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Scriptwriter Name: Poornima G

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Title: Isolation and Culture of Primary Marginal Cells of the Stria Vascularis from Neonatal Mice

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? **Yes**

If **Yes**, can you record movies/images using your own microscope camera?

No

If your protocol involves microscopy but you are not able to record movies/images with your microscope camera, JoVE will need to use our scope kit.

If your microscope does not have a camera port, the scope kit will be attached to one of the eyepieces and **you will have to perform the procedure using one eye.**

Olympus SZ51

If a dissection or stereo microscope is required for your protocol, please list all shots from the script that will be visualized using the microscope (shots are indicated with the 3-digit numbers, like 2.1.1, 2.1.2, etc.).

SCOPE SHOTS: 2.8.3, 2.8.4, 2.8.5, 2.9.1, 2.9.2, 2.9.3, 2.9.4, 2.9.5
2.10.1, 2.10.2, 3.5.3,

Videographer: Please film the above-mentioned shots using the scope kit

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. **No**

Current Protocol Length

Number of Steps: 18

Number of Shots: 55 (13 Scope)

Introduction

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**.

Answers will be slightly edited in accordance with the journal guidelines

INTRODUCTION:

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

- 1.1. **Yutong Bai:** We established a primary marginal cell model to investigate stria vascularis dysfunction in hearing loss.
 - 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. **Yutong Bai:** This protocol can be applied to investigate stria vascularis dysfunction in age-related, noise-induced, and hereditary hearing loss.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

CONCLUSION:

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

- 1.3. **Qingyan Zhou:** The key challenge while performing this protocol is preventing cell damage during dissection and optimizing purification timing and proportion.
 - 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?~~

- 1.4. **Qingyan Zhou:** Researchers can perform ROS detection, senescence staining, electrophysiology, as well as Western blot and qPCR analyses using this technique.

- 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. **Qingyan Zhou:** Future studies can explore co-culture systems, growth factor optimization, and immortalization of marginal cells.
 - 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.

Ethics Title Card

This research has been approved by the Animal Ethics Committee of Renmin Hospital of Wuhan University

Protocol

2. Dissection of Neonatal Mouse Temporal Bone and Isolation of Cochlear Stria Vascularis

Demonstrator: Yutong Bai

2.1. To begin, prepare the dissection tools by arranging 2 pairs of micro-dissection forceps, 1 pair each of standard forceps, surgical scissors, and micro-dissection scissors on the work surface [1]. Using an autoclave, sterilize all the tools and dry them at 60 degrees Celsius for 4 hours [2]. Then, place the sterilized instruments into the biological safety cabinet. [3]

2.1.1. WIDE: Talent arranging tools on the laboratory bench.

2.1.2. Close-up of the sterilized instrument box placed on the laboratory bench, with the autoclave tape clearly showing the sterilization indicator (dark stripes). ~~Talent placing all the dissection tools into the autoclave and closing the lid.~~

NOTE: Authors modified the shots 2.1.2 and 2.1.3 and VO is adjusted accordingly

2.1.3. ~~Talent placing the sterilized tools into a 60 degrees Celsius oven and setting the timer to 4 hours.~~ Talent placing the sterilized instruments into the biological safety cabinet.

2.2. Using 75 percent ethanol, disinfect the stereomicroscope stage and housing thoroughly [1]. Place the disinfected stereomicroscope inside a biological safety cabinet [2] and expose all items to ultraviolet light for 30 minutes to ensure complete sterility before initiating the dissection procedure [3].

