Journal of Cell Science

Downregulation of collagen XI during late postnatal corneal development is followed by upregulation after injury

Mei Sun, Devon Cogswell, Sheila Adams, Yasmin Ayoubi, Ambuj Kumar, Tea Reljic, Marcel Y. Avila, Curtis E. Margo and Edgar M. Espana
DOI: 10.1242/jcs.258694

Editor: Kathleen Green

Review timeline
Original submission: 24 March 2021
Editorial decision: 4 May 2021
First revision received: 21 July 2021
Editorial decision: 31 August 2021
Second revision received: 16 November 2021
Accepted: 18 November 2021

Original submission

First decision letter

MS ID#: JOCES/2021/258694

MS TITLE: Collagen XI recapitulation regulates fibrillogenesis after corneal injury

AUTHORS: Edgar M Espana, Mei Sun, Devon Cogswell, Sheila Adams, Yasmin Ayoubi, Marcel Avila, and Curtis Margo
ARTICLE TYPE: Research Article

We have now reached a decision on the above manuscript.

To see the reviewers' reports and a copy of this decision letter, please go to: https://submit-jcs.biologists.org and click on the 'Manuscripts with Decisions' queue in the Author Area. (Corresponding author only has access to reviews.)

As you will see, the reviewers raise a number of substantial criticisms that prevent me from accepting the paper at this stage. They suggest, however, that a revised version might prove acceptable, if you can address their concerns. If you think that you can deal satisfactorily with the criticisms on revision, I would be pleased to see a revised manuscript. We would then return it to the reviewers.

We are aware that you may be experiencing disruption to the normal running of your lab that makes experimental revisions challenging. If it would be helpful, we encourage you to contact us to discuss your revision in greater detail. Please send us a point-by-point response indicating where you are able to address concerns raised (either experimentally or by changes to the text) and where you will not be able to do so within the normal timeframe of a revision. We will then provide further guidance. Please also note that we are happy to extend revision timeframes as necessary.

Please ensure that you clearly highlight all changes made in the revised manuscript. Please avoid using 'Tracked changes' in Word files as these are lost in PDF conversion.

I should be grateful if you would also provide a point-by-point response detailing how you have dealt with the points raised by the reviewers in the 'Response to Reviewers' box. Please attend to all of the reviewers' comments. If you do not agree with any of their criticisms or suggestions please explain clearly why this is so.

Reviewer 1

Advance summary and potential significance to field

1) collagen XI is expressed in the stroma during development and following injury in adult mice 2) Collagen XI is a regulator of collagen fibrillogenesis in regenerating corneal tissue

Overall a well performed study that convincingly demonstrates the importance of Collagen XI in regulating collagen fibril diameter during corneal wound healing. It will be an important contribution to the corneal wound healing field.

Comments for the author

Introduction 1. "Collagens V and XI are fibril-forming collagens.(Blaschke et al., 2000; Fichard et al., 1995; Smith and Birk, 2012) These minor collagens can be considered different molecular forms of a single collagen type due to their structural similarities.(Fichard et al., 1995; Hoffman et al., 2010; Smith and Birk, 2012)." How do the functions of these two fibril-forming collagens differ? Why are there two that are so similar?
Results
1. In Fig. 2 it seems to the reviewer that cellular concentrations of the antigen should also be seen, not just diffuse stromal detection. What is the explanation for not detecting the cellular production?
2. Flg. 7, it would be better presented if each panel had inserts with higher magnification that more clearly shows the differences in fibril diameter. This is especially true if the final size of the figure is as it was in the review copy.
Discussion
1. "Cornealnstromal regeneration is not a widely accepted phenomenon. " Reword this sentence. Corneal stromal regeneration after corneal injury is not supported by published studies." or something like that.
2. "The entire field of laser refractive surgery of the cornea is based on the dogma that laser ablation of stromal tissue creates a predictable and irreversible permanent change in corneal shape." Provide references for this statement.

