

Submission ID #: 69839

Scriptwriter Name: Poornima G

Project Page Link: <https://review.jove.com/account/file-uploader?src=21300943>

Title: Biological Standardization of Human ASC-Based Biofabrication for Reproducible Macroscale Tissue Constructs

Authors and Affiliations:

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FINAL SCRIPT: APPROVED FOR FILMING



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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, 7 done**
- 3. Filming location:** Will the filming need to take place in multiple locations? **Yes**
If **Yes**, how far apart are the locations? **Two separate rooms next door to each other**
- 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: 24

Number of Shots: 60 (10 SC)

Commented [1]: Authors: The shots 2.5.1 and 2.5.2 have been modified. We can use the image in the results for 2.5.1 and shoot a representative scene for 2.5.2.

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
Some answers are shortened as per journal guidelines.

INTRODUCTION:

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

- 1.1. **Leandra Baptista:** This work presents a standardized biofabrication workflow that uses 3D-printed hydrogel templates to guide the assembly of spheroids into high-density cellular macrotissues.
 - 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. **Leandra Baptista:** The proposed approach produced transplantable tissue constructs, with or without scaffolds, advancing reproducible biofabrication methodologies for regenerative medicine. The approach used in this study biofabricated transplantable tissues with or without scaffolds, advancing the biofabrication field for regenerative medicine.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera **NOTE: The authors modified the statement on spot**

CONCLUSION:

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

- 1.3. **Aline Pimentel:** The main challenge in this protocol is ensuring reproducibility through uniform spheroid generation and accurate scaffold printing, which are essential for predictable fusion and macroscale tissue formation.
 - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

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

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- 1.4. **Larissa Resende:** Additional methods that can be performed following this procedure include mechanical testing and viability assays.

- 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. **Larissa Resende:** Future studies can investigate tissue maturation, functional differentiation, regenerative performance in vivo, and scalable biomanufacturing strategies.

- 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.

Authors: I moved the gelMA printing section later for a better flow

2. Preparation of Human Adipose-Derived Mesenchymal Stem Cells

Demonstrator: Bianca Montenegro

- 2.1. To begin, place the flask containing hADSC (*H-D-A-S-C*) monolayer in a biological safety cabinet and transfer the culture medium to a waste container [1-TXT]. **Authors:** Should we mention labeling the monolayer here, or is that unnecessary? If yes, please give some details about the label.

- 2.1.1. Talent placing the flask in a biosafety cabinet and transferring the medium to a waste container. **TXT: hADSC: Human Adipose-Derived Mesenchymal Stem Cells** **Videographer:** Please obtain CU shot as well for this shot.

NOTE: 2.1.1 TO 2.3.1: Recorded in the same shot

Videographer's NOTE: 3 shorts films 2.1.1 - 1st wide-angle, 2nd wrong FPS, 3rd action short

- 2.2. Wash the monolayer twice with 1× PBS [1] and discard the solution [2]. If required, label the cells with a suitable fluorescent dye [3].

- 2.2.1. Talent adding 1× PBS to the monolayer.

- 2.2.2. Talent discarding the PBS solution.

- 2.2.3. **Reuse 2.2.1** **Video editor:** If the tube from which the solution is taken is labeled as "PBS," then please blur that label or use a different angle. Basically we do not want to see 'PBS' for this shot 2.2.3.

- 2.3. Add a 0.125% trypsin EDTA solution sufficient to cover the monolayer and close the flask [1]. Incubate the flask in a humidified incubator at 37 degrees Celsius for up to 5 minutes [2]. After incubation, inactivate the trypsin by adding an equal volume of DMEM supplemented with 10% FBS [3].

- 2.3.1. Talent adding a 0.125% trypsin/ethylenediaminetetraacetic acid solution to the culture flask and closing the culture flask.

- 2.3.2. Talent placing the culture flask in a humidified incubator.

- 2.3.3. Talent adding Dulbecco's Modified Eagle Medium supplemented with 10% fetal

bovine serum to the culture flask.

NOTE: 2.3.3 TO 2.4.2: Recorded in the same shot

- 2.4. Collect the cell suspension using a serological pipette [1]. Transfer the cell suspension to centrifuge tubes [2] and spin at 400 *g* for 5 minutes at room temperature [3].

- 2.4.1. Talent collecting the cell suspension with a serological pipette.

- 2.4.2. Talent transferring the cell suspension into centrifuge tubes.

