

- 3.2.1. Talent attaching the PBS-filled syringe to one side port and attaching an empty sterile syringe to the opposite port.
- 3.2.2. Talent closing both end ports and opening both side ports.

3.3. Next, slowly inject PBS into the extra capillary space while holding the cartridge at a slight angle to minimize air bubbles [1]. If air is present in the extra-capillary space, open the right-end port and withdraw fluid into the empty syringe until the space is filled with liquid [2].

- 3.3.1. Talent slowly injecting PBS into the extra capillary space while holding the cartridge at a slight angle.
- 3.3.2. Talent opening the right end port and withdrawing fluid into the empty syringe.
- 3.4. Close both side-port slide clamps and open both end-port slide clamps [1]. Verify flow by gently compressing the pump tubing with fingers [2].
 - 3.4.1. Talent closing both side-port slide clamps and opening both end-port slide clamps.
 - 3.4.2. Talent gently compressing the pump tubing with fingers.
- 3.5. Leave the empty syringes attached to the side ports to serve as caps [1]. Replace the syringes with new ones every time they have been out of the laminar flow hood [2].
 - 3.5.1. Talent leaving the empty syringes attached to the side ports.
 - 3.5.2. Talent replacing the syringes with new syringes.

4. Fiber Coating and Cell Inoculation

- 4.1. Withdraw 20 milliliters of cell suspension or coating solution into a sterile syringe using a large-gauge needle [1-TXT]. Attach the syringe containing the solution to one side port and attach an empty sterile syringe to the other side port [2]. Close both end ports and open both side ports [3].
 - 4.1.1. Talent withdrawing cells or coating solution into a sterile syringe using a large-gauge needle. **TXT: 1×10^8 cells in condition medium or 100 - 500 μg matrix protein in PBS**
 - 4.1.2. Talent attaching the syringe containing the solution to one side port and attaching an empty sterile syringe to the other side port.
 - 4.1.3. Talent closing both end ports and opening both side ports.
- 4.2. Gently mix the solution by transferring fluid between the two syringes [1]. Leave half of the solution in each syringe and close both side ports [2].
 - 4.2.1. Talent transferring fluid between the two syringes.

- 4.2.2. Shot of 1/2 of the solution remaining in each syringe and closing both side ports.
- 4.3. Then, open the right end port and loosen the reservoir cap by one-quarter turn [1]. Inject the solution from one syringe into the extra capillary space and close the attached side port [2].
 - 4.3.1. Talent opening the right end port and loosening the reservoir cap 1/4 turn.
 - 4.3.2. Talent injecting the solution from one syringe into the extra capillary space and closing the attached side port.
- 4.4. Then, inject the solution from the other syringe, close the attached side port and open both end ports [1].
 - 4.4.1. Talent injecting the solution from the other syringe and closing the attached side port and opening both end ports.
- 4.5. If inoculating cells, leave the cartridge in a horizontal position for 30 minutes at room temperature [1]. Then, rotate it 180 degrees along the long axis and let it sit for an additional 30 minutes [2].
 - 4.5.1. Talent placing the cartridge in a horizontal position at room temperature.
 - 4.5.2. Talent rotating the cartridge 180 degrees along the long axis.
- 4.6. If coating the fibers with matrix proteins such as fibronectin or collagen, leave the cartridge overnight at room temperature [1-TXT]. After the coating or inoculation incubation is complete, begin placing the cartridge into the Duet pump system [2].
 - 4.6.1. Talent placing the cartridge aside at room temperature.
 - 4.6.2. Talent showing the cartridge and pointing to the Duet pump system.
- 4.7. Adjust the pump flow rate to the setting 25 on the control box and keep the pump running to make the cartridge easier to install [1]. Grasp the right edge of the flow path stand and slide the pump tubing into the pump tubing [2].
 - 4.7.1. Talent setting the pump flow rate to 25 on the control box while the pump is running.
 - 4.7.2. Talent grasping the right edge of the flow path, stands and slides the pump tubing into the pump tubing.

