

AWARD NUMBER: W81XWH-19-1-0778

TITLE: Early Intervention Stem Cell-Based Therapy (EISCBT) for Corneal Burns and Trauma

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REPORT DOCUMENTATION PAGE

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14. ABSTRACT The goal of this project is to validate new stem cell markers which can be used to assess the corneal stromal stem cell (CSSC) regenerative potential and develop a regenerative corneal bandage (ReCoBand) with CSSC embedded in or with a regenerative extracellular matrix that can be applied in the battlefield to prevent permanent scarring of the cornea and treat the corneal scarring after trauma, blast, or burn wounds to preserve vision. In the first year of this project we have: (1) validated stem cell markers with which CSSC possess the regenerative potential, (2) characterized a transgenic mouse model which the fibrotic cells turning to green when the cornea is scarred, (3) confirmed that CSSC can prevent corneal scarring on this transgenic Col3a1-EGFP mouse model, (4) discovered one of the mechanisms that CSSC prevent corneal scar formation, (5) proved that ReCoBand with verified CSSC can prevent corneal scar formation in an alkali burn rabbit model. These accomplishments meet the proposed milestones and predict success in meeting the overall goals of the project by the end of the 2 year time-course.						
15. SUBJECT TERMS cornea, scarring, stem cells, regeneration, plastic-compressed collagen, anti-inflammatory, RNAseq, gene expression						
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1. INTRODUCTION: *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

Corneal trauma and chemical burns lead to corneal scarring, producing a long-term reduction in vision, frequently blindness. Corneal scarring and decompensation are the second-most common causes of poor vision among ocular injuries in combat, commonly caused by explosions with fragmentary munitions and by chemical and thermal exposure. Although corneal transplantation is effective at restoring vision, it is not an ideal solution for the war fighter with long-term medication and possible rejection. The **subject** of this project is to discover a portable and effective treatment potential which can be simply used in the clinic of a battlefield to promote corneal regeneration and save soldiers' vision and preserve their normal ability in their routine life and as a soldier. The **purpose** is to validate new marker genes which can be used to assess the regenerative potential of corneal stromal stem cell (CSSC) strains and validate the effectiveness of a regenerative corneal bandage (ReCoBand) containing either CSSC or regenerative ECM in a large animal model. The scope of the research includes three Aims: **(1)** To validate new marker genes to assess the regenerative potential of CSSC by in vitro qPCR assessment and in vivo in our Col3-EGFP mouse model; **(2)** To evaluate the regenerative potential of CSSC in the ReCoBand with expressing of those identified new marker genes in an alkali burn rabbit model as immediate acute treatment and delayed treatment (1 week after alkali burn); **(3)** To evaluate the regenerative potential of a regenerative ECM in the ReCoBand in the same alkali burn rabbit model as acute and delayed treatment. The results will demonstrate how to select the correct CSSC with regenerative potential and demonstrate the regenerative potential of CSSC and ECM in the ReCoBand to treat corneal blindness which can be used clinically, especially in a combat field to save soldiers' vision. If success, it will dramatically reduce the need for corneal transplantation requiring donor tissues and significantly save vision in a timely manner.

2. KEYWORDS: *Provide a brief list of keywords (limit to 20 words).*

Cornea, Scarring, Blindness, Regeneration, Stem cells, Stem cell-based therapy, ReCoBand, ECM, Vision

3. ACCOMPLISHMENTS: *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?

List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Major goals of the project:

1. Validate new marker genes as a means of assessing the regenerative potential of CSSC
2. Proof of Concept and safety studies in a large animal model
3. Effectiveness of ECM in remediating corneal scars.

Milestones:

1. Identification of new stem cell markers to identify regenerative potential. (12m) **Achieved**
2. Proof of Concept for effectiveness of ReCoBand in a large animal model.(12m) **Achieved (because of COVID-19 with campus shutting down, it's a little delayed)**
3. Determination of effectiveness of delayed application of ReCoBand-SC therapy. (14m) **Achieved**
4. Demonstrate Effectiveness of ECM in ReCoBand format (ECM-ReCoBand).(20m) **Delayed and will start it soon (because of COVID with delayed ECM from the collaborator)**
5. Demonstration of Proof of Concept of effectiveness of ECM in corneal regeneration in a ReCoBand device. (24m) **Delayed and will finish it by the end of the NCE period.**

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

During the 2nd year of the project, we have made progress toward the goals.

Specific Aim 1: Validate new marker genes as a means of assessing the regenerative potential of CSSC.

Task 1: Correlate new marker gene expression of CSSC cell lines with regenerative potential.

Subtask 1: DHRPO approval to use cell lines derived from deidentified cadaveric human tissues. (To be obtained before starting work on project) (0-1m).

Subtask 2: ACURO approval for mouse protocols. (0-6m)

Subtask 3: Screen of CSSC lines from 12 donors to assess correlation of regenerative potential with marker gene expression.(6-10m)

Milestone: Identification of new stem cell markers to identify regenerative potential. (12m)

Accomplishment: This has been reported in the year 1 annual report.

Specific Aim 2: Proof of Concept and safety studies in a large animal model

Task 2: Demonstrate ReCoBand effectiveness in Rabbit Corneal Burn Model

Subtask 1: ACURO approval for rabbit experiments (0-10m)

Subtask 2: Examine ability of ReCoBand containing CSSC (SCReCoBand) to regenerate rabbit cornea after alkali burns. (10-12m)

Milestone: Proof of Concept for effectiveness of ReCoBand in a large animal model.(12m)

Accomplishment: As reported in the year 1 annual report, we had done several experiments confirming that ReCoBand was effective in a rabbit corneal wound model. But we had some problems since the results were not consistent. To optimize the rabbit alkali burn model, we compared different concentrations of NaOH and different time durations, we got the conclusion that **1N of NaOH** soaked in a filter paper on the corneal surface for **15 seconds** followed by 20ml of PBS wash is the best wound condition to generate rabbit corneal scar model. While CSSC were able to be cultured remaining stem cell characteristics, they had increased expression of fibrotic markers when they were cultured in the compressed collagen (CC) gel for 48 hrs. We did different culture duration and detected their gene expression, we decided to culture CSSC in the CC gel for **24 hrs** before we apply the CC gel containing CSSC onto rabbit corneas (reported in year 2 quarter2 report). We seeded 900 cells/mm² or 1800 cells/mm² in the CC gel and compared the results on the rabbit alkali burned corneas. We found that both the concentrations of the CSSC in the CC gel were effective to prevent corneal scar formation after alkali burn (reported in year 2 quarter1 report). We decided we will use **900 cells/mm²** for the following experiments since less cells less cost.

