

AWARD NUMBER: W81XWH-17-2-0061

TITLE: “Implantation of full-thickness skin columns to decrease hypertrophic scar formation after deep partial thickness burns”

PRINCIPAL INVESTIGATOR: Rodney Chan, MD

**CONTRACTING ORGANIZATION: Metis Foundation
San Antonio, TX 78205**

REPORT DATE: October 2018

TYPE OF REPORT: Annual

**PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012**

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14. ABSTRACT PURPOSE: The purpose of this study is to provide proof-of-concept, pre-clinical evidence for use of autologous full-thickness skin column implantation for the treatment of deep partial-thickness burn injury to prevent hypertrophic scar formation. This evidence will support a pivot into clinical trials of this strategy. OBJECTIVES: The long-term objective is to develop autologous full-thickness skin column implantation as an <i>improved, low-morbidity</i> methodology for the treatment of deep partial-thickness burn. The scientific objective of this proposal is to evaluate the early (pre-epithelialization) and late (post-epithelialization) efficacy of three different seeding ratios of <i>implanted</i> full-thickness skin columns in reducing hypertrophic scar formation by accelerating wound closure, improving skin quality parameters, reducing wound contraction and minimizing donor-site morbidity in a porcine model of deep partial thickness burn.						
15. SUBJECT TERMS Burn wound healing, skin graft, skin replacement therapy, skin substitute						
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Table of Contents

1. INTRODUCTION:	4
2. KEYWORDS:	4
3. ACCOMPLISHMENTS:	4
4. IMPACT:	8
5. CHANGES/PROBLEMS:	9
6. PRODUCTS:	10
7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS	11
8. SPECIAL REPORTING REQUIREMENTS	13
9. APPENDICES:	13

1. INTRODUCTION:

Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

SINGLE HYPOTHESIS: Autologous full-thickness skin columns implanted into the residual dermis of debrided deep partial-thickness burns will decrease hypertrophic scar formation with minimal donor site morbidity.

PURPOSE: The purpose of this study is to provide proof-of-concept, pre-clinical evidence for use of autologous full-thickness skin column implantation for the treatment of deep partial-thickness burn injury to prevent hypertrophic scar formation. This evidence will support a pivot into clinical trials of this strategy.

OBJECTIVES: The long-term objective is to develop autologous full-thickness skin column implantation as an *improved, low-morbidity* methodology for the treatment of deep partial-thickness burn. The scientific objective of this proposal is to evaluate the early (pre-epithelialization) and late (post-epithelialization) efficacy of three different seeding ratios of *implanted* full-thickness skin columns in reducing hypertrophic scar formation by accelerating wound closure, improving skin quality parameters, reducing wound contraction and minimizing donor-site morbidity in a porcine model of deep partial thickness burn.

PREDICTED OUTCOME: The predicted outcome of this study is the identification of an optimal seeding ratio for full-thickness skin column treatment of deep partial thickness burn and the development

2. KEYWORDS:

Provide a brief list of keywords (limit to 20 words).

Burn wound healing, skin graft, skin replacement therapy, skin substitute

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. **Red percentages/text represent cumulative progress under FY18.*

Specific Aim 1: Identify optimal implanted FTSC seeding density and treatment modality for early treatment of open, debrided deep partial-thickness burn	Timeline Months	% completed
Subtask 1: Obtain approval of local IACUC for porcine study	0-2	100%
Subtask 2: Obtain 2 nd level ACURO approval	2-3	100%
Subtask 3: Perform animal studies for Aim 1a: Optimization of FTSC seeding density for early treatment of deep partial-thickness burn.	3-9	100%
Subtask 4: Perform analysis of primary endpoint for Aim 1a	9-12	80%
Subtask 5: Perform analysis of secondary endpoints for Aim 1a	9-12	50%
<i>Milestones Achieved: Local IACUC approval at USAISR</i>	2	100%
<i>Milestone Achieved: ACURO approval</i>	3	100%
<i>Milestone Achieved: Optimized seeding ratio for early implantation</i>	9-12	80%
Subtask 1: Perform animal studies for Aim 1b: Comparative evaluation of early treatment of deep partial-thickness burn with implanted versus topical FTSCs	12-16	5%
Subtask 2: Perform analysis of primary endpoint for Aim 1b	16-19	
Subtask 3: Perform analysis of secondary endpoints for Aim 1b	16-19	
Subtask 4: Perform animal studies for Aim 2: Identify optimal implanted FTSC seeding density for late treatment of epithelialized deep partial-thickness burn	18-20	
Subtask 5: Perform analysis of primary endpoint for Aim 2	20-22	
Subtask 6: Perform analysis of secondary endpoint for Aim 1b	20-22	
<i>Milestone(s) Achieved: Question of topical vs. anatomic implantation</i>	16-19	
<i>Milestone(s) Achieved: Optimized seeding ratio for late implantation</i>	22-24	
Subtask 1: Perform animal studies for Aim 3: Determine donor skin quality after	3-9	5%

