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Title: A Protocol for Harvesting Single-Cell Suspension from Mouse Corneas

Authors and Affiliations:

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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? **Yes, all done**

SCOPE shots: 2.2.1, 2.2.2, 2.2.3, 2.2.4

3. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

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: **3/7/2026**

When you are ready to submit your video files, please contact our China Location Producer, [Yuan Yue](#).

Current Protocol Length

Number of Steps: 7

Number of Shots: 17 (4 Scope)

Introduction

Authors: Please note that **the questions will not appear on screen**. Please answer in **stand-alone full sentence with sufficient context**.

INTRODUCTION:

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

- 1.1. ~~Naikun Jiang~~~~Shangkun Ou~~: This protocol primarily introduces a method for obtaining high-quality single cells from intact mouse corneas.

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

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

- ~~1.2. Shangkun Ou: Conventional methods yield poor cell viability and low single-cell recovery. This protocol improves outcomes by optimizing digestive enzyme selection and timing based on corneal structure.~~

- 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera **NOTE: Not filmed**

~~Describe the research contexts or systems in which this protocol can be applied.~~

- 1.3. **Naikun Jiang**: Our method yields viable single cells with high RNA integrity, making them compatible with downstream applications such as single-cell RNA sequencing and flow cytometry.

- 1.3.1. 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?~~

- 1.4. **Chao Huang**: After obtaining single cells, we can characterize corneal cell function and surface marker expression. This facilitates the study of cell-cell interactions.

- 1.4.1. 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.~~

- 1.5. **Chao Huang:** A challenge in this method is precisely separating the cornea from other tissues.
 - 1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

Ethics Title Card

This research has been approved by the Experimental Animal Ethics Committee at Guizhou Medical University

Protocol

2. Preparation of Mouse Cornea for Isolation of Corneal Single Cells

Demonstrators: Chao Huang, Naikun Jiang

2.1. To begin, position a 6 to 8-week-old euthanised female C57BL-6 mouse on a sterile dissection table [1-TXT]. After rinsing the closed eyelids with PBS, use ophthalmic scissors to make an incision along the posterior pole of the eyeball [2] and carefully place the isolated eye into a Petri dish containing ice-cold Supplemental Hormonal Epithelial Medium [3].

2.1.1. WIDE: Talent placing the euthanized animal on the sterile work surface. **TXT: Euthanasia: CO₂ asphyxiation, followed by cervical dislocation**

2.1.2. Talent making an incision along the posterior pole using ophthalmic scissors.

2.1.3. Talent transferring the isolated eyeballs into a Petri dish containing ice-cold medium.

2.2. Rinse the eyeball 3 times with 1× PBS supplemented with 5% penicillin-streptomycin under sterile conditions [1]. Under a dissecting microscope, use ophthalmic scissors and forceps to make an incision from the posterior pole toward the corneal limbus [2]. Gently detach the cornea from the sclera and conjunctiva along the limbal border [3]. Then, using two pairs of forceps, completely remove the lens and iris [4].

2.2.1. SCOPE: 2.2.1-2.

2.2.2. SCOPE: 2.2.2-1 00:03-00:06 and 00:15-00:19.

2.2.3. SCOPE: 2.2.3-1 01:09-01:15.

2.2.4. SCOPE: 2.4.4-4. 00:11-0:14 and 00:31-00:33

2.3. After washing the corneal tissue in PBS, mince it into fragments of approximately 0.5 by 0.5 millimeters using ophthalmic scissors [1]. Incubate the tube containing the corneal fragments with collagenase A solution in a cell culture incubator at 37 degrees Celsius for 1 hour [2].

