

Submission ID #: 70579

Scriptwriter Name: Pallavi Sharma

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Title: Fabrication and Surgical Application of Enriched Photocrosslinked Gelatin–Riboflavin Hydrogels for Corneal Wound Repair in Rabbits

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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

☐ Incorrect

2. Interview statements: Which interview statement filming option is the most appropriate for your group? **Please select one.**



Interview Statements are read by JoVE's voiceover talent.

3. Proposed interview filming date: Please indicate the proposed date that your group will self-film interviews: Read by JoVE's voiceover talent.

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

Current Protocol Length

Number of Steps: 12

Number of Shots: 14

Introduction

NOTE to VO: Please record the interview and conclusion statements

INTRODUCTION:

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

- 1.1. This study develops and evaluates a blue-light-crosslinkable gelatin-riboflavin hydrogel to improve corneal wound repair and assess healing efficacy in rabbits

- 1.1.1. [2.2.1](#)

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

- 1.2. Existing methods lack suture-free, transparent, bioactive materials. This protocol uses light-activated hydrogels for precise, biocompatible corneal wound repair.

- 1.2.1. [2.7.1](#)

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. This protocol enables quantification of corneal healing, hydrogel performance, biocompatibility, and treatment efficacy in controlled rabbit models of corneal wounds.

- 1.3.1. [Figure 6](#)

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

- 1.4. The main challenge was ensuring the hydrogel remained in the wound bed during early healing, necessitating tarsorrhaphy to prevent self-rubbing.

- 1.4.1. [2.9.1](#)

Ethics Title Card

This research has been approved by the Animal Research Ethics Committee of the University of the Basque Country

Protocol

NOTE: The protocol section was drafted from available footage

2. Surgical Application of Hydrogel for Corneal Stromal Wound Repair

Demonstrator: NOTE: Not provided

2.1. To begin, using sterile technique, pipette the required volume of prepared gelatin–riboflavin hydrogel into the target well, mold, dish, or substrate [1].

2.1.1. LAB MEDIA: In vitro hydrogel synthesis.MOV: 0:00:06-0:00:20.

2.2. Position the Led-C (*Led-C*) curing lamp 2 centimeters above the hydrogel surface and initiate in situ crosslinking for a total of 2 minutes, delivered as six pulses of 20 seconds with 5 to 10 second pauses between pulses to prevent local heating [1-TXT].

2.2.1. LAB MEDIA: In vitro hydrogel synthesis.MOV: 0:00:25-0:00:35. TXT: Blue light (420–480 nm, 850–1000 mW/cm²)

2.3. Then, confirm complete gelation visually [1].

2.3.1. LAB MEDIA: In vitro hydrogel synthesis.MOV: 02:08-02:10

2.4. After placing the anesthetized animal in a lateral decubitus position on a sterile surgical platform and disinfecting the periocular region with povidone-iodine solution, place an eyelid speculum on the operative eye to maintain continuous exposure of the ocular surface during the procedure [1-TXT].

2.4.1. LAB MEDIA: Trephination and wound scraping.mp4: 0:00:00-0:00:33. TXT: Anesthesia: Ketamine: 1 mL/kg (50 mg/mL), Xylazine 0.3 mL/kg (20 mg/mL)

2.5. Center a 6.5-millimeter diameter vacuum trephine on the rabbit cornea [1]. Apply suction to stabilize the device and rotate the trephine three-quarter turns to achieve an approximate stromal depth of 187 micrometers [2].

2.5.1. LAB MEDIA: Trephination and wound scraping.mp4: 0:00:33-0:00:36.

2.5.2. LAB MEDIA: Trephination and wound scraping.mp4: 0:00:36-0:00:46.

2.6. Using a crescent blade, perform an anterior stromal keratectomy within the trephined area [1]. Remove the epithelial and anterior stromal layers until a smooth wound bed is obtained [2].

2.6.1. LAB MEDIA: Trephination and wound scraping.mp4: 0:01:45-0:02:10, 03:13-03:30

2.6.2. LAB MEDIA: Trephination and wound scraping.mp4: 0:03:45-0:04:15

2.7. Now, fill the corneal defect with 50 microliters of the Human amniotic membrane extract or autologous serum-enriched hydrogel formulation preheated to 32 degrees Celsius to ensure optimal viscosity [1-TXT].

2.7.1. LAB MEDIA: Hydrogel cxl.mp4: 0:00:05-0:00:15

2.8. Initiate in situ crosslinking by illuminating the corneal surface with blue light at 420 to 480 nanometers for 2 minutes, delivering six pulses of 20 seconds with 5 to 10 second pauses between pulses to limit local heating [1].