FTSC harvests		
Subtask 2: Perform analysis of primary endpoint for Aim 3	9-12	
Subtask 3: Perform analysis of secondary endpoints for Aim 3	9-12	
Milestone(s) Achieved: donor skin quality of FTSC harvests	9-12	
Milestone(s) Achieved: Complete statistical analysis of all data (Aims 1-3)	19-24	

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.

1) Major activities

Aim 1a porcine experiments.

2) Specific objectives

Evaluating FTSC implantation ratio under Aim 1a and planning to conduct Aim 1b.

3) Significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or

Porcine FTSC implantation have successfully been implanted under aim 1a. Figure 1 shows skin column progress over 90 days for all skin column ratios.

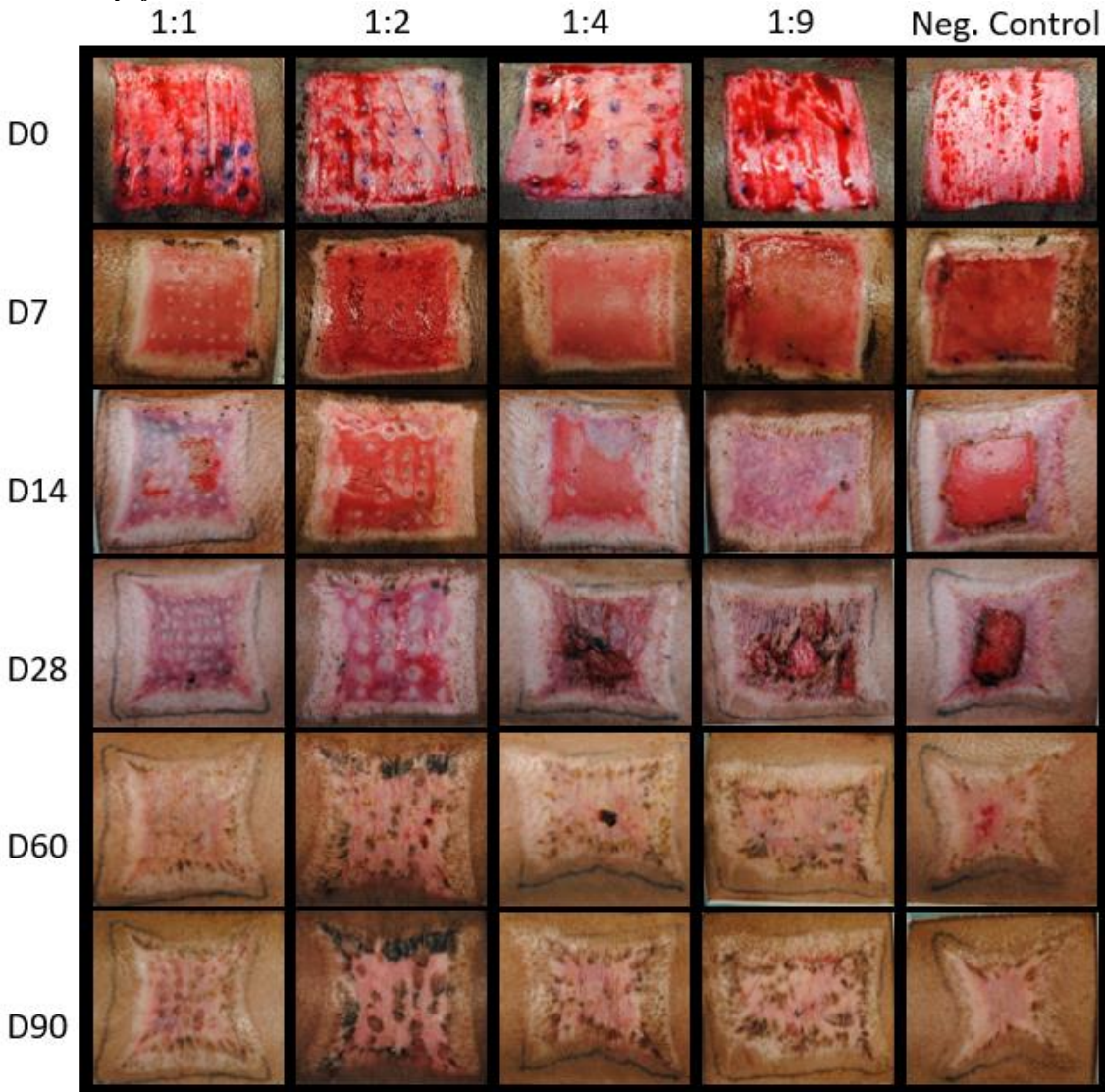


Figure 1. Visualization of FTSC implanted wounds at ratios 1:1, 1:2, 1:4, 1:9 and untreated control over 90 days. Briefly, deep-partial thickness burns were debrided and implanted with FTSC at different ratios. **The 1:9 ratio group has been replaced with the 1:2 ratio group.**

FTSC donor sites heal fast and without scarring shown in figure 2.

