

Submission ID #: 71037

Scriptwriter Name: Pallavi Sharma

Project Page Link: <https://review.jove.com/account/file-uploader?src=21666333>

Title: A Microfluidic System for Modeling Endothelial Dysfunction under Combined Physiological Pulsatile Shear Stress and Oscillatory Hyperglycemia

Authors and Affiliations:

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Kai-Rong Qin (krqin@dlut.edu.cn)

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. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **NO**

3. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **YES**

4. Proposed filming date: To help JoVE process and publish your video in a timely manner, please indicate the proposed date that your group will film here: **05/21/2026**

Current Protocol Length

Number of Steps: 37

Number of Shots: 63

Introduction

INTRODUCTION:

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

- 1.1. **Si-Yu Hu:** This study investigates how synchronized oscillatory hyperglycemia and pulsatile shear stress together drive endothelial dysfunction in diabetic vascular disease.
 - 1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B roll: 3.8.1*

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

- 1.2. **Si-Yu Hu:** Existing models use static high glucose or steady laminar flow; we investigate how pulsatile shear stress counters oscillatory glucose injury.
 - 1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.7.2*

CONCLUSION:

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

- 1.3. **Si-Yu Hu:** This protocol enables quantifying ROS levels and cell viability to study endothelial dysfunction under oscillatory hyperglycemia and pulsatile shear stress.
 - 1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 6.1.3*

~~Are there any additional methods or analyses that can be performed following this procedure?~~

- 1.4. **Si-Yu Hu:** Following this procedure, molecular analyses such as the Nrf2 pathway or eNOS signaling can be performed to elucidate endothelial protection mechanisms.
 - 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. **Si-Yu Hu:** Future studies could use this system to explore mechanobiological pathways, incorporate disturbed flow geometries, or smooth muscle cell co-culture.
 - 1.5.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

Protocol

2. Microfluidic Chip Fabrication for Studying Pulsatile Shear Stress

Demonstrator: Si-Yu Hu

- 2.1. To begin, use computer-aided design software to design the microfluidic chip structure [1]. Make the SU-8 master mold through a commercial company [2].
 - 2.1.1. WIDE: Talent at the computer screen with the microfluidic chip structure displayed on the monitor.
 - 2.1.2. Shot of the completed SU-8 master mold.
- 2.2. In a clean mixing cup, mix the polydimethylsiloxane curing agent and prepolymer at a 10 to 1 ratio by weight and stir the mixture vigorously by hand for 5 minutes [1]. Then place the container in a vacuum chamber at a pressure of minus 0.08 Megapascal for at least 15 minutes to remove air bubbles trapped during mixing [2].
 - 2.2.1. Talent adding the polydimethylsiloxane curing agent and prepolymer to a clean mixing cup and stirring it.
 - 2.2.2. Talent placing the container in the vacuum chamber.
- 2.3. Now, pour the polydimethylsiloxane onto the wafer placed in a glass Petri dish [1]. Degas the polydimethylsiloxane poured onto the wafer in a desiccator for an additional 15 minutes to remove any bubbles formed during casting [2].
 - 2.3.1. Talent pouring the polydimethylsiloxane onto the wafer in the glass Petri dish.
 - 2.3.2. Talent placing the glass Petri dish with the wafer and polydimethylsiloxane in the vacuum chamber.
- 2.4. Place the Petri dish containing the mold and polymer in a hot oven set to 80 degrees Celsius for 2 hours [1].
 - 2.4.1. Talent placing the Petri dish containing the mold and polymer in the oven.
- 2.5. Using a scalpel, carefully peel the solidified polymer slab from the master mold [1]. Using a core punch tool, create inlet and outlet ports at the designated channel ends on the polymer slab [2].
 - 2.5.1. Talent peeling the solidified polymer slab from the master mold with a scalpel.
 - 2.5.2. Talent creating the inlet and outlet ports on the polymer slab with the core punch tool.
- 2.6. Now, clean the glass coverslip and the patterned face of the polymer slab with adhesive tape [1].
 - 2.6.1. Talent cleaning the glass coverslip/polymer slab with adhesive tape.