2.8.1. LAB MEDIA: Hydrogel cxl.mp4: 0:00:20-0:00:54.

2.9. Then, to place the first suture, rasp the lateral upper eyelid margin with forceps. Pass the needle through the grey line at the eyelid margin from the skin side to the conjunctival side, taking a full-thickness bite while avoiding deep penetration into the tarsal plate [1].

2.9.1. LAB MEDIA: Tarsorrhaphy.mp4: 0:00:00-0:00:24

2.10. Now, pass the needle through the corresponding point of the lower eyelid margin from the conjunctival side to the skin side to create apposition of the lid margins [1].

2.10.1. LAB MEDIA: Tarsorrhaphy.mp4: 0:00:24-0:00:55

2.11. Then, tie the suture with a secure surgeon's knot and two square knots, positioning the knot on the skin surface slightly to the outer side of the eyelid margin rather than directly over it [1].

2.11.1. LAB MEDIA: Tarsorrhaphy.mp4: 0:00:55-0:01:19.

2.12. At last, trim the suture tails to 3 to 4 millimeters to reduce irritation while leaving enough length for later removal. Monitor and record ocular healing from day 3 to day 7 using slit-lamp microscopy and fluorescein staining [1].

2.12.1. LAB MEDIA: Tarsorrhaphy.mp4: 0:01:20-0:01:38

Results

3. Results

- 3.1. Fluorescein staining revealed a progressive reduction in wound area, with most defects fully re-epithelialized by day 5 [1]. At day 3, median wound areas were comparable among all groups with no significant differences [2].
 - 3.1.1. LAB MEDIA: Figure 6A. *Video editor: Show the sequence of eye images and highlight how the stained area becomes smaller over time until it disappears by day 5.*
 - 3.1.2. LAB MEDIA: Figure 6B. *Video editor: Highlight all four groups on the day 3 plot*
- 3.2. The control group showed variable wound stability, with 20 percent of corneas displaying persistent defects or reopening by day 7 [1] despite showing the fastest early re-epithelialization, with 60% of corneas achieving complete closure by day 5 [2].
 - 3.2.1. LAB MEDIA: Figure 6C–E. *Video editor: Highlight the control group at day 7 showing remaining or reappearing open areas.*
 - 3.2.2. LAB MEDIA: Figure 6D,E. *Video editor: Highlight the control group at day 5*
- 3.3. Hydrogels with autologous serum formulation showed accelerated epithelial repair, reaching a median wound area of 1.09 percent at day 5 and complete closure in 100 percent of corneas by day 7 [1]. Empty hydrogels and human amniotic membrane extract hydrogels promoted wound closure, with 90 percent of corneas healed by day 7, while 10 percent retained residual defects [2].
 - 3.3.1. LAB MEDIA: Figure 6C–E. *Video editor: Highlight the H-AS group at day 5 and 7 showing very small remaining wound area.*
 - 3.3.2. LAB MEDIA: Figure 6C–E. *Video editor: Highlight the H and H-HAMe groups at day 7 showing mostly closed corneas with a small number still open.*
- 3.4. At day 3, mild ocular irritation signs such as redness, swelling, and discharge were most frequently observed in the control and hydrogel groups [1]. The hydrogels with autologous serum formulation showed significantly lower irritation scores compared to the control at day 3 [2].
 - 3.4.1. LAB MEDIA: Figure 8A. *Video editor: Highlight the control and H eye images at day 3 showing visible redness and swelling.*
 - 3.4.2. LAB MEDIA: Figure 8B,C. *Video editor: Highlight the H-AS group on the day 3 score plot showing lower values than the control group.*
- 3.5. By day 7, irritation scores in the hydrogels with autologous serum formulation returned to 0, indicating full recovery [1].

