AWARD NUMBER: W81XWH-16-2-0063

TITLE: "Microfragmented Adipose Tissue and Blood Plasma-Based Hydrogels for Treatment of Combat-Associated Burn Injuries"

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REPORT DATE: MAY 2020

TYPE OF REPORT: Final Report

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

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1. REPORT DATE *(DD-MMM-YYYY)*
MAY 2020

2. REPORT TYPE
Final Report

3. DATES COVERED *(From - To)*
09/30/2016 - 01/29/2020

4. TITLE AND SUBTITLE
Microfragmented Adipose Tissue and Blood Plasma-Based Hydrogels for Treatment of Combat-Associated Burn Injuries

5a. CONTRACT NUMBER
W81XWH-16-2-0063

5b. GRANT NUMBER
MB150163

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)
USAMRDC

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES
Approved for Public Release; Distribution Unlimited

14. ABSTRACT
Following full thickness burn injuries, the subcutaneous adipose tissue often suffers severe damage. Even when the hypodermal fat layer is not fully involved, surgical intervention usually results in removal of the hypodermis down to muscle fascia. Although this avoids the complications of inadvertently leaving necrotic foci in the wound bed, it negates the benefit of retaining viable hypodermal adipose tissue and microvasculature. Grafting onto fat has been shown to reduce wound contraction, especially in extremity burns located near joints, resulting in better range of motion and improved sensation. This indicates that grafts onto fat may heal better and have improved innervation. In addition, the removal of the hypodermal tissue results in loss of vasculature and poor graft take, leading to wound contraction and scarring. The purpose of this study was to evaluate application of micro-fragmented adipose tissue (Lipogems) as a hypodermal skin substitute using hydrogels in a porcine full-thickness wound model. We hypothesize that early reconstruction of hypodermis using purified Lipogems and hydrogels will improve angiogenesis, healing, and scar appearance.

15. SUBJECT TERMS
Adipose Tissue Stem Cells; Lipogems; Plasma Hydrogels; Burn Wound Healing; Porcine Model

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON USAMRDC
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**	Unclassified	38	**19b. TELEPHONE NUMBER** *(include area code)*
Unclassified	Unclassified	Unclassified			

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

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1. INTRODUCTION

Write a narrative (one paragraph) that describes the subject, purpose and scope of the research.

Subject: Burn wound care is a major socio-economic problem in the United States (**1-3**). According to American Burn Association (ABA), approximately 500,000 burn patients require medical attention in the United States (**4**). Severe burns are mostly of full thickness category and few treatment options exist. If not appropriately treated, burn wounds may result in long-term healing complications, such as contraction, and scarring, which negatively affect the quality of life of an individual. The current standard of care to treat deep partial thickness (DPT) and full thickness (FT) burns involves excision of dead necrotic tissue until viable wound bed, followed by early coverage with a split thickness skin graft. In cases of large total body surface area (TBSA) burn, the surgical treatment becomes proportionately more difficult because of increasing extent and severity. Often resulting in the removal of subcutaneous adipose tissue, even when the hypodermal fat layer is not fully involved, down to muscle fascia to avoid the complications of inadvertently leaving necrotic foci in the wound bed (**1**). Most of the surgical interventions, autograft or allograft, does not replace hypodermal adipose tissue. Whereas, it has been shown previously grafting on to the fat layer to be beneficial resulting in better healing and range of motion (**5**). Moreover, currently available skin substitutes do not include the adipose tissue layer in their constructs. A recent clinical study shows that skin grafting on subcutaneous fat to have a substantial influence on reduction of repeated reconstructive surgeries and long term scarring which may reduce serious deformity complications (**6**). Therefore, our effort in this project focuses on reconstructing/replacing subcutaneous adipose tissue after a full-thickness skin injury. To harness the advantage of subcutaneous adipose we investigated the utility of a point-of-care FDA approved technology developed by Lipogems LLC. Lipogems is a sterile closed system in which lipoaspirate (adipose tissue collected during liposuction procedure) is treated only by mechanical processes and filtering methods without any enzymatic digestion. For clinical use, Lipogems can be isolated at the 'bed-side' in the surgical suite using aspirator and processed with this mechanical device. The processed fat essentially contains mesenchymal stem cells, pericytes, and endothelial cells in a cluster within the collagenous extracellular matrix (**7-9**). The processed adipose tissue also contains major structural proteins like collagen, actin and elastins which along with cells and soluble paracrine signals will results in generation of mature adipose tissue. In order to optimize the use of Lipogems we have taken a systematic approach to evaluate processed adipose tissue *in vitro* and then *in vivo*.

Purpose: The *major goal* of this project is to reconstruct full-thickness wound with microfragmented adipose tissue (Lipogems). Our *objective* is to replace the hypodermis of a full thickness burn wound after debridement before grafting. We *hypothesized* subcutaneous hypodermal replacement will augment revascularization of a full-thickness burn wound and improve healing and scar appearance. In order to accomplish this goal, we prepared a purified micro-fragmented adipose tissue called "Lipogems" and delivered using hydrogel-based formulations. In order to assess the feasibility, and efficacy of using Lipogems to treat full thickness skin loss, we have used a porcine full-thickness excision and burn wound models. Wherein, following excision or burn wound, autologous/ allogeneic adipose layer was spared to prepare a hydrogel-Lipogems formulation and used to cover the wounds along with autologous meshed split thickness skin graft (mSTSG).