Task 3: Test delayed application of ReCoBand (Aim 2B)

Milestone: Determination of effectiveness of delayed application of ReCoBand-SC therapy.

Accomplishment: With all the conditions determined as stated above (alkali burn model on rabbit corneas: 1N NaOH for 15 S, CSSC in CC gel for 24 hrs at the seeding concentration 900 cells/mm²), we generated rabbit alkali burn corneal scar model and waited for 1 week when the corneas had no inflammation and the scar had formed. At 1 week after wound, we randomly grouped the rabbits as **a**) wound only (Wnd), **b**) wound with compressed collagen gel without cells (Wnd+CC), **c**) wound plus CSSC strain 436 in CC (Wnd+436), **d**) wound plus CSSC strain 439 in CC (Wnd+439), **e**) naïve controls (the left eye of each rabbit without any treatment). We did OCT to scan the corneas before treatment, after wound, 1 week later before treatment, immediately after CC or CC + CSSC treatment, 10 days after CC or CC+CSSC treatment when we terminate the rabbits. We saw differences but the OCT data are under analysis, and we will have the data ready for the next quarterly report. **Appendix 1, Figure 1** shows the representative corneal pictures in

different treatment groups. The pictures indicate the corneal scarring was completely or partially cured by CSSC (436 and 439). qPCR results on the rabbit corneas (**Appendix 1, Figure 2**) indicate that CSSC 436 and 439 treatment successfully suppressed the expression of fibrotic markers FN-EDA and SPARC. CC only treatment was also reduced the fibrotic marker expression. Further investigation is needed. The results confirmed the effectiveness of delayed application of ReCoBand-SC therapy.

Specific Aim 3. Effectiveness of ECM in remediating corneal scars.

Task 4: Effectiveness of ECM in the ReCoBand format.

Subtask 1: Prepare ReCoBand gels containing ECM and examine physical properties.(12-14m)

Subtask 2: Compare effectiveness of ECM-ReCoBand to ECM hydrogel in C57/Bl6 model of debridement. (14-16m)

Subtask 3: Compare ECM-ReCoBand and SC-ReCoBand in alkali burn model using Col3a-EGFP mouse model. (16-18m)

Subtask 4: Dose Response and stability study with ReCoBand-ECM. (18-20m)

Milestone: Demonstrate Effectiveness of ECM in ReCoBand format (ECM-ReCoBand).(20m)

Task 5: Effectiveness of ReCoBand-ECM in large animal model.

Demonstrate effectiveness of ECM-ReCoBand in corneal regeneration of rabbit corneal after alkali burn. (20-24m)

Milestone: Demonstration of Proof of Concept of effectiveness of ECM in corneal regeneration in a ReCoBand device. (24m)

Accomplishment: Due to COVID, ECM from the collaborator Dr. Badyak was delayed. We have discussed the next step and we will have the ECM soon and will start the experiments on the proposed ECM related studies. At the meantime, we were working on testing the effectiveness of ReCoBand of different CSSC strains in CC gel in C57BL/6 mouse model with debridement as part of the Subtask2. Adult C57BL/6 mouse corneas were wounded by debridement using an algerbrush as we reported previously (Boote, Du, et al. IOVS 2012, 53:2786. PMID: 22467580). We tested different CSSC strains and human corneal fibroblasts (HCF, as negative control). Immediately after corneal debridement, we put the cells on the corneal surface as fibrin gel as we reported previously (Basu et al. Sci Transl Med. 2014, 6:266ra172. PMID: 25504883). We had the following groups: Naïve, Wound only, Wound+ Fibrin gel, Wound + CSSC strains 436, 437, 438, 455, 466P, 470L, Wound + HCF. We took OCT on the corneas before wound, immediately after wound or wound with different cell treatment, on Day 3 and Day 10 after the surgery and sacrificed the mice at Day 10. We took pictures of the corneas, did the wholmount staining on the corneas to stain the fibrotic markers Col3a1 and α SMA, we also extracted the RNAs from the corneas to do qPCR to detect the gene expression changes. For the OCT data, we partially analyzed the data. **Appendix 1, Figure 3** shows some of the OCT scans indicating the corneal thickness and light scatter (showing the transparency or scar formation). We will have complete analysis results in the next quarter report with statistical analysis. **Appendix 1, Figure 4** shows the corneal pictures indicating the corneal transparency or scar formation. Wholemenout staining shows the expression of Col3a (green) and α SMA (red). **Appendix 1, Figure 5** shows qPCR results on the expression of fibrotic genes α SMA, Col3a1, Tenascin C (TNC), SPARC, FN-EDA. All the results indicate that Wound and Wound + Fibrin gel were not able to prevent corneal scar formation with increased corneal scarring and increased fibrotic marker expression. Most corneas treated with CSSC were prevented with scar formation. Some corneas with HCF treatment had obvious scarring while some were almost transparency. This might be related to the quality of the HCF. We normally passage HCF at very high density and don't use them until passage 6 or above. This time we used passage 4 HCF which might contain some stem cells which had not differentiated into fibroblasts. We are going to do qPCR on the HCF to detect if they express some stem cell markers. For the following experiments, we will only use HCF on or beyond passage 6.

We will finish the proposed experiments within this NCE period.

We have published one invited paper "Regenerative therapy for the Cornea" in the Progress in Retinal and Eye Research with the support from DoD.

What opportunities for training and professional development has the project provided?

If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. “Training” activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. “Professional development” activities result in increased knowledge or skill in one’s area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Dr. Gary Yam was recruited as a research associate professor in the department of Ophthalmology at University of Pittsburgh. He has been working on this project since he came. He has been trained to take responsibilities for some experiments and he has submitted his own DoD grant and he will become an independent researcher in the corneal regeneration field.

