All of these are accomplished. I have written in red if I needed to respond.

**Editorial comments:**  
Changes to be made by the Author(s):  
1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.  
2. Please rephrase the Short Abstract to clearly describe the protocol and its applications in complete sentences between 10-50 words: “Here, we present a protocol to …”  
3. Please rephrase the Long Abstract to more clearly state the goal of the protocol.  
4. Please rephrase the Introduction to include a clear statement of the overall goal of this method.  
5. Please define all abbreviations before use.  
6. Please use SI abbreviations for all units: L, mL, µL, h, min, s, etc.  
7. Please include a space between all numbers and their corresponding units: 15 mL, 37 °C, 60 s; etc.  
8. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents. For example: Gibco, MP Biomedicals, Advance Biomatrix, Pel-freeze, Katena, Personna, Fisher, Falcon, Kim Wipe, Vector Laboratories, etc.  
9. Please include an ethics statement before your numbered protocol steps, indicating that the protocol follows the animal care guidelines of your institution. There are no animal studies in this manuscript. This does not apply.  
10. Please revise the protocol to be a numbered list: step 1 followed by 1.1, followed by 1.1.1, etc. Please refrain from using bullets, dashes, or indentations.  
11. Please revise the protocol text to avoid the use of any personal pronouns (e.g., "we", "you", "our" etc.).

12. Please revise the protocol to contain only action items that direct the reader to do something. The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as “could be,” “should be,” and “would be” throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a “Note.”  
13. Lines 112-128, 188-203: Please move the solutions, materials and equipment information to the Materials Table.  
14. Lines 130-141: Please write the text in the imperative tense.  
15. Line 147: Please describe how to remove excess tissue from the eye. What tool is used?  
16. 1.1-1.3: What is used to hold the globe, etc.?  
17. Lines 154-183, 207-211: The Protocol should be made up almost entirely of discrete steps without large paragraphs of text between sections. Please simplify the Protocol so that individual steps contain only 2-3 actions per step and a maximum of 4 sentences per step and that they are described in the imperative tense in complete sentences. Please move the discussion about the protocol to the Discussion.  
18. 1.15: Please add more details to your protocol step. Please ensure you answer the “how” question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action.  
19. Please include single-line spaces between all paragraphs, headings, steps, etc.  
20. After you have made all of the recommended changes to your protocol (listed above), please highlight 2.75 pages or less of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol. The protocol is not long. The entire protocol needs to be demonstrated.   
21. Please obtain explicit copyright permission to reuse any figures from a previous publication. Explicit permission can be expressed in the form of a letter from the editor or a link to the editorial policy that allows re-prints. Please upload this information as a .doc or .docx file to your Editorial Manager account. The Figure must be cited appropriately in the Figure Legend, i.e. “This figure has been modified from [citation].” (none). For Figure 1, an email giving permission from the website manager is uploaded.   
22. Figures 4-6: Please include a space between the numbers and their units and use the micro symbol µ instead of u (i.e., 100 µm and 50 µm).  
23. Please shorten the figure legends. The Discussion of the Figures should be placed in the Representative Results. Details of the methodology should not be in the Figure Legends, but rather the Protocol.   
24. Discussion: Please also discuss critical steps within the protocol, any modifications and troubleshooting of the technique.

25. Please ensure that the references appear as the following: [Lastname, F.I., LastName, F.I., LastName, F.I. Article Title. Source. Volume (Issue), FirstPage – LastPage (YEAR).] For more than 6 authors, list only the first author then et al.  
26. References: Please do not abbreviate journal titles. Please include volume and issue numbers for all references.

Thank you to the reviewers for their comments.

**Reviewers' comments:**  
  
  
  
Reviewer #1:  
  
Manuscript Summary:  
The paper deals with the description of an important model of wound healing using corneal organ cultures. The use of organ cultures allows studying live human tissue, which is a unique opportunity for an experimental system. The difference of the used model from the majority of other studies is related to stromal wounds that recapitulate the emergence of myofibroblasts and scarring, which are seen clinically. The authors present an organ culture model system for assessing scarring versus regenerative healing using rabbit, pig, and human eyes. Corneas still in the globe are wounded with a trephine removing central epithelium and anterior part of the stroma. After wounding, corneas are cut out, mounted on a collagen/agar base, and cultured for up to one month. This model can be used to assay improvement in healing, the effects of various anti-scarring agents and for toxicology studies. The authors also present their data on testing the effects of small molecule inhibitors and lipid-mediated siRNA transfection for gene knockdown. The paper is well written and this reviewer has only minor comments on the manuscript.  
  