2.7. Next, treat both the polymer slab and the glass coverslip with a plasma cleaner at 65 watts under room air for 60 seconds [1]. Immediately align the polymer channel face with the treated side of the glass coverslip, then press gently to create a permanently closed microfluidic channel [2].

2.7.1. Talent placing the polymer slab and glass coverslip into the plasma cleaner.

2.7.2. Talent aligning the polymer channel face with the treated side of the glass coverslip and pressing the polymer slab.

3. Endothelial Cell Preparation for Microfluidic Chamber Seeding

3.1. Place the bonded microfluidic chip in a boronized glass bottle filled with deionized water [1]. Sterilize by autoclaving at 121 degrees Celsius and 15 pounds per square inch for 20 minutes [2].

3.1.1. Talent placing the bonded microfluidic chip into a boronized glass bottle filled with deionized water.

3.1.2. Talent placing the bottle into the autoclave.

3.2. Place the sterilized chip into a 60-millimeter culture dish filled with an equimolar mixture of PBS and low glucose DMEM [1].

3.2.1. Talent placing the sterilized chip into the culture dish containing the medium mixture.

3.3. Then, using a 1-milliliter syringe, introduce 100 micrograms per milliliter fibronectin into the channel [1] and incubate at 37 degrees Celsius for 4 hours [2].

3.3.1. Talent introducing fibronectin into the microfluidic channel using a syringe.

3.3.2. Talent placing the chip for incubation.

3.4. Next, add 24.5 millimolar D-mannitol to low glucose DMEM to elevate osmotic pressure [1]. Add 5 millimolar D-glucose to high glucose DMEM to obtain a final concentration of 30 millimolar glucose [2]. Prepare normal glucose culture medium and high glucose culture medium supplemented with 20 percent FBS and 1 percent penicillin streptomycin [3].

3.4.1. Talent adding D-mannitol to low glucose Dulbecco's Modified Eagle Medium.

3.4.2. Talent adding D-glucose to high glucose Dulbecco's Modified Eagle Medium.

3.4.3. Talent adding FBS to the culture media.

3.5. Then, warm PBS, trypsin, normal glucose culture medium, and high glucose culture medium in a water bath at 37 degrees Celsius [1].

- 3.5.1. Talent placing containers of phosphate buffered saline, trypsin, and culture media into a water bath.
- 3.6. Harvest the human umbilical vein endothelial cells from the culture dish at 90 percent confluency [1]. Detach the cells using 0.25 percent trypsin-EDTA solution [2].
 - 3.6.1. Talent observing and collecting human umbilical vein endothelial cells from a culture dish at confluency.
 - 3.6.2. Talent adding trypsin-ethylenediaminetetraacetic acid solution to the culture dish.
- 3.7. After incubating the cells at 37 degrees Celsius for 30 seconds, count the harvested cells [1]. Adjust the concentration to 1×10^6 cells per milliliter in normal glucose medium and high glucose medium, respectively [2].
 - 3.7.1. Talent counting the harvested cells using cell counter.
 - 3.7.2. Talent adjusting the cell concentration in separate media.
- 3.8. Using a 1 milliliter syringe, inject 100 microliters of the cell suspension into the inlet port of the microfluidic chip with normal glucose medium for the control group and high glucose medium for the experimental group [1]. Place the microfluidic chips in a humidified incubator at 37 degrees Celsius with 5 percent carbon dioxide for 48 hours [2].
 - 3.8.1. Talent injecting the cell suspension into the inlet port of the microfluidic chip using a syringe.
 - 3.8.2. Talent placing the microfluidic chips into the incubator.