Scope: While there are emerging technologies and stem cell-based therapeutics to treat full thickness burn wound, still their FDA clearance as a biologic is still under scrutiny. We aim at providing a cost-effective, bedside treatment option to regenerate full-thickness burn wounds with better long term healing. To accomplish this goal, we proposed to deliver Lipogems using hydrogel-based biomaterial. Since Lipogems retains the essential cell populations necessary for wound regeneration, when delivered via hydrogel matrix will improve healing of full-thickness burn wounds with better scar outcomes. The Lipogems-hydrogel treatment will allow cells within the Lipogems to recapitulate the 3-D microenvironment and act as a conducive medium to foster better graft take and wound healing outcomes.

2. KEYWORDS

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

Lipogems, hydrogels, Porcine excision wound, deep partial thickens burn wound, meshed split thickness skin graft, adipose tissue

3. ACCOMPLISHMENTS

The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction.

What were the major goals of the project – (goals to be accomplished and status)

List the major goals of the project as stated in the approved Statement of Work (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. Only provide a STATUS update for either Major Task or Milestone Achieved for each Specific Aim.

Specific Aim 1: Optimization and characterization of Lipogems and hydrogel based formulations	**Timeline (Months)**	**Status**
Major Task 1: Human Lipogems-hydrogel based formulation	**Y1**	Start: Y1Q1 Completed:Y2Q2
Subtask 1: Lipogems-PEGylated PFP hydrogel formulation	9-12	Completed
Subtask 2: Lipogems-PEGylated fibrin hydrogel formulation	9-12	
Subtask 3: Lipogems-collagen hydrogel formulation	9-12	
Subtask 3: *In vitro* characterization Lipogems-hydrogel formulations	12-15	

Milestone(s):

1. *Human and porcine adipose tissue Lipogems preparation protocol established.*
2. *In vitro Lipogems and Lipogems-hydrogels characterization completed.*
3. *Consolidated manuscript with the results from porcine partial thickness wound model treated with Lipogems formulations (Draft in preparation).*

Specific Aim 2: Screening and evaluation of Lipogems in full-thickness porcine wound model	**Y2**	Start: Y2Q1 Completed:Y2Q4
Major Task 1: Full-thickness excision wound model (30cm^2 wounds)	7-24	Y2Q1-Y3Q1
Subtask 1: Autologous adipose tissue isolation	13-24	
Subtask 2: Surgical procedure	7-24	
Major Task 2: Treatment of full-thickness wounds with Lipogems and Lipogems-hydrogels	10-24	
Major Task 3: End point measurements	13-24	
Subtask 1: Photo-Documentation of Healing	13-24	
Subtask 2: Assessment of graft take and healing	13-24	
Major Task 4: Data Analysis	22-27	

Milestone(s) Achieved:

1. *Treatment protocol established to evaluate Lipogems and Lipogems-hydrogel formulation.*

2. *Effective dose of Lipogems and Lipogems-hydrogels identified.*
3. *Efficiency of Lipogems and Lipogems-hydrogel formulation evaluated.*

Specific Aim 3: Lipogems using full-thickness porcine burn wound model	**Y3**	Start: Y2Q4 Experiments Completed:Y3Q4
Major Task 1: Full-thickness burn excision wound model (5×5 cm wounds)	25-36	Completed Y3Q4
Subtask 1: IACUC approval	Y2Q4	
Subtask 2: ACURO approval	Y32Q1 (*Delayed*)	
Subtask 2: Allogeneic adipose tissue isolation	22-25	
Major Task 2: Treatment of full-thickness burn wounds with Lipogems and Lipogems-hydrogels	27-36	
Major Task 3: End point measurements	30-36	
Subtask 1: Photo-Documentation of Healing	30-36	
Subtask 2: Assessment of graft take and healing	30-36	
Major Task 4: Data Analysis	30-36	*Data analysis completed*
No Cost Extension approved to complete data analysis	*NCE: START January 2020*	*NCE Approved*
Data Analysis	*NCE:END May 2020*	*Completed*
Final Report	*May 28, 2020*	*Completed*

Milestone(s) Achieved:

4. *Burn treatment protocol established to evaluate Lipogems and Lipogems-hydrogel formulation.*
5. *Safety and efficacy of Lipogems and Lipogems-hydrogel formulation evaluated.*
6. *All the experiments completed as proposed.*
7. *Under preparation manuscript (Pending, due to COVID-19 pandemic-compilation of manuscript delayed). Since the project involved robust data sets from both in vivo, specific Aim 3 (N=6) and in vitro studies, co-ordination with project associated personnel and data-reach were difficult. The manuscript is currently drafted and expected to be completed by June 2020 and submitted for publication.*

What was accomplished under these goals – (detailed progress and results)

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.

Specific Aim 1: Optimization and characterization of Lipogems and hydrogel based formulations

- **Major Activities**:

- Process for isolating Lipogems from porcine and human subcutaneous adipose tissue was established during this reporting period.
- Method to isolate Lipogems (human and porcine) of desirable size range was established.
- Volume of human Lipogems and the media conditions were optimized to grow ASCs from the Lipogems *in vitro*.
- Lipogems were frozen and cryopreserved for later experimental use.
- Optimized volume fraction of human Lipogems to hydrogel (using PEG-PFP hydrogel) that allowed cells from the Lipogems to migrate and proliferate within the hydrogel microenvironment.