How were the results disseminated to communities of interest?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

The published paper cited the support of this grant which allows people know the scientific work, technologies, and the grant support. The animal models (mouse corneal debridement wound, rabbit corneal alkali burn wound model) will be shared if other researchers are interested and ask for it. Due to COVID-19, there were no scientific conferences recently but we will submit abstracts to scientific meetings when available.

What do you plan to do during the next reporting period to accomplish the goals?

If this is the final report, state “Nothing to Report.”

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

Now we have confirmed the CSSC cell lines/strains and we have confirmed with our results that ReCoBand are effective in both preventing and treating corneal scars in both mouse and rabbit models. We will do the experiments on the same rabbit models using the ReCoBand with the regenerative ECM on it and compare the results with ReCoBand with confirmed CSSC strains. We will be able to fulfill the proposed Specific Aim 3 and have more animal numbers with cells to confirm our previous results.

4. IMPACT: Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

We identified the specific stem cell genes with which the corneal stromal stem cells (CSSC) have strong regenerative potential. This illustrates an important fact that stem cells vary markedly in their potential according to their source and that defining criteria for assessing that potency will be essential for translating their use to the clinic.

We characterized the Col3a-EGFP mice which shows green cornea which the cornea has scar. This can be used to track the scar changes with treatment in vivo by directly looking at the green or not green corneas.

We optimized the rabbit alkali burn model which is suitable for studying corneal regeneration which will be shared to the public when we publish our findings in a scientific journal.

We found that CSSC seeded in the compressed collagen over 2 days can increase the expression of fibrotic markers while culturing in the compressed collagen for 24 hrs would remain good stem cell characteristics. After 24 hr culturing, they can be stored without further growing.

The ReCoBand we discovered, which contains compress collagen and verified CSSC, has been confirmed to have the regenerative potential, even in a relatively severe alkali burn rabbit model. It can prevent scar formation in an acute wound model and can reverse already formed corneal scarring in a chronic wound model. This exciting discovering illustrates a hope that a ReCoBand can be stored and used in a clinic at a combat field to save soldiers' vision right on the site.

What was the impact on other disciplines?

If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to Report

What was the impact on technology transfer?

If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to Report

What was the impact on society beyond science and technology?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to Report

- 5. CHANGES/PROBLEMS:** *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, “Nothing to Report,” if applicable:*

Changes in approach and reasons for change

Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Nothing to Report

Actual or anticipated problems or delays and actions or plans to resolve them

Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

We did have a problem with the rabbit alkali burn model. By optimizing the conditions, we solved the problem and now we have an optimized condition which can generate a stable corneal wound scar for studying corneal regeneration.
Due to COVID, we have had a difficulty to get the ECM from our collaborator. We have discussed it and we are going to start the experiments with ECM in this quarter.

Changes that had a significant impact on expenditures

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to Report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Nothing to Report

Significant changes in use or care of human subjects

Nothing to Report

Significant changes in use or care of vertebrate animals

Nothing to Report

Significant changes in use of biohazards and/or select agents

Nothing to Report

6. PRODUCTS: *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**

Report only the major publication(s) resulting from the work under this award.

Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume; year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

One manuscript citing DoD support was published in Prog Retin Eye Res as Appendix 2.

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to Report

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Nothing to Report

- **Website(s) or other Internet site(s)**

List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to Report

- **Technologies or techniques**

Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to Report

- **Inventions, patent applications, and/or licenses**

Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to Report

- **Other Products**

Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

- *data or databases;*
- *physical collections;*
- *audio or video products;*
- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate “no change”.

Example:

Name: Mary Smith
Project Role: Graduate Student
Researcher Identifier (e.g. ORCID ID): 1234567
Nearest person month worked: 5

Contribution to Project: Ms. Smith has performed work in the area of combined error-control and constrained coding.
Funding Support: The Ford Foundation (Complete only if the funding support is provided from other than this award.)

Name: Yiqin Du
Project Role: PI
Univ. Pitt. ID: 82941
Nearest person month worked: 3
Contribution to Project: YD designed the project and is directing it; she is in charge of animal protocols and regulations. She analyzes the results.

Name: Gary Yam
Project Role: Co-investigator
Univ. Pitt. ID: 195239
Nearest person month worked: 2.4
Contribution to Project: Dr. Yam does cell culture and animal work. He also helps to analyze data and write manuscripts.

Name: Moira Geary
Project Role: Animal Technician
Univ. Pitt. ID: 120308
Nearest person month worked: 8.40
Contribution to Project: Ms. Geary maintains the mouse breeding colony and genotyping. She helps for animal procedures, OCT and ELISA.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to Report

What other organizations were involved as partners?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:

Organization Name:

Location of Organization: (if foreign location list country)

Partner's contribution to the project (identify one or more)

- Financial support;
- In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);
- Facilities (e.g., project staff use the partner's facilities for project activities);
- Collaboration (e.g., partner's staff work with project staff on the project);
- Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and
- Other.

