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TITLE: Anti-scar Treatment for Deep Partial-thickness Burn Wounds

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CONTRACTING ORGANIZATION: The Geneva Foundation

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14. ABSTRACT
The FDA-approved drug pirfenidone is an anti-inflammatory/anti-fibrosis drug indicated for pulmonary fibrosis that we hypothesize can diminish scarring when applied topically to deep partial-thickness burn wounds in two animal models. The long-term objective is to learn to effectively use pirfenidone with regard to dosage, formulation and timing of treatment of burn wounds, such that animal studies will likely translate to the clinic. The objective of this proposal is to evaluate pirfenidone for efficacy in reducing fibrosis and scarring parameters in mouse and porcine models of deep partial-thickness burn wounds. The dosage formulation and schedule of treatment will be optimized and molecular markers of inflammation, angiogenesis, wound healing, and fibrosis will be correlated with scar reduction.

15. SUBJECT TERMS
Deep Partial-Thickness Burn; Pirfenidone; Hypertrophic Scar; Fibrosis; Formulations; mouse Burn Model; Porcine Burn Model; Topical; Inflammation; Granulation; Proliferation

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1. INTRODUCTION:

Deep Partial-thickness (DPT) burns frequently result in hypertrophic scars that can lead to severe functional impairment, psychological morbidity, and costly long term healthcare. Current treatment options lack effectiveness. The purpose of this research is to identify dosage formulations and treatment schedules for the FDA-approved drug Pf to evaluate it for use as a topical prophylactic and treatment against fibrotic scarring of DPT-burn wounds. The scope of the research is to evaluate pirfenidone for efficacy in reducing fibrosis and scarring parameters in mouse and porcine models of deep partial-thickness burn wounds. The dosage formulation and schedule of treatment will be optimized and molecular markers of inflammation, angiogenesis, wound healing, and fibrosis will be correlated with scar reduction.

2. KEYWORDS:

Deep Partial-Thickness Burn; Pirfenidone; Hypertrophic Scar; Fibrosis; Formulations; mouse Burn Model; Porcine Burn Model; Topical; Inflammation; Granulation; Proliferation

3. ACCOMPLISHMENTS:

What were the major goals of the project?

1. Identification of topical formulations and doses that effectively deliver Pirfenidone (Pf) to the dermis of deep partial-thickness (DPT)-burn wounds at each phase of healing and mitigate fibrosis of the closed wounds.
2. Optimization of the schedule of topical applications and uses this optimized schedule to determine detailed molecular changes in healing wounds resulting from Pf treatment.
3. Validation of the efficacy of Pf to reduce hypertrophic scarring in the Duroc porcine DPT-burn model.

What was accomplished under these goals?

Major accomplishments of Goals #1 and #3:

Aside from the development of a tool to quantify collagen arrangement in normal *vs*. burned skin as one of the endpoints to assess Pirfenidone (Pf) treatment effects, during the bulk of Year 5, we have experienced significant contract delays for obtaining Duroc pigs in order to determine the Pf treatment effects in reducing burn-induced hypertrophic scarring (Goal #3). The delays were further compounded by the COVID pandemic which caused the facility shut down from March to most part of July, 2020. The ISR animal facilities resumed some levels of large animal activity in August 2020 and the contract issues for pigs were also resolved. Consequently we were allowed to start limited quantities of large animal study. Two Duroc pigs were ordered in September 2020. Treatment study started on October 19, 2020. Below summarizes the major activities and accomplishments associated with the goals described above for 4th quarter, Year 5:

1. Pf Patch Fabrication and Characterization for Pirfenidone Treatment of Porcine Deep Partial-Thickness Burn Wounds for Scar Reduction