- **Specific objective(s)**:

- Optimize Lipogems isolation process from porcine and human adipose tissue.
- Cluster diameter of the Lipogems obtained from different extrusion methods was analyzed.
- Human Lipogems grown under standard media condition supplemented with fetal bovine serum or Mesenpro, a stem cell specific growth media.
- Determine the desirable amount of Lipogems to be incorporated within hydrogels to enable cells to sprout and proliferate.
- Human Lipogems with different hydrogel formulations, viz., Lipogems - collagen, PEGylated fibrin (FPEG) and PEGylated platelet free plasma (PEG-PFP) were formulated and screened for the hydrogel's (collagen, FPEG and PEG-PFP) ability to support cell growth from Lipogems was analyzed.
- PEG-PFP hydrogels incorporated with Lipogems (human and porcine) were further evaluated for
- Phenotype changes of the cells growing out from Lipogems were monitored for 10 days and light micrographs were recorded.
- Sprouting and network formation in two different growth conditions; Dulbecco's Minimal Essential Media (DMEM) basal media supplemented with 2.5% human platelet lysate (hPL) and Mesenpro basal media supplemented with Mesenpro growth supplements. Finally, sections were immunostained with alpha smooth muscle actin (αSMA).

Specific Aim 2: Screening and evaluation of Lipogems in full-thickness porcine wound model

- **MAJOR ACTIVITIES**:

- **Optimization and characterization of Human Lipogems and hydrogel based formulations** *(completed Y1 task).*
- **Screening and evaluation of Lipogems in full-thickness porcine wound model**

- **SPECIFIC OBJECTIVES**

- Optimized method to isolate Lipogems (human and porcine) of desirable size range was established.
- Optimized volume fraction of human Lipogems to hydrogel (using PEG-PFP, PEG-fibrin and Collagen hydrogels) that allowed cells from the Lipogems to migrate and proliferate within the hydrogel microenvironment.

- Treatment protocol established to evaluate Lipogems and Lipogems-hydrogel formulation in a porcine full thickness wound healing model.
- Effective dose of Lipogems and Lipogems-hydrogels identified.
- Efficiency of Lipogems and Lipogems-hydrogel formulation evaluated *in vivo*.

Specific Aim 3: Lipogems using full-thickness porcine burn wound model

- **MAJOR ACTIVITIES**

- **Evaluation of Lipogems in full-thickness burn porcine wound model**

- **SPECIFIC OBJECTIVES**

- Allogeneic Lipogems isolated and stored.
- Treatment protocol established to evaluate Lipogems and Lipogems-hydrogel formulation in a porcine full thickness burn wound healing model.
- Safety and efficiency of Lipogems and Lipogems-hydrogel formulation evaluated *in vivo*.

METHODS AND RESULTS (consolidated - all three specific Aims):

METHODS:

- **Isolation of Lipogems from porcine and human adipose tissue:** Lipogems from porcine and human subcutaneous adipose tissue was isolated using the kit supplied by LIPOGEMS, USA. Isolation process involves mechanical shearing of the adipose sample without addition of any enzymes. Briefly, emulsion of fragmented adipose tissue forms within the barrel and the saline flow washes the tissue, under gravitational force until saline barrel clears and allowing the processed adipose tissue (Lipogems) to float which are then extruded through the grey filter and collected for further experiments (**Figure 1**). The cluster of Lipogems were collected in a separate sterile conical tube and used further for preparing Lipogems-hydrogel (collagen, FPEG and PEG-PFP) formulations. Lipogems isolated from both human and porcine sources were cryopreserved using standard cryoprotectant media (Dulbecco's Minimal Essential Media (DMEM) containing 10% dimethyl sulfoxide and fetal bovine serum).
- **Lipogems Clusters:** Lipogems from porcine subcutaneous adipose tissue was isolated using the kit supplied by LIPOGEMS, USA and optimized for size range by collecting Lipogems using six different methods, clusters recovered from 500 µm diameter pore size filter of the kit; 500 µm diameter pore size filter of the kit followed by passing through 14 gauge needle; 1.5 mm diameter pore size filter of the kit; ~1.5 mm diameter pore size filter of the kit and passed through 14 gauge needle, 18 gauge needle, and 14 gauge needle and then through 18 gauge needle.
- **Stem cell quantification**: A known amount of porcine Lipogems was enzymatically processed using collagenase to isolate adipose derived stem cells and quantified. Briefly, the Lipogems (5g) was suspended in HBSS and centrifuged for 10 minutes at 500xg. The floating tissue was carefully collected; and to every 1 ml of the floating fraction tissue, 3500 units of collagenase type II was added and incubated for 45 minutes at 37 °C in an orbital shaker incubator at 125

rpm. The undigested tissue was removed by sequential passage through 100-mm and 70-mm nylon mesh filters. The final cell pellets were resuspended and plated on a 25 cm2 flask MesenPRO RS™ growth media and allowed to attach overnight (~16 hours). Attached cells were then trypsinized and counted. Cells isolated from adipose tissue (5g) using a similar method was used as a control. Cells from the clusters (passage 0) were harvested and passaged (P1). An aliquot of the cells were plated on a 2-well chamber slides and culture for 24 hours at 37 °C. After 24 hours, light microscopic pictures were obtained and cells were stained using BODIPY fluorescent lipid probe. Epifluorescent images were captured to visualize intracellular lipids with the P1 ASCs.

- **Preparation of Hydrogels:**

Collagen Hydrogel: Collagen hydrogels were by initiating fibril formation in type 1 collagen (900 µl, 5 mg/mL of rat tail tendon collagen; Travigen) by adjusting the pH to 6.8–7.0 using 100 µl of 10x Dulbecco's phosphate buffered saline (PBS) and 23 µl of 1N sodium hydroxide (NaOH). The fibrillated collagen was added to a 6-well cell culture insert and incubated for 30-40 min at 37 °C to allow complete gelation of collagen.