Reviewer 2

Advance summary and potential significance to field

Type XI collagen has not be reported in the mouse cornea previously. The authors look at expression in the post-natal cornea as it develops, in response to injury, and use an inducible knockout of the protein to show that removing collagen XI alters collagen fiber diameters in the wounded corneal stroma.

Comments for the author

The paper by Sun and colleagues titled "Collagen XI expression and recapitulation post corneal injury" looks at expression of collagen XI in the mouse corneal stroma during post-natal development, in response to two different types of wounds, and in the wounded corneas of mice whose expression of collagen XI has been inhibited using an ERT inducible Cre-lox mouse. The quality of the images presented is exceptional and the study is presented well. Yet, there are numerous issues that need to be addressed.
1. The word “recapitulation” used in the title is not helpful to readers. The authors appear to intend to evoke the idea of the recapitulation theory (ontogeny recapitulates phylogeny) since collagen XI is up-regulated during early post-natal development and in response to injury. The

recapitulation theory was largely disproved many years ago (see Wikipedia entry for Recapitulation Theory). There is no need to use a term some readers will not understand and others may rebel against. Perhaps a variation on a title like “Down-regulation of Collagen XI during late post-natal corneal development is followed by up-regulation in response to corneal injury” would be more helpful.

2. Figure 1 offers compelling data on the down-regulation of collagen XI seen during post-natal corneal development in the mouse at the mRNA and protein level using The Wes system to assess protein expression. There are no statistics indicated on the quantification shown in panels 1A and 1C. It would be useful to give readers the molecular mass of the protein shown in Figure 1B. Also, the extraction method provided in the methods section is inadequately described. Typically, buffers harsher than RIPA are used to extract collagens including collagen XI from tissues. The authors do not describe the use of any protease or MMP inhibitors in the extraction buffer. It would have been useful for the authors to have re-extracted the insoluble pellet as is typical for matrix extraction procedures. If collagen XI became incorporated into the matrix and became covalently cross-linked to other collagens over time with age, there may appear to be less soluble collagen even though there was more total collagen.
3. Figure 3 could be combined with Figure 4. There is no mag bar present in Figure 3A and B.
4. The methods described for the types of wounds made to the cornea are confusing and incomplete. The methods section describes a “partial thickness corneal laceration performed limbus to limbus”. The results section and legend to Figure 4 describes keratectomy and debridement wounds. There is no method described for the debridement wound. The terms used in the methods section, results, and legends for the laceration/keratectomy wounds need to be the same.
5. The experiments extracting RNA from control and wounded corneas involve removing the corneal epithelium as a sheet from the stroma using dispase. This allows the authors to quantify RNA from the remaining corneal button which includes the corneal stroma and endothelium. As shown in Figure 4A and C, the epithelium has grown into the stromal wound bed; it is unlikely that all of the epithelium and its RNA have been removed in the keratectomy wounded corneas. In the control and debridement wounded corneas, the dispase procedure will work as expected. This difference may bias the results from the keratectomy wounded corneas which will contain more epithelial RNA.
6. In Figure 5, injured corneal RNA samples are labeled with the letters “Inj”; the legend states that they are labeled “Inju”.
7. In Figure 6, the injury type presented in the images is confusing for the following reasons: The use of the terms I and R in Figure 6A implies that the data were obtained from keratectomy wounded mice. Yet, the images shown in Figure 6B appear morphologically more similar to those presented in Figure 4 for debridement rather than keratectomy. It would be more consistent if the images presented in Figure 6B and C morphologically appeared more similar to those presented in Figure 4A and C. Also, the time points used for these studies are not made explicitly clear to the readers.
8. In Figure 7, the quality of the TEM images is excellent. It would be useful to present images from the adjacent areas of the wounded corneas to show that the changes seen in collagen fiber diameter are due to wounding and not due to the deletion of the gene in sites where the stroma was left uninjured.