Major Concerns:  
None  
  
Minor Concerns:  
1. Please describe in more detail the markers used for the general reader including alpha-SMA, fibronectin ED-A and Spautin-1 (and why it is used).

Spautin targets USP10 non-specifically. We have added this information now to the results with the description of the siRNA, see below. We have added a description of a-SMA and FN-EDA to the results section of Figure 5.

2. It is unclear what is "experimental siRNA". Please just call it by the target, e.g., siRNA to fibronectin.

It has been added to the text for Figure 5. It had been omitted because the explanation of the target may be beyond the scope of the paper but hopefully it fits into the construct of the paper.

3. It is unclear why the epithelium stains for alpha-SMA. Is this a paraffin embedding problem? In fresh-frozen sections this does not happen.

True. We have tried to eliminate this but have not been successful. However, others have seen this as well in organ culture and when we induce “regenerative healing” with our USP10 siRNA, the staining is significantly reduced suggesting it is EMT. These concepts are published and now better explained and cited in this paper. Our studies have focused only on quantifying myofibroblast and fibrotic development in the stroma. We have made clear that our quantification is only of the stroma using this technique because of any background in the epithelium. I have augmented the prose on this entire issue in the Discussion.

4. JOVE has recently published one paper on human corneal organ cultures used for epithelial wound healing studies. The authors might like to cite it (Kramerov et al. Adenoviral gene therapy for diabetic keratopathy: effects on wound healing and stem cell marker expression in human organ-cultured corneas and limbal epithelial cells. J Vis Exp, 2016;110:e54058.

Inserted.   
  
  
  
Reviewer #2:  
  
Manuscript Summary:  
The authors present an organ culture, ex vivo model for creating, assessing, and investigating corneal wound healing.  
  
Major Concerns:  
-Why do the authors highlight the need for wounding prior to corneal removal/cut from the globe?

Please see the answer to the next question.

-Cant the model be used by scientists when only the corneal rim is available? and not the whole globe?

Yes. It may be a bit more difficult to wound as the wounding is performed in the globe because the pressure of the globe plus the hand pressure on the globe aids in creating a smooth, taut surface for wounding. However, we have added this possibility to the Discussion and cited a paper that used human corneas as the starting point of the experiment.   
  
Minor Concerns:  
-Short abstract: The authors state "organized 3D multi cellular environment". It is just the cornea. Please correct.

The cornea is a multi-cellular environment. Epithelial cells, keratocytes, endothelial cells, limbal cells, resident immune cells, etc…and the cornea is also highly innervated.

-It is not clear why the model is limited to trephine wounds? Unless there are problems with using other wounding protocols (burn, penetrating, and so on), the authors should highlight the flexibility of their model.

Thank you for the suggestion. Although we have not done this ourselves, we have added the possibility to the Discussion as an alternative wounding strategy.   
  
  
  
Reviewer #3:  
  
General comments:  
Authors describe a corneal wound healing model using porcine corneas stored in a Petri dish that aims to study "stromal scar" generation. Authors do not study "stromal scar formation" (extracellular matrix deposition, transparency,…), but only a surrogate that is myofibroblast formation in the stroma.

We have removed the word scar in many places and replaced it with myofibroblast development and fibrotic marker development..

They also used siRNA in this model but this part of the protocol is not fully described. The protocol is rather well described but certain details should be provided to enable others to reproduce the results. Several sentences and concepts described in the introduction and the discussion are oversimplified or incorrect.  
  
Specific comments:  
Introduction  
- Line 70  
o Epithelial wound healing primarily involves several factors not limited to "activation of limbal stem cells", such as migration of epithelial cells from the leading edge  
o Location of epithelial stem cells, especially in animal corneas is a debated subject (Majo et al. Nature 2008)  
o You should discuss these point as wound healing model are not that simple.  
- Line 78 to 90:  
o please provide reference for each assertion  
- Line 81:

This paper is not intended in any way to discuss the activation of limbal stem cells. We have added this prose.