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

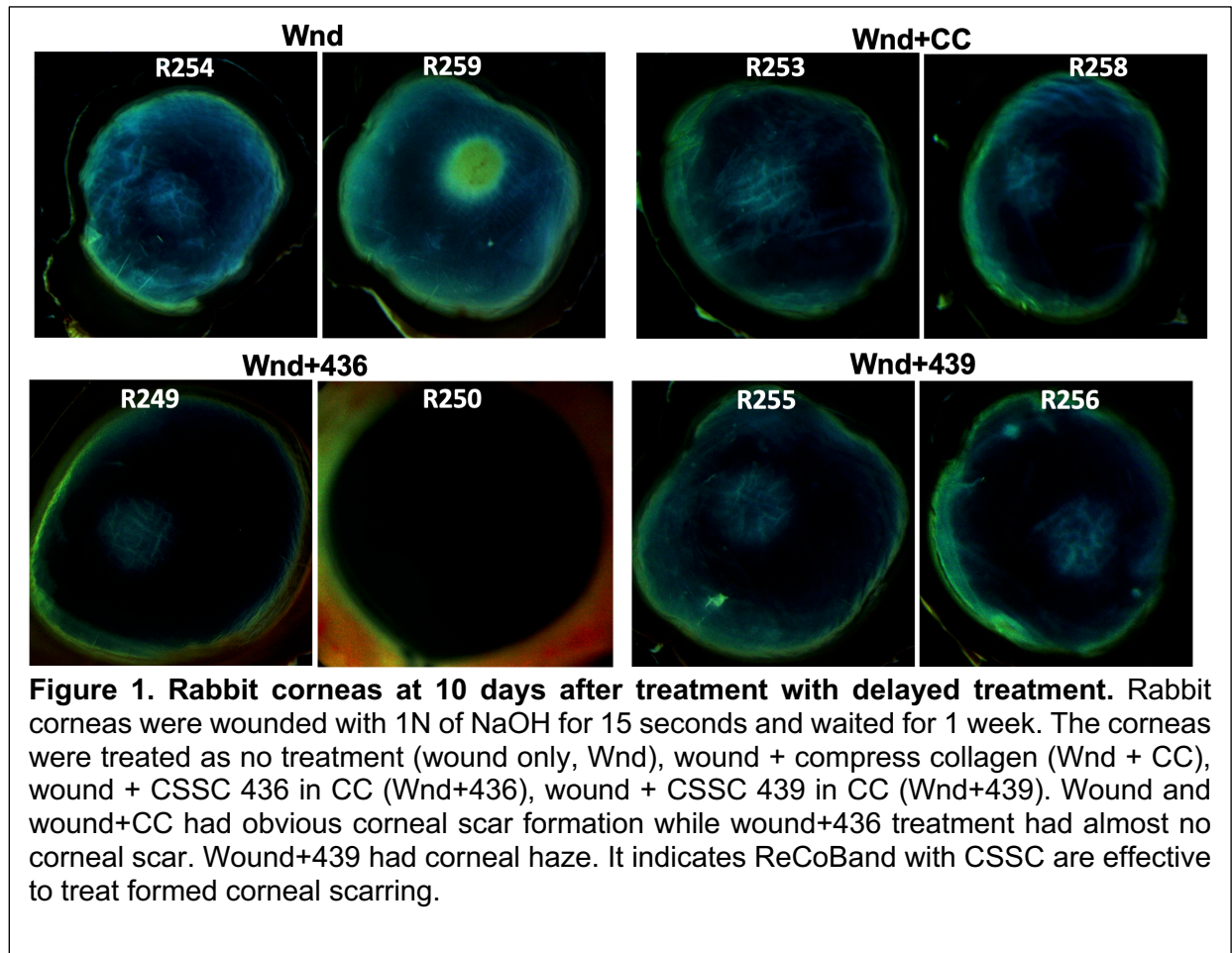
COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to <https://ers.amedd.army.mil> for each unique award.

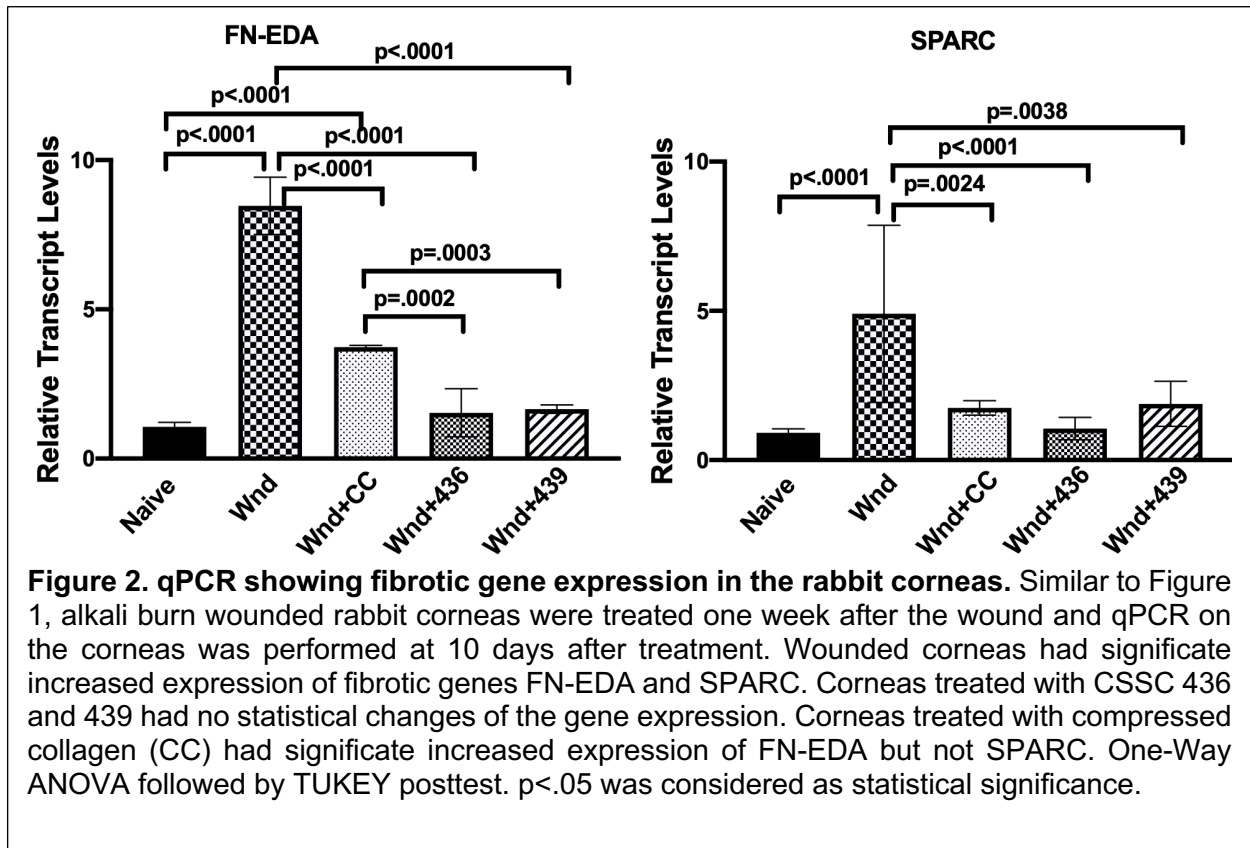
Nothing to Report

QUAD CHARTS: If applicable, the Quad Chart (available on <https://www.usamraa.army.mil>) should be updated and submitted with attachments.