Reviewer 3

Advance summary and potential significance to field

In this manuscript, the authors characterized the expression of collagen 11 in mouse cornea during wound healing. They observed an increase in collagen 11 expression at day 1 post injury and the expression went down to the normal level at 6 weeks post injury. Interestingly, knockout of collagen 11 resulted in abnormal fibril formation. This suggests that collagen 11 has a role in corneal wound healing.

Comments for the author

how loss of collagen 11 results in such abnormal fibrils remains unanswered.

Furthermore, it is unknown whether the abnormal fibrils have any impact on vision and corneal clarity. Overall, this manuscript is descriptive and lacks the mechanistic insight.

1. Fig4 described two injury models: keratotomy and debridement injury. It's not clear which one was conducted in Fig5 and 6.
2. Tamoxifen (TM) should be mentioned at the first place when it is mentioned.
3. In Methods, more detail about keratotomy and debridement injury are necessary. A cartoon may be more helpful to understand these two injury approaches.
4. Fig1C doesn't have standard deviation even though the figure legend says so.
5. Please carefully correct typos in this manuscript.
6. Fig 1 experiment design is confusing. The RNAs from stroma were used for PCR but proteins were isolated from whole cornea.
7. In Fig1, it's not sure how the authors normalize col XI protein levels to the total protein staining. What program was used?
8. In Fig2 the signal is faint and is difficult to distinguish from background. Thus, it is hard to interpret the data. A normal serum only control may be helpful to determine the background fluorescent signal.
9. Why no green DTAF in Fig 6b to indicate the injured area?
10. In Fig7, high mag images of controls are necessary.

First revision

Author response to reviewers' comments

Reviewer 1: Thank you for your comments and suggestions. We addressed your comments and made changes in the manuscript as recommended.

Reviewer 1 Advance Summary and Potential Significance to Field:
1) collagen XI is expressed in the stroma during development and following injury in adult mice
2) Collagen XI is a regulator of collagen fibrillogenesis in regenerating corneal tissue

Overall a well performed study that convincingly demonstrates the importance of Collagen XI in regulating collagen fibril diameter during corneal wound healing. It will be an important contribution to the corneal wound healing field.

Reviewer 1 Comments for the Author:
Introduction
1. "Collagens V and XI are fibril-forming collagens.(Blaschke et al., 2000; Fichard et al., 1995; Smith and Birk, 2012) These minor collagens can be considered different molecular forms of a single collagen type due to their structural similarities.(Fichard et al., 1995; Hoffman et al., 2010; Smith and Birk, 2012)." How do the functions of these two fibril-forming collagens differ? Why are there two that are so similar?

We are not sure how the functions of these two collagens differ. Collagen I form fibrils with collagen V. Collagen XI form fibrils with collagen II. It could be possible that during development or after injury, collagen II could be temporarily expressed in the stroma. Expression of collagen II has been shown in keratocytes in vitro and in developing mammal corneas. Collagen XI form fibrils with collagen III which is highly expressed during fibrillogenesis after corneal injury.
It is difficult to explain why collagens V and XI are so similar. The genes for collagens V and XI are on different chromosomes, 9 and 6, respectively. The regulatory functions of collagen XI are unknown, but it is becoming evident in the field of matrix biology that collagens and matrix components are not just structural fibrils but proteins with regulatory functions. The areas of collagen XI and V that are different most have different but necessary functions. The differences in collagens V and XI structure may relate to as yet undetermined regulatory functions.

Results

1. In Fig. 2 it seems to the reviewer that cellular concentrations of the antigen should also be

seen, not just diffuse stromal detection. What is the explanation for not detecting the cellular production?

We can see hints of collagen XI inside the cytoplasm of cells occasionally. Keratocyte shape, being stellate and spread between lamellae with thin cytoplasm, makes it hard to differentiate intracellular expression vs matrix deposition. In injured tissue, cells are more disorganized in shape and intracellular expression is more easily noticed. We added a picture showing green is DTAF and areas of cytoplasmic collagen XI are red, after injury, findings that support this phenomenon. (Fig 2)

[*NOTE: We have removed unpublished data that had been provided for the referees in confidence*.]