Donor sites

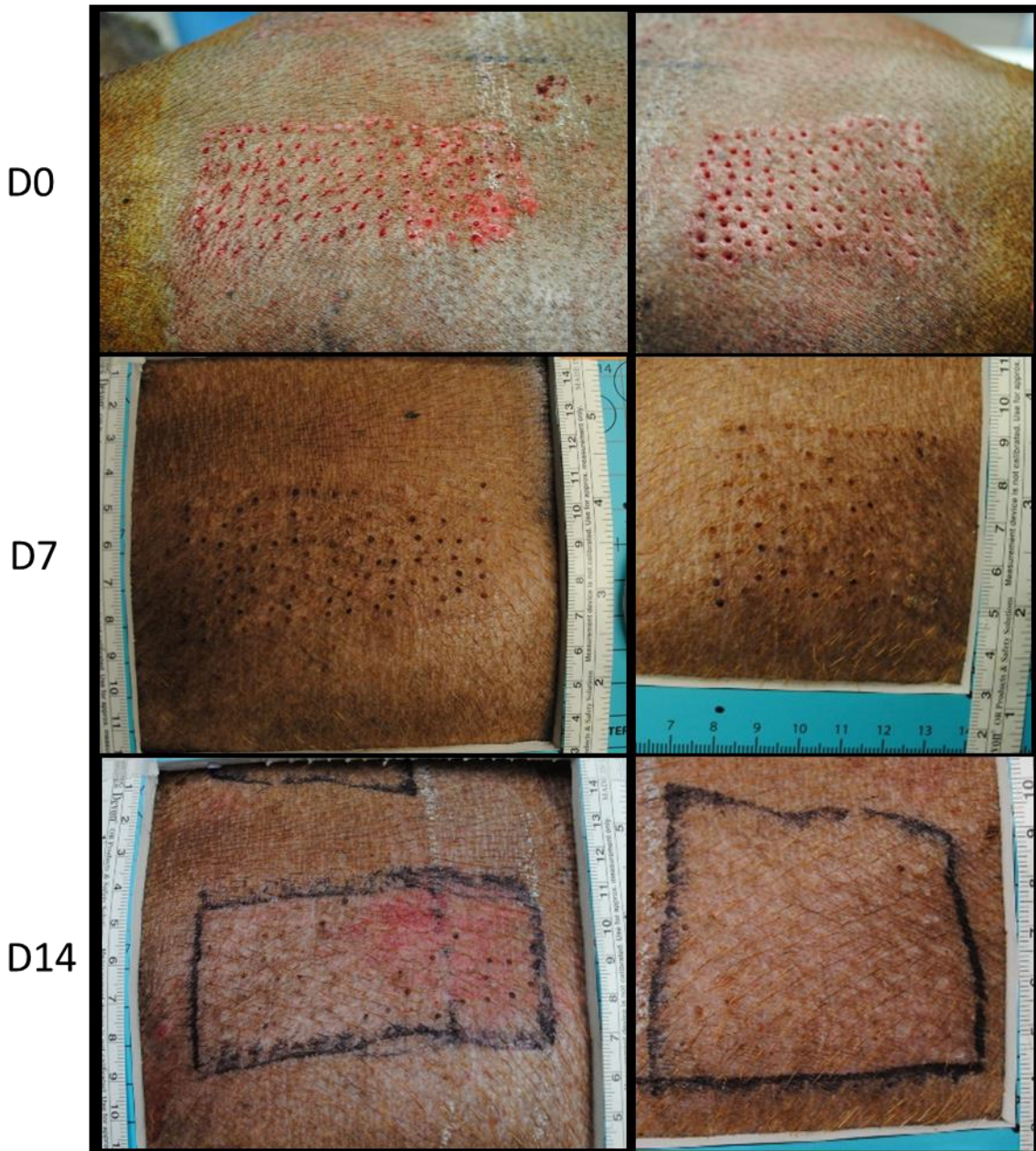


Figure 2. Shows 2 different donor sites over 14 days after harvest. FTSC donor sites healed fast and without obvious morbidity. See figure 3 for Histology and verification of findings.

