

Submission ID #: 71578

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Title: Preparation of Decellularized Human Peripheral Nerve Grafts Using Sodium Hydroxide and Sodium Deoxycholate

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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? **No**

3. Filming location: Will the filming need to take place in multiple locations? **Yes**

The filming may take place in two laboratory areas located on the 2nd and 3rd floors of the same building. The locations are easily accessible by elevator or stairs.

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: 16

Number of Shots: 35

Interviews

Videographer: Obtain headshots for all authors available at the filming location.

INTRODUCTION:

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

- 1.1. **Subin Kim:** Our research develops decellularized human nerve grafts to achieve effective cellular removal while preserving the native extracellular matrix architecture.
 - 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. **Jiyeon Mun:** Conventional detergents can damage the extracellular matrix and leave cytotoxic residues; controlled NaOH, mild detergent, and continuous washing address both limitations.
 - 1.2.1. 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?~~

- 1.3. **Jiyeon Mun:** This protocol enables evaluation of decellularization efficiency, extracellular matrix preservation, residual detergent, and cytocompatibility in human nerve grafts.
 - 1.3.1. 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.~~

- 1.4. **Jiyeon Mun:** The main challenge is maintaining uniform solution exchange by keeping nerves suspended and preventing tissue folding or adhesion during processing.
 - 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. **Subin Kim:** Future studies can evaluate process scale-up, donor variability, longer grafts, and regenerative performance under clinically relevant conditions.
 - 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.*

2. Preparation of Solutions and Peripheral Nerve Segments

Demonstrator: Subin Kim

- 2.1. Begin by preparing all the decellularization solutions [1] and storing them in separate sterile glass bottles [2].
 - 2.1.1. WIDE: Talent in the lab preparing the solutions. **TXT- Refer to the text protocol for decellularization solution details**
 - 2.1.2. WIDE: Talent transferring the prepared solutions into separate sterile and labeled glass bottles.
- 2.2. Open the packaged peripheral nerve aseptically after disinfecting the exterior [1]. Rinse the nerve several times with sterile distilled water [2].
 - 2.2.1. WIDE: Talent opening the peripheral nerve package aseptically, after disinfecting the exterior
 - 2.2.2. CU: Talent rinsing the peripheral nerve with sterile distilled water.
- 2.3. Using sterile scissors and forceps, remove residual adipose tissue, connective tissue, unnecessary branches, and damaged nerve tissue [1]. Rinse the nerve again with sterile distilled water [2]. Cut the nerve into 2 to 5-centimeter segments and group them by length [3-TXT].
 - 2.3.1. CU: Talent trimming residual adipose tissue, connective tissue, unnecessary branches, and damaged tissue from the nerve.
 - 2.3.2. CU: Talent rinsing the nerve again using sterile distilled water.
 - 2.3.3. CU: Talent cutting the nerve into 2 to 5-centimeter segments and arranging them by length. **TXT: Refer to the text protocol for nerve segment groupings**

3. Peristaltic Pump System Set-Up

- 3.1. Attach chemically resistant silicone tubing of sufficient length to both ends of the chromatography column [1]. Connect one end of the tubing to the peristaltic pump [2]. Place the remaining free end of the tubing and the pump tubing into a 1-liter glass bottle of isopropyl alcohol to create a closed-loop circulation system [3].

- 3.1.1. WIDE: Talent attaching silicone tubing to both ends of the chromatography column.
- 3.1.2. CU: Talent connecting one end of the tubing to the peristaltic pump.
- 3.1.3. WIDE: Talent connecting the free ends of the tubing to a 1-liter glass bottle containing IPA.

4. De-lipidization of the Peripheral Nerve

Demonstrator: Jiyeon Mun

- 4.1. On Day 1, open one end of the chromatography column and partially fill the column with IPA (*i-p-a*) [1]. Using sterile forceps, gently grasp the outer tissue at one end of a prepared nerve segment, without compressing the main body, and lower the segment into the solution [2].
 - 4.1.1. CU: Talent partially filling the open chromatography column with IPA.
 - 4.1.2. ECU: Talent grasping the outer tissue at one end of the nerve segment and carefully lowering the segment into the column.
- 4.2. Gently straighten the nerve segment and position it longitudinally, parallel to the direction of fluid flow, ensuring that the segment is not folded, coiled, or pressed against the column wall or outlet filter [1]. Close the column and position it at a slight upward incline, such that the outlet is higher than the inlet [2].
 - 4.2.1. CU: Talent straightening the nerve segment longitudinally within the column.
 - 4.2.2. WIDE: Talent positioning the closed column at a slight upward incline.
- 4.3. Connect the IPA reservoir to the peristaltic pump system [1] and perform continuous solution exchange for a duration proportional to the nerve segment's length [2-TXT]. Finally, drain the IPA from the column [3].
 - 4.3.1. WIDE: Talent connecting the IPA reservoir to the peristaltic pump.
 - 4.3.2. WIDE: Shot of IPA circulating continuously through the chromatography column. **TXT- Refer to the text protocol for exact solution exchange durations**
 - 4.3.3. CU: Talent draining the IPA from the chromatography column after the procedure.
- 4.4. Wash the nerve by adding 40 milliliters of sterile distilled water to the column [1], then gently invert or shake the column [2]. Drain the water and repeat this wash four more times [3 -TXT].