Corneal epithelial wounding that does not breach the epithelial basement membrane normally closes within 24-72 h 9. Soon after wounding, the cells at the edge of the epithelium start spreading and migrating into the epithelial free surface, to reestablish epithelial barrier function. This activity is sequentially followed by activation of corneal basal cell proliferation first and, in a later stage, of precursor cells located at the outer limbal zone to achieve recovery of epithelial cell mass 10,11.

o "inducing integrin mediated myofibroblast differentiation"  
o Not only Integrin mediated, several factors could be involved, TGFß, CD147, etc…

The word integrin was removed. We had already named other factors.   
o Please rephrase  
o Provide reference  
- Line 98 to 102:

I have added a reference to our paper.

o "The corneas are cultured in serum-free media plus vitamin C. Neither the addition of serum nor growth factors are needed to induce scar formation."  
o Discuss the addition of Vitamin C

We have added the reason for adding stabilized vitamin C and referenced this point.   
  
Methods  
- Line 170  
o "Before use, the agar should come to a warm (not hot) temperature."  
o Temperature is critical, please specify the range of temperature used.  
- Line 176

We have now listed “(approximately 25 ºC)”

o Use International nomenclature for volumes. "mL"  
- Line 178

done  
o "Wet the corneal surface every day to maintain moisture."  
o Specify how many times

“once” is added  
o Specify incubation environment

5% CO2 and 37°C is added.

o Specify if you placed a lid  
- Line 181-182:

“cover with the lid” was added.   
o "For gene knockdown, the wound is treated with experimental or control siRNA that is complexed to Lipofectamine 2000 (Invitrogen) by the standard protocol."  
o As it is a protocol, please specify what is the "standard" protocol" and how you proceeded for "wound treatment"  
- Line 227 & 234:

We have removed the phrase by the standard protocol and added the method. We are not allowed to state the name of the company in the text.

o Specify the antibody and the dilution that were used

added 1:250  
- Did you estimated the depth of the trephination?

This wound is not a “perfectly controlled wound” like PRK as reviewer 4 suggests and is stated in the Discussion. Although after performing hundreds of these wounds, we seem to produce the approximate same depth every time. It is about the top 1/3 of the cornea if one follow’s our procedure. Wounding mechanically is not a perfect science. We view this as an advantage to the model as clinical wounds, (unless it is in a clinician’s office) are irregular in nature. Just as burning a cornea with NaOH or other agents is done routinely in vivo, it can’t be completely controlled. This method is reproducible and has been routinely taught to junior staff, who mastered it on the first try.

Figure 1.  
Line 302-303 "An intact membrane separating the epithelium from the stroma"  
- Specify "Basement membrane" as it is not clear if you refer to the Bowman layer or to the Basement membrane

I was purposely vague because I am generalizing to all mammals. I have added an intact Bowman’s membrane or basement membrane separating the epithelium from the stroma is a necessary to prevent scarring in all mammals.

Figure 2.  
On your schematic representation, in F you represent myofibroblast in the epithelium and a restoration of the Bowman layer. Can you correct or provide a reference for this?

We have redone this to continue a “break” in the membrane.

After epithelial closure, the TGFß signal is reduced. As you emphasized TGFß comes from tears, epithelial cells and an autocrine loop of myofibroblast. Do you have a reference to emphasize the persistence of such autocrine loop after epithelial closure and basement membrane restoration? After epithelial closure, the drop in TGFß level in the stroma induces apoptosis of myofibroblast but indeed not all of them. This point should be discussed in the discussion.

We have added in the Discussion with references that restoration of the basement membrane reduces fibrotic outcomes.

- Can you provide the result of your staining after 4 weeks?

It is the same, but I have taken out the words up to 4 weeks as we are not showing it here.