- 2.4.3. Talent placing the centrifuge tubes in the centrifuge.

- 2.5. After discarding the supernatant, resuspend the cell pellet in DMEM [1]. Homogenize the suspension [2] and take an aliquot for cell counting and viability assessment [3].

NOTE: 2.5.1 TO 2.5.3: Recorded in the same shot

- 2.5.1. Talent resuspending the cell pellet in Dulbecco's Modified Eagle Medium.

- 2.5.2. Talent homogenizing the cell suspension. **Authors: You homogenize by pipetting up and down several times, is it?**

- 2.5.3. Talent transferring an aliquot of the cell suspension for cell counting and viability assessment.

3. Generation of Spheroids in Agarose Micromolds

Demonstrator: Isabelle Diederichs

- 3.1. ~~Obtain an agarose mold containing 81 circular recesses [1-TXT]. Aliquot 1×10^6 of the prepared hADSCs into a 15-milliliter centrifuge tube [2].~~

- ~~3.1.1. Shot showing the agarose mold. **TXT: Refer to the text manuscript for agarose mold preparation**~~ **NOTE: Shot and VO moved to step 3.5**

- 3.1.2. Talent adding the required number of cells into a 15 milliliter centrifuge tube.

- 3.2. To wash the cells, add PBS to the tube [1] and spin at 500 *g* for 5 minutes at room temperature [2]. Discard the supernatant [3] and add fresh PBS [4]. Repeat the washing procedure two more times [5].

- 3.2.1. Talent adding 1× PBS to the centrifuge tube.

- 3.2.2. Talent placing the centrifuge tube in the centrifuge.

Videographer: Please obtain multiple reusable shots for step 4.2.2. It will be used

again

3.2.3. Talent discarding the supernatant.

NOTE: 3.2.3 TO 3.2.4 Recorded in the same shot

3.2.4. Talent adding fresh 1× PBS to the centrifuge tube.

3.2.5. *Reuse 3.2.2*

3.3. After the third centrifugation, carefully discard the supernatant [1]. Dry the tube opening with sterile gauze [2] and place the tube vertically [3].

3.3.1. Talent discarding the supernatant after the third centrifugation.

3.3.2. Talent drying the tube opening with sterile gauze.

3.3.3. Talent placing the centrifuge tube in a vertical position.

NOTE: 3.3.1 TO 3.4.2 Recorded in the same shot

3.4. Resuspend the cell pellet in 120 microliters of 3D culture medium [1]. Homogenize the suspension [2]. Adjust the suspension volume to 200 microliters with additional 3D culture medium if necessary [3].

3.4.1. Talent resuspending the cell pellet in 120 microliters of 3D culture medium.

3.4.2. Talent homogenizing the cell suspension. **Authors: Do you homogenize by pipetting up and down?**

3.4.3. Talent adding an additional 3D culture medium to adjust the suspension volume to 200 microliters.

3.5. Obtain an agarose mold containing 81 circular recesses [1-TXT]. Aspirate 200 microliters of the cell suspension [2] and plate it in the center of the mold, avoiding bubble formation [3]. Leave the plate in the biological safety cabinet for 5 minutes to let the cells settle into the recesses [4].

3.1.1. Shot showing the agarose mold. **TXT: Refer to the text manuscript for agarose mold preparation.** **NOTE: Place shot 3.1.1 here**

3.5.1. Talent aspirating 200 microliters of the cell suspension.

NOTE: 2.5.1 TO 3.5.2: Recorded in the same shot

3.5.2. Talent plating the cell suspension in the center of the mold.

3.5.3. Talent placing the plate aside in the biological safety cabinet.

3.6. Then, place the plate in a humidified incubator at 37 degrees Celsius for 40 minutes [1].

After incubation, carefully add 2 milliliters of 3D medium to each well [2] and return the plate to the incubator for about 24 hours [3].

NOTE: 3.5.3 TO 3.6.1: Recorded in the same shot

- 3.6.1. Talent placing the plate in a humidified incubator.
- 3.6.2. Talent adding 2 milliliters of 3D medium to each well.
- 3.6.3. Talent placing the plate back into the humidified incubator.

4. Morphometric Analysis of Spheroid Diameter and Sphericity

Demonstrator: Larissa Fontes

- 4.1. Use the 10× (10-X) objective of an inverted optical microscope to capture images of the spheroids positioned along the two diagonals of the agarose micromold [1]. Calibrate the image scale using the 100-micrometer scale bar before measuring the diameters [2].