4.4.1. WIDE: Talent adding 40 milliliters of sterile distilled water of the column.

Videographer: Please obtain multiple reusable takes of 4.4.1. as these will be used later in the protocol

4.4.2. Talent gently inverting or shaking the column to wash the nerve tissue.

Videographer: Please obtain multiple reusable takes of 4.4.2. as these will be used later in the protocol

4.4.3. CU: Talent draining the distilled water from the column.

4.5. Next, connect a reservoir containing 5 liters of sterile distilled water and perform continuous solution exchange overnight [3]. After the overnight wash, store the nerve fully submerged in sterile distilled water at 4 degrees Celsius for up to 24 hours [4].

4.5.1. WIDE: Talent connecting a 5-liter sterile-distilled-water reservoir to start the overnight solution exchange.

4.5.2. Talent storing the nerve segment fully submerged in sterile distilled water at 4 degrees Celsius.

5. First Decellularization with Sodium Hydroxide

5.1. On Day 2, connect the 0.25-normal sodium hydroxide solution to the peristaltic pump system [1] and perform continuous solution exchange for a duration proportional to the nerve segment's length [2]. Then drain the sodium hydroxide solution from the column [3].

5.1.1. WIDE: Talent connecting the sodium hydroxide reservoir to the peristaltic pump.

5.1.2. WIDE: Shot of Sodium hydroxide solution circulating continuously through the chromatography column.

5.1.3. CU: Talent draining the sodium hydroxide solution from the chromatography column.

5.2. Next, repeatedly wash the nerve 5 times by adding 40 milliliters of sterile distilled water and gently inverting or shaking the column, as demonstrated [1]. Connect a 5-liter sterile distilled water reservoir to perform continuous exchange overnight [2]. After the overnight wash, store the nerve fully submerged in sterile distilled water at 4 degrees Celsius for up to 24 hours [3].

5.2.1. *Reuse 4.4.1 and 4.4.2*

- 5.2.2. WIDE: : Talent connecting a 5-liter sterile-distilled-water reservoir to start the overnight solution exchange.
- 5.2.3. Talent storing the nerve segment fully submerged in sterile distilled water at 4 degrees Celsius.

6. Second Decellularization with SDC

- 6.1. Next day, connect the 4 percent weight-per-volume SDC (*s-d-c*) solution to the peristaltic pump system [1] and perform continuous solution exchange for a duration proportional to the nerve segment's length [2]. Then drain the SDC solution from the column [3].
 - 6.1.1. WIDE: Talent connecting the 4 percent weight-per-volume SDC solution to the peristaltic pump.
 - 6.1.2. WIDE: Shot of SDC solution circulating continuously through the chromatography column.
 - 6.1.3. CU: Talent draining the SDC solution from the chromatography column.
- 6.2. Wash the nerve by adding 40 milliliters of sterile distilled water, as demonstrated, and repeat the washes 5 times [1-TXT]. Connect a 5-liter sterile distilled water reservoir and perform continuous solution exchange overnight [2]. After the overnight wash, store the nerve fully submerged in sterile distilled water at 4 degrees Celsius for up to 24 hours [3].
 - 6.2.1. *Reuse 4.4.1 and 4.4.2*
 - 6.2.2. WIDE: Talent connecting the 5-liter sterile-distilled-water reservoir to begin the overnight solution exchange.
 - 6.2.3. Talent storing the nerve segment fully submerged in sterile distilled water at 4 degrees Celsius.

7. Sterilization of the Decellularized Peripheral Nerve

- 7.1. Remove a small volume of distilled water from the column [1]. Carefully retrieve the decellularized peripheral nerve while keeping it suspended in the remaining liquid [2]. Package and seal the peripheral nerve together with sterile distilled water [3-TXT].
 - 7.1.1. Talent removing a small amount of water from the column.
 - 7.1.2. ECU: Talent gently retrieving the suspended decellularized nerve from the column.

7.1.3. CU: Talent sealing the packaged decellularized peripheral nerve kept in sterile distilled water. **TXT: Package sterilized by gamma irradiation at 25 kGy**

Results

8. Results

8.1. Hematoxylin and eosin staining showed abundant nuclei in native peripheral nerve tissue, whereas nuclei and other cellular components were largely absent following decellularization [1]. Residual DNA content was substantially reduced in decellularized nerve tissue compared with native tissue, confirming effective decellularization [2].