- Did you assess the deposition of extracellular matrix? The goal would be to assess if the aSMA cells are functionally active

Figure 5D, E is fibronectin-EDA. This is a splice variant of fibronectin, also called cellular fibronection. It is secreted from cells under fibrotic conditions, whereas “normal” plasma fibronectin circulates through the body. Thus, FN-EDA serves as one marker of fibrotic ECM expression. As reviewer 1 suggests as well, we have added text about FN-EDA and other markers that are routinely used.

- Can you provide a reference assessing the migration of fibroblast in the depopulated stroma after wound induction?

Inserted:

After corneal stromal wounding, the stroma is populated with cells of multiple origins including differentiated resident stromal cells as well as bone marrow-derived fibrocytes 3-5.

- A strong staining is observed in the epithelium, can you provide the negative control of each staining?

We have published this control (*Gillespie, et al Journal of Cell Science, 2017*). I have more thoroughly addressed this issue in the Discussion as stated above.

- Line 260 you say it could be related to EMT ("There is also an increase in epithelial reactivity that may suggest EMT transition."). Have you made other staining to confirm that hypothesis? Can you provide references from other team to emphasize this observation? Can you provide images in the unwounded area to compare the staining? Also provide the negative control images as it could be some background staining. Please see the new prose in the Discussion, which addresses these concerns.   
  
Line 270  
- The corneal surface is wetted every day and media is changed every two days.  
- Contradicts line 177?

Added:  
5.3 Wet the corneal surface once every day by adding 1 drop of SSFM from the conditioned media in the dish to maintain moisture. (Take the dish out of the incubator, put under the hood, remove the lid, wet surface with media from dish using a sterile pipette, cover again and put it back at the incubator).

Please provide specification for the SiRNA and the control used.  
Done.   
Figure 4 and 5  
You said you quantified the immunostaining. Can you provide the results?

The results are published with extensive quantification (*Gillespie, et al Journal of Cell Science, 2017*). Fig 4-6 are examples of what can be done with this method. We have added quantification of Figure 5 (Fig 5G) with permission, amended and reproduced from the *JCS* article.   
  
Figure 4-5-6  
Can you also explain your findings and provide pictures in the center of the wound at distance of the wound edge? Was the aSMA stained cells distributed evenly?

We have added two images to Figure 4 at 10X (Fig 4G,H) demonstrating the margins of the wound, and the gradient of a-SMA activation. These are fluorescent images so we have added this method of staining to the protocol.   
  
Line 277:  
- "fibronectin-EDA"  
- Provide antibody reference, dilution in the method section

We have taken this out of the Figure legend and listed the antibodies with dilutions in the Methods  
  
Line 280:  
- "In addition, performing qRT-PCR can assure gene knockdown in the tissue"  
- Can you provide these results?

We started doing this for in vivo studies (beyond the scope of this paper). We have done it for other targets in organ culture studies. Although I don’t have the data for the USP10 study, I think it is worth mentioning as it is absolutely standard and anyone that can isolate RNA, can do this.   
  
Did you observe a modification in stromal organization and matrix deposition?

Yes, we state this in the Results and we have shown the staining for FN-EDA.   
  
Line 282-286:  
- For RNA isolation, specify what part of the tissue you use or if you get rid of the sclera, endothelium, epithelium

There is no sclera. We have inserted:

**Methods:**

1. **Preparing the Tissue:**
   1. After a two week incubation if using some of the tissue for qRT-PCR analysis, before fixing, cut the cornea in half through the wound.
   2. Put this half or only ¼ (either is enough tissue) into stabilizing RNA protect reagent.
   3. Using a standard isolation kit, isolate RNA and perform qRT-PCR. Note: Alternatively, the wounded part only, can be isolated and tested for gene expression.
   4. For the other half of the cornea, place into Tissue Path Cassettes and submerge in fixative (10% formalin) for 2-4 days at Room Temperature (RT).

Discussion  
Line 366:  
You don't provide results in rabbit corneas, consequently you can't say the results are equivalent.

I didn’t say that the results are equivalent. I am saying that others have performed the trephine wound in rabbits in vivo and so you can perform organ culture with rabbit corneas and determine IF they are equivalent.   
  