- 4.1.1. Talent positioning the sample under the inverted microscope and capturing images of the spheroids.

- 4.1.2. SCREEN: 7.1.1, 7.1.2, 7.2.1, 7.2.2.mp4 - 0:21 *Video editor: Hold frame and highlight the 100 µm scale bar at the bottom right of the screen*

- 4.2. Determine the major diameter of each photographed spheroid using the software's Line tool [1]. Measure the minor diameter by making a second measurement at approximately 90 degrees to the first [2]. Calculate the sphericity and the mean spheroid diameter [3].

- 4.2.1. SCREEN: 7.1.1, 7.1.2, 7.2.1, 7.2.2.mp4 - 0:22-00:29

- 4.2.2. SCREEN: 7.1.1, 7.1.2, 7.2.1, 7.2.2.mp4 - 0:30-00:38.

- 4.2.3. SCREEN: 7.1.1, 7.1.2, 7.2.1, 7.2.2.mp4 - 01:56-01:59

NOTE: Step 4.3 was not provided

- ~~4.3. Calculate sphericity from the two perpendicular diameters [3].~~

- ~~- 4.3.1. **SCREEN:** Show the calculation of the mean diameter from the two perpendicular diameter measurements.~~

- ~~- 4.3.2. **SCREEN:** Show the calculation of spheroid sphericity using the largest and smallest diameters.~~

4.3.3. **SCREEN:** Hover the cursor over the sphericity values ~ 1 .

5. 3D Printing and Photocrosslinking of Gelatin Methacryloyl (GelMA) Scaffolds

Demonstrators: Larissa Resende and Maria Domingos

5.1. To prepare the printer for scaffold fabrication, move the printhead to the home position [1] and load the previously generated G-code file [2-TXT].

5.1.1. Talent moving the printhead to the home position.

5.1.2. **SCREEN:** 2.1,2, 2.2.1, 2.2.(new) mp4 - 0:02-00:10 and 00:18-0:23 **TXT: Refer to the text manuscript for generating the G-code file**

5.2. Using the software's manual controls, extrude a small amount of hydrogel from the syringe to ensure continuous flow through the nozzle [1]. Then, start the printing process [2].

5.2.1. **SCREEN:** 2.1,2, 2.2.1, 2.2.(new) mp4 - 0:023-0:40 .

5.2.3. Added shot: Shot of Printing Process

5.3. After printing, photocrosslink the scaffold using the 3D bioprinter's ultraviolet light system for 5 minutes at a light intensity of 20 milliwatts per square centimeter [1].

5.3.1. Talent turning on the UV light (or placing the printed scaffold in UV chamber if it is a separate chamber) for photocrosslinking.

NOTE: Separated into 3 short parts: 1st giving the command, 2nd giving the UV signal, and 3rd giving the shutdown command.

5.4. Use depolymerized 2% agarose solution to attach the 3D-printed scaffolds to the wells of a 12-well culture plate [1]. Secure the scaffolds to the bottoms of the wells to prevent displacement during spheroid seeding and subsequent culture [2].

NOTE: 5.4.1 TO 5.4.2: Recorded in the same shot

5.4.1. Talent applying the depolymerized 2% agarose solution to the wells of a 12-well culture plate.

5.4.2. Talent securing the scaffolds to the bottoms of the wells.

5.5. Capture stereomicroscope images of the scaffolds with a ruler or caliper reference to

assess printing quality and scaffold structure [1]. Calibrate the images and measure the 3D-printed scaffold and pore dimensions accurately [2]. **Authors:** These steps are summarized as we are already over the 55-shot limit

5.5.1. LAB MEDIA: Figure 3D.

5.5.2. SCREEN: 5.5.2-NEW.mp4 00:34-00:40 and 01:00-01:01

6. Spheroid Seeding into GelMA Scaffolds and Bioassembly

Demonstrator: Larissa Fontes

6.1. Collect the spheroids from the agarose micromolds using a pipette with wide-bore tips [1] and transfer them into 2 milliliter microtubes [2].

6.1.1. Talent collecting the spheroids from the agarose micromolds using a pipette with wide-bore tips.

6.1.2. Talent transferring the spheroids into 2 milliliter microtubes.

6.2. When the spheroids settle at the bottom of the microtubes, discard the supernatant [1]. Resuspend the spheroids in 50 microliters of 3D Medium [2].