4. Dynamic Microfluidic Flow Setup

- 4.1. Calculate the flow rate required to achieve the target pulsatile shear stress using the presented equation [1-TXT].
 - 4.1.1. TEXT ON A PLAIN BACKGROUND
$$\tau = (6\mu Q)/(wh^2)$$
$$\mu:$$
 viscosity, w : channel width, and h : channel height
- 4.2. Connect the pressure source to the pressure controller [1]. Then, link the pressure controller to the valve controller connected to the computer for automated control of pressure waveforms. [2].
 - 4.2.1. Talent connecting the pressure source to the pressure controller.
 - 4.2.2. Talent connecting the pressure controller to the valve controller and computer.
- 4.3. Fill two 100 milliliter reservoirs with normal glucose culture medium and high glucose culture medium, respectively [1]. Connect the reservoirs to the corresponding channels of the pressure controller [2].

- 4.3.1. Talent filling two reservoirs with normal glucose and high glucose culture media.
- 4.3.2. Talent connecting the reservoirs to the pressure controller.
- 4.4. Then, link the reservoirs and the two-position three-way magnetic valve using silicone tubing [1].
 - 4.4.1. Talent connecting the reservoirs and a magnetic valve with silicone tubing.
- 4.5. Set the target physiological pulsatile shear stress corresponding to a flow rate of approximately 200 microliters per minute [1].
 - 4.5.1. SCREEN: 4.5.1.mp4: 00:06-00:18
- 4.6. Program the programmable air pump to generate a sine wave function to maintain the pulsatile shear stress [1]. Next, program the valve controller to switch channels every 5 minutes to generate a square wave glucose oscillation during dynamic stimulation [2].
 - 4.6.1. SCREEN: 4.6.1.mp4: 00:06-00:32 *Video editor: Please speed it up!*
 - 4.6.2. SCREEN: 4.6.2.mp4: 00:10-00:32.
- 4.7. Link the two-position three-way magnetic valve and the microfluidic chip using silicone tubing [1]. Start the programmable pump system to deliver combined oscillatory glucose and pulsatile shear stress for a total of 30 minutes [2].
 - 4.7.1. Talent connecting the magnetic valve and microfluidic chip with silicone tubing.
 - 4.7.2. Talent starting the programmable pump system.
- 4.8. Using 1.3-millimeter diameter 90-degree angled stainless steel connectors, attach the inlet and outlet ports of the microfluidic chip to the tubing [1]. Connect the microfluidic chip outlet to the waste reservoir [2].
 - 4.8.1. Talent attaching the tubing to the inlet and outlet ports of the microfluidic chip using angled stainless steel connectors.
 - 4.8.2. Talent connecting the outlet tubing of the microfluidic chip to the waste reservoir.

5. Assessment of Cell Viability and Intracellular ROS Using Fluorescent Probes

- 5.1. After performing flow field verification, prepare the Calcein-AM and propidium iodide working solution according to the manufacturer's instructions at the experimental endpoint [1].
 - 5.1.1. Talent adding the Calcein-AM and propidium iodide into labelled tube containing the diluent.
- 5.2. Once the channel is rinsed one time with PBS, introduce the Calcein-AM and propidium iodide working solution into the channel [1] and incubate at 37 degrees Celsius with 5 percent carbon dioxide for 30 minutes in the dark [2].

