Animal Models of Corneal Injury

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[Abstract] The cornea is an excellent model system to use for the analysis of wound repair because of its accessibility, lack of vascularization, and simple anatomy. Corneal injuries may involve only the superficial epithelial layer or may penetrate deeper to involve both the epithelial and stromal layers. Here we describe two well-established *in vivo* corneal wound models: a mechanical wound model that allows for the study of re-epithelialization and a chemical wound model that may be used to study stromal activation in response to injury (Stepp *et al.*, 2014; Carlson *et al.*, 2003).

Materials and Reagents

Note: All reagents may be maintained at room temperature.

1. FVB mouse (5-10 weeks old)
2. Isoflurane (Abbott Laboratories, catalog number: 5260-04-05)
3. Proparacaine hydrochloride ophthalmic solution (0.5%) (Bausch & Lomb, NDC: 24208-730- 06)
4. Fluorescein sodium and benoxinate hydrochloride ophthalmic solution (0.25%/0.4%) (Akorn, NDC: 17478-640-10)
5. Weck-cel cellulose eye spears (Medtronic, catalog number: 0008680)
6. NaOH solution (see Recipes)

Chemical injury

7. Sterile water
8. 1x sterile phosphate buffered saline buffer (1x PBS)
9. Sodium hydroxide (NaOH) 1.0 N (normal) solution (Sigma-Aldrich, catalog number: S2770)
10. Fluorescein solution (see Recipes)

Equipment

1. Heating pad for mouse
2. Algerbrush II with 0.5 mm Burr (Katena, catalog number: K 2-4900)

3. Trephine with handle (1.5 mm) (Beaver-visitec, catalog number: 9748)
4. Alcohol swabs - isopropyl alcohol 70% (BD, catalog number: 326895)
5. Filter paper (Thermo Fisher Scientific, catalog number: 09-795AA)
6. Ear punch (Roboz, catalog number: 65-9902)
7. Forceps (Dumont #5)
8. Pipette with tips (1 ml)
9. Timer
10. Stereo microscope (for scratch/epithelial injury, need filter set to visualize GFP; for both injuries, camera attachment is optional) (Leica, catalog number: MZ16F)
11. Anesthesia machine with nose cone attachment appropriate for mice (Summit Anesthesia Solutions, catalog number: AS-01-0007)

Procedure

Ethical statement: All procedures discussed here are in accordance with and were approved by the University of California, San Francisco Institutional Animal Care and Use Committee.

For scratch/epithelial injury

1. Set up the surgical area.
   a. Place the isoflurane chamber near the microscope and place the anesthesia platform with the nose cone under the microscope objective. Place a mouse heating pad by the nose cone.
   b. Place the Proparacaine, Algerbrush, weck-cels, and trephine on the lab bench near the surgical area. Have 20 µl of 1:40 diluted Fluorescein solution drawn up in a pipette (see Recipes).
   c. Clean off the Algerbrush burr by rubbing with an alcohol swab.
2. Anesthetize the mouse in the isoflurane chamber. A typical approach for anesthesia involves placing the animal in an induction chamber connected to an oxygen source and isoflurane vaporizer, and adjusting oxygen flow to 0.9 liters/min and the isoflurane vaporizer to 1-2%. As soon as the mouse becomes unresponsive and has shallow breathing, it may be transferred to the anesthesia platform with the nose cone and placed on a heating pad. Position the mouse head so that the eye to be injured is facing up towards the microscope objective.
3. Squeeze the bottle of Proparacaine and place 1 drop of Proparacaine on the cornea. Wait 30 sec.
4. Use a weck-cel to dry off the cornea by gently sweeping across cornea once and dabbing both corners of the eye.
5. Apply periocular pressure with one hand to proptose the mouse eye. (Optional step: Take a brightfield picture of the cornea prior to injury.)

6. Use the other hand to mark the cornea with the trephine as central as possible with gentle pressure. Hold the handle of trephine with your thumb and second fingers and place the entire circular edge on the cornea. Gently turn the trephine with mild pressure approximately 3 clock hours to mark the cornea. Avoid making multiple marks.
7. Turn the Algerbrush on and make an epithelial defect in the center of the cornea by applying gentle pressure in a circular manner and observing a break in the surface epithelial cell layer. Carefully extend the epithelial defect close to the trephine mark.
8. Turn the Algerbrush off and use the dull blades of the Algerbrush burr to gently remove the remaining corneal epithelium out to the trephine mark. Apply Fluorescein solution to the cornea and confirm size of epithelial defect using the microscope GFP filter.
9. Optional step: Apply periocular pressure to proptose the mouse eye. Take a fluorescence picture of the wounded cornea after applying Fluorescein solution and using the GFP filter (Figure 1).

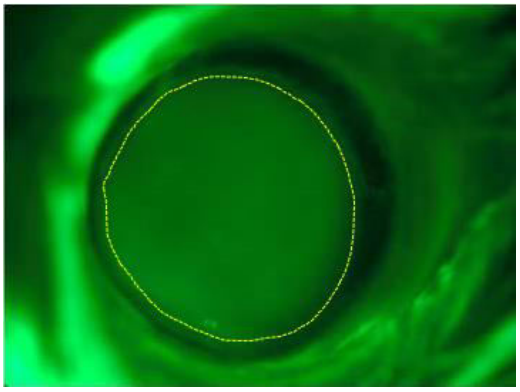

Figure 1. Corneal scratch/epithelial injury. Photograph of an epithelial defect of the central cornea after scratch injury stained with Fluorescein solution. The border of the scratch wound is in shown in yellow. [adapted from Figure 2 of Chan *et al.* (2013)]

10. Place 1 drop of Proparacaine on the cornea.
11. Remove mouse from the nose cone and allow mouse to awaken in a recovery cage. Monitor the mouse for pain and eye infections.