2.2.1. Talent wiping the stereomicroscope stage and housing with 75 percent ethanol.

2.2.2. Talent placing the disinfected stereomicroscope inside a biological safety cabinet.

2.2.3. Talent closing the sash and turning on the ultraviolet light inside the biological safety cabinet.

2.3. Carefully disinfect the neonatal mouse pup anesthetized with 4% isoflurane using 75% ethanol [1]. Place the disinfected pup on a 6 cm dish inside the biosafety cabinet for dissection [2-TXT]. **NOTE:** Authors modified the shots and VO is adjusted accordingly

2.3.1. Talent carefully and thoroughly spraying the anesthetized pup with 75 percent ethanol. **Authors:** Please place a fully anesthetized animal. We do not want to show the process of inducing anesthesia or decapitating the animal

- 2.3.2. Talent placing the disinfected pup on a 6 cm dish inside the biosafety cabinet.
TXT: Euthanize the animal following institutionally approved protocol
- 2.4. Immobilize the head firmly with straight forceps [1]. Using micro-dissection scissors, open the scalp along the sagittal suture [2] and separate the scalp bilaterally with scissors to excise it completely [3].
- 2.4.1. Talent stabilizing the neonatal mouse head with straight forceps.
- 2.4.2. Close-up of micro-dissection scissors cutting along the sagittal suture.
- 2.4.3. Talent pulling the scalp apart with scissors and removing it. **NOTE: Authors modified the shots and VO is adjusted accordingly**
- 2.5. Next, using micro-dissection scissors, bisect the neonatal mouse head along the midline [1]. With forceps, remove the brain tissue carefully to expose the temporal bones [2].
- 2.5.1. Talent cutting the head along the midline with micro-dissection scissors.
- 2.5.2. Talent removing brain tissue with forceps to reveal the temporal bones.
- 2.6. Observe the bilateral temporal bones at the cranial base [1] and remove unnecessary surrounding bones while retaining the temporal bone tissue containing the cochlea [2].
- 2.6.1. Close-up of the cranial base showing both temporal bones.
- 2.6.2. Talent carefully removing unnecessary surrounding bone with scissors while preserving the cochlear-containing temporal bone.
- 2.7. Transfer one temporal bone into a 35-millimeter sterile dish containing fresh Hank's Balanced Salt Solution placed on ice [1].
- 2.7.1. Talent placing one temporal bone into a 35 millimeter sterile dish filled with fresh Hank's Balanced Salt Solution on ice.
- 2.8. Transfer the other temporal bone under the stereomicroscope [1-TXT]. Using one pair of forceps, secure the temporal bone tissue firmly [2]. Using another pair of forceps, perform blunt dissection to remove surface muscle tissue until the white semicircular ossicle ring becomes visible [3]. Confirm the cochlear position visually [4] and continue blunt dissection downward until translucent cochlear tissue is observed [5].
- 2.8.1. Talent placing the second temporal bone under the stereomicroscope on an ice-cooled surface. **TXT: Perform the following procedures on ice**

- 2.8.2. Close-up of one pair of forceps holding the temporal bone steady.
- 2.8.3. SCOPE: Close-up of another pair of forceps performing blunt dissection to expose the white semicircular ossicle ring. *Videographer: Please film the above-SCOPE shots using the scope kit*
- 2.8.4. SCOPE: Clear view of the cochlear position under the stereomicroscope.
- 2.8.5. SCOPE: Continued blunt dissection revealing translucent cochlear tissue.

- 2.9. Using micro-dissection forceps, carefully remove a small piece of the bone wall of the cochlea while keeping the tissue on ice [1]. Gently insert one side of the forceps into the cochlea [2] and carefully separate the bone wall from the membranous labyrinth [3]. Remove the detached bone wall [4] and extend the membranous labyrinth tissue into fresh Hank's Balanced Salt Solution [5].
 - 2.9.1. SCOPE: Micro-dissection forceps removing a small piece of bone wall of the cochlea .
 - 2.9.2. SCOPE: One side of the forceps being gently inserted into the cochlea.
 - 2.9.3. SCOPE: Separation of the bone wall from the membranous labyrinth.
 - 2.9.4. SCOPE: Removal of the loosened bone wall.
 - 2.9.5. SCOPE: transferring and spreading the membranous labyrinth tissue into fresh Hank's Balanced Salt Solution on ice. **NOTE: Authors modified this shot into a scope shot**

