

Submission ID #: 72929

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Project Page Link: <https://review.jove.com/account/file-uploader?src=22243393>

Title: A Microfluidic Platform for Studying Roles of Mechanical Compression in Tumor-Immune Cell Interactions in a 3D Extracellular Matrix.

Authors and Affiliations:

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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**

3. Filming location: Will the filming need to take place in multiple locations? **Yes**

If **Yes**, how far apart are the locations? Locations are two rooms across the hall from one another.

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: 21

Number of Shots: 49

Interviews

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

INTRODUCTION:

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

- 1.1. **Faith Muriuki**: Our goal is to use mechanical signals to improve the ability of immune cells to kill tumor cells effectively.
 - 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. **Faith Muriuki**: This protocol can be applied to investigate compressive stress-mediated regulation of immune cell killing across diverse immune cell types and target cell models.
 - 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. **Faith Muriuki**: By making slight modifications, this protocol can examine how compression affects interactions between tumor cells and other cell types, such as fibroblasts.
 - 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. **Faith Muriuki**: The main consideration when executing this protocol is ensuring proper alignment of the pressure control unit with Layer 1 for accurate and consistent compressive stress application.
 - 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. **Faith Muriuki**: Future studies can leverage this platform to identify the molecular mechanisms that enable immune cells to adapt to compressive stress.
 - 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.

Protocol

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

2. Surface Activation of the Sample Loading Chamber

Demonstrator: Faith Muriuki

- 2.1. To begin, place a mask over the sample-loading chamber so that only the wells are exposed [1]. Expose the masked Layer 1 to oxygen plasma for 15 seconds at the high-power setting [2], then place Layer 1 in a Petri dish [3].
 - 2.1.1. WIDE: Talent placing a mask over the sample-loading chamber with only the wells exposed.
 - 2.1.2. Talent placing the masked Layer 1 into the oxygen plasma system.
 - 2.1.3. Talent placing Layer 1 in a Petri dish.
- 2.2. Treat each well with 10 microliters of 1% polyethylenimine [1]. After 10 minutes, remove the polyethylenimine from the wells by vacuum aspiration from the four corners of each well to prevent scratching the glass surface [2].
 - 2.2.1. Talent pipetting 10 microliters of 1% polyethylenimine into each well.
 - 2.2.2. Talent removing the polyethylenimine from the four corners of each well by vacuum aspiration.
- 2.3. Wash the wells with 10 microliters of PBS [1] and remove the wash by vacuum aspiration from the side [2]. Then, treat the wells with 10 microliters of 0.1% glutaraldehyde for 30 minutes [3]. Remove the glutaraldehyde from the corners using vacuum aspiration [4].
 - 2.3.1. Talent pipetting 10 microliters of phosphate-buffered saline into each well.
 - 2.3.2. Talent removing the phosphate-buffered saline from the side of each well by vacuum aspiration.
 - 2.3.3. Talent pipetting 10 microliters of 0.1% glutaraldehyde into each well.
 - 2.3.4. Talent removing the glutaraldehyde from the corners of each well by vacuum aspiration.
- 2.4. Next, wash the wells with PBS 3 times, removing the wash from the corners after each wash [1]. Then, wrap the Petri dish containing Layer 1 in foil [2] and leave it in the biosafety cabinet overnight [3].
 - 2.4.1. Talent washing the wells with PBS.
 - 2.4.2. Talent wrapping the Petri dish containing Layer 1 in foil.

2.4.3. Talent placing the wrapped Petri dish in the biosafety cabinet. NOTE: 2.4.3 Was not slated but was filmed 2 shots of and is located within the shot labeled 2.4.2

2.5. Submerge the 38 by 88-millimeter metal frame and the 12 M2.5 (*M-Two-Point-Five*) pan-head screws in 70% ethanol overnight at room temperature for sterilization [1].