6.2.1. Talent removing and discarding the supernatant from the microtube. **NOTE:** there's a close-up of this step that isn't identified at the beginning

6.2.2. Talent resuspending the spheroids in 50 microliters of 3D Medium.

6.3. Using a pipette with wide-bore tips, collect the spheroids [1] and gently embed them in the GelMA (*Gel-M-A*) scaffold pores [2-TXT]. Wait 20 to 40 minutes to let the spheroids settle [3]. Then, add 1 milliliter of 3D Medium to each well [4]. Immediately after seeding on day 0, acquire phase-contrast and stereomicroscope images of the spheroids [5]. Then, incubate the samples in a humidified incubator [6].

NOTE: 6.3.1 TO 6.3.2: Recorded in the same shot

6.3.1. Talent collecting the spheroids with a pipette fitted with wide-bore tips.

6.3.2. Shot of gently embedding the spheroids into the GelMA scaffold pores. **TXT:** GelMA: Gelatin Methacryloyl

NOTE: 6.3.3 TO 3.6.4: Recorded in the same shot

6.3.3. Talent placing the scaffold containing the spheroids aside to allow settling.

6.3.4. Talent adding 1 milliliter of 3D Medium to each well.

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- 6.3.5. Talent positioning the spheroid fusion within the GelMA scaffold pores under a phase-contrast or stereomicroscope. *Video editor: Show LAB MEDIA 6.7.2--NEW.mp4 - 0:04 still frame as an inset when VO says "day zero by....."*
- 6.3.6. Talent placing the samples in a humidified incubator. **Authors: This step is added, please check if this is correct**
- 6.4. After 24 hours, add 1 milliliter of 3D Medium to each well [1].
 - 6.4.1. Talent adding 1 milliliter of 3D Medium to each well.
- 6.5. Monitor the fusion of the spheroids within the pores [1] of the GelMA scaffolds every 24 hours until day 3 [2].
 - 6.5.1. Talent acquiring images at the microscope unit. **NOTE: This was already recorded in part 4.1.1**
 - 6.5.2. SCREEN: 6.7.2—NEW.mp4 00:25 and 00:40 *Video editors: Show the still frames side by side*

Results

7. Results

Authors: We will use the manuscript figures directly submitted by you for the results section

- 7.1. Human ADSCs cultured in a monolayer [1] were successfully induced to form compact, well-defined spheroids within 24 hours after seeding into agarose micromolds [2].
 - 7.1.1. LAB MEDIA: Figure 2C.
 - 7.1.2. LAB MEDIA: Figure 2D. *Video editor: Highlight the single round cell cluster in the center.*
- 7.2. The morphometric analysis revealed a mean spheroid diameter of about 239.8 micrometers at 24 hours [1] and a sphericity value close to 1 [2].
 - 7.2.1. LAB MEDIA: Figure 2F. .
 - 7.2.2. LAB MEDIA: Figure 2E.
- 7.3. The CAD (*Kad*) model of the two-pore 3D scaffold was successfully used [1] to print the GelMA hydrogel scaffolds [2].
 - 7.3.1. LAB MEDIA: Figure 3A and C.
 - 7.3.2. LAB MEDIA: Figure 3D and E.
- 7.4. No significant differences were detected between the dimensions of the 3D model and those of the printed scaffolds for the scaffold diameter [1], the pore diameter [2], and the scaffold thickness [3].
 - 7.4.1. LAB MEDIA: Figure 3F.
 - 7.4.2. LAB MEDIA: Figure 3G.
 - 7.4.3. LAB MEDIA: Figure 3H.
- 7.5. The spheroids were distributed within the scaffold pore regions immediately after transfer [1]. They showed partial coalescence after 48 hours [2], and extensive fusion after 72 hours [3].
 - 7.5.1. LAB MEDIA: Figure 4A.
 - 7.5.2. LAB MEDIA: Figure 4B.

7.5.3. LAB MEDIA: Figure 4C.

7.6. After 72 hours, a macroscale biological construct was visible under stereomicroscopy and was estimated to occupy about 80% of the entire pore diameter [1].