For chemical injury

1. Set up the surgical area.
   a. Place the isoflurane chamber near the microscope and place the anesthesia platform with the nose cone under the microscope objective.
   b. Place the Proparacaine, filter paper, forceps, weck-cels, and NaOH on the lab bench near the surgical area.

c. Have the pipette with 500 µl PBS drawn up readily available. (Optional: Have 20 µl Fluorescein solution drawn up in a pipette.)
d. Prepare 2 mm filter paper discs using the ear punch.

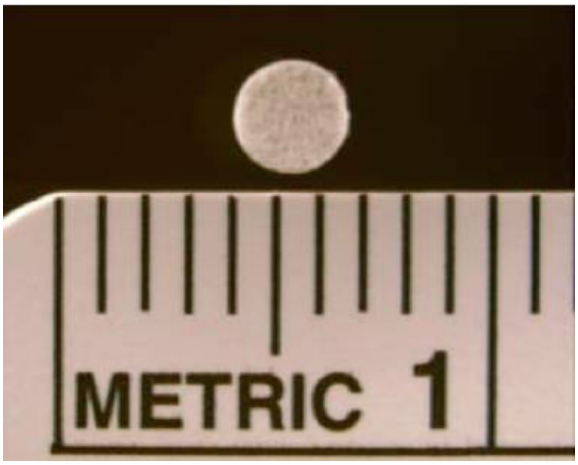

Figure 2. Filter paper disc. An ear punch can be used to create uniform 2 mm filter paper discs.

e. Set the timer for 10 sec and 30 sec.

2. Anesthetize the mouse in the isoflurane chamber. A typical approach for anesthesia involves placing the animal in an induction chamber connected to an oxygen source and isoflurane vaporizer, and adjusting oxygen flow to 0.9 L/min and the isoflurane vaporizer to 1-2%. As soon as the mouse becomes unresponsive and has shallow breathing, it may be transferred to the anesthesia platform with the nose cone and placed on a heating pad. Position the mouse head so that the eye to be injured is facing up towards the microscope objective.
3. Place 1 drop of Proparacaine on the cornea. Wait 30 sec.
4. Use a weck-cel to dry off the cornea by gently sweeping across cornea once and dabbing both corners of the eye.
5. Use the forceps to submerge the filter paper into the NaOH solution for exactly 10 sec.
6. Apply periocular pressure with one hand to proptose the mouse eye. (Optional step: Take a brightfield picture of the cornea prior to injury.)
7. Use forceps in the other hand to apply the NaOH-soaked filter paper to the center cornea for exactly 30 sec. Perform this step using the stereomicroscope to precisely place the filter paper as central as possible.
8. Remove the filter disc from the cornea using forceps.
9. Immediately flush the eye with 500 µl PBS to wash away residual NaOH (apply PBS, dry with weck-cel, apply PBS, dry with weck-cel, repeat until all 500 µl PBS has been used).

10. Optional step: Apply periocular pressure to proptose the mouse eye. Take a fluorescence picture of the wounded cornea after applying Fluorescein solution and using the GFP filter.

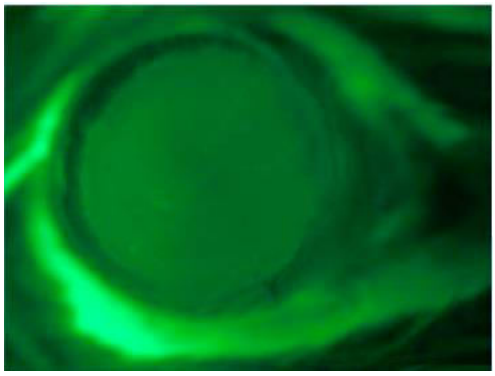

Figure 3. Corneal chemical injury. Photograph of an epithelial defect of the central cornea after chemical injury stained with Fluorescein solution [adapted from Figure 1 of Chan *et al.* (2013)]

11. Place 1 drop of Proparacaine on the cornea.
12. Remove mouse from the nose cone and allow mouse to awaken in a recovery cage. Monitor mouse for pain and eye infections.

Notes

1. When using the scratch injury model to compare epithelial repair rates between mice, it is recommended that littermate and/or age-matched mice are used.

Recipes

1. NaOH solution
   Mix the following ingredients in a 1.5 ml Eppendorf tube the same day of the procedure: 50 µl 1 N NaOH and 450 µl sterile H_2O to gain a total volume of 500 µl.
2. Fluorescein solution (optional for chemical injury)
   Prepare the 1:40 dilution in a 1.5 ml Eppendorf tube the same day of the procedure: 2 µl Fluorescein sodium/benoxinate hydrochloride ophthalmic solution and 78 µl sterile PBS to gain a total volume of 80 µl.

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