4.8. Slide the base of the flow path into the slot on the pump base [1]. Ensure that the rectangular register snaps into the cut-out on the flow path stand [2]. Place the reservoir bottle in the indentation on the side of the pump base [3]. Fit the thin power cord between the gasket on the incubator and attach the control box to the side of the incubator using the magnet [4].

4.8.1. Talent sliding the base of the flow path into the slot on the pump base.

4.8.2. Shot of the rectangular register snapping into the cut-out on the flow path stand.

4.8.3. Talent placing the reservoir bottle in the indentation on the side of the pump base.

4.8.4. Talent fitting the thin power cord between the gasket on the incubator and attaching the control box to the side of the incubator using the magnet.

5. Harvest Preparation and Collection of Conditioned Medium

5.1. Attach an empty sterile syringe fitted with a 3-way stopcock and sterile filter to the right-side port [1]. Set the 3-way stopcock to vent through the sterile filter, and attach a second empty sterile syringe to the left-side port [2].

5.1.1. Talent attaching an empty sterile syringe with a 3-way stopcock and sterile filter to the right side port.

5.1.2. Talent setting the 3-way stopcock to allow venting through the sterile filter and attaching a second empty sterile syringe to the left side port.

5.2. Open the right-side port and maintain sterile venting through the filter [1].

5.2.1. Talent opening the right-side port while maintaining sterile venting through the filter.

5.3. Next, tilt the right end of the cartridge upward and withdraw conditioned medium into the left syringe [1]. Completely drain the contents of the extra capillary space into the left syringe [2].

5.3.1. Talent tilting the right end of the cartridge upward and withdrawing conditioned medium into the left syringe.

5.3.2. Shot of the extra capillary space contents draining completely into the left

syringe.

- 5.4. Close the right and the left side ports [1], remove the syringe, and transfer the harvest to a 50-milliliter conical tube [2].
 - 5.4.1. Talent closing the right and left side ports.
 - 5.4.2. Talent removing the syringe and collecting the harvest into a 50-milliliter conical tube.
- 5.5. Replace the syringe on the left side port and open the left side port [1]. Then open the right-end port and unscrew the reservoir cap by one-quarter turn [2].
 - 5.5.1. Talent replacing the syringe on the left side port and opening the left side port.
 - 5.5.2. Talent opening the right end port and unscrewing the reservoir cap 1/4 turn.
- 5.6. Tilt the left end of the cartridge upward and start filling the extra capillary space by withdrawing on the syringe connected to the left side port [1]. Fill the space until the liquid meniscus is in the middle of the side port tubing [2].
 - 5.6.1. Talent tilting the left end of the cartridge upward and withdrawing on the syringe connected to the left side port.
 - 5.6.2. Shot of the liquid meniscus in the middle of the side port tubing.
- 5.7. After closing the right end port [1], open the right-side port and set the 3-way stopcock to vent through the filter [2]. Then open the left-side port and tilt the cartridge to the right [3]. Drain the extra capillary space completely into the left syringe [4].
 - 5.7.1. Talent closing the right end port.
 - 5.7.2. Talent opening the right-side port and setting the 3-way stopcock to vent through the filter.
 - 5.7.3. Talent opening the left side port and tilting the cartridge to the right.
 - 5.7.4. Talent draining the extra capillary space completely into the left syringe.
- 5.8. Close the left side port, remove the syringe, and collect the harvested extra capillary space contents into the 50-milliliter conical tube [1]. Reconnect the syringe into the left side port, close the right-side port, and open the right-end port [2].
 - 5.8.1. Talent closing the left side port, removing the syringe, and collecting the harvested extra capillary space contents into the 50-milliliter conical tube.

5.8.2. Talent replacing the syringe into the left side port, closing the right-side port, and opening the right-end port.