2.5.1. Talent submerging the 38 millimeter × 88 millimeter metal frame and the 12 M2.5 pan-head screws in 70% ethanol.

3. Priming of the Pressure Control Unit

3.1. Expose the pressure control unit to oxygen plasma for 30 seconds using the high-power setting [1]. Submerge the pressure control unit, with the features facing downward, in de-ionized water to fill the channels and chambers and leave it overnight [2].

3.1.1. Talent placing the pressure control unit into the oxygen plasma system.

3.1.2. Talent submerging the pressure control unit with the features facing downward in de-ionized water.

3.2. The following day, remove the pressure control unit from the de-ionized water [1]. Fill a microfluidic reservoir with 50 milliliters of de-ionized water [2].

3.2.1. Talent removing the pressure control unit from the de-ionized water.

3.2.2. Talent filling the microfluidic reservoir with 50 milliliters of de-ionized water.

3.3. Connect a syringe filled with air to the pneumatic tubing line [1]. Slowly depress the syringe plunger to pressurize the reservoir and drive the de-ionized water through the fluid delivery tubing [2] until the tubing is filled and water begins to drip from the tube [3].

3.3.1. Talent connecting an air-filled syringe to the pneumatic tubing line.

3.3.2. Talent depressing the syringe plunger

3.3.3. Shot of the filled fluid delivery tubing with water dripping from the tube.

3.4. Connect the pre-filled, dripping fluid-delivery tubing through the polycarbonate manifold to the 1.5-millimeter inlet on the back of the pressure control unit [1]. Allow the pressure control unit and manifold assembly to air-dry for 2 hours [2].

3.4.1. Talent connecting the pre-filled dripping fluid delivery tubing through the polycarbonate manifold and then to the 1.5 millimeter inlet on the back of the pressure control unit.

3.4.2. Shot of the dried pressure control unit.

4. Preparation of Cell-Embedded Collagen

4.1. Remove the metal frame and the screwdrivers from the 70% ethanol and dry them [1]. In the biosafety cabinet, place 3 microtubes with a 600-microliter capacity on ice [2]

and label them as "N", "sp" (*S-P*), sp+NK (*S-P-plus-N-K*) [2].

4.1.1. Talent removing the metal frame and the screwdrivers from the 70% ethanol.

4.1.2. Talent placing 3 microtubes with a 600 microliter capacity on ice inside the biosafety cabinet.

4.1.3. TEXT ON A PLAIN BACKGROUND

N: Neutralization

sp: MCF-7 spheroids

sp+NK: MCF-7 spheroids with NK cells

4.2. Prepare the neutralization mixture by adding 10x (*10-X*) the required volume of Medium 199 (*One-Nine-Nine*) and sodium hydroxide to the "N" tube [1]. Transfer the appropriate volume of the Medium 199 to sodium hydroxide mixture to the "sp" and "sp+NK" tubes to achieve a final concentration of 1x (*One-X*) [2].

4.2.1. Talent adding Medium 199 and sodium hydroxide to the "N" tube. NOTE: 4.2.1 and 4.2.2 were merged into one shot and it is tail slated 4.2.1 and 4.2.2

4.2.2. Talent transferring the neutralization mixture into the "sp" and "sp+NK" tubes.

4.3. Then, harvest the MCF-7 (*M-C-F-Seven*) tumor spheroids [1] and centrifuge at 141 g for 3 minutes to allow them to settle to the bottom of the tube [2]. Carefully remove the supernatant [3] and resuspend the spheroids in 400 microliters of equimolar MCF-7 and natural killer complete medium [4].

4.3.1. Talent harvesting the tumor spheroids.

4.3.2. Talent removing the supernatant from the tube.

4.3.3. Talent resuspending the tumor spheroids in 400 microliters of 50:50 MCF-7 and natural killer complete medium.

4.4. Next, collect the natural killer cells in a 50-milliliter conical tube [1]. Centrifuge at 141 g for 4 minutes [2] and count the cells [3].