PEGylated Fibrin hydrogel (FPEG): PEGylated fibrin hydrogel was prepared by mixing 250 µl of succinimidyl glutarate modified polyethylene glycol (SG-PEG-SG) (8 mg/ml in tris buffered saline; TBS) with 500 µl of fibrinogen stock (40 mg/ml in TBS) incubated for 20 min in a 5% CO_2 humidified incubator at 37 °C. After incubation, the PEGylated fibrinogen wase cross-linked with 1 ml of thrombin stock (25 U/ml in 40 mM of calcium chloride ($CaCl_2$) at a final concentration of 10 U/mL in a cell-culture insert (6-well format), the mixture was incubated in a 5% CO_2 humidified incubator at 37 °C for 10 min to allow for complete gelation.

PEGylated platelet free plasma (PEG-PFP) hydrogel: PEG-PFP hydrogels were prepared from PFP isolated from both human and porcine whole blood. Briefly, PEG (8mg/ml) was mixed with a PFP at a 1:20 v/v ratio. This mixture was then incubated for 10 minutes in a 5% CO_2 humidified incubator at 37°C. Gelation of the PEG-PFP liquid mixture was then initiated using human thrombin (10U/ml of PEG-PFP) and incubated for 20 minutes in a 5% CO_2 humidified incubator at 37°C to obtain PEGylated PFP hydrogels.

- **Hydrogel-Lipogems formulations:** Different hydrogels (collagen, FPEG and PEG-PFP) was prepared by mixing Lipogems clusters of different volumes (50µl, 100µl and 250µl /ml of PEGylated PGP hydrogel). Briefly, Lipogems cluster of different concentrations were mixed with collagen, PEGylated fibrinogen PEGylated PFP solution in a 12-well format cell culture insert and gelled as mentioned above. Lipogems incorporated hydrogels (1.1 cm^2 surface; 12mm diameter and ~ 0.5 to 0.7 cm thick) were maintained in MesenPRO RS™growth media for 10 days and photomicrographs were recorded. In addition, post day 10, Lipogems-hydrogels were harvested, fixed with 4% paraformaldehyde for 20 minutes, treated with a gradient sucrose (5 to 20%) and cryo-embedded using sucrose: histoprep embedding media (1:2 v/v ratio). The cryopreserved specimens were frozen slowly using liquid nitrogen cooled isopentane. Frozen sections were cut (7 µm thick), mounted on a slide and stained using with wheat germ agglutinin (WGA) and counterstained with DAPI nuclei stain to overall cell distribution within the hydrogels.

Immunohistochemical analysis of human Lipogems- PEGylated PFP hydrogels: After 10 days of *in vitro* incubation, human Lipogems- PEGylated PFP hydrogels were harvested, fixed, cryo-embedded using sucrose: histoprep protocol (as above 3.c). The cryopreserved specimens were frozen slowly using liquid nitrogen cooled isopentane. Frozen sections were cut (7 µm thick), mounted on a slide and stained using αSMA antibody to observe the cytoskeletal morphology of cells that has sprouted within PEGylated PFP hydrogel. The sections were counter stained using DAPI to observe cell nuclei.