- 5.2.1. Talent introducing the staining solution into the channel.
- 5.2.2. Talent placing the device for incubation.
- 5.3. Immediately capture fluorescent images of the stained cells using an inverted fluorescence microscope equipped with a 10 times objective lens [1]. Use the fluorescein isothiocyanate green filter for Calcein-AM and the tetramethylrhodamine or rhodamine red filter for propidium iodide [2].
 - 5.3.1. SCREEN: 5.3.1.mp4: 00:03-00:07
 - 5.3.2. Talent switching between FITC and TRITC or Rhodamine filters using the inverted fluorescence microscope.
- 5.4. Prepare the live cell oxidative stress fluorescent probe working solution at a concentration specified by the manufacturer in PBS [1].
 - 5.4.1. Talent preparing the live cell oxidative stress fluorescent probe solution in phosphate buffered saline.
- 5.5. After flushing the channel three times with PBS, introduce the live cell oxidative stress fluorescent probe solution into the channel and incubate as demonstrated earlier [2].
 - 5.5.1. Talent introducing the probe solution into the channel and placing the chip for incubation.
- 5.6. Flush the channel three times with PBS to remove the unbound probe [1]. Immediately capture fluorescent images using the far-red filter set through a 20x (Axe) objective lens, while maintaining identical exposure parameters across all groups [2].
 - 5.6.1. Talent flushing the microfluidic channel with phosphate buffered saline.
 - 5.6.2. SCREEN: 5.6.2.mp4: 00:07-00:15

6. Quantitative Analysis and Evaluation of Oxidative Stress and Cellular Response

Video editor: Notify Pallavi when the video is ready so she can add translations for the Chinese icons being clicked..

- 6.1. Open the acquired Calcein-AM green and propidium iodide red images in ImageJ [1]. Manually adjust the threshold for each channel to distinguish fluorescent signals from background [2]. Use the cell counter to quantify fluorescent cells, adjusting the minimum cell size based on observations to determine live and dead cell counts across multiple fields of view.[3].
 - 6.1.1. SCREEN: 6.1.1.mp4: 00:05-00:15
 - 6.1.2. SCREEN: 6.1.2.mp4: 00:04-00:29
 - 6.1.3. SCREEN: 6.1.3.mp4: 00:04-00:13, 00:18-00:26
- 6.2. Now, calculate the percentage of live cells using spreadsheet software for each

experimental condition [1]. Then, calculate the mean and standard deviation across multiple fields of view using statistical analysis software to compare groups [2].

6.2.1. SCREEN: 6.2.1.mp4: 00:04-00:15

6.2.2. SCREEN: 6.2.2.mp4: 00:05-00:40

6.3. Drag the image of choice into ImageJ. Set the scale by calibrating the pixel size using the microscope metadata by selecting **Analyze** and choosing **Set Scale** [1].

6.3.1. SCREEN: 6.3.1.mp4: 00:02-00:24

6.4. Then, remove the non-specific uniform background signal from the entire image using the background subtraction function by selecting **Process** and choosing **Subtract Background** [1].

6.4.1. SCREEN: 6.4.1.mp4: 00:03-00:10

6.5. To manually adjust the threshold slider to segment and define the intracellular reactive oxygen species signal, select **Image** and choose **Adjust**, followed by **Threshold** [1]. Set the measurements by selecting **Analyze** and choosing **Set Measurements** to include area and mean gray value [2]. Determine the minimum cell size based on observed cell dimensions, then measure the mean intensity of the segmented signal within the cell monolayer ROI by selecting **Analyze** and **Analyze Particles**. [3].

6.5.1. SCREEN: 6.5.1.mp4: 00:02-00:22

6.5.2. SCREEN: 6.5.2.mp4: 00:07-00:15

6.5.3. SCREEN: 6.5.3.mp4: 00:02-00:28

6.6. Report the reactive oxygen species level for each experimental condition as the mean of measured values derived from at least three randomly selected fields of view per chip [1].

6.6.1. SCREEN: 6.6.1.mp4: 00:06-00:18

6.7. Input the quantitative results into data analysis and graphing software [1].

6.7.1. SCREEN: 6.7.1.mp4: 00:04-00:07

6.8. Determine statistical significance by comparing experimental groups with the control group using two-way analysis of variance followed by Tukey post hoc test with a significance level of 0.05 [1]. Use the software to perform the analysis and generate plots with error bars representing standard deviation [2].