4.4.1. Talent collecting the natural killer cells in a 50 milliliter conical tube.

4.4.2. Talent places the cells in the centrifuge.

4.4.3. Talent counting the natural killer cells using cell counter.

4.5. Resuspend the resulting pellet in 200 microliters of complete natural killer medium [1]. Add the appropriate volume of the collagen stock solution to each microcentrifuge tube [2]. Then, add the appropriate volume of the cell suspension to each tube to obtain a final volume of 200 microliters containing 1.5 milligrams per milliliter collagen [3].

4.5.1. Talent resuspending the cell pellet in 200 microliters of complete natural killer medium. NOTE: 4.5.1 was not slated but was filmed

- 4.5.2. Talent adding the collagen stock solution to each microcentrifuge tube.
- 4.5.3. Talent adding the cell suspension to each microcentrifuge tube.
- 4.6. Mix the suspension gently by pipetting to ensure an even distribution of the cells and collagen while minimizing bubble formation [1].
- 4.6.1. Talent gently pipetting the suspension to mix the cells and collagen. **NOTE: 4.6.1b was added as a continuation of 4.6.1 and demonstrates mixing and adding NK cells**

5. Device Assembly and Cell Loading

- 5.1. Place Layer 1 inside the dried metal frame [1] and load 8 microliters of the collagen mixture into each well [2].
 - 5.1.1. Talent placing Layer 1 inside the dried metal frame.
 - 5.1.2. Talent loading 8 microliters of the collagen mixture into each well.
- 5.2. Align the pressure control unit and manifold assembly with the cell chambers on Layer 1 using a microscope [1]. Insert the dried screws through the manifold and into the metal frame to secure the pressure control unit and Layer 1 [2].
 - 5.2.1. SCREEN: Talent aligning the pressure control unit and manifold assembly with the cell chambers on Layer 1. *Videographer: Please record the computer screen for this shot*
 - 5.2.2. Talent inserting the dried screws through the manifold into the metal frame to secure the pressure control unit and Layer 1.
- 5.3. Place the assembled device in a 37 degrees Celsius and 5% carbon dioxide incubator for 45 minutes to allow collagen polymerization [1]. Add 1 millimolar MCF-7 and NK complete cell culture medium through the 4 media ports [2].
 - 5.3.1. Talent placing the assembled device into the incubator.
 - 5.3.2. Talent adding the complete cell culture medium through the 4 media ports.

6. Imaging of Tumor-Immune Interactions

- 6.1. Place the assembled device inside the stage incubator mounted on an inverted epifluorescence microscope for live-cell imaging [1]. Place a secondary housing box over the assembled device to maintain the internal environment throughout the imaging period [2].
 - 6.1.1. Talent placing the assembled device inside the stage incubator mounted on the inverted epifluorescence microscope.
 - 6.1.2. Talent placing the secondary housing box over the assembled device.
- 6.2. Connect the device to the pressure controller using the pneumatic tubing [1]. Capture

brightfield and GFP images of tumor spheroids prior to compression to assess their deformation after compression [2]. Apply a constant pressure of 120 millibars throughout the imaging period [3].

6.2.1. Talent connecting the device to the pressure controller using the pneumatic tubing.

6.2.2. LAB MEDIA: 1B2 *Video editor: please show the left image only*

6.2.3. Talent initiating pressure application through the pressure controller.

6.3. Begin time-lapse imaging by acquiring brightfield and GFP images every 10 minutes for 18 hours [1].

6.3.1. SCREEN: SCREEN.Time-lapse-image-acquisition.mp4: 01:57-02:01, 02:13-02:17

Videographer: Please film the computer screen as a back up

Results

7. Results

7.1. Spheroids subjected to compression exhibited a larger projected area than uncompressed spheroids, confirming successful transmission of mechanical load from the deformable membrane to the tumor spheroid [1].

7.1.1. LAB MEDIA: Figure 1B2. *Video editor: Highlight the image labelled as "compressed".*

7.2. Over time, a progressive loss of GFP fluorescence was observed in spheroids containing NK cells [1], as well as in spheroids subjected to compression and those exposed to both compression and NK cells, indicating NK cell-mediated tumor cell killing [2].