2.3.1. Talent mincing corneal tissue into small fragments inside a tube using ophthalmic scissors.

2.3.2. Talent placing the sample in the incubator.

- 2.4. Inspect the tissue every 10 minutes during the digestion [1]. When no large tissue fragments remain, centrifuge the sample at 300 *g* for 5 minutes at room temperature [2]. Then, carefully aspirate the supernatant [3].
 - 2.4.1. Talent visually checking the digestion tube.
 - 2.4.2. Talent placing the tube into the centrifuge.
 - 2.4.3. Talent aspirating and removing the supernatant from the centrifuged sample.
- 2.5. Next, resuspend the pellet in 200 microliters of 0.25 percent trypsin EDTA solution [1].
 - 2.5.1. Talent adding trypsin ethylenediaminetetraacetic acid solution to the pellet and gently resuspending.
- 2.6. Place the tube in a cell culture incubator set to 37 degrees Celsius for 10 minutes to digest remaining tissue fragments [1].
 - 2.6.1. Talent placing the tube into a cell culture incubator.
- 2.7. Finally, use a P-1000 pipette fitted with a wide-bore tip to gently triturate the mixture to obtain a single-cell suspension [1]. Centrifuge the cell suspension as demonstrated earlier and resuspend the cell pellet in PBS containing 0.04% BSA [2]
 - 2.7.1. 2.7.1 00:05-00:10.
 - 2.7.2. *Reuse 2.4.2 00:05-00:13 and 2.7.1 00:01-00:03*

Results

3. Results

- 3.1. Upon counting the isolated corneal cells using a hemocytometer [1], it was found that a total of 1.05×10^5 cells were obtained from 8 corneas, with an average cell viability of 94.6% and a single-cell ratio exceeding 96.3%, confirming the suspension's suitability for downstream applications [2].

3.1.1. LAB MEDIA: Figure 3

3.1.2. LAB MEDIA: Figure 3. *Video editor: Highlight the round unstained white cells. DO NOT highlight the blue cells pointed by red arrows*

- 3.2. Immunofluorescence analysis showed that the cell suspension comprised 83.5% Pan-CK-positive epithelial cells [1], 13.5% keratocan-positive stromal cells [2], and approximately 3% cells negative for both markers [3].

3.2.1. LAB MEDIA: Figure 4. *Video editor: Highlight the green-stained areas in B.*

3.2.2. LAB MEDIA: Figure 4. *Video editor: Highlight the red-stained areas in A.*

3.2.3. LAB MEDIA: Figure 4.

- 3.3. The RNA extracted from the single-cell suspension exhibited a high RNA integrity number of around 8.8 [1] and a 28S by 18S ribosomal RNA ratio of about 2.15, confirming excellent RNA quality [2].

3.3.1. LAB MEDIA: Figure 5.

3.3.2. LAB MEDIA: Figure 5. *Video editor: Highlight the peaks 1905 and 4572*

1. Ophthalmic

Pronunciation

link:

[[https://www.merriam-](https://www.merriam-webster.com/dictionary/ophthalmic)

[webster.com/dictionary/ophthalmic](https://www.merriam-webster.com/dictionary/ophthalmic)]([https://www.merriam-](https://www.merriam-webster.com/dictionary/ophthalmic)

