Video Article

A Mouse Model of the Cornea Pocket Assay for Angiogenesis Study

Zhongshu Tang[1], Fan Zhang[1], Yang Li[1], Pachiappan Arjunan[1], Anil Kumar[1], Chunsik Lee[1], Xuri Li[1]

[1]National Eye Institute

Correspondence to: Xuri Li at lixur@nei.nih.gov

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Abstract

A normal cornea is clear of vascular tissues. However, blood vessels can be induced to grow and survive in the cornea when potent angiogenic factors are administered [1]. This uniqueness has made the cornea pocket assay one of the most used models for angiogenesis studies. The cornea composes multiple layers of cells. It is therefore possible to embed a pellet containing the angiogenic factor of interest in the cornea to investigate its angiogenic effect [2,3]. Here, we provide a step by step demonstration of how to (I) produce the angiogenic factor-containing pellet (II) embed the pellet into the cornea (III) analyze the angiogenesis induced by the angiogenic factor of interest. Since the basic fibroblast growth factor (bFGF) is known as one of the most potent angiogenic factors [4], it is used here to induce angiogenesis in the cornea.

Video Link

The video component of this article can be found at https://www.jove.com/video/3077/

Protocol

All equipments and reagents used are sterile. The protocol was approved by the Animal Care and Use Committee (ACUC) at the NEI/NIH (animal study protocol NEI-553), and was performed according to the NIH guidelines and regulations.

1. Producing the angiogenic factor-containing pellets

In this part, the pellets containing the angiogenic factor of interest are prepared.

1. Make 10% (w/v) sucralfate solution with PBS. Store under room temperature.
2. Make 12% (w/v) poly-HEMA with absolute ethanol. Store under room temperature.
3. Make 1 µg/µl bFGF solution. Store in -80 °C.
4. To make 50 pellets, mix 5 µl of poly-HEMA, 1µl of sucralfate and 4 µl of bFGF stock solution, vortex thoroughly.
5. Place a piece of clean parafilm in a Petri dish, expose it under UV light for 15 min in a tissue culture hood. Drop 0.2 µl of the solution mixture on the parafilm for one pellet. Thus, 10 µl of the solution mixture should yield 50 pellets. Let the pellets dry at room temperature for 1-2 hours. The dried pellets can be stored in 4 oC for several days, or -80 °C for several months.
6. Upon usage, pry and pick up the pellets with the tips of a pair of forceps. Be extremely careful not to break them or make them spring away.

2. Performing the mouse cornea pocket assay

In this part, we will demonstrate how to make a pocket in the mouse cornea, and how to insert a pellet into the pocket. The angiogenic factor contained in the pellet will be released gradually to the surrounding areas of the cornea (Fig. 1). In this demonstration, we use 8-week female C57/Bl6 mice.

1. The mouse is deeply systematically anesthetized with the mixture of ketamine (75 mg/kg) and xylazine (5mg/kg, i.p. injection). This will keep the mouse under an anesthesia state for 30-60 min. Topical anesthetic (0.5% proparacaine HCl) is applied to the cornea. After 5 min, fix one eye under a dissecting microscope.
2. A very gentle cut is made in the middle of the cornea 1.2 -1.4 mm from the corneal limbus (as illustrated in Figure 1) with a von Graefe cataract knife. Be extremely careful not to cut through the cornea.
3. A pocket is made under the epithelium layer of the cornea by horizontally inserting the knife into the middle of the cornea and extending the knife toward the limbus carefully (Fig.1). The size of the pocket has to be big enough to accommodate one angiogenic factor-containing pellet. Be extremely careful not to puncture through the cornea.
4. Insert a pellet with a pair of forceps into the pocket slowly. Try to make the pellet as flat as possible.
5. After implantation of the pellet, a topical ophthalmic antibiotic ointment is applied to the eye. Instruments are wiped with 70% alcohol pads between animals.

6. Move the mouse to a warm and dry area and monitor it, until it is able to maintain an upright posture and then return it to its home cage. Systemic analgesics (e.g., buprenorphine, 2 mg/kg, i.p. injection) and topical antibiotic ointment (e.g. gentamicin) are given twice daily for 2 days after the surgery and the mouse is closely monitored for 3 days. Healing is expected to complete within the first 48 hours post-surgery. The eye with the pocket will be examined under a slit-lamp biomicro scope between days 4-10 after pellet implantation.

3. Representative results

1. Seven days after the pellet implantation, the mouse is anesthetized systemically and topically as described in 2.1.
2. Observe the blood vessels in the cornea induced by the angiogenic factor under a dissecting microscope. In the cornea treated with vehicle, no blood vessel growth can be observed (Fig. 2A, vehicle-treated cornea). In the cornea treated with bFGF, robust angiogenesis can be seen (Fig. 2B). The blood vessel growth can be assessed using the maximum length of the blood vessels (from the normal limbal vessels to the top of the new vessels), and the total blood vessel areas (Fig. 2B, delineated with a red dashed line in the bFGF-treated cornea). Other quantification methods have also been described [2,5].