6.8.1. SCREEN: 6.8.1-6.8.2.mp4: 00:11-00:20, 00:35-00:43, 00:50-01:08

6.8.2. SCREEN: 6.8.2.mp4: 00:03-00:10

Results

7. Results

- 7.1. Representative fluorescence images showed a high proportion of viable cells across all groups [1], with negligible cell death observed in the normal glucose and normal glucose with pulsatile shear stress groups [2], while the oscillatory glucose and oscillatory glucose with pulsatile shear stress groups exhibited a visible increase in dead cells [3].
 - 7.1.1. LAB MEDIA: Figure 3A. *Video editor: Show the full set of fluorescence images for all groups*
 - 7.1.2. LAB MEDIA: Figure 3A. *Video editor: Highlight the rows labeled NG and NG+PSS showing mostly green cells and very few red cells*
 - 7.1.3. LAB MEDIA: Figure 3A. *Video editor: Highlight the rows labeled OG and OG+PSS showing more red cells compared to NG groups*
- 7.2. Quantitative analysis showed that oscillatory glucose exposure resulted in a significant reduction in cell viability compared to the control group [1], and pulsatile shear stress application under oscillatory glucose conditions significantly increased viability compared to oscillatory glucose alone [2].
 - 7.2.1. LAB MEDIA: Figure 3B. *Video editor: Highlight the bar labeled OG and compare it to the NG group, showing a significantly lower value*
 - 7.2.2. LAB MEDIA: Figure 3B. *Video editor: Highlight the OG+PSS group and compare it to OG group, showing a higher value*
- 7.3. Under normal glucose conditions, intracellular reactive oxygen species levels were low [1] and were further reduced following the application of shear stress [2], whereas oscillatory glucose conditions caused a marked increase in red fluorescence intensity, indicating elevated reactive oxygen species [3].
 - 7.3.1. LAB MEDIA: Figure 4A. *Video editor: Highlight the NG panel under None showing low red signal*
 - 7.3.2. LAB MEDIA: Figure 4A. *Video editor: Highlight the NG panel under PSS showing reduced red signal compared to None*
 - 7.3.3. LAB MEDIA: Figure 4A. *Video editor: Highlight the OG panel under None showing strong red fluorescence*
- 7.4. The oscillatory glucose-induced increase in intracellular reactive oxygen species was reduced following the application of shear stress [1].
 - 7.4.1. LAB MEDIA: Figure 4A. *Video editor: Compare the OG panels under None and PSS, showing reduced red signal in PSS*

7.5. Quantitative analysis revealed that shear stress significantly reduced levels of reactive oxygen species under normal glucose conditions [1]. In contrast, oscillatory glucose conditions led to a significant increase in reactive oxygen species levels compared to normal glucose [2]. Furthermore, shear stress significantly decreased reactive oxygen species levels, even under oscillatory glucose conditions. [3].

7.5.1. LAB MEDIA: Figure 4B. *Video editor: Highlight the NG bars for None and PSS showing a lower value in PSS*

7.5.2. LAB MEDIA: Figure 4B. *Video editor: Highlight the OG None bar showing a higher value compared to NG*

7.5.3. LAB MEDIA: Figure 4B. *Video editor: Highlight the OG PSS bar showing a lower value than OG None*

1. Microfluidic
Pronunciation link: <https://www.merriam-webster.com/dictionary/microfluidics>
IPA: /ˌmaɪkroʊfluˈɪdɪk/
Phonetic Spelling: my·kroh·floo·id·ik
2. Computer-aided
Pronunciation link: <https://www.merriam-webster.com/dictionary/computer-aided>
IPA: /kəmˈpjʊːtər ˈeɪdɪd/
Phonetic Spelling: kuhm·pyoo·tur ay·did
3. SU-8
Pronunciation link: No confirmed link found
IPA: /ˌɛsˌjuː ˈeɪt/
Phonetic Spelling: ess·yoo·ayt
4. Polydimethylsiloxane
Pronunciation link: <https://www.howtopronounce.com/polydimethylsiloxane>
IPA: /ˌpɑːliˌdaɪˌmɛθəlsɪˈləːksɪn/
Phonetic Spelling: pol·ee·dye·meth·uhl·sih·lok·sayn
5. Prepolymer
Pronunciation link: <https://www.howtopronounce.com/prepolymer>
IPA: /ˌpriːˈpɑːlɪmər/
Phonetic Spelling: pree·pol·ih·mur

