

Submission ID #: 70923

Scriptwriter Name: Sri Lakshmi Priya

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Title: Novel Combination of Antioxidants to Restore Healthy Intercellular Communication in an In Vitro Model Using Conditioned Medium from Senescent Cells

Authors and Affiliations:

Alfredo Martínez-Gutierrez, Helena Cami

Biotechnology Unit, Mesoesthetic Pharma Group

Corresponding Authors:

Alfredo Martinez-Gutierrez amartinez@mesoesthetic.com

Email Addresses for All Authors:

Alfredo Martinez-Gutierrez amartinez@mesoesthetic.com
Helena Cami hcam@mesoesthetic.com

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

☐ Incorrect

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? **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: **29/05/2026**

When you are ready to submit your video files, please contact our Content Manager, [Utkarsh Khare](#).

Current Protocol Length

Number of Steps: 16

Number of Shots: 32

Interviews

INTRODUCTION:

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

- 1.1. **Alfredo Martínez-Gutiérrez:** Our research is focused on developing a novel in vitro method to characterize altered intercellular communication between senescent and healthy dermal cells.
 - 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. **Alfredo Martínez-Gutiérrez:** This protocol can be applied to study the influence of compounds on key hallmarks of skin aging.
 - 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. **Alfredo Martínez Gutiérrez:** The most important consideration is achieving a successful senescent state to obtain the full secretory phenotype characteristic of skin aging.
 - 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. **Alfredo Martínez Gutiérrez:** Additionally, ELISA or Western Blot can be performed using this protocol to measure specific protein changes in dermal cells.
 - 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. **Alfredo Martínez-Gutiérrez:** Future studies can build on this work by investigating novel dysregulated markers, including mitochondrial alterations and specific aging pathway damage.

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

Ethics Title Card

Human dermal fibroblasts utilized in this protocol were commercially sourced; therefore, Institutional Review Board approval was not required for this study

Protocol

2. Senescence Induction in Dermal Fibroblasts Using UVB

Demonstrator: Laia Grau, Biotechnology Unit, mesoesthetic Pharma Group, Viladecans

- 2.1. To begin, plate human dermal fibroblasts in a 6-well plate at a density of 60000 cells per well using Dulbecco's Modified Eagle Medium or DMEM (*D-M-E-M*) supplemented with 10% FBS [1].
 - 2.1.1. WIDE: Talent adding human dermal fibroblasts into a 6-well plate containing DMEM.
- 2.2. After 24 hours, remove the cell culture medium [1] and add 1-milliliter of PBS to each well [2].
 - 2.2.1. Talent aspirating the cell culture medium from the 6-well plate.
 - 2.2.2. Talent adding PBS into the well.
- 2.3. Irradiate the cells with ultraviolet B light or UVB (*U-V-B*) at 25 millijoules per square centimeter to induce cellular damage associated with senescence [1].
 - 2.3.1. Talent placing the 6-well plate inside the ultraviolet B irradiation device.
- 2.4. Remove the PBS from the wells [1]. Add 2 milliliters of fresh cell culture medium to each well [2].
 - 2.4.1. Talent aspirating PBS from the 6-well plate.
 - 2.4.2. Talent adding fresh cell culture medium into the well.
- 2.5. After 72 hours, remove the cell culture medium from the wells [1]. Add 1 milliliter of PBS to each well [2]. Repeat UVB irradiation as previously demonstrated [3].
 - 2.5.1. *Reuse 2.2.1*
 - 2.5.2. *Reuse 2.2.2*
 - 2.5.3. *Reuse 2.3.1*
- 2.6. Following 72 hours, remove the cell culture medium from the wells [1]. Add 0.5 milliliters of 0.05% EDTA to each well [2]. Incubate the plate at 37 degrees Celsius for 1 to 2 minutes [3]. Harvest the cells using 1.5 milliliters of fresh cell culture medium [4].
 - 2.6.1. Talent aspirating the cell culture medium from the wells.
 - 2.6.2. Talent adding trypsin-EDTA solution into the well.
 - 2.6.3. Talent placing the 6-well plate in the incubator.

2.6.4. Talent adding fresh cell culture medium and collecting the detached cells from the 6-well plate.

2.7. Plate the harvested cells in a 6-well plate at a density of 80000 cells per well using cell culture medium [1]. Induce cellular senescence after plating the cells [2].

2.7.1. Talent plating the harvested cells into a new 6-well plate containing cell culture medium.

2.7.2. Shot of the plated senescent dermal fibroblasts in the 6-well plate

3. Compound Treatment and Conditioned Medium Transfer

3.1. After 24 hours, remove the cell culture medium from the wells [1]. Add 2 milliliters of cell culture medium containing the compounds of interest to each well [2].