Line 378-379:  
- "In terms of translation to in vivo studies, this same procedure can be accomplished in rabbits and thus organ culture results can be directly compared to in vivo results"  
- You can't directly compare two completely different experiments (in vivo vs ex vivo).  
- Wound healing involves tears, immune system, neurotrophic factors, etc…  
- You don't provide in vivo results to sustain this assertion  
- In mouse model as much as 70% of myofibroblast comes from bone marrow-derived precursor cells, what is not reproduced in an ex-vivo setting. Discuss that limitation  
- Reformulate your sentence.

I removed the word “directly”. No model system is perfect. The elegant work of Dr. Wilson shows that in mice a large percentage of the myofibroblasts derive from bone marrow. However, these are mice and thus if held to this standard we must also note that we do not know if this is true in humans. Furthermore, as Dr. Wilson writes “No differences in function between myofibroblasts derived from keratocytes and myofibroblasts derived from bone marrow-derived cells have been discovered.” *(Gustavo et al.J Refract Surg. 2016;32(2):118-125.)*

We already say that : “Since the organ culture corneas lack a functional limbal vasculature, tears, and aqueous humor, each investigator much assess if this will be a useful model for their studies. Resident activation of immune cells has been demonstrated, but the exact parallel to *in vivo* studies is not yet clear 6.”

We are demonstrating a 3D cellular model system that has been extremely consistent for producing myofibroblasts after wounding. Given the number of papers using TGFb to stimulate myofibroblast development in cell culture, a system that itself is flawed given the concentrations used (as an example), it seems quite reasonable to publish an assay in which wounded tissue without growth factor and serum addition produces myofibroblast-rich tissue and one in which experimental drugs can be easily applied to test if myofibroblast development and persistence can be regulated.   
  
Line 388-391:  
- "However, if assaying for fibrotic endpoints, or the effects on reepithelialization, bacterial infection, proliferation, migration, or apoptosis, the exact depth of the wound is not critical as long as the wound penetrating the anterior stroma is similar between corneas."  
- In corneal wound in vivo model using PRK, differences in myofibroblast activation could be observed if the stromal bed is regular or irregular, or between 2 stromal wounds with a difference in the depth of the wound of only 50 to 100µm  
- Most wound healing model trying to reproduce corneal scar study anterior stroma wounds. The density of keratocytes is uneven between the anterior and posterior part of the cornea  
- Please discuss that point and correct your statement

To satisfy this request we have changed it to:

A limitation of this model is that use of a trephine to produce a wound is uneven and cannot be reproduced identically from cornea to cornea compared to PRK laser-induced wounds 7. However, naturally occurring wounds are not all equivalent in depth and a large body of data suggest that any breach in the basement membrane generates myofibroblast development and haze in the stroma, whereas regeneration of the basement membrane leads to diminished scarring 8-10. Our pig corneal organ culture model employs a severe wound in which the basement membrane is removed within the area of the trephine. Development of myofibroblasts and fibrotic markers in the corneal stroma have been consistently and reproducibility achieved using this model system.  
  
Line 391:  
- "In addition, we have found that with the ex vivo organ culture tissue, using paraffin embedding has been more successful than frozen sections."  
- Please explain

We have chosen to remove this.   
  
End of comments.  
  
  
  
  
Reviewer #4:  
  
Manuscript Summary:  
This manuscript describes the process of wounding a cornea and maintaining it as an ex vivo organ culture model for the testing of interventions for modulating corneal wound healing, etc.  
  
Major Concerns:  
The description of this protocol at first glance seems reasonable, but gong through it in detail gives rise to many questions about the repeatability of this process. I have no doubt that the authors have had success developing and maintaining this organ culture system, however, if a reader were to attempt to replicate this procedure, there would be much trial and error to develop their own protocol. There is not enough detail in this manuscript for someone else to be able to replicate it.

We have now made it as clear as possible. It is very easy to do and to replicate.

Abstract does not have nearly enough background to introduce the model or the goals or the outcomes that are to be measured.

We have rewritten the abstract.   
  
If human corneas are used that are not of sufficient quality to be used in in vivo transplantation for human patients, how can they reliably demonstrate mechanistically the responses to wounding and healing that a normal cornea would have - this needs to be addressed as a limitation. There are considerable differences between different species (cell layers, layer thickness, lack of or robust nature of Bowman's layer, corneal diameter and curvature, overall thickness) that needs to be discussed and the differences in protocol (determining depth of wound, how much agarose necessary, etc.) need to be outlined specifically.