6. Vacuum Chamber
Pronunciation link: <https://www.merriam-webster.com/dictionary/vacuum>
IPA: /'vækju:m 'tʃeɪmbər/
Phonetic Spelling: vak·yoom chaym·bur
7. Megapascal
Pronunciation link: <https://www.merriam-webster.com/dictionary/megapascal>
IPA: /'megə,pæskəl/
Phonetic Spelling: meg·uh·pas·kuhl
8. Degas
Pronunciation link: <https://www.merriam-webster.com/dictionary/degas>
IPA: /di:'gæs/
Phonetic Spelling: dee·gas
9. Desiccator
Pronunciation link: <https://www.merriam-webster.com/dictionary/desiccator>
IPA: /'desə,ketər/
Phonetic Spelling: dess·uh·kay·tur
10. Scalpel
Pronunciation link: <https://www.merriam-webster.com/dictionary/scalpel>
IPA: /'skælpəl/
Phonetic Spelling: skal·puhl
11. Plasma Cleaner
Pronunciation link: <https://www.merriam-webster.com/dictionary/plasma>
IPA: /'plæzmə 'kli:nər/
Phonetic Spelling: plaz·muh klee·nur
12. Boronized
Pronunciation link: <https://www.howtopronounce.com/boronized>
IPA: /'bɔ:rənaɪzd/
Phonetic Spelling: bor·uh·nyzd
13. Autoclaving
Pronunciation link: <https://www.merriam-webster.com/dictionary/autoclave>
IPA: /'ɔ:toʊ,kleɪv/
Phonetic Spelling: aw·toh·klayv
14. Equimolar
Pronunciation link: <https://www.howtopronounce.com/equimolar>
IPA: /,i:kwɪ'moʊlər/
Phonetic Spelling: ee·kwee·moh·lur
15. Fibronectin
Pronunciation link: <https://www.merriam-webster.com/medical/fibronectin>
IPA: /,faɪbroʊ'nektɪn/
Phonetic Spelling: fy·broh·nek·tin
16. Osmotic
Pronunciation link: <https://www.merriam-webster.com/dictionary/osmotic>
IPA: /ɑ:z'mɑ:tɪk/
Phonetic Spelling: oz·mot·ik