7.2.1. LAB MEDIA: Figure 2A. *Video editor: Sequentially highlight the row labeled '+NK' from 0 hours to 18 hours.*

7.2.2. LAB MEDIA: Figure 2A. *Video editor: Sequentially highlight the row labeled '+comp+NK' from 0 hours to 18 hours*

7.3. The spheroid-only condition and the spheroid-with-compression showed a general upward trend in GFP fluorescence intensity over time, indicating continued tumor growth or maintenance of viability [1].

7.3.1. LAB MEDIA: Figure 2B. *Video editor: Highlight the black curve labeled 'sp' and the purple curves labeled '+comp'*

7.4. Both NK cell-treated spheroids and spheroids subjected to compression with NK cells showed a progressive decrease in GFP fluorescence intensity, indicating tumor cell death [1].

7.4.1. LAB MEDIA: Figure 2B. *Video editor: Highlight the red and blue curves.*

7.5. The compression-positive spheroids exhibited the lowest degree of GFP loss, suggesting that compression alone had a minimal effect on spheroid viability.

7.5.1. LAB MEDIA: Figure 2C. *Video editor: Highlight the purple data points labeled '+comp'.*

7.6. Spheroids containing NK cells showed significantly greater GFP loss than the compression-only condition [1]. Similarly, spheroids exposed to both compression and NK cells exhibited significantly greater GFP loss, indicating that NK cells were the primary drivers of tumor cell death [2].

7.6.1. LAB MEDIA: Figure 2C. *Video editor: Compare the red and purple data points*

7.6.2. LAB MEDIA: Figure 2C. *Video editor: Compare the purple and blue data points*

7.7. The spheroids with both compression and NK cells exhibited a greater average GFP loss than the NK cell-treated spheroids, but this difference was not significantly different [1].

7.7.1. LAB MEDIA: Figure 2C. *Video editor: Compare the blue and red data points*

1. Oxygen plasma
Pronunciation link: No confirmed link found
IPA: /'ɑ:ksɪdʒən 'plæzmə/
Phonetic Spelling: OK·sih·juhn PLAZ·muh
2. Polyethylenimine
Pronunciation link: No confirmed link found
IPA: /,pɑ:li,eθə'li:nɪ'mi:n/
Phonetic Spelling: pol·ee·eth·uh·LEEN·ih·meen
3. Aspiration
Pronunciation link: <https://www.merriam-webster.com/dictionary/aspiration>
IPA: /,æspə'reɪʃən/
Phonetic Spelling: as·puh·RAY·shuhn
4. Glutaraldehyde
Pronunciation link: <https://www.merriam-webster.com/dictionary/glutaraldehyde>
IPA: /,glu:tə'rældə'haɪd/
Phonetic Spelling: gloo·tuh·RAL·duh·hyde
5. Biosafety
Pronunciation link: No confirmed link found
IPA: /,baɪəʊ'seɪfti/
Phonetic Spelling: by·oh·SAYF·tee
6. Sterilization
Pronunciation link: <https://www.merriam-webster.com/dictionary/sterilization>
IPA: /,sterələ'zeɪʃən/
Phonetic Spelling: stair·uh·luh·ZAY·shuhn
7. De-ionized
Pronunciation link: <https://www.merriam-webster.com/dictionary/deionize>