- 2.10. Using one pair of forceps, secure the basilar membrane [1]. Next, using another pair of forceps, carefully separate the stria vascularis [2-TXT].
 - 2.10.1. SCOPE: One pair of forceps holding the basilar membrane steady.
 - 2.10.2. SCOPE: Second pair of forceps separating the stria vascularis from surrounding tissue. **TXT: Repeat the harvesting procedure on the contralateral side**

3. Enzymatic Disaggregation for Obtaining Marginal Cells of Stria Vascularis

Demonstrator: Qingyan Zhou

- 3.1. Transfer the isolated stria vascularis into a 1.5-milliliter microcentrifuge tube containing 1 milliliter of collagenase type II (2) solution placed on ice [1].
 - 3.1.1. Talent placing the isolated stria vascularis into a 1.5 milliliter microcentrifuge

tube containing collagenase type II solution on ice.

- 3.2. Place the sample in a 37 degrees Celsius incubator for 30 minutes [1]. Next, using a centrifuge, spin the tube at 300 *g* for 8 minutes [2].
 - 3.2.1. Talent placing the 1.5 milliliter microcentrifuge tube containing tissue in collagenase type II into a 37 degrees Celsius incubator.
 - 3.2.2. Talent placing the microcentrifuge tube into the centrifuge and closing the lid.
- 3.3. Carefully remove the supernatant from the tube [1] and add 1 milliliter of culture medium to the pellet [2]. Gently triturate to terminate digestion [3] and centrifuge the tube again at 300 *g* for 8 minutes [4].
 - 3.3.1. Close-up of a pipette aspirating the supernatant without disturbing the pellet.
 - 3.3.2. Talent adding 1 milliliter of culture medium into the tube.
 - 3.3.3. Talent gently pipetting up and down to resuspend the cells.
 - 3.3.4. Talent placing the tube back into the centrifuge and closing the lid.
- 3.4. Next, aspirate and discard the supernatant [1]. Add 1 milliliter of fresh culture medium to the pellet [2]. Triturate gently to obtain a single-cell suspension [3]. Transfer the suspension into a 15-milliliter conical tube [4] and add additional culture medium to adjust the total volume to 4 milliliters [5-TXT].
 - 3.4.1. Close-up of supernatant being carefully removed from the tube.
 - 3.4.2. Talent adding 1 milliliter of fresh culture medium into the tube.
 - 3.4.3. Talent gently pipetting to create a single-cell suspension.
 - 3.4.4. Talent transferring the cell suspension into a 15 milliliter conical tube.
 - 3.4.5. Talent adding additional culture medium to reach a total volume of 4 milliliters.
TXT: Pool tissues harvested from 12 neonatal mice
- 3.5. Mix the cell suspension thoroughly by repeated pipetting [1]. Transfer 1 milliliter of the suspension into each well of a 6-well plate [2] and add DMEM supplemented with 2% FBS, 1% Epi-CGS-a, and 1% penicillin-streptomycin [3]. Place the plate in a 37 degrees Celsius incubator with 5 percent carbon dioxide to culture the cells [4].
 - 3.5.1. Talent pipetting the suspension up and down repeatedly in the 15 milliliter conical tube.
 - 3.5.2. Talent dispensing 1 milliliter of the suspension into each well of a 6 well plate.

- 3.5.3. Talent adding supplemented culture medium to each well until the total volume reaches 2 milliliters.
- 3.5.4. Talent placing the 6 well plate into a 37 degrees Celsius incubator set to 5 percent carbon dioxide.
- 3.6. Aspirate the old medium from the well using a pipette [1]. Add fresh Hank's Balanced Salt Solution to wash and then aspirate it [2]. Add fresh culture medium [3]. Monitor the cell status regularly under a microscope [4]. Observe clustered growth of marginal cells approximately 2 days after plating [5].
 - 3.6.1. Talent aspirating old medium from a well.
 - 3.6.2. Added shot: Talent adding fresh Hank's Balanced Salt Solution to wash and then aspirating it.
 - 3.6.3. Added shot: Talent adding fresh culture medium.
 - 3.6.4. Talent placing the culture plate under a microscope for observation.
 - 3.6.5. LAB MEDIA: Figure2Aa.