I removed human cornea. Typically the human corneas that one receives for research have no mechanical issues. They have infiltrates or the reason for donor death is not clear. In terms of the differences between species, if the basement (and/or Bowman’s membrane) is breached and doesn’t heal, persistent myofibroblasts will be present. The species doesn’t matter. I have many references to this in all of the reviews and we have inserted (as reviewer 3 suggested) references to show that when the basement membrane heals, scarring is diminished.   
  
The use of vitamin C is not discussed, just a passing mention, and this does not recapitulate the normal wounded state and degree of resultant fibrosis. This is induced fibrosis that is not natural.

The reason for the addition of Vitamin C is discussed above and added to the text. We are not using serum or growth factors to induce myofibroblast development. We are comfortable with adding vitamin c in supplemented serum-free media and using this as a model system. It does not compromise the results as others have shown. This is now better cited in the text.   
  
Protocol text: Agar solution - what temperature, how long?

Inserted.

Give a specific description and name to your surgical instruments (not just a catalogue number).

There is only one real surgical instrument, a trephine. This is named. There is nothing else to name.

How is the chopping board prepared? Is it sterilized and how? What material is it made of? How big? Need exacts volumes or weights of ingredients for supplemented serum-free media.

We have added ethanol sterilized chopping board. It doesn’t matter how big or how small. An eye isn’t that big. As per the journal’s instructions, we have put the SSFM recipe in the Methods.   
  
Methods description: Need much more detail about preparation of globe? What tissues are removed? How much? How long are they dipped in things? What do you mean by hold globe with Kim-Wipe and create pressure. Description is lacking here. Description of wounding is imprecise - "about 5 times"? Need to have a better method of determining depth? A precise trephine that only goes to a certain depth perhaps? How are you determining depth beyond "eyeballing it"? The goal should be to keep wound depth precise and repeatable between globes. Also, a razor blade is very crude for removing the wound button? Consider a Martinez dissector or something that will more precisely separate the lamellae. Where are you making short incision in the globe/cornea to remove the cornea from the globe? I assume you mean sclera posterior to the limbus, but it does not read this way? Also, how far posterior to the limbus?

We have stated exactly what we do. I am hoping that it is clear now. As I have said, the depth is not exactly precise as PRK (of course) but it is very easy and it works every time. Anyone I have taught can do it on the first or second try. Use of this technique has also been repeatedly published.

Need exact temperature of agar and volume necessary (will vary between species).

“Until full” . Temp is now stated.

Incubation - how much media is necessary during a change? How did you determine that changing once every other day is sufficient? What are the outcomes, factors you are assessing here? What are you moistening the cornea with - how much, how often? Once daily dose not mimic the in vivo state and adds the tremendous stress of exposure to the organ which does not recapitulate normal wound healing state. Are you leaving the cornea to sit in any experimental drugs you are applying topically? This also does not mimic what would happen with pulse therapy in the living animal. Need volumes that would need to be ordered to perform this protocol and how long those amounts would likely last.

I have clearly stated how to do this technique.   
  
Histo prep: RT = room temperature (?). How are you maintaining corneal curvature in your processed corneas? Need more precise volumes of fixatives and reagents.

A core facility inserts the cornea into a paraffin block. This is not an issue. We explained the orientation.   
  
Representative results: Background is lacking as are goals and outcomes assessments? The methods you describe do not evaluate time to re-epithelialization (rate of), so making an argument about using your methods for evaluating this ins imprecise and spurious.

I have been as clear as possible. Hopefully the changes will satisfy these requests.

Need much more detail about what you are assessing with Image J - are you assessing wound size, depth character, area, intensity of scar (and if so, how?). Be very specific if you expect someone to be able to repeat your protocol. If you are going to discuss this particular experiment as an example, it needs much more background regarding what you are looking to assess, what the agents you are using are expected to do and what the results were, how they were determined, assessed and evaluated. Need more discussion and explanation of a "regeneratively healed wound" and the difference between a normal wound - how you are assessing this and how it is achieved.