3.1.1. Talent aspirating the cell culture medium from the 6-well plate.

3.1.2. Talent adding cell culture medium into the well.

3.2. Following an additional 24 hours, plate a separate batch of human dermal fibroblasts in a 6-well plate as demonstrated previously [1].

3.2.1. Talent plating human dermal fibroblasts into a 6-well plate containing cell culture medium.

3.3. At 48 hours after compound treatment of senescent cells and 24 hours after plating healthy fibroblasts, collect 2 milliliters of conditioned medium from each well of the senescent cells [1]. Transfer the conditioned medium to the healthy fibroblasts [2].

3.3.1. Talent collecting conditioned medium from the senescent fibroblast wells.

3.3.2. Talent transferring conditioned medium into the wells containing healthy fibroblasts.

3.4. Add 1 milliliter of PBS to the senescent cells [1]. Harvest the cells using trypsin as previously demonstrated [2]. Using an ELISA (*ee-LYE-zuh*) or qPCR (*Q-P-C-R*), analyze the harvested cells for senescence or inflammation markers [3].

3.4.1. Talent adding PBS to the senescent cell wells.

3.4.2. Talent adding trypsin and harvesting the senescent cells.

3.4.3. Shot of harvested cells being analyzed using ELISA or qPCR.

3.5. Place the healthy fibroblasts receiving conditioned medium at 37 degrees Celsius with 5% carbon dioxide for 24 hours [1].

3.5.1. Talent placing the 6-well plate containing healthy fibroblasts into the incubator.

4. Analysis of Extracellular Matrix Gene Expression

- 4.1. Harvest the healthy fibroblasts using trypsin as previously demonstrated [1].
 - 4.1.1. Talent adding trypsin and harvesting healthy fibroblasts.
- 4.2. With an RNA (*R-N-A*) extraction kit, extract RNA from the cell pellets [1].
 - 4.2.1. Talent sitting with the RNA extraction kit, pipette and a tray of tubes.
- 4.3. Using a reverse transcription kit [1], perform reverse transcription of 200 nanograms of RNA [2].
 - 4.3.1. Talent sitting with the reverse transcription kit.
 - 4.3.2. Talent adding 200 nanograms of RNA into the tube containing reverse transcription reaction.
- 4.4. Prepare the qPCR master mix [1]. Run the qPCR reaction [2] to analyze extracellular matrix gene expression [3].
 - 4.4.1. Talent adding reagents into a tube.
 - 4.4.2. TEXT ON A PLAIN BACKGROUND:
95°C - 30 sec
40 cycles: 95°C - 5 sec
60°C - 30 sec
 - 4.4.3. SCREEN: 70923_screenshot_1.jpg

Results

5. Results

- 5.1. After repeated UVB irradiation, senescent fibroblasts exhibited characteristic senescent features [1], including increased expression of CDKN2A (*C-D-K-N-two-A*) [2] and CDKN1A (*C-D-K-N-one-A*) [3] compared to non-irradiated cells, confirming the senescent phenotype [4].
 - 5.1.1. LAB MEDIA: Figure 2A-B. *Video editor: Emphasize figure B.*
 - 5.1.2. LAB MEDIA: Figure 2C. *Video editor: Emphasize the blue bar labelled CDKN2A under UVB.*
 - 5.1.3. LAB MEDIA: Figure 2C. *Video editor: Emphasize the orange bar labelled CDKN1A under UVB.*
 - 5.1.4. LAB MEDIA: Figure 2C. *Video editor: Emphasize the blue and orange bars under No UVB.*
- 5.2. The photoaging-associated cytokines Interleukin-18 or IL-18 (*I-L-eighteen*) [1] and SCF (*S-C-F*) were upregulated in senescent dermal fibroblasts after UVB irradiation [2] compared to the non-irradiated control group [3].
 - 5.2.1. LAB MEDIA: Figure 3. *Video editor: Emphasize the blue bar labelled “IL-18” in the “Control (+) UVB” group.*
 - 5.2.2. LAB MEDIA: Figure 3. *Video editor: Emphasize the orange bar labelled “SCF” in the “Control (+) UVB” group*
 - 5.2.3. LAB MEDIA: Figure 3. *Video editor: Emphasize the blue and orange bars under control Control (-) UVB” group*
- 5.3. Treatment with vitamin C and idebenone reversed the UVB-induced increase in IL-18 [1] and SCF expression in senescent fibroblasts [2].
 - 5.3.1. LAB MEDIA: Figure 3. *Video editor: Emphasize the blue bar labelled “IL-18” in the “Ideb+VitC” group.*
 - 5.3.2. LAB MEDIA: Figure 3. *Video editor: Emphasize the orange bar labelled “SCF” in the “Ideb+VitC” group.*
- 5.4. Senescent fibroblasts exhibited altered levels of the epigenetic marker H3K27me3 (*H-three-K-twenty-seven-me-three*) after UVB irradiation [1].
 - 5.4.1. LAB MEDIA: Figure 4. *Video editor: Emphasize the bar labelled as control and Ideb+Vit C in the (+) UVB group.*
- 5.5. Treatment with vitamin C and idebenone reversed the altered H3K27me3 levels in senescent fibroblasts [1].