9. **APPENDICES:** Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

Appendix 1: Figures





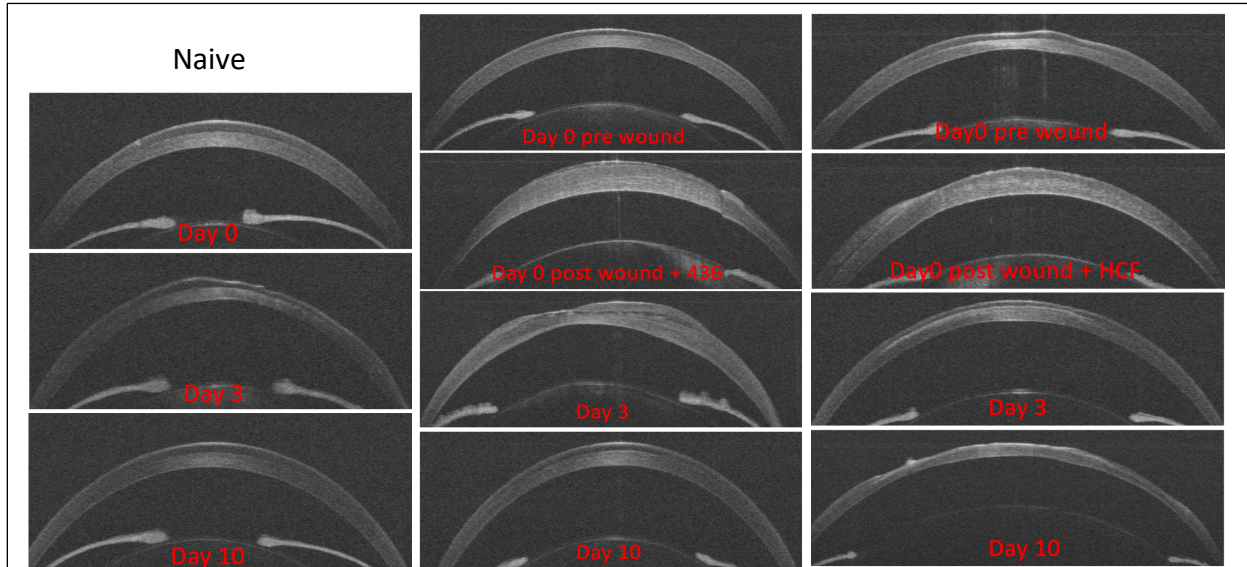
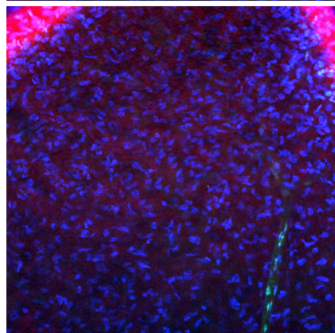
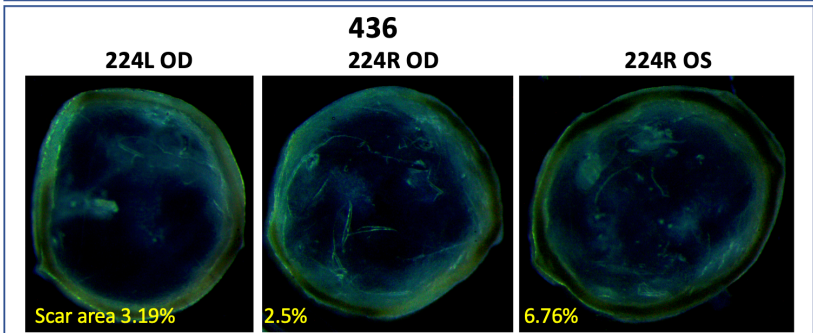
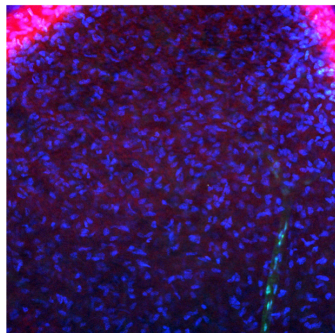
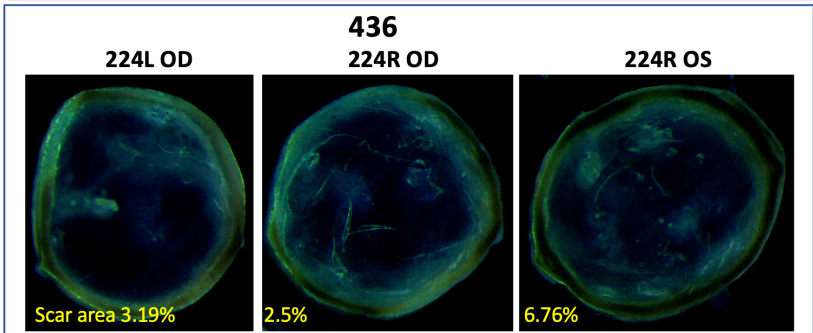
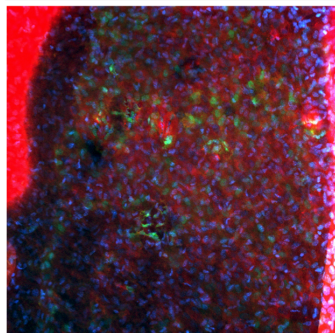
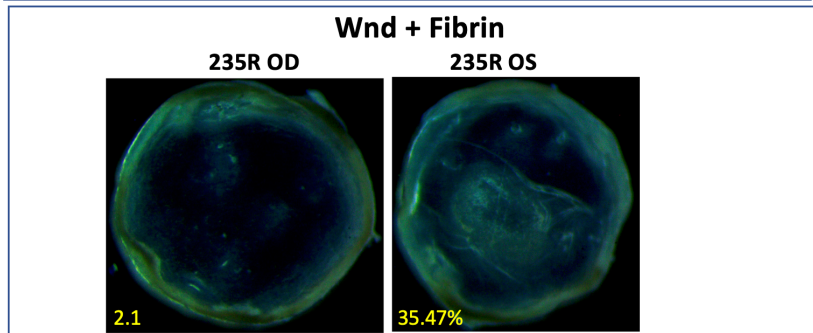
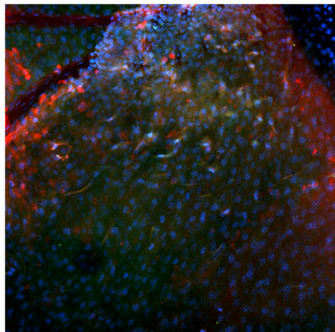
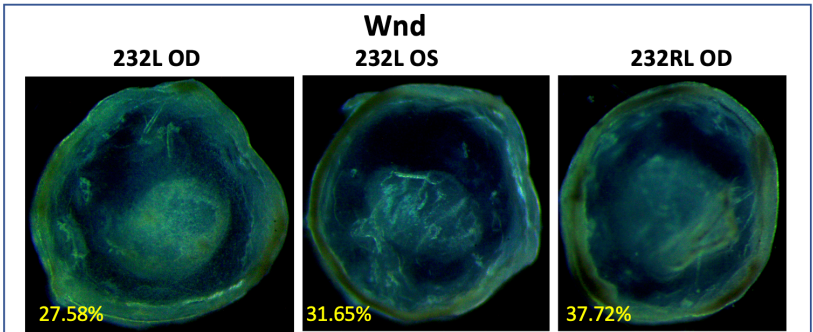