We have added the steps of quantification to the protocol and added a graph of the quantification of Figure 5, now Fig 5G.   
  
Minor Concerns:  
Figure 1 does not illustrate what it is purported to in the text.

???

Figure 3 A and B are not noted in the body of the text.

Yes, they are there.

A few minor grammatical and punctuation errors. Figure legends - It is not true that primates and chickens are the only species that have a Bowman's layer.

I have asked 3 other leaders in the field. This is the answer that I consistently received. If you disagree please tell us what is the other species with the reference.

What tissues are you removing when you are preparing the globes - be specific. Discuss quantification of corneal staining with Image J in detail. This method as described is not useful for determining effects on wound re-epithelialization as described - there need to be a method for determining time to complete re-epithelialization. Need to discuss how you would assess this (fluorescein at different time points, sacrificing representative samples at different time points, confocal imagery? Also, if epithelium is to be assessed only after fixation and processing, there are major concerns for artifact formation. Discuss in detail your camera settings for photography (focal distance, aperture, etc.)? Is there any post-production necessary? How are the images analyzed?

Most of this has been addressed. Imaging is done with a microscope at any setting to image histological samples.

The references below are for this response only. The numbers don’t coordinate with the numbers in the manuscript.

1 Ljubimov, A. V. & Saghizadeh, M. Progress in corneal wound healing. *Prog Retin Eye Res.* **49** 17-45, doi:10.1016/j.preteyeres.2015.07.002, (2015).

2 Echevarria, T. J. & Di Girolamo, N. Tissue-regenerating, vision-restoring corneal epithelial stem cells. *Stem Cell Rev.* **7** (2), 256-268, doi:10.1007/s12015-010-9199-1, (2011).

3 Wilson, S. E., Mohan, R. R., Hong, J. W., Lee, J. S. & Choi, R. The wound healing response after laser in situ keratomileusis and photorefractive keratectomy: elusive control of biological variability and effect on custom laser vision correction. *Arch Ophthalmol.* **119** (6), 889-896, doi:emo10005 [pii], (2001).

4 Zieske, J. D., Guimaraes, S. R. & Hutcheon, A. E. Kinetics of keratocyte proliferation in response to epithelial debridement. *Exp Eye Res.* **72** (1), 33-39 (2001).

5 Lassance, L., Marino, G. K., Medeiros, C. S., Thangavadivel, S. & Wilson, S. E. Fibrocyte migration, differentiation and apoptosis during the corneal wound healing response to injury. *Exp Eye Res.* **170** 177-187, doi:10.1016/j.exer.2018.02.018, (2018).

6 Stepp, M. A. *et al.* Wounding the cornea to learn how it heals. *Exp Eye Res.* **121C** 178-193, doi:S0014-4835(14)00044-X [pii]

10.1016/j.exer.2014.02.007, (2014).

7 Sharma, A., Mehan, M. M., Sinha, S., Cowden, J. W. & Mohan, R. R. Trichostatin a inhibits corneal haze in vitro and in vivo. *Invest Ophthalmol Vis Sci.* **50** (6), 2695-2701, doi:iovs.08-2919 [pii]

10.1167/iovs.08-2919, (2009).

8 Marino, G. K., Santhiago, M. R., Torricelli, A. A., Santhanam, A. & Wilson, S. E. Corneal Molecular and Cellular Biology for the Refractive Surgeon: The Critical Role of the Epithelial Basement Membrane. *J Refract Surg.* **32** (2), 118-125, doi:10.3928/1081597X-20160105-02, (2016).

9 Marino, G. K., Santhiago, M. R., Santhanam, A., Torricelli, A. A. M. & Wilson, S. E. Regeneration of Defective Epithelial Basement Membrane and Restoration of Corneal Transparency After Photorefractive Keratectomy. *J Refract Surg.* **33** (5), 337-346, doi:10.3928/1081597X-20170126-02, (2017).

10 Marino, G. K. *et al.* Epithelial basement membrane injury and regeneration modulates corneal fibrosis after pseudomonas corneal ulcers in rabbits. *Exp Eye Res.* **161** 101-105, doi:10.1016/j.exer.2017.05.003, (2017).