IPA: /äf'thal-mik/

Phonetic Spelling: aaf·THAL·mik

2. Posterior

Pronunciation link: https://www.merriam-webster.com/dictionary/posterior

IPA: /pō'stir-ē-ər/

Phonetic Spelling: poh·STEER·ee·er

3. Euthanasia

Pronunciation link: https://www.merriam-webster.com/dictionary/euthanasia

IPA: /,yü-thə-'nā-zhə/

Phonetic Spelling: yoo·thuh·NAY·zhuh

4. Asphyxiation

Pronunciation link: https://www.merriam-webster.com/dictionary/asphyxiation

IPA: /as-'fik-sē-'ā-shən/

Phonetic Spelling: as·fik·see·AY·shun

5. Cervical

Pronunciation link: https://www.merriam-webster.com/dictionary/cervical

IPA: /'sər-vi-kəl/

Phonetic Spelling: SUR·vi·kul

6. Corneal

Pronunciation link: https://www.merriam-webster.com/dictionary/corneal

IPA: /'kôr-nē-əl/

Phonetic Spelling: KOR·nee·ul

7. Limbus

Pronunciation link: https://www.merriam-webster.com/dictionary/limbus

IPA: /'lim-bəs/

Phonetic Spelling: LIM·bus

8. Sclera

Pronunciation link: https://www.merriam-webster.com/dictionary/sclera

IPA: /'sklir-ə/

Phonetic Spelling: SKLEER·uh

9. Conjunctiva

Pronunciation link: https://www.merriam-webster.com/dictionary/conjunctiva

IPA: /,kän-jəŋ(k)-'tī-və/

Phonetic Spelling: kahn·jungk·TY·vuh

10. Collagenase

Pronunciation link: https://www.merriam-webster.com/dictionary/collagenase

IPA: /kə-'la-jə-,nās/

Phonetic Spelling: kuh·LAJ·uh·nays

11. Centrifuge

Pronunciation link: https://www.merriam-webster.com/dictionary/centrifuge

IPA: /'sen-trə-,fyüj/

Phonetic Spelling: SEN·truh·fyooj

12. Aspirate

Pronunciation link: [https://www.merriam-

webster.com/dictionary/aspirate](https://www.merriam-webster.com/dictionary/aspirate)

IPA: /'as-pə-,rāt/

Phonetic Spelling: AS·puh·rayt

13. Supernatant

Pronunciation link: https://www.merriam-webster.com/dictionary/supernatant

IPA: /,sü-pər-'nā-tənt/

Phonetic Spelling: soo·per·NAY·tunt

14. Microliters

Pronunciation link: https://www.merriam-webster.com/dictionary/microliter

IPA: /'mī-krō-,lē-tər/

Phonetic Spelling: MY·kroh·lee·ter

15. Trypsin

Pronunciation link: https://www.merriam-webster.com/dictionary/trypsin

IPA: /'trip-sən/

Phonetic Spelling: TRIP·sun

16. Ethylenediaminetetraacetic

Pronunciation link: No confirmed link found

IPA: /,εθə,li:n,darə,mi:n,tetrə'si:tɪk/

Phonetic Spelling: eth·uh·leen·dy·uh·meen·teh·truh·uh·SEE·tik

17. Pipette

Pronunciation link: https://www.merriam-webster.com/dictionary/pipette

IPA: /pī-'pet/

Phonetic Spelling: py·PET

18. Triturate

Pronunciation link: https://www.merriam-webster.com/dictionary/triturate

IPA: /'trɪch-ə-,rāt/

Phonetic Spelling: TRICH·uh·rayt

19. Dissociate

Pronunciation link: https://www.merriam-webster.com/dictionary/dissociate

IPA: /di-'sō-shē-,āt/

Phonetic Spelling: di·SOH·shee·ayt

20. Hemocytometer

Pronunciation link: https://www.merriam-webster.com/dictionary/hemocytometer

IPA: /,hē-mō-sī-'tä-mə-tər/

Phonetic Spelling: hee·moh·sy·TAH·muh·ter

21. Viability

Pronunciation link: https://www.merriam-webster.com/dictionary/viability

IPA: /,vī-ə-'bi-lə-tē/

Phonetic Spelling: vy·uh·BIH·luh·tee

22. Immunofluorescence

Pronunciation link: https://www.merriam-webster.com/dictionary/immunofluorescence

IPA: /,i-myə-nō-,flūr-'e-sʰn(t)s/

Phonetic Spelling: im·yuh·noh·floor·ES·unts

23. Epithelial

Pronunciation link: https://www.merriam-webster.com/dictionary/epithelial

IPA: /,e-pə-'thē-lē-əl/

Phonetic Spelling: ep·uh·THEE·lee·ul

24. Keratocan

Pronunciation link: No confirmed link found

IPA: No confirmed IPA found

Phonetic Spelling: No confirmed phonetic spelling found

25. Stromal

Pronunciation link: https://www.merriam-webster.com/dictionary/stromal

IPA: /'strō-məl/

Phonetic Spelling: STROH·mul

26. Ribosomal

Pronunciation link: https://www.merriam-webster.com/dictionary/ribosomal

IPA: /,rī-bə-'sō-məl/

Phonetic Spelling: ry·buh·SOH·mul