8.1.1. LAB MEDIA: Figure 3 *Video Editor: In Panel A on the left, please highlight the purple-stained nuclei in the 'native' nerve image using an arrow, while simultaneously showing the absence of nuclei in the adjacent 'decellularized' nerve image.*

8.1.2. LAB MEDIA: Figure 3 *Video Editor: In the graph in Panel B on the right, please highlight the significantly lower DNA level in the 'decellularized' tissue using an arrow.*

8.2. SEM analysis showed that the fibrous organization and microarchitecture of the extracellular matrix remained clearly visible after decellularization, indicating preservation of the native nerve ECM architecture [1].

8.2.1. LAB MEDIA: Figure 4. *Video Editor: Please highlight with an arrow the fibrous extracellular matrix architecture in both the 'native' nerve image on the left and the 'decellularized' nerve image on the right, at the same time.*

1. Decellularization

Pronunciation link: No confirmed link found

IPA: /diːˌsɛljələɾəˈzeɪʃən/

Phonetic Spelling: dee·sel·yuh·ler·uh·ZAY·shuhn

2. Peripheral

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

IPA: /pəˈrɪfərəl/

Phonetic Spelling: puh·RIF·uh·ruhl

3. Aseptically

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

- IPA: /er'septikli/
Phonetic Spelling: ay·SEP·tik·lee
4. Adipose
Pronunciation link: <https://www.merriam-webster.com/dictionary/adipose>
IPA: /'ædəˌpouz/
Phonetic Spelling: AD·uh·pohs
 5. Peristaltic
Pronunciation link: <https://www.merriam-webster.com/dictionary/peristaltic>
IPA: /ˌperə'stɔːltɪk/
Phonetic Spelling: pair·uh·STAWL·tik
 6. Silicone
Pronunciation link: <https://www.merriam-webster.com/dictionary/silicone>
IPA: /'sɪlɪˌkoon/
Phonetic Spelling: SIL·ih·kohn
 7. Chromatography
Pronunciation link: <https://www.merriam-webster.com/dictionary/chromatography>
IPA: /ˌkroʊmə'tɑːgrəfi/
Phonetic Spelling: kroh·muh·TOG·ruh·fee
 8. Isopropyl
Pronunciation link: <https://www.merriam-webster.com/dictionary/isopropyl>
IPA: /ˌaɪsoʊˈproʊpəl/
Phonetic Spelling: eye·soh·PROH·puhl
 9. De-lipidization
Pronunciation link: No confirmed link found
IPA: /diˌlɪpɪdəˈzeɪʃən/
Phonetic Spelling: dee·lip·ih·duh·ZAY·shuhn
 10. Longitudinally
Pronunciation link: <https://www.merriam-webster.com/dictionary/longitudinal>
IPA: /ˌlɑːndʒəˈtuːdənəli/
Phonetic Spelling: lon·juh·TOO·duh·nuh·lee
 11. Sodium hydroxide
Pronunciation link: <https://www.merriam-webster.com/dictionary/sodium%20hydroxide>
IPA: /'soʊdiəm haɪˈdrɔːkˌsaɪd/
Phonetic Spelling: SOH·dee·uhm hy·DROK·side
 12. Decellularized
Pronunciation link: No confirmed link found
IPA: /diˌseljələˈraɪzd/
Phonetic Spelling: dee·SEL·yuh·luh·ryzed
 13. Irradiation
Pronunciation link: <https://www.merriam-webster.com/dictionary/irradiation>
IPA: /ˌɪˌreɪdiˈeɪʃən/
Phonetic Spelling: ih·ray·dee·AY·shuhn
 14. Hematoxylin
Pronunciation link: <https://www.merriam-webster.com/dictionary/hematoxylin>

IPA: /hi:mə'tɑ:ksəlɪn/

Phonetic Spelling: hee·muh·TOK·suh·lin

15. Eosin

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

IPA: /'i:əsɪn/

Phonetic Spelling: EE·uh·sin

16. Nuclei

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

IPA: /'nu:kliˌaɪ/

Phonetic Spelling: NOO·klee·eye

17. Extracellular

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

IPA: /,ɛkstrə'seljələr/

Phonetic Spelling: ek·struh·SEL·yuh·ler

18. Microarchitecture

Pronunciation link: No confirmed link found

IPA: /,maɪkroʊ'ɑ:rkəˌtektʃər/

Phonetic Spelling: my·kroh·AR·kuh·tek·cher

19. Fibrous

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

IPA: /'faɪbrəs/

Phonetic Spelling: FY·bruhs

20. Architecture

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

IPA: /'ɑ:rkəˌtektʃər/

Phonetic Spelling: AR·kuh·tek·cher