2. FIg. 7, it would be better presented if each panel had inserts with higher magnification that more clearly shows the differences in fibril diameter. This is especially true if the final size of the figure is as it was in the review copy.

Thank you for the suggestion. Done.

Discussion

1. "Corneal stromal regeneration is not a widely accepted phenomenon. " Reword this sentence. Corneal stromal regeneration after corneal injury is not supported by published studies." or something like that.

Thank you for the suggestion, done. See first paragraph page 7.

2. "The entire field of laser refractive surgery of the cornea is based on the dogma that laser ablation of stromal tissue creates a predictable and irreversible permanent change in corneal shape." Provide references for this statement.

We are toning down this statement and providing two references.

Stromal changes after LASIK remain controversial, one study indicated a minor (insignificant) increase over time: ErieJC, PatelSV, McLarenJW, et al. Effect of myopic laser in situ keratomileusis on epithelial and stromal thickness: a confocal microscopy study. Ophthalmology. 2002;109:1447–1452.
Other study reports stability. PatelSV, ErieJC, McLarenJW, BourneWM. Confocal microscopy changes in epithelial and stromal thickness up to 7 years after LASIK and photorefractive keratectomy for myopia. J Refract Surg. 2007;23:385–392.

Reviewer 2 Advance Summary and Potential Significance to Field:
Type XI collagen has not been reported in the mouse cornea previously. The authors look at expression in the post-natal cornea as it develops, in response to injury, and use an inducible knockout of the protein to show that removing collagen XI alters collagen fiber diameters in the wounded corneal stroma.

Reviewer 2 Comments for the Author:
The paper by Sun and colleagues titled "Collagen XI expression and recapitulation post corneal injury" looks at expression of collagen XI in the mouse corneal stroma during post-natal development, in response to two different types of wounds, and in the wounded corneas of mice whose expression of collagen XI has been inhibited using an ERT inducible Cre-lox mouse. The quality of the images presented is exceptional and the study is presented well. Yet, there are numerous issues that need to be addressed.

Thank you for your comments and suggestions. We addressed your comments as follows:

1. The word “recapitulation” used in the title is not helpful to readers. The authors appear to intend to evoke the idea of the recapitulation theory (ontogeny recapitulates phylogeny) since collagen XI is up-regulated during early post-natal development and in response to injury. The

recapitulation theory was largely disproved many years ago (see Wikipedia entry for Recapitulation Theory). There is no need to use a term some readers will not understand and others may rebel against. Perhaps a variation on a title like "Down-regulation of Collagen XI during late post-natal corneal development is followed by up-regulation in response to corneal injury" would be more helpful.

We are following your suggestion. Thank you. Term recapitulation was removed from the entire manuscript. We changed the title and followed the journal instructions not to exceed 120 words.

2. Figure 1 offers compelling data on the down-regulation of collagen XI seen during post-natal corneal development in the mouse at the mRNA and protein level using The Wes system to assess protein expression. There are no statistics indicated on the quantification shown in panels 1A and 1C. It would be useful to give readers the molecular mass of the protein shown in Figure 1B. Also, the extraction method provided in the methods section is inadequately described. Typically, buffers harsher than RIPA are used to extract collagens including collagen XI from tissues. The authors do not describe the use of any protease or MMP inhibitors in the extraction buffer**. It would have been useful for the authors to have re-extracted the insoluble pellet as is typical for matrix extraction procedures. If collagen XI became incorporated into the matrix and became covalently cross-linked to other collagens over time with age, there may appear to be less soluble collagen even though there was more total collagen.**

We update the Materials and Methods section. Figure 1C is updated with SD bars. We also added in the molecular weight marker for the WES image to indicate the size of the collagen XI. Thank you for pointing out the oversight we inadvertently made regarding protein extraction. Actually, we did use other lysis buffer to extract protein for collagen XI protein expression. The buffer is the same buffer we used for collagen V extraction from cornea as previously published in this Journal: Collagen V is a dominant regulator of collagen fibrillogenesis: dysfunctional regulation of structure and function in a corneal-stroma-specific Col5a1-null mouse model. Journal of Cell Science, 2011 Dec 1;124(Pt 23):4096-105. The reference is updated.