Fabrication

For patch fabrication, all layers were cast by employing a doctor blade caster with adjustable blade height based on a layer-by-layer process. Layer-by-layer fabrication is the process of direct coating of a layer over

previous layers. This is in contrast with the lamination process which involves casting layers separately and combining them after they have individually dried/cured. First, the backing layer was prepared by casting a solution of 12.5 wt% polyurethane (PY-PT72AE) in tetrahydrofuran (THF) solvent over a release liner at a gap width of 150 µm. The layer was dried by allowing THF to evaporate for 30 minutes under ventilation. Next, a solvent-based solution of the acrylic drug matrix (DuT 87-2852), additionally composed of 15 wt% Pirenidone (Pf), was directly cast over the dried backing layer at a gap width of 600 µm. The drug matrix layer was dried overnight under ventilation. Finally, a two-part platinum-catalyzed silicone adhesive (MG 7-9850) was mixed using a dual asymmetric centrifuge for 2 minutes at 3,000 RPM. The mixed silicone adhesive (wound contact layer) was cast directly over the dried drug matrix layer at an application height of 400 µm and allowed to cure at 70˚C for 20 minutes. The multi-layered patch was then removed from the original liner and placed silicone-side down over a fluorosilicone coated polyester liner (3M™ Scotckpak™ 9709) that is compatible with silicone adhesives and can be easily removed. The composition of different layers of the Pf anti-scar patch is summarized in Table 1.

Table 1. Composition of Pf multi-layer patch

Layer	Component	Solution wt%[1]	Dry wt%[2]
Backing	Polyurethane (PY-PT72AE, Lubrizol)	12.5%	100.0%
Gap Width[3]: 150µm	Tetrahydrofuran	87.5%	-
Drug Matrix	Acrylic Solids (DURO-TAK 87-2852, Henkel)	70.4%	85.0%
Gap Width: 600µm	Acrylic Solvent Mix	25.1%	-
	Pirfenidone (Tyche Industries)	4.4%	15.0%
Adhesive	Silicone Adhesive, Part A (MG7-9850, DuPont)	50.0%	50.0%
Gap Width: 400µm	Silicone Adhesive, Part B (MG7-9850, DuPont)	50.0%	50.0%

[1]Solution wt% is the w/w of each component within the casting solution of each patch layer, not the patch as a whole

[2]Dry wt% is the w/w of each component after removal of solvents within each patch layer, not the patch as a whole

[3]Gap Width is the set height of the casting blade with direct coating over previous layers. Order: 1) Backing, 2) Drug Matrix, 3) Adhesive

Characterization

For the burn treatment study, high dose patches (15 wt% Pf in the drug matrix) and vehicle controls (blank patches without Pf) were produced according to Table 1. Five (5) sheets (6×20") were produced which yields 15 doses/sheet of 1.5" diameter for treatments, and 8 samples of 9/16" diameter for patch characterization. Overall patch area weights were comparable between high dose patches (29.9 ± 0.4 mg/cm^2; n=40) and control patches (29.3 ± 0.6 mg/cm^2; n=40) (Table 2). Variability across the area of the sheets were under a target of 10% with an overall relative standard deviation (RSD) of 1.35% and 2.17% for high dose and control patches, respectively. Pf loading for high dose patches was 2,282.7 ± 91.5 µg/cm^2 (n=30) with an acceptable RSD of 4.0%.

Table 2. Patch area weights and Pf loading

Characterization	High Dose Patch	Control Patch
Patch Area Weight (mg/cm^2)*	29.9 ± 0.4***	29.3 ± 0.6***
Pf Loading (µg/cm^2)**	2282.7 ± 91.5***	--

* Area weight, n = 40 (mean ± standard deviation)

** Pf loading, n = 30

*** Mean ± standard deviation

In Vitro Release Test (IVRT) of high dose Pf patches showed controlled release over 48 hours (Fig. 1). On average, 41.6% of Pf was released after 12 hours, 63.2% after 24 hours, and 80.0% after 48 hours (Fig. 1).

Release profiles are reproducible from batch to batch (n=5 batches, n=2 samples/batch) and are consistent with previous experiments of the same patch design.

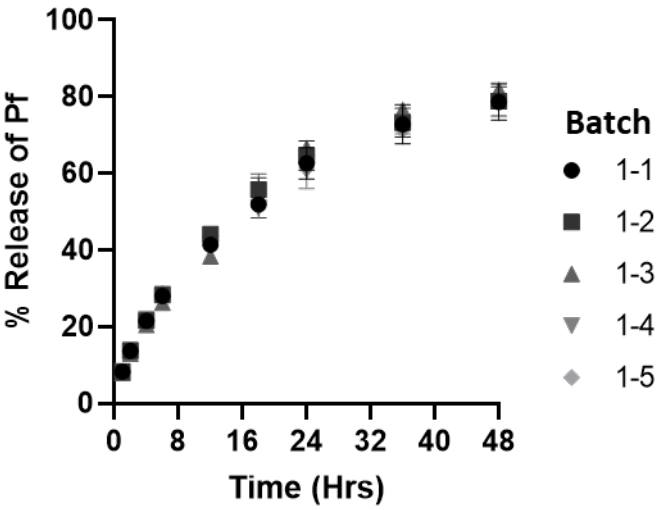

Figure 1. *In Vitro* Release Test (IVRT) of Pf from multiple batches of patches. N=5 batches, n=2 samples/batch; Bars: Mean ± standard deviation.