- IPA: /di:'aɪə,narzd/
Phonetic Spelling: dee·EYE·uh·nyzed
8. Microfluidic
Pronunciation link: <https://www.merriam-webster.com/dictionary/microfluidic>
IPA: /,maɪkroʊflu'ɪdɪk/
Phonetic Spelling: my·kroh·floo·ID·ik
9. Pneumatic
Pronunciation link: <https://www.merriam-webster.com/dictionary/pneumatic>
IPA: /nu:'mætɪk/
Phonetic Spelling: noo·MAT·ik
10. Polycarbonate
Pronunciation link: <https://www.merriam-webster.com/dictionary/polycarbonate>
IPA: /,pɑːli'kɑːrbəˌneɪt/, /,pɑːli'kɑːrbənət/
Phonetic Spelling: pol·ee·KAR·buh·nayt / pol·ee·KAR·buh·nuht
11. Manifold
Pronunciation link: <https://www.merriam-webster.com/dictionary/manifold>
IPA: /'mænəˌfoʊld/
Phonetic Spelling: MAN·uh·fohd
12. Neutralization
Pronunciation link: <https://www.merriam-webster.com/dictionary/neutralization>
IPA: /,nuːtrələ'zeɪʃən/
Phonetic Spelling: noo·truh·luh·ZAY·shuhn
13. Spheroid
Pronunciation link: <https://www.merriam-webster.com/dictionary/spheroid>
IPA: /'sfɪrˌɔɪd/
Phonetic Spelling: SFEER·oyd
14. Sodium hydroxide
Pronunciation link: <https://www.merriam-webster.com/dictionary/sodium%20hydroxide>
IPA: /'soʊdiəm haɪ'drɔːkˌsaɪd/
Phonetic Spelling: SOH·dee·uhm hy·DROK·side
15. Centrifuge
Pronunciation link: <https://www.merriam-webster.com/dictionary/centrifuge>
IPA: /'sentrəˌfjuːdʒ/
Phonetic Spelling: SEN·truh·fyooj
16. Supernatant
Pronunciation link: <https://www.merriam-webster.com/dictionary/supernatant>
IPA: /,suːpər'neɪtənt/
Phonetic Spelling: soo·per·NAY·tuht
17. Resuspend
Pronunciation link: No confirmed link found
IPA: /,riːsə'spend/
Phonetic Spelling: ree·suh·SPEND
18. Equimolar
Pronunciation link: <https://www.merriam-webster.com/dictionary/equimolar>

- IPA: /,i:kwə'moulər/, /,ekwə'moulər/
Phonetic Spelling: ee·kwuh·MOH·ler / eh·kwuh·MOH·ler
19. Collagen
Pronunciation link: <https://www.merriam-webster.com/dictionary/collagen>
IPA: /'kɑ:lədʒən/
Phonetic Spelling: KOL·uh·juhn
20. Microcentrifuge
Pronunciation link: No confirmed link found
IPA: /,maɪkroʊ'sentrə,fju:dʒ/
Phonetic Spelling: my·kroh·SEN·truh·fyooj
21. Polymerization
Pronunciation link: <https://www.merriam-webster.com/dictionary/polymerization>
IPA: /pə,lɪmə'reɪʒən/
Phonetic Spelling: puh·lim·er·uh·ZAY·shuhn
22. Millimolar
Pronunciation link: No confirmed link found
IPA: /,mɪlɪ'moulər/
Phonetic Spelling: mil·ih·MOH·ler
23. Epifluorescence
Pronunciation link: No confirmed link found
IPA: /,ɛpɪflʊə'resəns/
Phonetic Spelling: ep·ih·floor·ESS·uhns
24. Brightfield
Pronunciation link: No confirmed link found
IPA: /'braɪt,fi:ld/
Phonetic Spelling: BRYTE·feeld
25. Deformation
Pronunciation link: <https://www.merriam-webster.com/dictionary/deformation>
IPA: /,di:fɔ:r'meɪʃən/
Phonetic Spelling: dee·for·MAY·shuhn
26. Millibar
Pronunciation link: <https://www.merriam-webster.com/dictionary/millibar>
IPA: /'mɪlə,bɑ:r/
Phonetic Spelling: MIL·uh·bar
27. Fluorescence
Pronunciation link: <https://www.merriam-webster.com/dictionary/fluorescence>
IPA: /flʊ'resəns/
Phonetic Spelling: floor·ESS·uhns
28. Viability
Pronunciation link: <https://www.merriam-webster.com/dictionary/viability>
IPA: /,vaɪə'bɪləti/
Phonetic Spelling: vy·uh·BIL·uh·tee
29. Compression
Pronunciation link: <https://www.merriam-webster.com/dictionary/compression>

IPA: /kəmˈpreʃən/

Phonetic Spelling: kuhm·PRESH·uhn