- 5.5.1. LAB MEDIA: Figure 4. *Video editor: Emphasize the bar labelled as Ideb+Vit C in the (+) UVB group.*
- 5.6. Conditioned medium from senescent cells dysregulated the expression of extracellular matrix proteins ELN (*E-L-N*) and FBN1 (*F-B-N-one*) [1], growth factors CTGF (*C-T-G-F*) and IGF1 (*I-G-F-one*) [2], and proinflammatory cytokines Interleukin-6 or IL-6 (*I-L-six*) and Interleukin-8 or IL-8 (*I-L-eight*) in healthy fibroblasts after 24 hours of incubation [3].
 - 5.6.1. LAB MEDIA: Table 2. *Video editor: Emphasize the values in the rows labelled ELN and FBN1 under Conditioned medium from senescent cells in the control column.*
 - 5.6.2. LAB MEDIA: Table 2. *Video editor: Emphasize the values in the rows labelled CTGF and IGF1 under Conditioned medium from senescent cells in the control column.*
 - 5.6.3. LAB MEDIA: Table 2. *Video editor: Emphasize the values in the rows labelled IL-6 and IL-8 under Conditioned medium from senescent cells in the control column.*
- 5.7. Treatment with a combination of vitamin C and idebenone restored the altered expression of extracellular matrix proteins and growth factors by upregulating ELN, FBN1, CTGF, and IGF1 [1], while downregulating the proinflammatory cytokines IL-6 and IL-8 [2].
 - 5.7.1. LAB MEDIA: Table 2. *Video editor: Highlight the rows labelled ELN, FBN1, CTGF, and IGF1 under Conditioned medium from senescent cells in the vitamin C and idebenone column.*
 - 5.7.2. LAB MEDIA: Table 2. *Video editor: Highlight the rows labelled IL-6 and IL-8 under Conditioned medium from senescent cells in the vitamin C and idebenone column.*

Pronunciation Guides:

1. Dulbecco's
 Pronunciation link: <https://www.merriam-webster.com/dictionary/Dulbecco>
 IPA: /duːlˈbekoʊ/
 Phonetic Spelling: dool·BEK·oh
2. Fibroblasts
 Pronunciation link: <https://www.merriam-webster.com/dictionary/fibroblast>
 IPA: /ˈfaɪbrəˌblæst/
 Phonetic Spelling: FYE·broh·blast
3. Dermal
 Pronunciation link: <https://www.merriam-webster.com/dictionary/dermal>

- IPA: /'dɜ:məl/
Phonetic Spelling: DUR·muhl
4. Dulbecco's Modified Eagle Medium
Pronunciation link: <https://www.merriam-webster.com/dictionary/medium>
IPA: /'mi:diəm/
Phonetic Spelling: MEE·dee·uhm
5. PBS
Pronunciation link: No confirmed link found
IPA: /,pi:.bi:'es/
Phonetic Spelling: PEE·BEE·ESS
6. Ultraviolet
Pronunciation link: <https://www.merriam-webster.com/dictionary/ultraviolet>
IPA: /,ʌltrə'vaɪələt/
Phonetic Spelling: UL·truh·VYE·uh·luht
7. UVB
Pronunciation link: No confirmed link found
IPA: /,ju:.vi:'bi:/
Phonetic Spelling: YOO·VEE·BEE
8. Senescence
Pronunciation link: <https://www.merriam-webster.com/dictionary/senescence>
IPA: /sɪ'nɛsəns/
Phonetic Spelling: sih·NESS·uhns
9. EDTA
Pronunciation link: <https://www.merriam-webster.com/dictionary/EDTA>
IPA: /,i:.di:ti:'eɪ/
Phonetic Spelling: EE·DEE·TEE·AY
10. Trypsin
Pronunciation link: <https://www.merriam-webster.com/dictionary/trypsin>
IPA: /'trɪpsɪn/
Phonetic Spelling: TRIP·sin
11. Conditioned
Pronunciation link: <https://www.merriam-webster.com/dictionary/conditioned>
IPA: /kən'dɪʃənd/
Phonetic Spelling: kuhn·DISH·uhnd
12. ELISA
Pronunciation link: <https://www.merriam-webster.com/dictionary/ELISA>
IPA: /ɪ'laɪzə/
Phonetic Spelling: ih·LYE·zuh
13. qPCR
Pronunciation link: No confirmed link found
IPA: /,kju:.pi:.si:'ɑr/
Phonetic Spelling: KYOO·PEE·SEE·AR