2. Pf Treatment of Porcine Deep Partial-Thickness Burn Wounds for Scar Reduction

Pf treatment scheme

The results of Pf treatment of mouse deep partial-thickness burn wounds showed that Pf treatment during the inflammatory and remodeling phase decreases inflammation and improves fibrosis in these mouse burn wounds (Medina et al. 2019). A similar treatment schedule focusing on inflammatory and remodeling phase of healing was used to test Pf anti-scar effects on porcine burn wounds (Fig. 2).

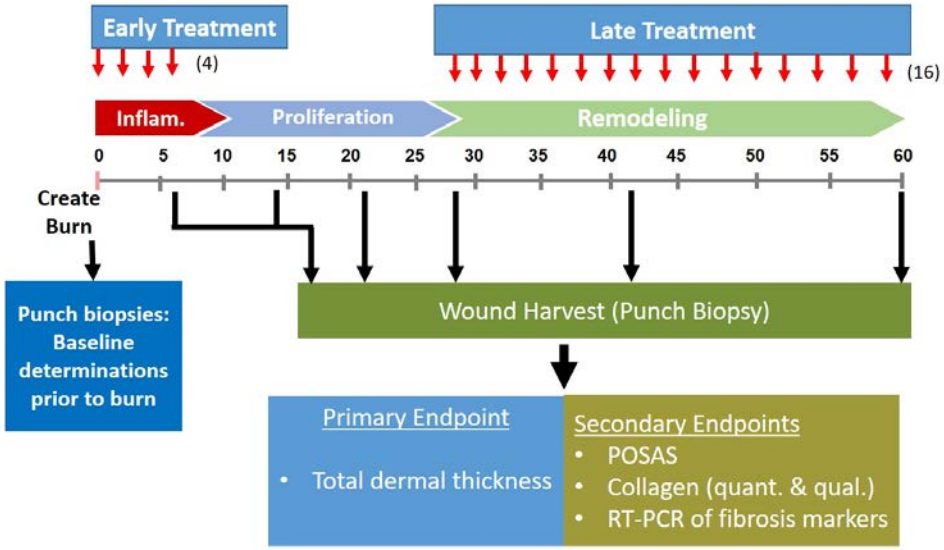

Figure 2. Study design for determining anti-scar effectiveness of Pf patches applied during the inflammatory and remodeling phase. The wound treatments are shown by the red arrows. At each red arrow, Pf patches (Pf at 15 wt%) and the blank patches (Patches without Pf) are tested. On Day 0, patches (control and Pf) are applied to wounds within 1 hour post-burn. The wounds treated with Pf are compared to those treated with blank patches to determine the effect of Pf on fibrosis, contracture and scarring parameter endpoints such as dermal thickness.

Layout, induction, and treatment of porcine deep partial-thickness burn wounds

Three cm diameter burn wounds were demarcated with a skin marker to the mid regions of the animal's back prior to burn wound induction. The placement of burn wounds was divided into the left and right region per the diagram of the wound layout (Fig. 3). Two 3.0 cm unburned wounds with an additional 5.0 cm diameter growth control to normalize burn wound size to the natural growth and weight gain of animals were created above and below the mid-region. Wounds on the left region received blank patches (no Pf) whereas wounds on the right region received Pf patches (15%wt Pf) within one hour of burn injury (Figs. 4 & 5). Burn wounds will be harvested on days 7, 14, 21, 28, 42, and 60 post burn as denoted. Thus far, patches adhered well onto burn wounds and their removal during patch changes did not cause any damage to the burn eschar. At the time of the submission of this progress report, we have completed 2 treatments. Treatments will continue and results will be reported in the next reporting period.