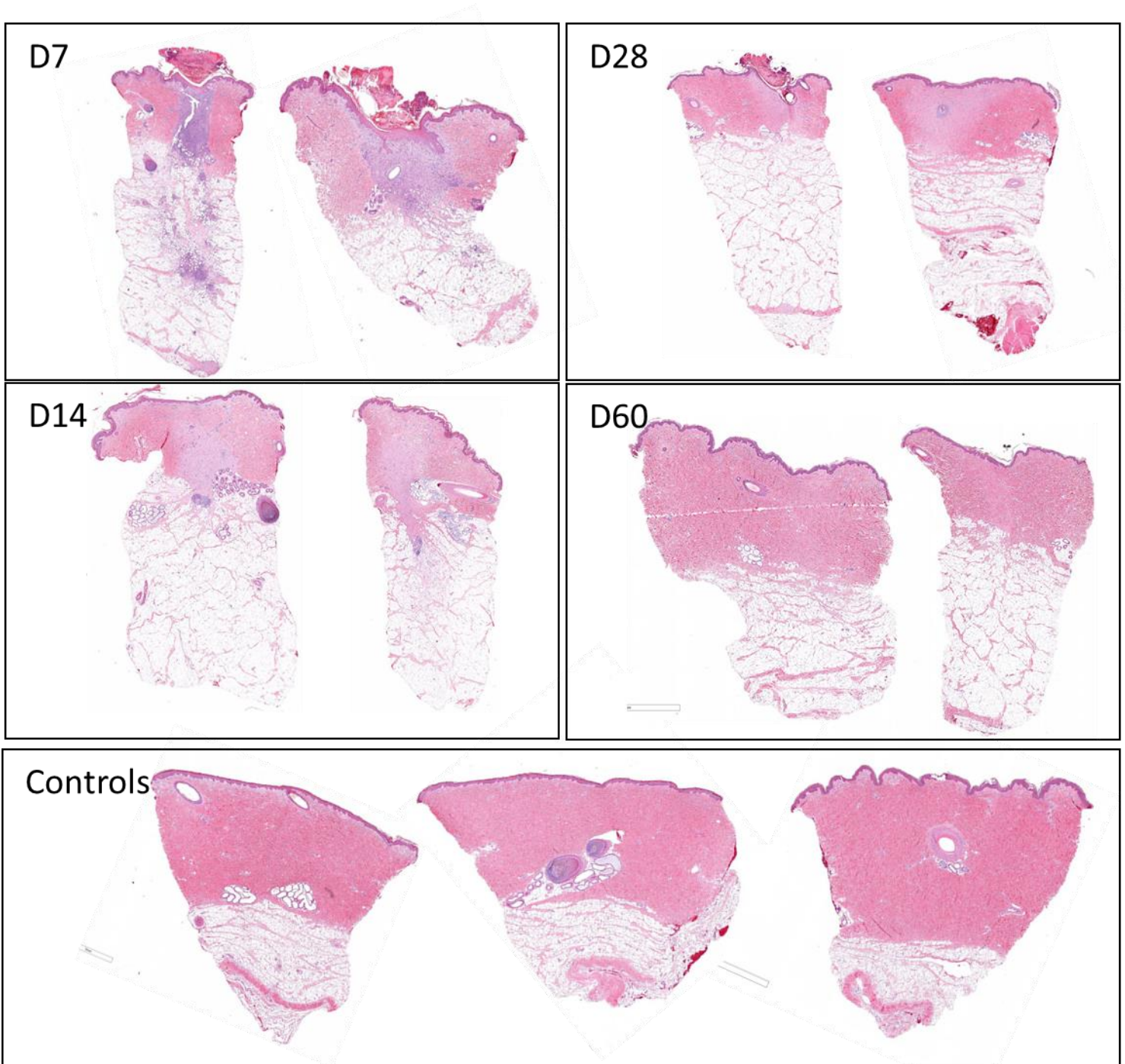


Figure 3. Shows H&E stained histology from 2 different donor sites and normal controls over 60 days after harvest. FTSC donor sites healed well. At day 28 some minor dermal re-modulation can be observed. The histology confirms the findings that the donor sites heal fast and without obvious morbidity..

Development: the 1:9 FTSC ratio performed poorly and has been replaced with a 1:2 ratio group. The 1:2 ratio group performs well based on preliminary data.

Summary: Aim 1a experiments are progressing well. We previously reported very limited effect of the 1:9 ratios and replaced that group with a 1:2 ratio group. Preliminary data on the 1:2 ratio group look good and seems to have been the right decision. We can now show histology and contraction data from the first animals and are currently working on assessing primary and secondary endpoints for the second set of animals.

4 Other achievements. Include a discussion of stated goals not met.

Experiments (aim 1a and 1b) are progressing as planned.

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.

This project allows for several opportunities of training and professional development. Most opportunities are in smaller study groups or at the individual level. Examples of training opportunities for residents, post-docs and techs include; 1) Animal ethics & porcine animal studies, 2) Porcine pain assessments and pain management in research animals, 3) Porcine burns and how to conduct dressing changes, wound & scar assessments etc. 4) The importance of adherence and maintenance of IACUC/ACURO protocols.

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.

Nothing to Report

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.

The main focus will be on completing Aim 2 (**Identify optimal implanted FTSC seeding density for late treatment of epithelialized deep partial-thickness burn**) and Aim 3 (**Determine donor skin quality after FTSC harvests**). The progress for FY 18 and plan forward for FY19 have been outlined under 3. Accomplishments.

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).

Nothing to Report

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.

Replacement of the 1:9 ratio group for the 1:2 ratio group. Reason: The 1:9 ratio group have too few skin columns to have a positive effect on wound healing and reduction of scarring. This was discovered in the first set of animals and amendments could quickly be made to the animal protocol to allow for the update. The new groups include the following ratios: 1:1, 1:2 and 1:4.