3. Figure 3 could be combined with Figure 4. There is no mag bar present in Figure 3A and B.

A new image has been added that clarifies the technique. Mag bar was added.

The methods described for the types of wounds made to the cornea are confusing and incomplete. The methods section describes a "partial thickness corneal laceration performed limbus to limbus". The results section and legend to Figure 4 describes keratectomy and debridement wounds. There is no method described for the debridement wound. The terms used in the methods section, results, and legends for the laceration/keratectomy wounds need to be the same.

Thank you for your comment and apologize for not being clear in the surgical technique description. We are making sure terms are consistent in the entire manuscript. We performed both types of injury in the same eye. We have just published this type of injury, T3, in a recent paper referenced in materials and methods: Creation and grading of experimental corneal scars in mice models. Cogswell D, Sun M, Greenberg E, Margo CE, **Espana EM.** Ocul Surf. 2021 Jan;19:53-62. In this current manuscript we used a combination of limbus to limbus keratotomy and debridement around the keratotomy.

5. The experiments extracting RNA from control and wounded corneas involve removing the corneal epithelium as a sheet from the stroma using dispase. This allows the authors to quantify RNA from the remaining corneal button which includes the corneal stroma and endothelium. As shown in Figure 4A and C, the epithelium has grown into the stromal wound bed; it is unlikely that all of the epithelium and its RNA have been removed in the keratectomy wounded corneas. In the control and debridement wounded corneas, the dispase procedure will work as expected. This difference may bias the results from the keratectomy wounded corneas which will contain more epithelial RNA.

We apologize for the confusion created with our description of the injury technique used and hope that the new Figure 3 better explains how we injured the corneas. It is expected that Dispase

digests basement membranes so we assumed that the “epithelium grown into the stromal wound bed” was removed since this cornea was injured 3 weeks prior. However, we agree that some epithelium may not be completely removed from keratotomy wounds and it could influence the results when using actin for normalization. To make sure results are consistent, we have repeated the same experiment using the entire cornea without epithelial removal and found similar results. Epithelium does not express collagen XI. We added a brief comment to Discussion about these findings.

6. In Figure 5, injured corneal RNA samples are labeled with the letters “Inj”; the legend states that they are labeled “Inju”.

Thank you, The typo was corrected.

7. In Figure 6, the injury type presented in the images is confusing for the following reasons: The use of the terms I and R in Figure 6A implies that the data were obtained from keratectomy wounded mice. Yet, the images shown in Figure 6B appear morphologically more similar to those presented in Figure 4 for debridement rather than keratectomy. It would be more consistent if the images presented in Figure 6B and C morphologically appeared more similar to those presented in Figure 4A and C. Also, the time points used for these studies are not made explicitly clear to the readers.

We have updated images in Figure 6. Tissue was collected 3 weeks after injury. This was added to the manuscript, Fig Legends.

8. In Figure 7, the quality of the TEM images is excellent. It would be useful to present images from the adjacent areas of the wounded corneas to show that the changes seen in collagen fiber diameter are due to wounding and not due to the deletion of the gene in sites where the stroma was left uninjured.

New photomicrographs were obtained and added to figure 6.

Reviewer 3 Advance Summary and Potential Significance to Field:
In this manuscript, the authors characterized the expression of collagen 11 in mouse cornea during wound healing. They observed an increase in collagen 11 expression at day 1 post injury and the expression went down to the normal level
at 6 weeks post injury. Interestingly, knockout of collagen 11 resulted in abnormal fibril formation. This suggests that collagen 11 has a role in corneal wound healing.