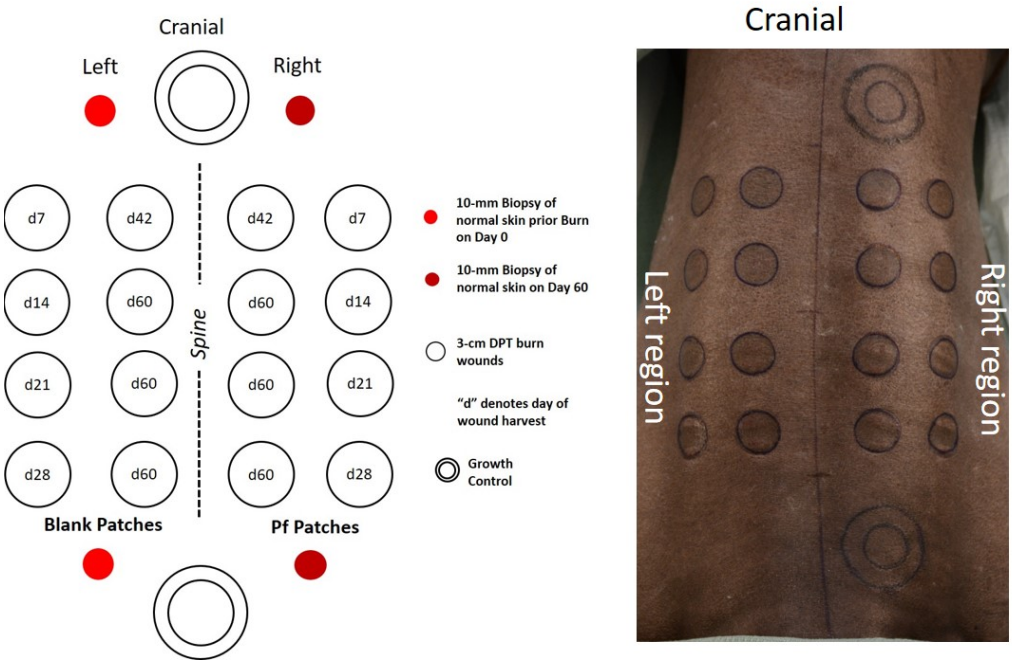

Figure 3. The layout of burn wounds and distribution of induced deep partial-thickness burn wounds in the mid-region of the pig's back. The diagrammatic layout of wounds (left) and the overview of the induced burn wounds in the mid region of the pig's back post-burn (right).