5.9. Then, tilt the left side of the cartridge upward and fill the extra capillary space by withdrawing on the left syringe [1]. Fill the extra capillary space until the liquid meniscus is in the middle of the side port tubing [2].

Author's NOTE: Left side and left end are the same. Cell inoculation and harvesting are similar but not identical. I would keep it

5.9.1. Talent tilting the left side of the cartridge upward and withdrawing on the left syringe.

5.9.2. Shot of the liquid meniscus in the middle of the side port tubing.

5.10. Finally, close the left side port, open the left end port, and tighten the reservoir cap [1]. Confirm system flow by manually compressing the pump tubing before returning the cartridge to the Duet pump [2].

5.10.1. Talent closing the left side port, opening the left end port, and tightening the reservoir cap.

5.10.2. Talent manually compressing the pump tubing and returning the cartridge to the Duet pump.

5.11. For glucose monitoring, use a 1-milliliter pipette to remove a 1-milliliter sample of medium from the reservoir bottle [1]. Place a small drop of the sampled medium onto the test strip of a compatible glucose meter to measure the glucose concentration [2].

5.11.1. Talent removing a 1-milliliter sample of medium from the reservoir bottle using a 1-milliliter pipette.

5.11.2. Talent placing a small drop of sampled medium onto the glucose meter test strip to measure glucose concentration.

Results

6. Results

6.1. Extracellular vesicle concentration reached a maximum after 24 hours, making 24-hour intervals between harvests optimal [1].

6.1.1. LAB MEDIA: Figure 4. *Video editor: Highlight the x-axis label '24' and the corresponding green bar.*

6.2. Typical exosome concentrations in harvested medium at 24 hours was about 1×10^{12} particles per milliliter [1].

6.2.1. LAB MEDIA: Figure 4. *Video editor: Highlight 1×10^{12} and the green bar.*

1.

Reservoir

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

IPA: /'rezər,vwɑ:r/, /'rezər,vɒə/

Phonetic Spelling: REZ·er·vwar, REZ·er·vor

2. Cartridge

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

IPA: /'kɑ:rtɪdʒ/

Phonetic Spelling: KAR·trij

3. Stainless-steel

Pronunciation link: <https://www.merriam-webster.com/dictionary/stainless%20steel>

IPA: /'stemləs 'sti:l/

Phonetic Spelling: STAYN·luhs STEEL

4. Sterility

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

IPA: /stə'riləti/

Phonetic Spelling: stuh·RIL·uh·tee

5. Autoclave

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

IPA: /'ɔ:tə,kleɪv/

Phonetic Spelling: AW·tuh·klayv

6. Conical

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

- IPA: /'kɑ:nɪkəl/
Phonetic Spelling: KON·ih·kuhl
7. Luer
Pronunciation link: No confirmed link found
IPA: /'lu:ər/
Phonetic Spelling: LOO·er
 8. Kinking
Pronunciation link: <https://www.merriam-webster.com/dictionary/kink>
IPA: /'kɪŋkɪŋ/
Phonetic Spelling: KING·king
 9. Capillary
Pronunciation link: <https://www.merriam-webster.com/dictionary/capillary>
IPA: /'kæpə,leri/
Phonetic Spelling: KAP·uh·lair·ee
 10. Extra capillary space (ECS)
Pronunciation link: No confirmed link found
IPA: /'ekstrə 'kæpə,leri speɪs/
Phonetic Spelling: EK·struh KAP·uh·lair·ee spays
 11. Syringe
Pronunciation link: <https://www.merriam-webster.com/dictionary/syringe>
IPA: /sə'rɪndʒ/
Phonetic Spelling: suh·RINJ
 12. Gauge
Pronunciation link: <https://www.merriam-webster.com/dictionary/gauge>
IPA: /geɪdʒ/
Phonetic Spelling: gayj
 13. Disinfect
Pronunciation link: <https://www.merriam-webster.com/dictionary/disinfect>
IPA: /,dɪsən'fekt/
Phonetic Spelling: diss·in·FEKT
 14. Laminar
Pronunciation link: <https://www.merriam-webster.com/dictionary/laminar>
IPA: /'læmənər/
Phonetic Spelling: LAM·uh·ner
 15. Inoculation
Pronunciation link: <https://www.merriam-webster.com/dictionary/inoculation>
IPA: /ɪˌnɑ:kjə'leɪʃən/
Phonetic Spelling: ih·NOK·yuh·LAY·shuhn
 16. Matrix
Pronunciation link: <https://www.merriam-webster.com/dictionary/matrix>
IPA: /'mertrɪks/
Phonetic Spelling: MAY·triks
 17. Fibronectin
Pronunciation link: <https://www.merriam-webster.com/medical/fibronectin>