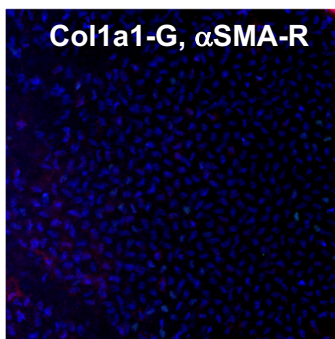
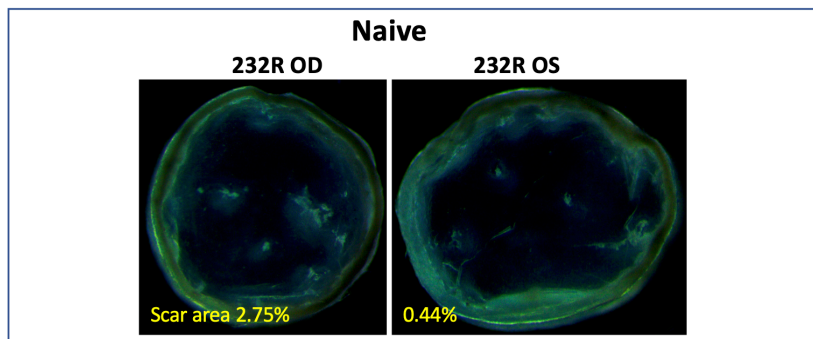
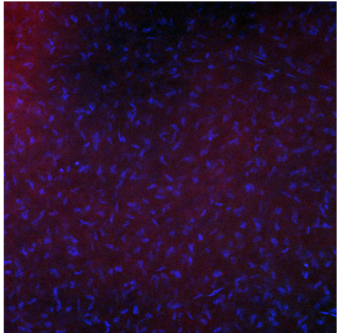
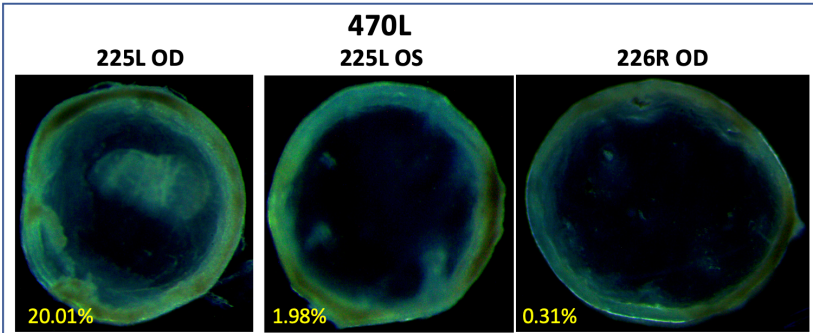
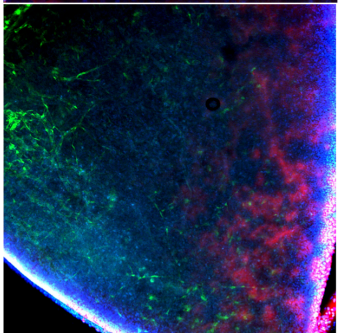
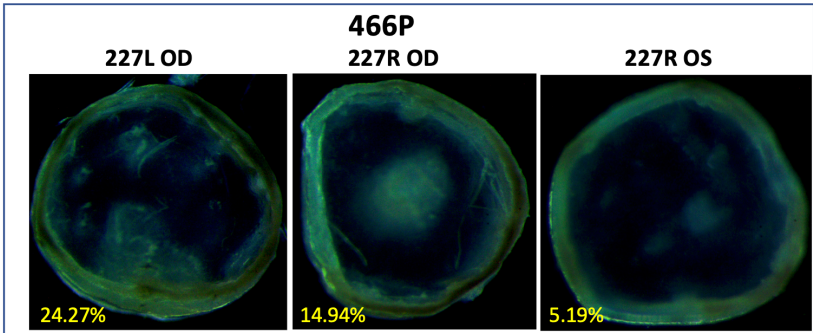
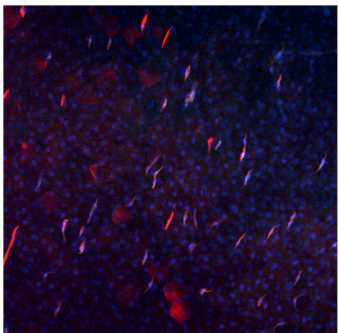
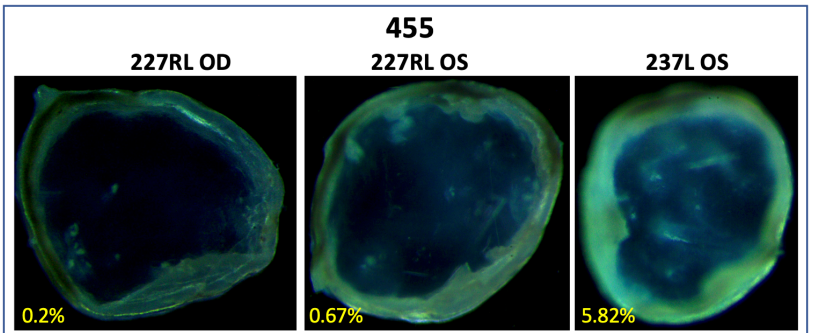
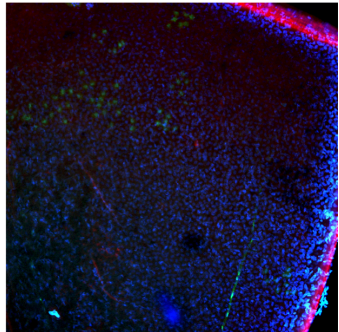
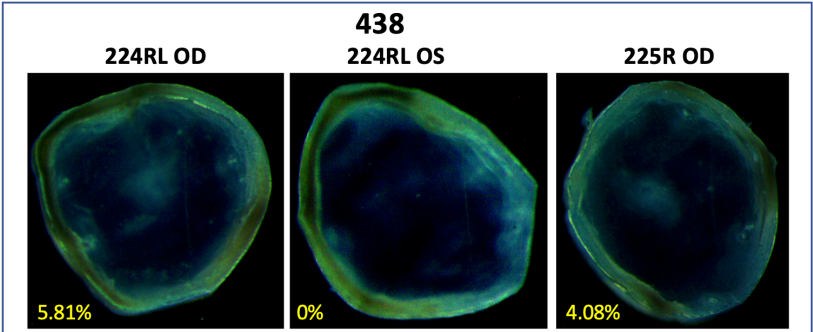
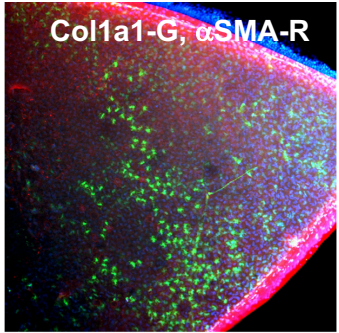
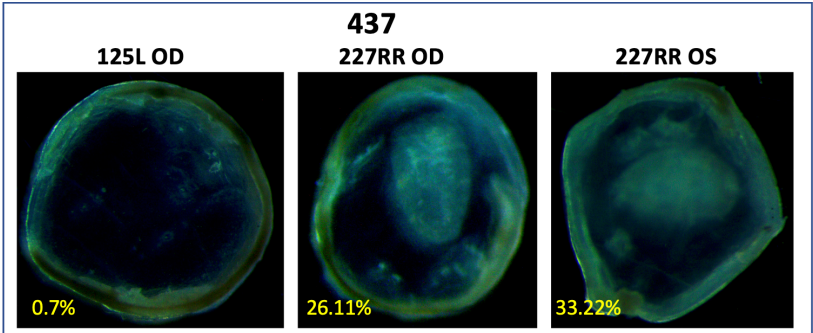