17. D-mannitol
 Pronunciation link: <https://www.merriam-webster.com/medical/mannitol>
 IPA: /'mæni,tə:l/
 Phonetic Spelling: man·ih·tawl
18. D-glucose
 Pronunciation link: <https://www.merriam-webster.com/dictionary/glucose>
 IPA: /'gluː,kʊs/
 Phonetic Spelling: gloo·kohs
19. Dulbecco's
 Pronunciation link: <https://www.howtopronounce.com/dulbecco>
 IPA: /dʊl'bɛkʊʊ/
 Phonetic Spelling: dul·bek·oh
20. Umbilical
 Pronunciation link: <https://www.merriam-webster.com/dictionary/umbilical>
 IPA: /ʌm'bɪlɪkəl/
 Phonetic Spelling: um·bil·ih·kuhl
21. Endothelial
 Pronunciation link: <https://www.merriam-webster.com/dictionary/endothelial>
 IPA: /,endʊ'θiːliəl/
 Phonetic Spelling: en·doh·thee·lee·uhl
22. Confluency
 Pronunciation link: <https://www.howtopronounce.com/confluency>
 IPA: /kən'fluːənsi/
 Phonetic Spelling: kuhn·floo·uhn·see
23. Trypsin-EDTA
 Pronunciation link: <https://www.merriam-webster.com/dictionary/trypsin>
 IPA: /'trɪpsɪn ,ɪːdiːtiː'ei/
 Phonetic Spelling: trip·sin ee·dee·tee·ay
24. Ethylenediaminetetraacetic
 Pronunciation link: <https://www.howtopronounce.com/ethylenediaminetetraacetic-acid>
 IPA: /,ɛθɪliːn,dai,æmɪn,tetrə,siːtɪk/
 Phonetic Spelling: eth·uh·leen·dye·am·ih·neen·tet·ruh·see·tik
25. Pulsatile
 Pronunciation link: <https://www.merriam-webster.com/dictionary/pulsatile>
 IPA: /'pʌlsə,tail/
 Phonetic Spelling: pul·suh·tyle
26. Shear Stress
 Pronunciation link: <https://www.merriam-webster.com/dictionary/shear>
 IPA: /ʃɪr stres/
 Phonetic Spelling: sheer stress
27. Physiological
 Pronunciation link: <https://www.merriam-webster.com/dictionary/physiological>
 IPA: /,fɪziə'lɑːdʒɪkəl/
 Phonetic Spelling: fiz·ee·uh·loj·ih·kuhl

28. Oscillation
Pronunciation link: <https://www.merriam-webster.com/dictionary/oscillation>
IPA: /ˌɑːsəˈleɪʃən/
Phonetic Spelling: os·uh·lay·shuhn
29. Calcein-AM
Pronunciation link: <https://www.howtopronounce.com/calcein>
IPA: /ˈkælsiːn eɪ ɛm/
Phonetic Spelling: kal·see·een ay·em
30. Propidium Iodide
Pronunciation link: <https://www.howtopronounce.com/propidium-iodide>
IPA: /ˈproʊˈpɪdiəm ˈaɪoʊdaɪd/
Phonetic Spelling: proh·pid·ee·uhm eye·uh·dyde
31. Fluorescein Isothiocyanate
Pronunciation link: <https://www.howtopronounce.com/fluorescein-isothiocyanate>
IPA: /ˌflʊəˈresiːn ˌaɪsoʊˈθaɪoʊˈsaɪənɛt/
Phonetic Spelling: floor·ess·een eye·soh·thye·oh·sy·uh·naye
32. Tetramethylrhodamine
Pronunciation link: <https://www.howtopronounce.com/tetramethylrhodamine>
IPA: /ˌtɛtrəˈmɛθəlˈroʊdəmiːn/
Phonetic Spelling: tet·ruh·meth·uhl·roh·duh·meen
33. Oxidative
Pronunciation link: <https://www.merriam-webster.com/dictionary/oxidative>
IPA: /ˈɑːksɪˌdeɪtɪv/
Phonetic Spelling: ok·sih·day·tiv
34. Intracellular
Pronunciation link: <https://www.merriam-webster.com/dictionary/intracellular>
IPA: /ˌɪntrəˈseljələr/
Phonetic Spelling: in·truh·sel·yuh·lur
35. Reactive Oxygen Species
Pronunciation link: <https://www.howtopronounce.com/reactive-oxygen-species>
IPA: /rɪˈæktɪv ˈɑːksɪdʒən ˈspiːʃiːz/
Phonetic Spelling: ree·ak·tiv ok·sih·juhn spee·sheez
36. Monolayer
Pronunciation link: <https://www.merriam-webster.com/dictionary/monolayer>
IPA: /ˈmɑːnoʊˌleɪər/
Phonetic Spelling: mon·oh·lay·ur
37. Tukey
Pronunciation link: <https://www.merriam-webster.com/dictionary/Tukey>
IPA: /ˈtuːki/
Phonetic Spelling: too·kee