4. Purification of Marginal Cells

- 4.1. On day 3, identify numerous spindle-shaped fibroblasts surrounding the cobblestone-like clusters of marginal cells [1].
 - 4.1.1. LAB MEDIA: Figure2Ba (upper panel).
- 4.2. Using a 1 milliliter pipette, gently pipette to partially remove the spindle-shaped fibroblasts based on their different adhesion rates [1]. Gently flush D-Hanks balanced salt solution over large areas in each of the 4 quadrants of the culture dish [2]. Examine the fibroblast density microscopically to later adjust the cell density. [3-TXT].
 - 4.2.1. Close-up of a 1 milliliter pipette gently aspirating medium to partially detach spindle-shaped fibroblasts.
 - 4.2.2. Talent flushing D-Hanks balanced salt solution across each of the 4 quadrants of the culture dish.
 - 4.2.3. LAB MEDIA: Figure2Bb **TXT: Repeat the large-area flushing procedure 2x in total to achieve uniform fibroblast distribution across the dish**

4.3. ~~Finally, cease purification once fibroblasts appear sparsely distributed among marginal cell clusters under microscopic examination [1].~~ Using a pipette, remove the spent medium [2] and add 2 milliliters of fresh culture medium to continue cultivation [3-TXT]. Perform imaging following immunostaining marginal cells with anti-CK18 and KCNQ1 antibodies [3], and treat the marginal cells with D-galactose to induce cellular senescence [4].

4.3.1. ~~SCOPE: View showing sparsely distributed fibroblasts among marginal cell clusters.~~

4.3.2. Close-up of a pipette aspirating and removing the media from the culture dish.

4.3.3. Talent adding 2 milliliters of fresh culture medium into the culture dish.

4.3.4. *Reuse 3.6.4*

4.3.5. *Reuse 4.2.2 or 3.5.3 and 3.5.4* **NOTE:** These 2 shots are added at the postshoot stage to replace the on-screen text with a shot to bridge the results

Results

5. Results

5.1. On day 2 after cell purification, immunofluorescence staining revealed CK18 [1] and KCNQ1 expression in the cultured cells [1].

5.1.1. LAB MEDIA: Figure 2C. *Video editor: Highlight the green staining labeled CK18.*

5.1.2. LAB MEDIA: Figure 2D. *Video editor: Highlight the green staining labeled KCNQ1.*

5.2. The half maximal inhibitory concentration for hydrogen peroxide was determined from the dose-response curve after assessing cell viability at different hydrogen peroxide concentrations using the CCK-8 assay [1].

5.2.1. LAB MEDIA: Figure 3A B.

5.3. Compared to the untreated control group [1], treatment with 1 millimolar hydrogen peroxide markedly increased intracellular green fluorescence, indicating increased reactive oxygen species accumulation [2].

5.3.1. LAB MEDIA: Figure 3C D. *Video editor: Highlight the CM-H2DCFH-DA image for Con and C bar. Please use the new updated image available on the text review page*

5.3.2. LAB MEDIA: Figure 3C D. *Video editor: Highlight green CM-H2DCFH-DA image for H2O2 and H bar. Please use the new updated image available on the text review page*

5.4. D-galactose-treated marginal cells showed a significant increase in senescence-associated beta-galactosidase-positive staining, indicating cellular senescence [1], compared to untreated controls [2].