Figure 3. OCT of mouse corneas at different time points. C57BL/6 mice were wounded by an algerbrush and treated with cells in a fibrin gel. OCT was taken before and immediately after treatment and at Day 3 and Day 10. This representative OCT pictures show Naïve mouse corneas (left) were transparency, the cornea wounded with CSSC 436 treatment (middle) recovered as transparency at Day 10, and the cornea wounded with HCF treatment (right) still remained corneal scarring at Day 10.





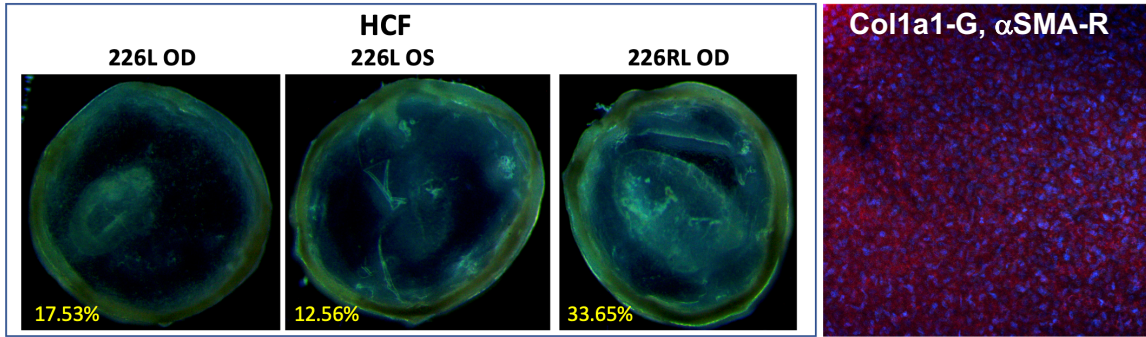


Figure 4. Mouse corneas with different treatment and wholemout staining. Similar to Figure 3, the mice were wounded and treated. Corneal pictures were taken at Day 10 after the treatments and corneal wholemouts were stained with fibrotic markers Col3a1 as red and α SMA as red. DAPI stains nuclei blue. Yellow numbers in the corneal pictures indicate the percentage of scar formation of the whole corneal area. The corneas with wound and wound + fibrin gel only had dramatical corneal scar formation with increased expression of Col3a1 and α SMA. The corneas treated with HCF had increased scar formation while treated with CSSC had reduced corneal scar formation.