- IPA: /ˌfaɪbroʊˈnektɪn/
Phonetic Spelling: FY·broh·NEK·tin
18. Collagen
Pronunciation link: <https://www.merriam-webster.com/dictionary/collagen>
IPA: /ˈkɑːlədʒən/
Phonetic Spelling: KOL·uh·juhn
19. Incubation
Pronunciation link: <https://www.merriam-webster.com/dictionary/incubation>
IPA: /ˌɪŋkjəˈbeɪʃən/
Phonetic Spelling: ing·kyuh·BAY·shuhn
20. Duet pump
Pronunciation link: No confirmed link found
IPA: /duˈeɪ pʌmp/
Phonetic Spelling: doo·AY pump
21. Flow path
Pronunciation link: No confirmed link found
IPA: /floʊ pæθ/
Phonetic Spelling: floh path
22. Gasket
Pronunciation link: <https://www.merriam-webster.com/dictionary/gasket>
IPA: /ˈgæskət/
Phonetic Spelling: GAS·kiht
23. Incubator
Pronunciation link: <https://www.merriam-webster.com/dictionary/incubator>
IPA: /ˌɪŋkjəˈbeɪtər/
Phonetic Spelling: ING·kyuh·bay·ter
24. Harvested
Pronunciation link: <https://www.merriam-webster.com/dictionary/harvest>
IPA: /ˈhɑːrvəstɪd/
Phonetic Spelling: HAR·vuh·stid
25. Conditioned medium
Pronunciation link: No confirmed link found
IPA: /kənˈdɪʃənd ˈmiːdiəm/
Phonetic Spelling: kuhn·DISH·uhnd MEE·dee·uhm
26. Stopcock
Pronunciation link: <https://www.merriam-webster.com/dictionary/stopcock>
IPA: /ˈstɑːp kɑːk/
Phonetic Spelling: STOP·kok
27. Venting
Pronunciation link: <https://www.merriam-webster.com/dictionary/vent>
IPA: /ˈventɪŋ/
Phonetic Spelling: VEN·ting
28. Meniscus
Pronunciation link: <https://www.merriam-webster.com/dictionary/meniscus>

IPA: /məˈnɪskəs/

Phonetic Spelling: muh·NISS·kuhs

29. Extracellular

Pronunciation link: <https://www.merriam-webster.com/medical/extracellular>

IPA: /ˌɛkstrəˈseljələr/

Phonetic Spelling: EK·struh·SEL·yuh·ler

30. Vesicle

Pronunciation link: <https://www.merriam-webster.com/medical/vesicle>

IPA: /ˈvesɪkəl/

Phonetic Spelling: VESS·ih·kuhl

31. Exosome

Pronunciation link: <https://www.merriam-webster.com/medical/exosome>

IPA: /ˈɛksouˌsoʊm/

Phonetic Spelling: EK·soh·sohm

32. Concentration

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

IPA: /ˌkɒːnsənˈtreɪʃən/

Phonetic Spelling: KON·suhn·TRAY·shuhn