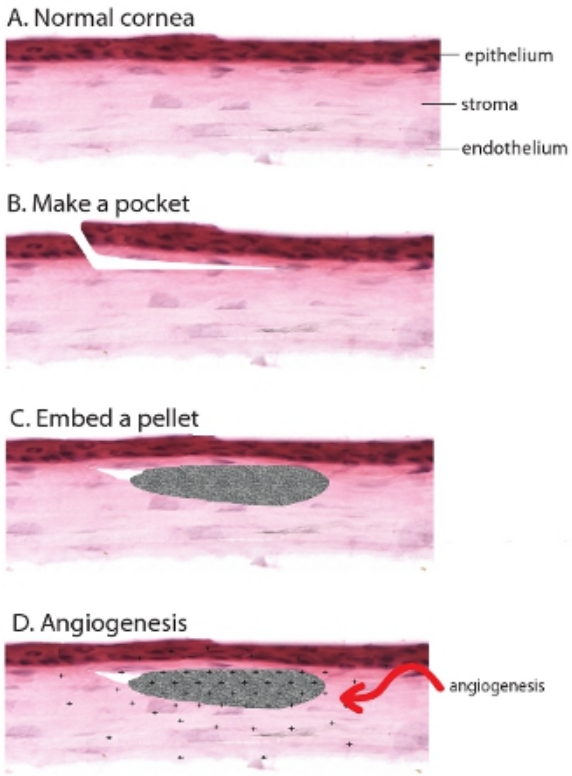

Figure 1. A. Normal cornea with H&E staining. No blood vessel exists in a normal cornea. **B**. Make a pocket underneath the epithelium layer of the cornea. **C**. Embed a pellet into the pocket. **D**. The angiogenic factor is released from the pellet, and new blood vessels grow from the normal limbal vessels.

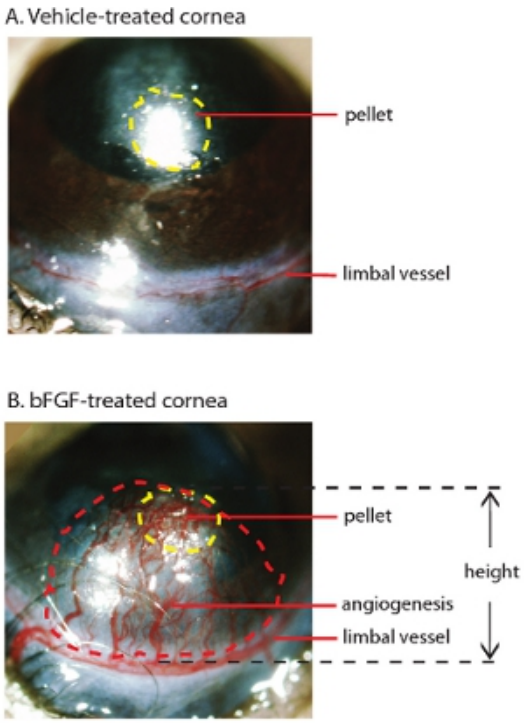

Figure 2. Representative result five days after implantation of the pellet. **A**. Cornea with a pellet containing the vehicle only. No new blood vessel formation in the cornea. Only the pre-existing normal limbal vessels are visible. **B**. Cornea with a pellet containing bFGF. Robust new blood vessels (dot-lined) grew from the pre-existing normal limbal vessels.

Discussion

The mouse cornea pocket assay model was first described in 1979 by Muthukkaruppan VR and Auerbach R [6]. Detailed protocols have been published by different laboratories [2,3,7,8]. Our laboratory has modified and used this method in our studies for many years [4]. The mouse cornea pocket assay is a relatively simple model with great reproducibility. Another advantage of this model is that since the cornea normally lacks blood vessels, there is minimum amount of background in this assay. Therefore, this model is commonly used by the field to study the angiogenic effect of different factors and molecules. Compared with the eyes of some larger animals, such as rats and rabbits, the mouse eyes are smaller and difficult to handle. Special care is therefore required when performing this model, especially during the processes of making the pocket in the cornea and inserting the pellet. The knife must be handled in a precisely controlled manner so that it will not puncture the cornea.

Disclosures

No conflicts of interest declared.

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References

1. Cao, Y., Linden, P., Shima, D., Browne, F., & Folkman, J. *In vivo* angiogenic activity and hypoxia induction of heterodimers of placenta growth factor/vascular endothelial growth factor. *The Journal of clinical investigation*. **98**, 2507-2511 (1996).
2. Rogers, M.S., Birsner, A.E., & D'Amato, R.J. The mouse cornea micropocket angiogenesis assay. *Nature protocols*. **2**, 2545-2550 (2007).
3. Poche, R.A., Saik, J.E., West, J.L., & Dickinson, M.E. The mouse cornea as a transplantation site for live imaging of engineered tissue constructs. *Cold Spring Harbor protocols*. **2010**, pdb prot5416 (2010).
4. Zhang, F., *et al.* VEGF-B is dispensable for blood vessel growth but critical for their survival, and VEGF-B targeting inhibits pathological angiogenesis. *Proceedings of the National Academy of Sciences of the United States of America*. **106**, 6152-6157 (2009).
5. Tong, S. & Yuan, F. Dose response of angiogenesis to basic fibroblast growth factor in rat corneal pocket assay: I. Experimental characterizations. *Microvascular research*. **75**, 10-15 (2008).
6. Muthukkaruppan, V. & Auerbach, R. Angiogenesis in the mouse cornea. *Science*. **205**, 1416-1418 (1979).
7. Ziche, M. Corneal assay for angiogenesis. *Methods in molecular medicine*. **46**, 131-142 (2001).
8. Ziche, M. & Morbidelli, L. The corneal pocket assay. *Methods in molecular biology*. **467**, 319-329 (2009).