5.4.1. LAB MEDIA: Figure 4A. *Video editor: Highlight the cells in the D-gal image.*

5.4.2. LAB MEDIA: Figure 4A. *Video editor: Highlight the cells in the Con image.*

1. Autoclave

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

- IPA: /'ɔ:.tuʊ.kleɪv/
Phonetic Spelling: aw·toh·klayv
2. Sterilize
Pronunciation link: <https://www.merriam-webster.com/dictionary/sterilize>
IPA: /'ster.ə.laɪz/
Phonetic Spelling: ster·uh·lyze
 3. Biological
Pronunciation link: <https://www.merriam-webster.com/dictionary/biological>
IPA: /,baɪ.ə'lɑ:.dʒɪ.kəl/
Phonetic Spelling: by·uh·laa·juh·kuhl
 4. Ethanol
Pronunciation link: <https://www.merriam-webster.com/dictionary/ethanol>
IPA: /'eθ.ə.nɔ:l/
Phonetic Spelling: eth·uh·nawl
 5. Stereomicroscope
Pronunciation link: <https://www.merriam-webster.com/dictionary/stereomicroscope>
IPA: /,ster.i.oo'maɪ.krə.skəʊp/
Phonetic Spelling: ster·ee·oh·my·kruh·skoʊp
 6. Ultraviolet
Pronunciation link: <https://www.merriam-webster.com/dictionary/ultraviolet>
IPA: /,ʌl.trə'vaɪ.ə.lət/
Phonetic Spelling: ul·truh·vy·uh·luht
 7. Neonatal
Pronunciation link: <https://www.merriam-webster.com/dictionary/neonatal>
IPA: /,ni:.oo'nei.təl/
Phonetic Spelling: nee·oh·nay·tuhl
 8. Isoflurane
Pronunciation link: <https://www.howtopronounce.com/isoflurane>
IPA: /,aɪ.sou'flʊr.əm/
Phonetic Spelling: eye·soh·floor·ayn
 9. Biosafety
Pronunciation link: <https://www.merriam-webster.com/dictionary/biosafety>
IPA: /,baɪ.oo'seɪf.ti/
Phonetic Spelling: by·oh·sayf·tee
 10. Immobilize
Pronunciation link: <https://www.merriam-webster.com/dictionary/immobilize>
IPA: /ɪ'moʊ.bə.laɪz/
Phonetic Spelling: ih·moh·buh·lyze
 11. Sagittal
Pronunciation link: <https://www.merriam-webster.com/dictionary/sagittal>
IPA: /'sædʒ.ə.təl/
Phonetic Spelling: saj·uh·tuhl
 12. Bilaterally
Pronunciation link: <https://www.merriam-webster.com/dictionary/bilaterally>

- IPA: /ˌbɑːlˈlæt.ə.ə.li/
Phonetic Spelling: by·lat·er·uh·lee
13. Temporal
Pronunciation link: <https://www.merriam-webster.com/dictionary/temporal>
IPA: /ˈtem.pə.rəl/
Phonetic Spelling: tem·pruhl
14. Cochlea
Pronunciation link: <https://www.merriam-webster.com/dictionary/cochlea>
IPA: /ˈkɑːk.li.ə/
Phonetic Spelling: kok·lee·uh
15. Cochlear
Pronunciation link: <https://www.merriam-webster.com/dictionary/cochlear>
IPA: /ˈkɑːk.li.ə/
Phonetic Spelling: kok·lee·er
16. Ossicle
Pronunciation link: <https://www.merriam-webster.com/dictionary/ossicle>
IPA: /ˈɑː.sɪ.kəl/
Phonetic Spelling: ah·si·kuhl
17. Membranous
Pronunciation link: <https://www.merriam-webster.com/dictionary/membranous>
IPA: /ˈmem.brə.nəs/
Phonetic Spelling: mem·bruh·nuhs
18. Labyrinth
Pronunciation link: <https://www.merriam-webster.com/dictionary/labyrinth>
IPA: /ˈlæb.ə.rɪnθ/
Phonetic Spelling: lab·uh·rinth
19. Basilar
Pronunciation link: <https://www.merriam-webster.com/dictionary/basilar>
IPA: /ˈbæs.ə.lə/
Phonetic Spelling: bass·uh·ler
20. Vascularis
Pronunciation link: <https://www.howtopronounce.com/vascularis>
IPA: /ˌvæs.kjəˈler.ɪs/
Phonetic Spelling: vas·kyuh·lair·iss
21. Enzymatic
Pronunciation link: <https://www.merriam-webster.com/dictionary/enzymatic>
IPA: /ˌen.zəˈmæt.ɪk/
Phonetic Spelling: en·zy·mat·ik
22. Disaggregation
Pronunciation link: <https://www.merriam-webster.com/dictionary/disaggregation>
IPA: /ˌdɪs.æɡ.rɪˈɡeɪ.ʃən/
Phonetic Spelling: dis·ag·ri·gay·shuhn
23. Collagenase
Pronunciation link: <https://www.howtopronounce.com/collagenase>