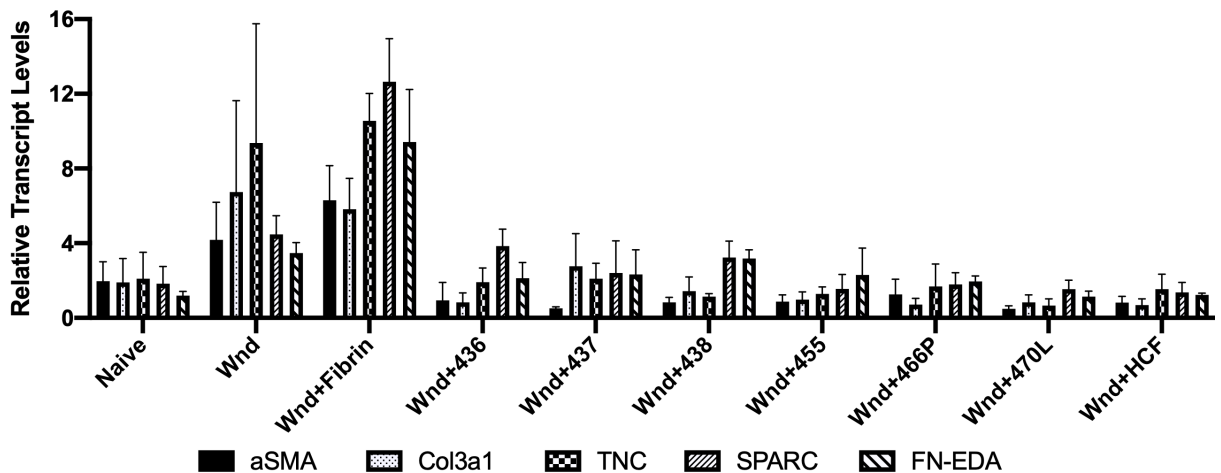


Figure 5. qPCR results on mouse corneas after wound and treatments at Day 10. Similar to Figure 3 and 4, mouse corneas were treated as different groups and the corneas were extracted for RNA at Day 10. qPCR was performed on the mouse corneas to detect the fibrotic marker expression. The corneas of both wound and wound + fibrin gel had increased expression of the fibrotic markers as compared to naïve controls. Most of the CSSC strains were able to suppress the fibrotic marker expression which indicates the ability to prevent scar formation, in consistent with the results as corneal pictures and wholemout staining shown in Figure 4. While some corneas treated with HCF had increased corneal scar formation, the gene expression was not increased. It may be because of the existence of stem cells in early passaged HCF or the mouse corneal individual differences. Two-Way ANOVA followed by TUKEY posttest.

Early Intervention Stem Cell-Based Therapy (EISCBT) for Corneal Burns and Trauma
Log# VR180189, W81XWH1910778



PI: Yiqin Du

Org: University of Pittsburgh

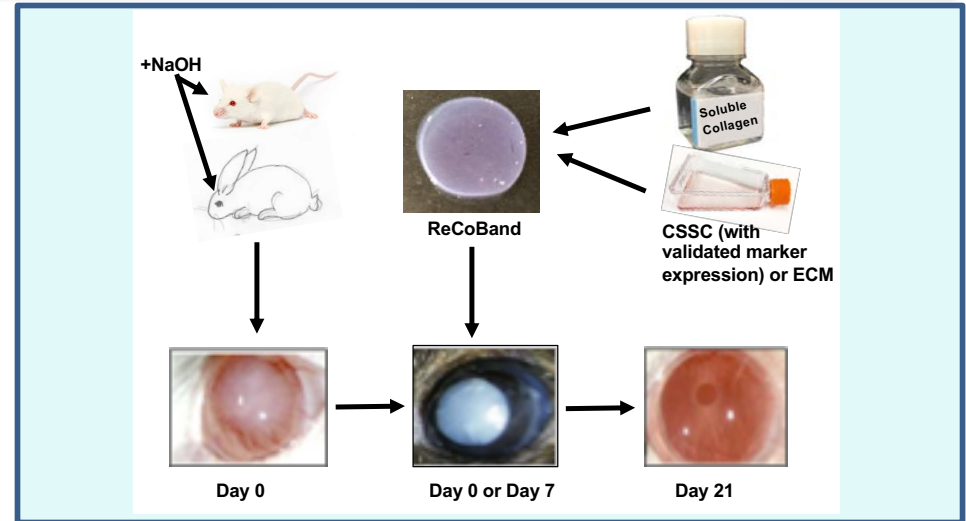
Award Amount: \$760,352

Study/Product Aim(s)

- **Aim 1.** Validate new marker genes as a means of assessing the regenerative potential of CSSC.
- Aim 2.** Provide proof of Concept and safety studies in a large animal model (rabbit alkali burn wounds)
- Aim 3.** Compare the regenerative efficacy of ECM in a ReCoBand device with that of CSSC

Approach

These experiments will test scar suppression/tissue regeneration of two promising therapeutic agents (CSSCs in ReCoBand and ECM ReCoBand) in a rabbit chemical burn model to provide Proof of Concept for their efficacy.



Accomplishment: Confirmed those 4 stem cell markers and validated the marker expression in cultured cells by staining.

Timeline and Cost

Activities	CY	19	20	21	22
Correlate marker gene expression with CSSC regenerative potential in vivo.					
Demonstrate ReCoBand effectiveness in Rabbit Corneal Burn Model					
Delayed application of ReCoBand					
Effectiveness of ECM in the ReCoBand format					
Estimated Budget (\$K)		\$42	\$254	\$284	\$180

Updated: (October 14, 2021)

Goals/Milestones

CY19 Goal – Submission protocols for ACURO approval; Identification of new stem cell markers

- Both mouse and rabbit protocols submitted and rabbit ACURO approved; NanoString assay and staining to test the markers.

CY20 Goals – Validation new markers correlated to CSSC regenerative potential; Proof of concept and safety studies in a rabbit model.

- Investigate the specific marker expression CSSCs have strong scar suppressing effect in a mouse model.

- Use the confirmed CSCS lines to test the concept in a rabbit alkali burn model

CY21 Goal – Proof of Concept in a rabbit model with delay application and validation of ECM effect

- Validate CSSC ReCoBand with delayed application after wound

CY21 Goal –

- Compare regenerative effect of ECM and CSSC

Comments/Challenges/Issues/Concerns

Budget Expenditure to Date

Projected Expenditure: \$760k

Actual Expenditure: \$580k