Reviewer 3 Comments for the Author:
How loss of collagen 11 results in such abnormal fibrils remains unanswered. Furthermore, it is unknown whether the abnormal fibrils have any impact on vision and corneal clarity. Overall, this manuscript is descriptive and lacks
the mechanistic insight.

Reviewer 3: Thank you for your comments and suggestions. We addressed your comments as follows:

Fig 4 described two injury models: keratotomy and debridement injury. It's not clear which one was conducted in Fig 5 and 6.

We apologize for the abbreviated description on how these injuries were created. We performed both injuries, keratotomy and debridement injuries in the same eye. The technique has been recently published: Creation and grading of experimental corneal scars in mice models. Cogswell D, Sun M, Greenberg E, Margo CE, Espana EM. Ocul Surf. 2021 Jan;19:53-62. The image shows a representative keratotomy area and a representative debridement area. We added a new picture explaining how the surgical technique was done to clarify the surgical technique used.

2. Tamoxifen (TM) should be mentioned at the first place when it is mentioned.

Fixed. Thank you.

3. In Methods, more detail about keratotomy and debridement injury are necessary. A cartoon may be more helpful to understand these two injury approaches.

We added a new image to Figure 3 illustrating how the injury technique was performed.

4. Fig 1C doesn't have standard deviation even though the figure legend says so.

Thank you for the suggestion. Standard deviation added.

5. Please carefully correct typos in this manuscript.

We carefully reviewed the manuscript again.

6. Fig 1 experiment design is confusing. The RNAs from stroma were used for PCR but proteins were isolated from whole cornea.

We used beta actin as internal control for RNA but total protein for WES which should be less affected by cell number. We were also concerned with the use of Dispase to remove the epithelium since this enzyme may digest stromal/ECM proteins. We assume based in pilot studies that epithelium does not express collagen XI.

7. In Fig1, it's not sure how the authors normalize col XI protein levels to the total protein staining. What program was used?

We added the following to the manuscript: Quantification by densitometry was performed using the area of the targeted protein and normalized to total protein amount, which was analyzed by loading an equal amount of protein to a separate capillary cartridge and detected with the Wes total protein detection module. Data analyses were performed using the Compass Software (ProteinSimple) and the normalization is calculated with Excel.

8. In Fig 2 the signal is faint and is difficult to distinguish from background. Thus, it is hard to interpret the data. A normal serum only control may be helpful to determine the background fluorescent signal.

It was added and the figure format is now on RGB to improve image visualization.

9. Why no green DTAF in Fig 6b to indicate the injured area?

New samples were prepared and stained and new Figures were added.

10. In Fig7, high mag images of controls are necessary.

Figures were added.

Second decision letter

MS ID#: JOCES/2021/258694

MS TITLE: Collagen XI recapitulation regulates fibrillogenesis after corneal injury

AUTHORS: Edgar M Espana, Mei Sun, Devon Cogswell, Sheila Adams, Yasmin Ayoubi, Marcel Avila, and Curtis Margo
ARTICLE TYPE: Research Article

We have now reached a decision on the above manuscript.

To see the reviewers' reports and a copy of this decision letter, please go to: https://submit-jcs.biologists.organd click on the 'Manuscripts with Decisions' queue in the Author Area. (Corresponding author only has access to reviews.)

As you will see, the reviewers overall felt that you responded well to the issues raised in the first round of review. Referee #3 has raised some critical issues, however. In particular they commented on statistical analysis. While I see that you did include additional information in the paper, overall as editor I have some concerns about the statistics. JCS is currently striving to ensure that data is presented in as transparent fashion as possible, including inclusion of all the data points and encouraging use of super plots where appropriate (in contrast to simple bar graphs). I also note that the p values for some of your graphs seem lower than I would expect based on the standard deviations. Therefore, I would ask that you consider showing all data points and also describe exactly what statistical tests were used and how the p values were defined. In addition, you have included quantitative data in a selective way, and I would strongly encourage you to include some sort of quantitative analysis in parallel with images that currently lack quantification.