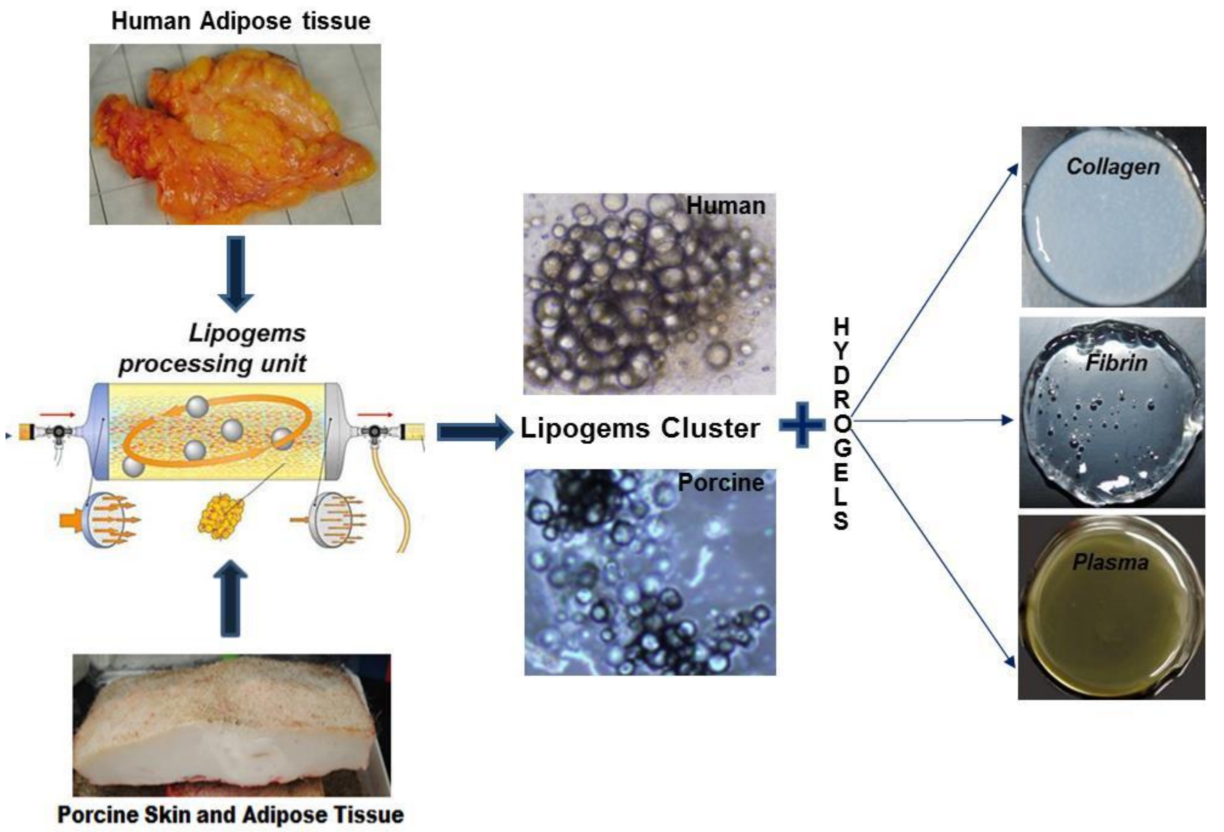


Figure 1: Schematic representation of Lipogems isolation process from porcine and human adipose tissue and formulation of Lipogems-hydrogels

The animal studies were carried out with ACURO (MB150163) and IACUC approval, and has been conducted in compliance with the Animal Welfare Act, the implementing Animal Welfare Regulations, and the principles of the Guide for the Care and Use of Laboratory Animals.

- **Full-thickness excision wound model**: Prior to excision and treatment, 100 ml of blood was obtained and used for preparing PEG-PFP hydrogels. Full thickness skin loss was performed using a surgical blade down to fat/fascia, with appropriate pain control and anesthetic protocols. The area excised was approximately a 28-36 cm^2 dermal piece from the central dorsum of the pig. A total of 50 6 cm diameter wounds (10 per animal; N=5 pigs) were created. Of 10 wounds, 9 were excised to fascia and treated while one wound was carefully excised to only remove the epidermis and dermis, leaving the subcutaneous fat layer intact. All wound edges and two equally large growth control areas were tattooed. The experimental timeline, collection of tissue biopsies, and end point measurements are shown in **Figure 2**.

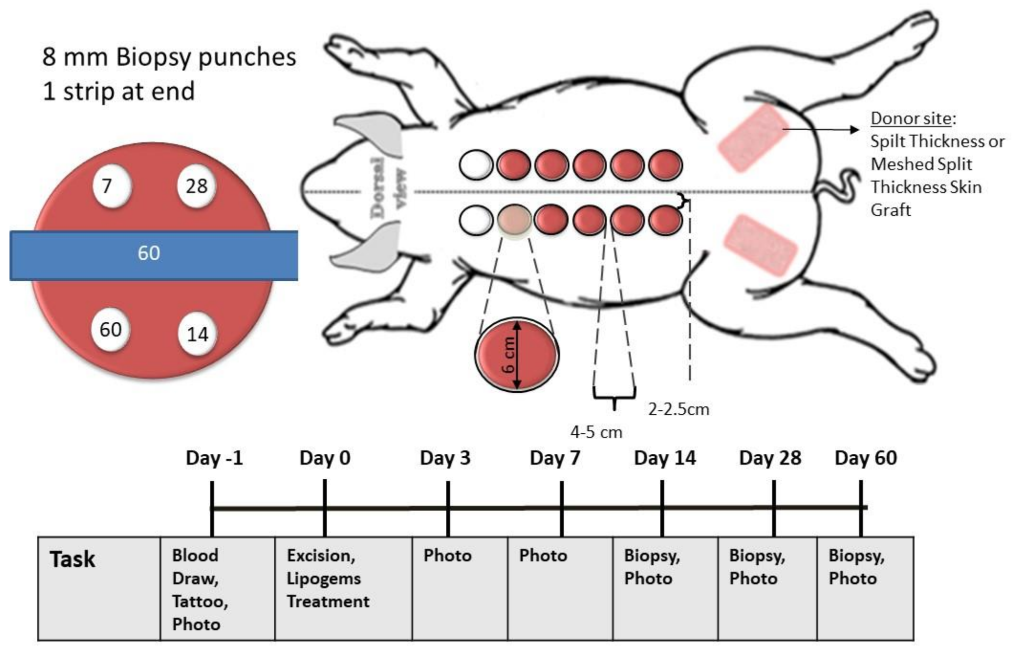


Figure 2: Schematic diagram of experimental wounds on the dorsum, time-line for treatment and assessment protocol, and biopsy procedure of pigs are represented above.

***In vivo* efficiency of porcine Lipogems and PEG-PFP-Lipogems**: PEG-PFP hydrogels-Lipogems were prepared by mixing the Lipogems extracted from the surgically excised adipose tissue with hydrogel solution prior to gelation. Briefly, PEG-PFP hydrogels were prepared from PFP isolated from porcine blood. PEG-PFP with Lipogems clusters of different volumes (1 ml and 2 ml/ 8mL of PEG-PFP hydrogel) were mixed with PEG-PFP solution in a 6 cm diameter sterile aluminum molds and gelled, the wounds were randomized (n=8/pig) and treated with the PEG-PFP hydrogels ± Lipogems of different concentration represented in **Figure 3A**. After application PEG-PFP hydrogels ± Lipogems (**Figure 3B**), all the wounds were covered with a split-thickness skin graft (STSG) that was harvested from the hind legs using a Zimmer pneumatic dermatome (Zimmer Inc, Warsaw, IN) set at the thickness of 12 thousandths of an inch (0.012") and meshed using a skin mesher at 1.5:1 ratio to get a mSTSG (**Figure 3C**). One of the wounds up to fat served as positive control and the other excised till fascia served as a negative control. Each wound was then covered with sterile antimicrobial Telfa wrapped gauze and overlaid with plain gauze. Additional dressing using tape, Ace bandages, stockinette and/or cloth jacket were applied. Punch biopsies (8 mm) were be taken on post treatment days with a strip through the middle also collected on the day of euthanasia (day 60).