3.5.1. LAB MEDIA: Figure 8C. *Video editor: Highlight the H-AS group at day 7 where the values reach 0 on the vertical axis.*

1. Riboflavin
Pronunciation link: <https://www.merriam-webster.com/dictionary/riboflavin>
IPA: /ˌraɪboʊˈfleɪvɪn/
Phonetic Spelling: rye·boh·flay·vin
2. Hydrogel
Pronunciation link: <https://www.merriam-webster.com/dictionary/hydrogel>
IPA: /ˈhaɪdroʊˌdʒəl/
Phonetic Spelling: hy·droh·jel
3. Crosslinking
Pronunciation link: <https://www.merriam-webster.com/dictionary/cross-link>
IPA: /ˈkrɒːsˌlɪŋk/
Phonetic Spelling: kraws·lingk
4. Gelation
Pronunciation link: <https://www.merriam-webster.com/dictionary/gelation>
IPA: /dʒəˈleɪʃən/
Phonetic Spelling: juh·lay·shuhn
5. Anesthetized
Pronunciation link: <https://www.merriam-webster.com/dictionary/anesthetize>
IPA: /əˈnesθəˌtaɪzd/
Phonetic Spelling: uh·nes·thuh·tyzed
6. Decubitus
Pronunciation link: <https://www.merriam-webster.com/dictionary/decubitus>
IPA: /dɪˈkjuːbɪtəs/
Phonetic Spelling: dih·kyoo·buh·tuhs
7. Periocular
Pronunciation link: No confirmed link found
IPA: /ˌpəriˈɑːkjələr/
Phonetic Spelling: peh·ree·ah·kyuh·ler
8. Povidone-iodine
Pronunciation link: <https://www.merriam-webster.com/dictionary/povidone>
IPA: /ˈpɑːvɪˌdoʊn ˈaɪəˌdiːn/
Phonetic Spelling: pah·vih·dohn eye·uh·deen
9. Speculum
Pronunciation link: <https://www.merriam-webster.com/dictionary/speculum>
IPA: /ˈspɛkjələm/
Phonetic Spelling: spek·yuh·luhm
10. Trephine
Pronunciation link: <https://www.merriam-webster.com/dictionary/trephine>
IPA: /trɪˈfaɪn/
Phonetic Spelling: trih·fyn

11. Stromal
 Pronunciation link: <https://www.merriam-webster.com/dictionary/stromal>
 IPA: /'stroʊmə/
 Phonetic Spelling: stroh·muhl
12. Keratectomy
 Pronunciation link: <https://www.merriam-webster.com/dictionary/keratectomy>
 IPA: /ˌkɛrə'tɛktəmi/
 Phonetic Spelling: keh·ruh·tek·tuh·mee
13. Epithelial
 Pronunciation link: <https://www.merriam-webster.com/dictionary/epithelial>
 IPA: /ˌɛpɪ'tiːliəl/
 Phonetic Spelling: eh·pih·thee·lee·uhl
14. Microliters
 Pronunciation link: <https://www.merriam-webster.com/dictionary/microliter>
 IPA: /'maɪkroʊˌliːtər/
 Phonetic Spelling: my·kroh·lee·ter
15. Amniotic
 Pronunciation link: <https://www.merriam-webster.com/dictionary/amniotic>
 IPA: /ˌæmni'ɑːtɪk/
 Phonetic Spelling: am·nee·ah·tik
16. Autologous
 Pronunciation link: <https://www.merriam-webster.com/dictionary/autologous>
 IPA: /ɔː'tɑːləɡəs/
 Phonetic Spelling: aw·tol·uh·guhs
17. Nanometers
 Pronunciation link: <https://www.merriam-webster.com/dictionary/nanometer>
 IPA: /'nænəˌmiːtər/
 Phonetic Spelling: nan·uh·mee·ter
18. Conjunctival
 Pronunciation link: <https://www.merriam-webster.com/dictionary/conjunctival>
 IPA: /kənˌdʒʌŋ(k)'taɪvəl/
 Phonetic Spelling: kuh·jungk·tye·vuhl
19. Tarsal
 Pronunciation link: <https://www.merriam-webster.com/dictionary/tarsal>
 IPA: /'tɑːrsəl/
 Phonetic Spelling: tar·suhl
20. Tarsorrhaphy
 Pronunciation link: <https://www.merriam-webster.com/dictionary/tarsorrhaphy>
 IPA: /ˌtɑːrsə'ræfi/
 Phonetic Spelling: tar·suh·raf·ee
21. Fluorescein
 Pronunciation link: <https://www.merriam-webster.com/dictionary/fluorescein>
 IPA: /ˌflɔr'esiːn/
 Phonetic Spelling: floor·eh·seen
22. Re-epithelialized
 Pronunciation link: No confirmed link found

IPA: /,riːˌɛpɪˈθiːliələɪzd/

Phonetic Spelling: ree·eh·pih·thee·lee·uh·lyzed

23. Corneas

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

IPA: /ˈkɔːrniə/

Phonetic Spelling: kor·nee·uh

24. Residual

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

IPA: /rɪˈzɪdʒuəl/

Phonetic Spelling: rih·zij·oo·uhl