We are aware that you may be experiencing disruption to the normal running of your lab that makes experimental revisions challenging. If it would be helpful, we encourage you to contact us to discuss your revision in greater detail. Please send us a point-by-point response indicating where you are able to address concerns raised (either experimentally or by changes to the text) and where you will not be able to do so within the normal timeframe of a revision. We will then provide further guidance. Please also note that we are happy to extend revision timeframes as necessary.

Please ensure that you clearly highlight all changes made in the revised manuscript. Please avoid using 'Tracked changes' in Word files as these are lost in PDF conversion.

I should be grateful if you would also provide a point-by-point response detailing how you have dealt with the points raised by the reviewers in the 'Response to Reviewers' box. Please attend to all of the reviewers' comments. If you do not agree with any of their criticisms or suggestions please explain clearly why this is so.

Reviewer 1

Advance summary and potential significance to field

Provides convincing evidence that Collagen XI regulates fibrillogenesis after corneal injury

Comments for the author

Good response to reviewer’s comments

Reviewer 2

Advance summary and potential significance to field

Type XI collagen has not be reported in the mouse cornea previously. The authors look at expression in the post-natal cornea as it develops, in response to injury, and use an inducible knockout of the protein to show that removing collagen XI alters collagen fiber diameters in the wounded corneal stroma.

Comments for the author

The authors have addressed this reviewers concerns.

Reviewer 3

Advance summary and potential significance to field

The authors answered some of the comments.

Comments for the author

The authors answered some of the comments but didn't respond to the most important questions: "How loss of collagen 11 results in such abnormal fibrils remains unanswered. Furthermore, it is unknown whether the abnormal fibrils have any impact on vision and corneal clarity." Furthermore, the authors still didn't show any statistical analysis for the data.

Second revision

Author response to reviewers' comments

Reviewer 3: Thank you for your comments and suggestions.

The authors answered some of the comments but didn't respond to the most important questions: "How loss of collagen 11 results in such abnormal fibrils remains unanswered. Furthermore, it is unknown whether the abnormal fibrils have any impact on vision and corneal clarity." Furthermore, the authors still didn't show any statistical analysis for the data.

We agree that we cannot provide a definite answer on the mechanisms by which collagen XI regulates fibril diameter. Our laboratory continues to work on this question. We think that collagen XI expression and function is significant only after stromal injury. As we have mentioned in our discussion, hybrid collagen fibrils made of I/XI molecules could be formed during severe injury or even fibrils of collagens II/XI molecules. We have *preliminary data* showing temporary expression of collagen II during stromal injury. We have a new inducible mouse model to specifically down-regulate collagen XI in the corneal keratocytes during development. We do not see any appreciable morphological findings in the corneas of the model either macroscopically or on electron microscopy examination. These findings suggest that collagen XI plays a significant role in fibrillogenesis only after injury.

Interestingly, we find that collagen XI is not expressed by adult keratocytes *in vitro* but its expression is up-regulated by serum in culture. Collagen XI is upregulated severely by TGF beta 1 *in vitro* too. Based on these data, our primitive conclusion is that collagen XI is basically a back-up or supplemental molecule for extreme situations where high quantities of new collagen fibrils are needed.

Ambuj Kumar MD PhD and his team analyzed the data in this manuscript to make sure that statistical analyses were appropriate. Dr Kumar is the director of the Methodology and Biostatistics Core at the University of South Florida, College of Medicine and has more than 450 publications where he has helped with data analysis.

Third decision letter

MS ID#: JOCES/2021/258694

MS TITLE: Collagen XI recapitulation regulates fibrillogenesis after corneal injury

AUTHORS: Edgar M Espana, Mei Sun, Devon Cogswell, Sheila Adams, Yasmin Ayoubi, Marcel Avila, Curtis Margo, Ambuj Kumar, and Tea Teljic
ARTICLE TYPE: Research Article

I am happy to tell you that your manuscript has been accepted for publication in Journal of Cell Science, pending standard ethics checks.