End point measurements:

Histology: The wound samples were fixed in 10% neutral buffered formalin, blocked in paraffin wax and 5μm section were cut and stained using Masson's Trichrome stain (MTS). Light microscopic images were taken using a Leica microscope (DMI 3000, Buffalo Grove).

Measurement of healing rate: Wound contraction were measured using pictures taken at different day pre and post treatment by Silhouette star (Aranz Medical), a non-contact device. Effect of Lipogems formulations on wound healing were calculated with measurements of the wound size compared to their original size. The unwounded growth control areas were used to normalize the wound size.

Treatment Groups

A

	Groups
1	No treatment control (excised to fascia)
2	No treatment control (excised to fat)
3	PEG-PFP hydrogel
4	Lipogems 1mL
5	Lipogems 2 mL
6	Lipogems 1mL+PEG-PFP hydrogel
7	Lipogems 2mL+PEG-PFP hydrogel

B

C

Figure 3: Experimental groups used (Table A), Lipogems processed from excised porcine adipose tissue and applied on the wounds (B) and covered with meshed split thickness autograft (C).

Measurement of blood perfusion: The blood flow microcirculation on the surface of the wound bed was measured using a commercially available Laser Speckle Imaging (LSI) system (moorFLPI-1, Moor Instruments). The blood flow perfusion of the entire wound was measured at high spatial and temporal resolution using a standardized setup. Briefly, laser speckle lens was aimed vertically and exactly perpendicular to the wound surface, the device focus and zoom dials were adjusted according to manufacturer's recommendations to achieve optimal image resolution in the field of view. After optimal adjustment, the wound along with tattoo was captured. High-resolution speckle images were acquired using a charge-coupled device camera (CCD). Perfusion data analysis expressed in laser speckle perfusion units (LSPU) was performed offline using the moorFLPI analysis software tool.

Melanin and Erythema Content: Pigmentation and vascularity of wounds treated with Lipogems±PEG-PFP groups and the normal skin site was measured over 60 days using the DermaLab Combo device (Cortex Technology, Denmark). To measure the pigmentation and vascularity, the color probe was place on the wound surface over the clear front and illuminated by the white LED lights. Spectrophotometry readings were recorded at 550±30 nm and 660 nm±60nm for hemoglobin and melanin, respectively. Pigmentation and erythema was determined using the melanin and hemoglobin values, respectively.

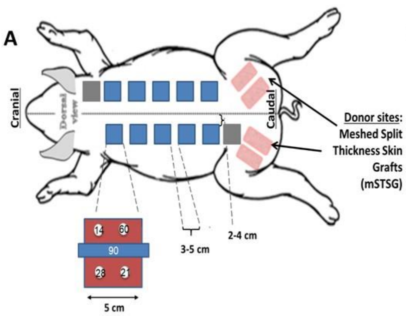
- **Treatment of 25 cm^2 full-thickness porcine burn wound with Lipogems + hydrogels:**

In this study, 5x5 cm^2 full thickness burns (25 sec) were created by placing a thermocoupled brass block heated to 100°C on the dorsum of each anesthetized pig at a constant pressure (~0.4 kg/cm2). Five wounds were placed on each side, separated by 3-5 cm, at same distance (2-4 cm) from the spine in the same anatomical location. Two unburned locations with tattoo were used as a controls (**Figure 4A**). Prior to burning on day -4, up to 200 ml of blood was obtained and used for preparing PEGylated plasma hydrogels. Full thickness burns were created on the dorsum as described above. On day 0, the wounds were sharp debrided to remove all necrotic tissue. Full-thickness skin loss was performed using a surgical blade down to fat for all wounds. The wounds were treated with the PEG-PFP hydrogels ± Lipogems. Widely meshed split thickness skin grafts will be applied to all wounds in addition to the designated treatments. After application PEG-PFP hydrogels ± Lipogems (**Figure 4B**), all the wounds were covered with a split-thickness skin graft (STSG) that was harvested from the hind legs using a Zimmer pneumatic dermatome (Zimmer Inc, Warsaw, IN) set at the thickness of 12 thousandths of an inch (0.012") and meshed using a skin mesher at 4:1 ratio to get a mSTSG. One of the wounds up to fat served as positive control and the other excised till fascia served as a negative control. Each wound was then covered with sterile antimicrobial Telfa wrapped gauze and overlaid with plain gauze. Additional dressing using tape, Ace bandages, stockinette and/or cloth jacket were applied. Punch biopsies (8 mm) were taken on p. and overlaid with plain gauze. Additional dressing using tape, Ace bandages, stockinette and/or cloth jacket were applied. Punch biopsies (8 mm) were be taken on post treatment days with a strip through the middle also collected on the day of euthanasia (day 90). On day 90 a strip through the middle will be collected on the day of euthanasia (**Figure 4C**).

End point measurements:

Histology: The wound samples were fixed in 10% neutral buffered formalin, blocked in paraffin wax and 5µm section were cut and stained using Masson's Trichrome stain (MTS). immunohisto Light microscopic images were taken using a Leica microscope (DMI 3000, Buffalo Grove).