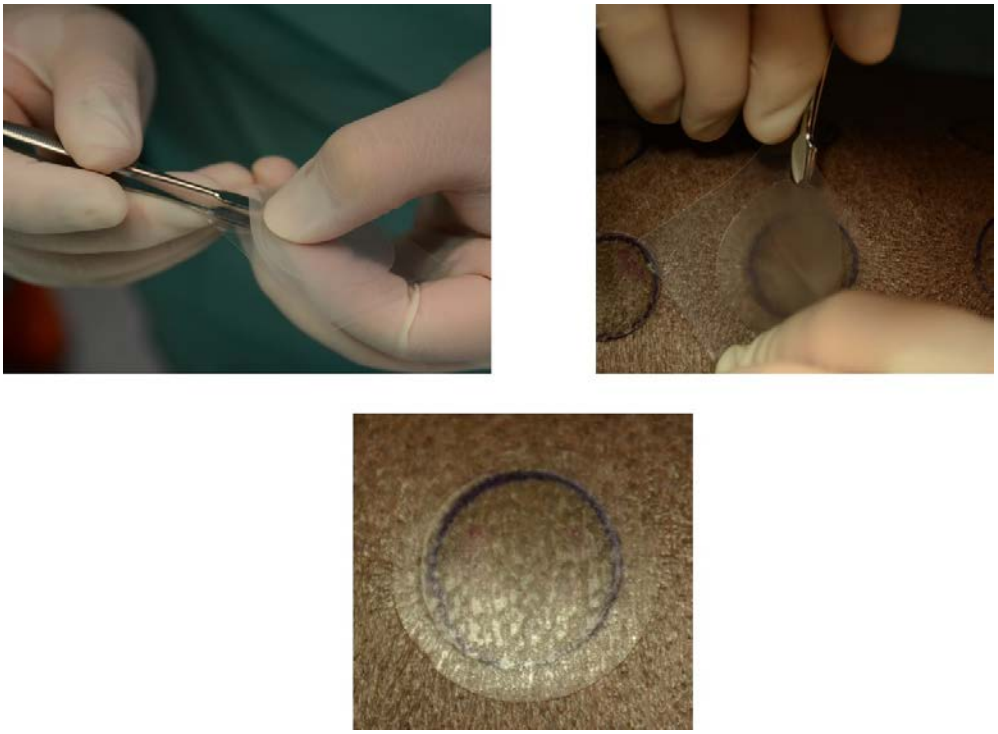

Figure 4. Application of Pf and blank patches to burn wounds. The patch was removed from the plastic liner (A) and placed directly onto the burn wound (B & C).

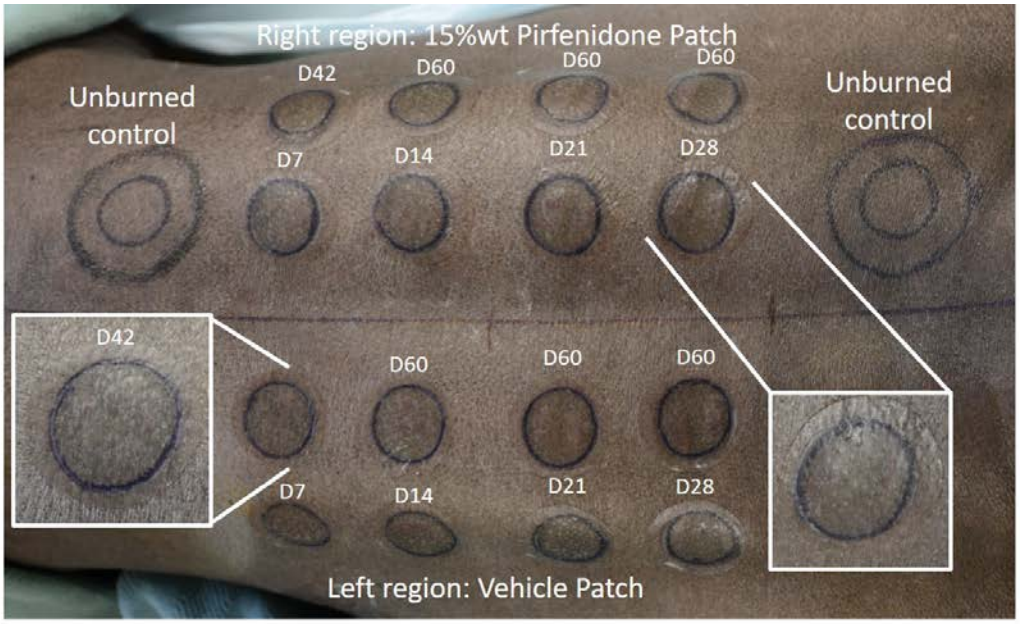

Figure 5. Overview of the burn wounds with applied patches. The burn wounds of the left flank were treated with control patches (no Pf). The burn wounds of the right flank were treated with Pf (15 wt%) patches. Unburned wound controls were not treated.

References:

Medina JL, Sebastian EA, Fourcaudot AB, Dorati R, **Leung KP**. Pirfenidone ointment modulates the burn wound bed in C57BL/6 mice by suppressing Inflammatory Responses. Inflammation. 2019. **42**(1):45-53. PMID: 30120654.

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

The study has provided a good training opportunity for Dr. Jesse Nguyen, who was trained as a molecular microbiologist in his graduate studies, to become proficient with large animal work. His participation also allows Dr. Nguyen to be familiar with the hypertrophic scarring process both at cellular and molecular levels.

How were the results disseminated to communities of interest?

During this reporting period, we have reported our findings in peer-reviewed journals as a means to disseminate the results to reach the members of research communities who are interested in developing therapeutic solutions to reduce fibrosis and scarring. See the list of publications below.

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

Continue to test Pf treatment effects in reducing DPT burn wound induced hypertrophic scarring in a red Duroc hypertrophic scarring model of DPT burn wounds. Continue to characterize the shallow and DPT burn wounds in red Duroc pigs.

4. IMPACT:

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

From the studies conducted, we have established a prototype antiscar patches containing Pirfenidone that can be deployed to treat porcine deep partial-thickness burn wounds.