14. Inflammation
Pronunciation link: <https://www.merriam-webster.com/dictionary/inflammation>
IPA: /ˌɪnfləˈmeɪʃən/
Phonetic Spelling: in·fluh·MAY·shuhn
15. Carbon dioxide
Pronunciation link: <https://www.merriam-webster.com/dictionary/carbon%20dioxide>
IPA: /ˌkɑrbən daɪˈɑksaɪd/
Phonetic Spelling: KAR·buhn dye·OK·side
16. RNA
Pronunciation link: <https://www.merriam-webster.com/dictionary/RNA>
IPA: /ˌɑr.ənˈeɪ/
Phonetic Spelling: AR·EN·AY
17. Transcription
Pronunciation link: <https://www.merriam-webster.com/dictionary/transcription>
IPA: /trænˈskrɪpʃən/
Phonetic Spelling: tran·SKRIP·shuhn
18. Extracellular
Pronunciation link: <https://www.merriam-webster.com/dictionary/extracellular>
IPA: /ˌɛkstrəˈsɛljələr/
Phonetic Spelling: EK·struh·SEL·yuh·luhr
19. Phenotype
Pronunciation link: <https://www.merriam-webster.com/dictionary/phenotype>
IPA: /ˈfiːnəˌtaɪp/
Phonetic Spelling: FEE·nuh·type
20. Cytokines
Pronunciation link: <https://www.merriam-webster.com/dictionary/cytokine>
IPA: /ˈsaɪtəˌkaɪn/
Phonetic Spelling: SYE·toh·kyne
21. Interleukin
Pronunciation link: <https://www.merriam-webster.com/dictionary/interleukin>
IPA: /ˌɪntərˈluːkɪn/
Phonetic Spelling: IN·ter·LOO·kin
22. Idebenone
Pronunciation link: <https://www.howtopronounce.com/idebenone>
IPA: /ˌaɪdebəˈnoʊn/
Phonetic Spelling: eye·DEB·uh·nohn
23. Epigenetic
Pronunciation link: <https://www.merriam-webster.com/dictionary/epigenetic>
IPA: /ˌɛpɪdʒəˈnɛtɪk/
Phonetic Spelling: ep·ih·juh·NET·ik
24. H3K27me3
Pronunciation link: No confirmed link found

IPA: /eɪtʃ θriː keɪ ˈtwɛnti ˈsevən ɛm iː θriː/

Phonetic Spelling: AITCH·three·KAY·twen·tee·SEV·uhn·EM·EE·three

25. ELN

Pronunciation link: No confirmed link found

IPA: /iː.ɛlˈɛn/

Phonetic Spelling: EE·EL·EN

26. FBN1

Pronunciation link: No confirmed link found

IPA: /ɛf.biːˈɛn wʌn/

Phonetic Spelling: EF·BEE·EN·one

27. CTGF

Pronunciation link: No confirmed link found

IPA: /siː.tiː.dʒiːˈɛf/

Phonetic Spelling: SEE·TEE·JEE·EF

28. IGF1

Pronunciation link: No confirmed link found

IPA: /aɪ.dʒiːˈɛf wʌn/

Phonetic Spelling: EYE·JEE·EF·one

29. CDKN2A

Pronunciation link: No confirmed link found

IPA: /siː.diː.keɪ ɛn tuː ˈeɪ/

Phonetic Spelling: SEE·DEE·KAY·EN·two·AY

30. CDKN1A

Pronunciation link: No confirmed link found

IPA: /siː.diː.keɪ ɛn wʌn ˈeɪ/

Phonetic Spelling: SEE·DEE·KAY·EN·one·AY

31. SCF

Pronunciation link: No confirmed link found

IPA: /ɛs.siːˈɛf/

Phonetic Spelling: ESS·SEE·EF

32. Vitamin

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

IPA: /ˈvaɪtəˌmɪn/, /ˈvɪtəˌmɪn/

Phonetic Spelling: VYE·tuh·min; VIT·uh·min