- IPA: /kəˈlædʒ.ə.neɪs/
Phonetic Spelling: kuh·laj·uh·nays
24. Microcentrifuge
Pronunciation link: <https://www.howtopronounce.com/microcentrifuge>
IPA: /ˌmaɪ.kroʊˈsen.trɪ.fjuːdʒ/
Phonetic Spelling: my·kroh·sen·truh·fyooj
25. Incubator
Pronunciation link: <https://www.merriam-webster.com/dictionary/incubator>
IPA: /ˈɪn.kjəˌbeɪ.tər/
Phonetic Spelling: in·kyuh·bay·ter
26. Centrifuge
Pronunciation link: <https://www.merriam-webster.com/dictionary/centrifuge>
IPA: /ˈsen.trəˌfjuːdʒ/
Phonetic Spelling: sen·truh·fyooj
27. Supernatant
Pronunciation link: <https://www.merriam-webster.com/dictionary/supernatant>
IPA: /ˌsuː.pəˈneɪ.tənt/
Phonetic Spelling: soo·per·nay·tuhtnt
28. Triturate
Pronunciation link: <https://www.merriam-webster.com/dictionary/triturate>
IPA: /ˈtraɪ.tʃəˌreɪt/
Phonetic Spelling: try·chuh·rayt
29. Suspension
Pronunciation link: <https://www.merriam-webster.com/dictionary/suspension>
IPA: /səˈspen.ʃən/
Phonetic Spelling: suh·spen·shuhn
30. Conical
Pronunciation link: <https://www.merriam-webster.com/dictionary/conical>
IPA: /ˈkɑː.nɪ.kəl/
Phonetic Spelling: kah·ni·kuhl
31. Supplemented
Pronunciation link: <https://www.merriam-webster.com/dictionary/supplement>
IPA: /ˈsʌp.lə.mənt/
Phonetic Spelling: sup·luh·ment
32. Fibroblasts
Pronunciation link: <https://www.merriam-webster.com/dictionary/fibroblast>
IPA: /ˈfaɪ.broʊˌblæst/
Phonetic Spelling: fy·broh·blast
33. Adhesion
Pronunciation link: <https://www.merriam-webster.com/dictionary/adhesion>
IPA: /ədˈhiː.zən/
Phonetic Spelling: ad·hee·zhuhn
34. Immunostaining
Pronunciation link: <https://www.howtopronounce.com/immunostaining>

IPA: /ɪˌmjuː.nooˈsteɪ.nɪŋ/

Phonetic Spelling: ih·myoo·noh·stay·ning

35. Senescence

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

IPA: /siˈnes.əns/

Phonetic Spelling: sih·nes·uhns

36. Immunofluorescence

Pronunciation link: <https://www.howtopronounce.com/immunofluorescence>

IPA: /ɪˌmjuː.noo.fluˈres.əns/

Phonetic Spelling: ih·myoo·noh·flu·res·uhns

37. Inhibitory

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

IPA: /ɪnˈhib.əˌtɔːr.i/

Phonetic Spelling: in·hib·uh·tor·ee

38. Intracellular

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

IPA: /ɪnˌtrəˈsel.jə.lə/

Phonetic Spelling: in·truh·sel·yuh·ler

39. Fluorescence

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

IPA: /fluˈres.əns/

Phonetic Spelling: flu·res·uhns

40. Galactose

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

IPA: /gəˈlæk.toʊs/

Phonetic Spelling: guh·lak·tohs