The development of the porcine burn model establishes the clinically relevant endpoints for assessing Pf treatment efficacy in reducing hypertrophic scarring.

What was the impact on other disciplines?

Nothing to report.

What was the impact on technology transfer?

Office of Technology Transfer at MRDC has prepared a one-pager synopsis describing the antiscar technology for potential licensing to laboratories and industry for further product development.

What was the impact on society beyond science and technology?

Nothing to report.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

Nothing to report.

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

Due to contract issues (prior COVID) and COVID pandemic, we experienced significant delays in obtaining Duroc pigs to test pirfenidone treatment effect on scar reduction of deep partial-thickness burn wounds. The contract issues were resolved and ISR has partially opened the animal facilities in July 2020. In September 2020 we were allowed to order 2 Duroc pigs for the proposed Pirfenidone treatment study.

Changes that had a significant impact on expenditures

Nothing to report.

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

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:

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

Journal publications.

1. Chung E, Wells A, Kiamco MM, and Leung K. Dual asymmetric centrifugation efficiently produces a poloxamer-based nanoemulsion gel for topical delivery of Pirfenidone. 2020. AAPS PharmSciTech. 21(7):265. PMID: 33006045.
2. Wells AR and **Leung KP**. Pirfenidone attenuates the profibrotic contractile phenotype of human dermal myofibroblasts. 2020. Biochem. Biophys. Res. Commun. **521**(3):646-651. PMID: 31679692.
3. Evani SJ, Rajasekhar Karna SL, Seshu J, and **Leung KP**. Pirfenidone regulates LPS mediated hyper activation of neutrophils. 2020. Sci Reports. Accepted

Books or other non-periodical, one-time publications.

Nothing to report.

Other publications, conference papers and presentations.

Nothing to report.

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

Nothing to report.

- **Technologies or techniques**

We have developed a layer-by-layer fabrication technique for producing topical Pirfenidone (Pf) patches that can adhere to burn wounds and provide a sustained delivery of the anti-scar agent –Pf into burn wounds.

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

Docket No. ISR 19-13-WO01: PCT/US2020/044251 filed 30 Jul 2020, “Layered Composite for Scar Treatment and Prevention,” by Leung, Dr. Kai P. (ISR).

This PCT claims priority of:
Docket No. ISR 19-13X: 62/880,396 filed 30 Jul 2019, “Tri-Layer Laminated Pirfenidone-Containing Film For Scar Treatment And Prevention” by Leung, Dr. Kai P. (ISR).

- **Other Products**

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

What individuals have worked on the project?

Name:	Kai Leung
Project Role:	PI
Nearest person month worked:	3
Contribution to Project:	Dr. Leung is responsible for insuring compliance with all regulatory requirements. He has chosen the following personnel to assist him in the proposed studies because of their expertise in animal surgery and in the field of molecular biology, histology, histochemistry, and immunohistochemistry, PCR array analysis, as well as wound healing analysis.
Name:	Jesse Nguyen
Project Role:	Postdoctoral Fellow
Nearest person month worked:	10.8
Contribution to Project:	Dr. Nguyen is responsible for the animal surgical procedures and work with the PI to plan and execute the animal model required for this proposed research. He has coordinated with other team members and successfully determined the burn time required to produce porcine deep partial-thickness (DPT) burn wounds.
Name:	Andrea Fourcaudot
Project Role:	Research Associate
Nearest person month worked:	9.6
Contribution to Project:	Ms. Fourcaudot has assisted in the development of the porcine DPT burn wound model. She has processed burn tissue and perform tissue sectioning for
Name:	Kishan Evani
Project Role:	Post-Doctoral Fellow
Nearest person month worked:	9.6
Contribution to Project:	Using LPS-stimulated human neutrophils Dr. Evani has determined the anti-inflammatory properties of Pf.
Name:	Adrienne Wells
Project Role:	Post-Doctoral Fellow
Nearest person month worked:	9.6
Contribution to Project:	Using TGF beta-stimulated human dermal fibroblasts, Dr. Wells has determined the anti-fibrotic properties of Pf.

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

Nothing to report.

What other organizations were involved as partners?

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: N/A

QUAD CHARTS: Attached.

9. APPENDICES: N/A