7.6.1. LAB MEDIA: Figure 4D. Video editor: Highlight the white mass at the center.

7.7. Confocal imaging confirmed the formation of a single fused construct within the pore after 72 hours [1].

7.7.1. LAB MEDIA: Figure 4E. Video editor: Highlight the continuous green structure occupying the image area.

7.8. The resulting construct measured 3,711.08 micrometers in length [1] and 4,258.97 micrometers in width [2]. **Authors:** The image shows that 3711 was width and 4258 was length (vertical measurement), should we change this or should we simply rotate the image by 90 degrees?

Author's NOTE: Simply rotate the image by 90 degrees

7.8.1. LAB MEDIA: Figure 4E. *Video editor: Rotate the image by 90degrees clockwise and hide the label E. Highlight the measurement label showing 3,711.08 micrometers.*

7.8.2. LAB MEDIA: Figure 4E. *Video editor: Rotate the image by 90degrees clockwise and hide the label E. Highlight the measurement label showing 4,258.97 micrometers.*

1. hADSC
Pronunciation link: No confirmed link found
IPA: /ˌeɪtʃˈeɪ diː ɛs ˈsiː/
Phonetic Spelling: aych·ay·dee·ess·see
2. Monolayer
Pronunciation link: <https://www.merriam-webster.com/dictionary/monolayer>
IPA: /ˈmɑːnoʊˌleɪər/
Phonetic Spelling: mon·oh·lay·er
3. Biological
Pronunciation link: <https://www.merriam-webster.com/dictionary/biological>
IPA: /ˌbaɪəˈlɑːdʒɪkəl/
Phonetic Spelling: bye·uh·laa·juh·kuhl
4. Biosafety
Pronunciation link: <https://www.merriam-webster.com/dictionary/biosafety>
IPA: /ˌbaɪoʊˈseɪfti/
Phonetic Spelling: bye·oh·sayf·tee

5. Fluorescent
Pronunciation link: <https://www.merriam-webster.com/dictionary/fluorescent>
IPA: /flɔːˈresənt/
Phonetic Spelling: flor·ess·uhnt
6. Trypsin
Pronunciation link: <https://www.merriam-webster.com/dictionary/trypsin>
IPA: /ˈtrɪpsɪn/
Phonetic Spelling: trip·sin
7. Ethylenediaminetetraacetic
Pronunciation link: <https://www.howtopronounce.com/ethylenediaminetetraacetic-acid>
IPA: /ˌɛθəˌliːn daɪˌæməˌniː tetrəˈsiːtɪk/
Phonetic Spelling: eth·uh·leen·dye·am·uh·nee·tet·ruh·uh·see·tik
8. Incubator
Pronunciation link: <https://www.merriam-webster.com/dictionary/incubator>
IPA: /ˈɪŋkjəˌbeɪtər/
Phonetic Spelling: ing·kyuh·bay·ter
9. Dulbecco
Pronunciation link: <https://www.howtopronounce.com/dulbecco>
IPA: /dɒlˈbekoʊ/
Phonetic Spelling: duhl·bek·oh
10. Centrifuge
Pronunciation link: <https://www.merriam-webster.com/dictionary/centrifuge>
IPA: /ˈsentrəˌfjuːdʒ/
Phonetic Spelling: sen·truh·fyooj
11. Supernatant
Pronunciation link: <https://www.merriam-webster.com/dictionary/supernatant>
IPA: /ˌsuːpərˈneɪtənt/
Phonetic Spelling: soo·per·nay·tuhnt
12. Resuspend
Pronunciation link: <https://www.merriam-webster.com/dictionary/resuspend>
IPA: /ˌriːsəˈspend/
Phonetic Spelling: ree·suh·spend
13. Aliquot
Pronunciation link: <https://www.merriam-webster.com/dictionary/aliquot>
IPA: /ˈæliˌkwɑːt/
Phonetic Spelling: al·ih·kwaht
14. Viability
Pronunciation link: <https://www.merriam-webster.com/dictionary/viability>
IPA: /ˌvaɪəˈbɪləti/
Phonetic Spelling: vy·uh·bil·uh·tee
15. Agarose
Pronunciation link: <https://www.merriam-webster.com/dictionary/agarose>
IPA: /ˈæɡəˌroʊs/
Phonetic Spelling: ag·uh·rohs