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.

1. Received pigs that were much smaller than stated on the order. We received 3 purebred Durocs that were just over 20kg when they were delivered. Normally, we receive pigs in the 40-45 kg range. This was resolved by just letting the pigs grow in our vivarium for 2-3 months. Since other parts of the project have been progressing faster than anticipated there is not actual impact of this delay.
2. Long wait for histopathologic assessment/scoring. The turnaround time for the ISR core histopathology assessments is very slow. We are working together with them to figure out how this can be improved.

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

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.

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).*

Nothing to Report -Yet

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.*

Submitted to ABA 2019, Las Vegas.

- **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.)

<i>Name:</i>	Rodney Chan, MD.
<i>Project Role:</i>	Principal Investigator
<i>Researcher Identifier (e.g. ORCID ID):</i>	https://orcid.org/0000-0002-5061-847X https://scholar.google.com/citations?user=Cu787kYAAAAJ&hl=sv
<i>Nearest person month worked:</i>	4
<i>Contribution to Project:</i>	Dr. Chan is the PI of the award.
<i>Funding Support:</i>	The Metis Foundation
<i>Name:</i>	Anders Carlsson, PhD.
<i>Project Role:</i>	Co-Principal Investigator
<i>Researcher Identifier (e.g. ORCID ID):</i>	https://orcid.org/0000-0002-4846-108X https://scholar.google.com/citations?user=WdVK3Z8AAAAJ
<i>Nearest person month worked:</i>	3
<i>Contribution to Project:</i>	Dr. Carlsson is the Co-PI of the award.
<i>Funding Support:</i>	The Metis Foundation

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.

Since the contract was initially awarded, W81XWH-13-CCCRP-CCIBPII has been completed and the PI’s current total commitment is at 78%. There is no anticipation for any over commitment during the anticipated period of performance for this award.

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.

Organization Name: The Metis Foundation

Location of Organization: 300 Convent Street, Suite 1330. San Antonio, TX 78205

Partner's contribution to the project : In-kind support, Collaboration, Personnel exchanges

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.*

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.