A



B

Group number	Treatment
1	Positive Control (Normal debridement to fat)
2	Negative Control (Debridement to fascia)
3	PEGylated PFP hydrogel
4	Lipogems
5	Lipogems Embedded in PEGylated PFP hydrogels

C

Day -4	Day 0	Days 3, 7, 10, 14	Days 21, 28, 60	Day 90
1) Blood draw 2) Tattoo 3) Burn 4) NIM	1) NIM 2) Setup sterile field 3) Debride wounds 4) Harvest skin grafts 5) Apply Treatments 6) Apply primary and secondary dressings	1) Remove bandaging 2) Clean wounds 3) NIM 4) Biopsy (D14 only) 5) Reapply Lipogems and Hydrogels 6) Rebandage	1) Remove bandaging 2) Clean wounds 3) NIM 4) Biopsy 5) Rebandage	1) Remove bandaging 2) Clean wounds 3) NIM 4) Biopsy 5) Euthanasia

Figure 4: Schematic diagram of,) Experimental wounds on the dorsum, time-line for treatment and assessment protocol (B), and biopsy procedure of pigs are represented above (C).NIM: non-invasive imaging.

Immunohistochemistry: Biopsies collected at various time points (Figure 4C) were, fixed, paraffin embedded and sections were cut (4-5µm thick), mounted on a slide and stained using αSMA antibody to observe the blood vessel formation and matrix remodeling. The sections were counter stained using DAPI to observe cell nuclei.

Measurement of healing rate and blood perfusion: Wound contraction and blood flow microcirculation on the surface of the wound bed were measured using similar methods mentioned above in section 6. The unwounded growth control areas were used to normalize the wound size and perfusion density.

KEY RESULTS

Lipogems clusters possess ASCs and forms tubular network within PEG-PFP hydgrogels

We were able to successfully isolate microfragmented adipose tissue (Lipogems) from both porcine and human porcine subcutaneous adipose tissue. Lipogems from porcine subcutaneous adipose tissue isolated using different methods was in size range between ~350µm-700µm. Lipogems cluster were then plated and culture for up to 10 days in both α-MEM supplemented with 10% FBS and MesenPRO stem cell growth media. ASCs in both media (P0 cells; (**Figure 5A and 5B**) showed spindle shaped morphology. Further, ASCs were passaged and P1 cells positively stained with BODIPY, showing presence of cytosolic neutral lipids (**Figure 5C**).

Lipogems fraction of subcutaneous tissue yielded ~ 1.1×10^5 cells/g in comparison to 1.45×10^5 cells/g of minced adipose tissue (**Figure 5D**). We then analyzed the characteristics of cells sprouting out from human Lipogems clusters (100µl/ml of hydrogels) into three different hydrogels, collagen; FPEG and PEG-PFP. Post 10 day of culture the frozen sections of Lipogems-hydrogels were stained with WGA and DAPI. PEG-PFP hydrogel facilitated robust cells to migrate and proliferate from the Lipogems clusters. In FPEG hydrogel less number of cells were observed in the hydrogel region, while collagen hydrogels were least favored by the cells to grow out from the Lipogems clusters. These results showed Lipogems within PEG-PFP hydrogels favor to liberate faster cell sprouting and growth.

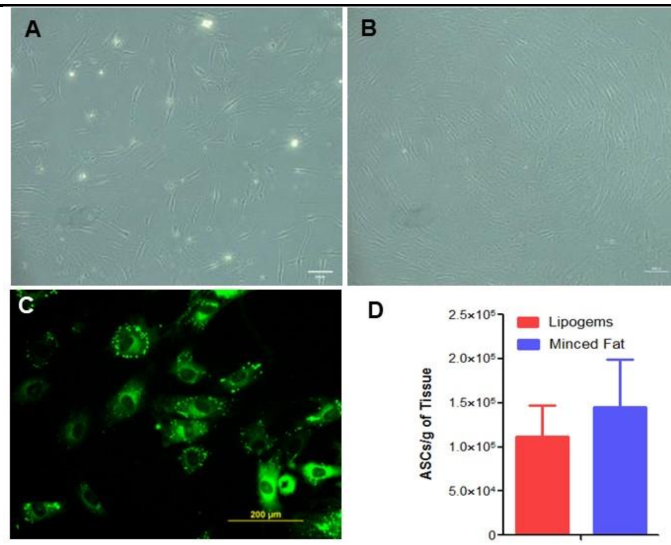


Figure 5: ASCs cultured in α-MEM media with 10% FBS, B: ASCs cultured MesenPRO stem cell growth media.ASCs show spindle shaped morphology and stained positive with c: BODPY, D: Comparative graph showing the yield of ASCs/gram of enzymatically digested adipose tissue and from freshly isolated Lipogems