16. Micromolds
Pronunciation link: No confirmed link found
IPA: /'maɪkroʊˌmoʊldz/
Phonetic Spelling: my·kroh·mohldz
17. Spheroids
Pronunciation link: <https://www.merriam-webster.com/dictionary/spheroid>
IPA: /'sfɪrɔɪd/
Phonetic Spelling: sfeer·oydz
18. Microliters
Pronunciation link: <https://www.merriam-webster.com/dictionary/microliter>
IPA: /'maɪkroʊˌliːtər/
Phonetic Spelling: my·kroh·lee·ter
19. Morphometric
Pronunciation link: <https://www.howtopronounce.com/morphometric>
IPA: /ˌmɔːrfəˈmetrɪk/
Phonetic Spelling: mor·fuh·met·rik
20. Sphericity
Pronunciation link: <https://www.howtopronounce.com/sphericity>
IPA: /sfəˈrɪsɪti/
Phonetic Spelling: sfuh·riss·ih·tee
21. Gelatin
Pronunciation link: <https://www.merriam-webster.com/dictionary/gelatin>
IPA: /ˈdʒələtɪn/
Phonetic Spelling: jel·uh·tin
22. Methacryloyl
Pronunciation link: <https://www.howtopronounce.com/methacryloyl>
IPA: /ˌmɛθəˈkrɪləɪl/
Phonetic Spelling: meth·uh·kril·oyl
23. GelMA
Pronunciation link: No confirmed link found
IPA: /ˈdʒɛl mɑː/
Phonetic Spelling: jel·mah
24. Hydrogel
Pronunciation link: <https://www.merriam-webster.com/dictionary/hydrogel>
IPA: /ˈhaɪdrəˌdʒɛl/
Phonetic Spelling: hy·druh·jel
25. Nozzle
Pronunciation link: <https://www.merriam-webster.com/dictionary/nozzle>
IPA: /ˈnɑːzəl/
Phonetic Spelling: noz·uhl
26. Photocrosslink
Pronunciation link: No confirmed link found
IPA: /ˌfoʊtoʊˈkrɔːsˌlɪŋk/
Phonetic Spelling: foe·toh·kraws·lingk

27. Ultraviolet
 Pronunciation link: <https://www.merriam-webster.com/dictionary/ultraviolet>
 IPA: /ˌʌltrəˈvæələt/
 Phonetic Spelling: ul·truh·vye·uh·luht
28. Depolymerized
 Pronunciation link: <https://www.merriam-webster.com/dictionary/depolymerize>
 IPA: /diːpəˈlɪməˌraɪzd/
 Phonetic Spelling: dee·puh·lim·uh·ryzd
29. Stereomicroscope
 Pronunciation link: <https://www.howtopronounce.com/stereomicroscope>
 IPA: /ˌsteriəʊˈmaɪkrəˌskoʊp/
 Phonetic Spelling: stair·ee·oh·my·kruh·skohp
30. Scaffold
 Pronunciation link: <https://www.merriam-webster.com/dictionary/scaffold>
 IPA: /ˈskæfəld/
 Phonetic Spelling: skaf·uhld
31. Bioassembly
 Pronunciation link: <https://www.howtopronounce.com/bioassembly>
 IPA: /ˌbaɪəʊəˈsɛmbli/
 Phonetic Spelling: bye·oh·uh·sem·blee
32. Phase-contrast
 Pronunciation link: <https://www.merriam-webster.com/dictionary/phase-contrast>
 IPA: /ˈfeɪz ˌkɑːntræst/
 Phonetic Spelling: fayz·kon·trast
33. Coalescence
 Pronunciation link: <https://www.merriam-webster.com/dictionary/coalescence>
 IPA: /ˌkoʊəˈlesəns/
 Phonetic Spelling: koh·uh·less·uhns
34. Macroscale
 Pronunciation link: <https://www.merriam-webster.com/dictionary/macroscale>
 IPA: /ˈmækroʊˌskeɪl/
 Phonetic Spelling: mak·roh·skayl
35. Confocal
 Pronunciation link: <https://www.merriam-webster.com/dictionary/confocal>
 IPA: /kənˈfoʊkəl/
 Phonetic Spelling: kuhn·foh·kuhl
36. Micrometers
 Pronunciation link: <https://www.merriam-webster.com/dictionary/micrometer>
 IPA: /maɪˈkraːmɛtər/
 Phonetic Spelling: my·krom·uh·ter
37. CAD
 Pronunciation link: <https://www.merriam-webster.com/dictionary/CAD>
 IPA: /ˌsiː ɛr ˈdiː/
 Phonetic Spelling: see·ay·dee

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