Full thickness Skin Columns (FTSC) Implantation to Decrease Hypertrophic Scar after Partial-Thickness Burn

W81XWH-17-2-0061



PI: Rodney Chan, MD/Anders Carlsson, PhD Org: Metis Foundation/USAISR

Award Amount: \$749,432

Study/Product Aim(s)

Study Goal: Accelerate wound closure and improve regenerated skin quality using FTSC implantation after deep-partial thickness burn (DPTB).

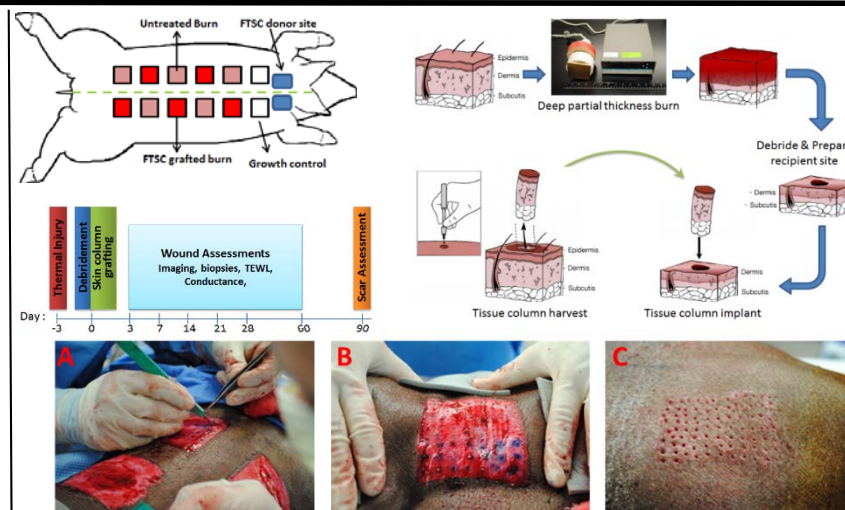
AIM 1: Establish optimal **early** FTSC seeding density for treatment of DPTB. This aim will simulate the treatment of acute burns before re-epithelialization.

AIM 2: Establish optimal **late** FTSC seeding density for treatment of DPTB. This aim will simulate the treatment of burns after re-epithelialization.

Approach

Using established porcine models of DPTB we will compare wound kinetics, restoration integumentary function and regenerated skin quality after treatment with or without implanted FTSC. Treatment strategies will include three different ratios of FTSCs implantation in residual dermis following DPTB.

Comparisons between treatment groups will be made by assessing wound closure, degree of contraction, distribution of pigmentation, scar hypertrophy, transepidermal water loss and patient observer scar scale (POSAS). Histopathology and expression analysis will be investigated to establish mechanism of action



A) visualize the skin column implantation/technique. B) Shows a close up on a debrided partial-thickness burn after implantation of skin columns. C) Shows the skin column donor site on the shoulder/neck portion of the animal.

Timeline and Cost

AIM	TASKS AND MILESTONES	FY	18	19
	Regulatory approvals (IACUC)			
1	Evaluate FTSC treatment of DPTB Establish seeding density for early FTSC treatment			
2	Evaluate FTSC treatment of DPTB - 2a: seeding density for late FTSC treatment			
3	Data analysis, review, publication			
4	Study close-out			
Proposal Budget (\$K)		TOTAL: \$749	\$369.5	\$379.5

Goals/Milestones

FY18 Goal – Begin FTSC seeding studies in DPTB.

- ✓ Obtain IACUC approval for and validate DPTB models
- ✓ Validate FTSC harvest technique and device
- ✓ Finalize protocol for FTSC treatment of DPTB
- ✓ **Begin AIM 1, early treatment** of DPTB seeding density experiments
- ✓ **Begin AIM 2, late treatment** of DPTB seeding density experiments

FY19 Goals – Complete DPTB experiments, data collection/analysis, publish and close-out.

- Complete AIM 1, early treatment experiments
- Complete AIM 2, late treatment experiments
- Complete data collection/analysis for AIM 1 and AIM 2 data.

Comments/Challenges/Issues/Concerns: The new 1:2 ratio group (replacement for 1:9 ratio) performs well based on preliminary data.

Budget Expenditure to Date

Projected Expenditure: Year 1: \$312,559
Actual Expenditure: Year 1: \$156,096.00

Updated: 29 October 2018