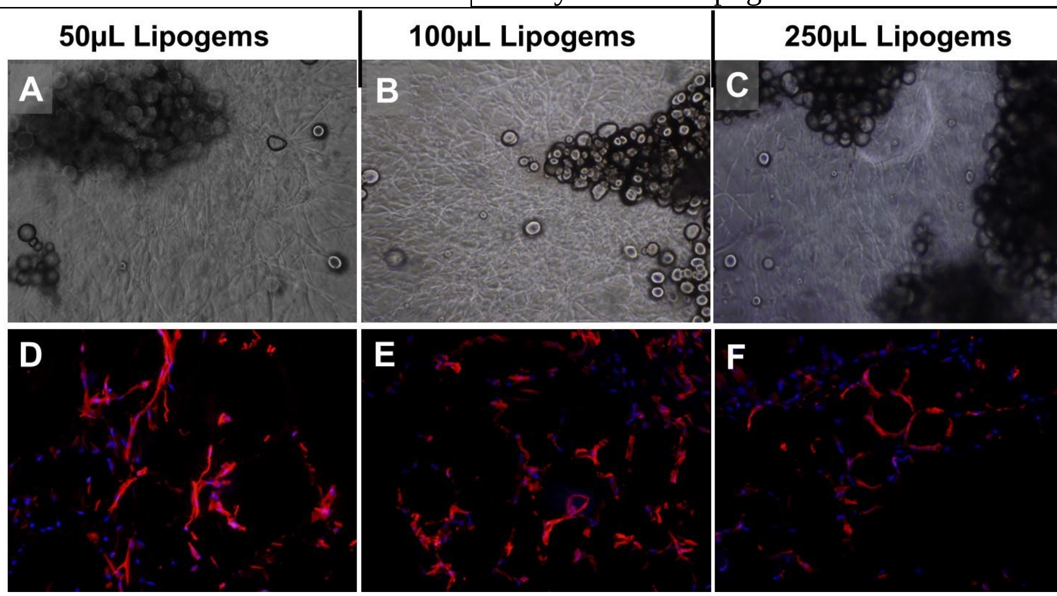


Figure 6: Morphological characterization of cells sprouting from Lipogems clusters within the PEG-PFP hydrogel. A-C: Day 12 light micrographs of cells sprouting within the PEGylated-PFP hydrogel embedded with different concentrations of Lipogems in MesenPRO culture media. Images original magnification: ×40. D-F:. Immunoflurescence image of cryosections stained with human αSMA Epifluorescence images of PEGylated-PFP hydrogel embedded with different concentrations of Lipogems in MesenPRO media shows tubular network formation. Sections were counterstained with DAPI to identify cell nuclei. Images original magnification: ×100.

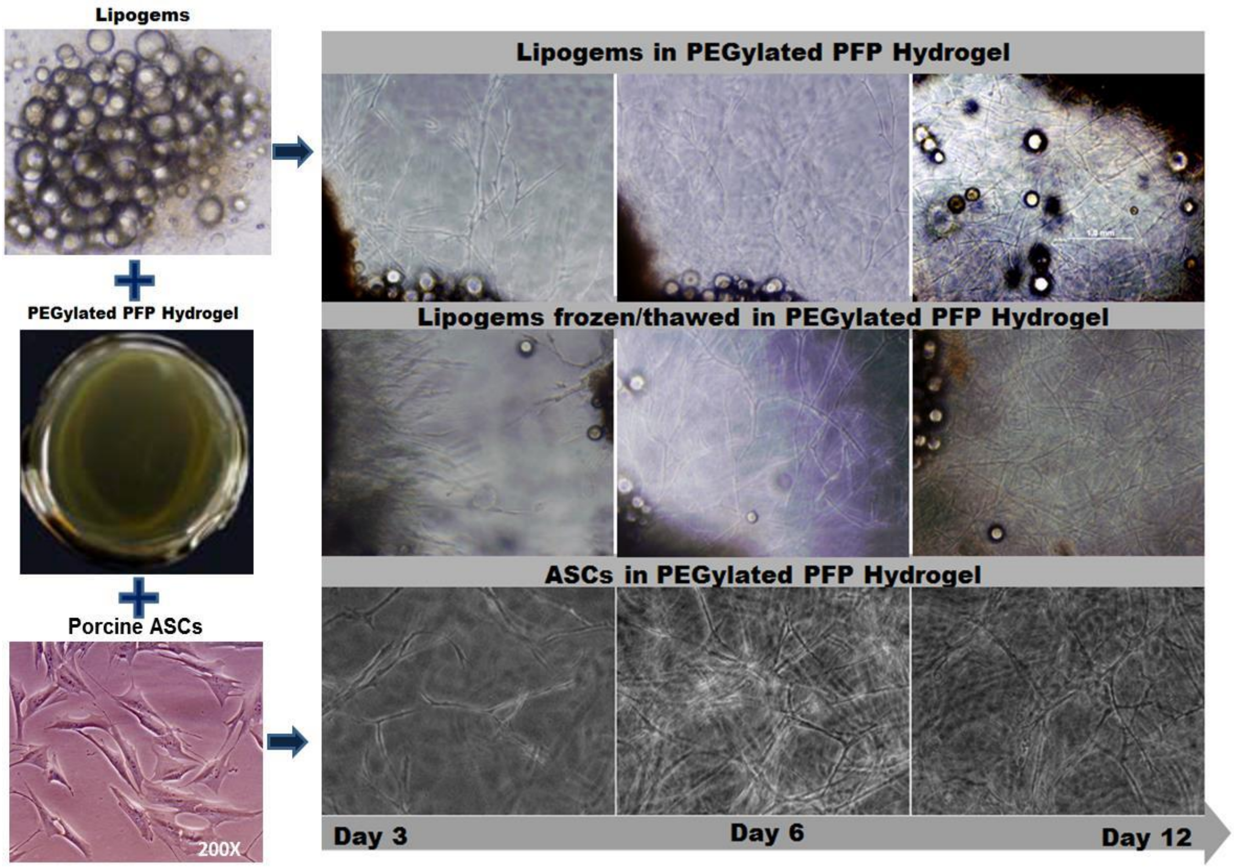


Figure 7: Porcine Lipogems, freshly isolated and frozen stock vs adipose stem cells (ASCs) forming tubular structures in PEG-PFP hydrogels: Lipogems and ASCs formed tubular structures within PEG-PFP hydrogel. Images ASCs and Lipogems (fresh and frozen) = ×100; light micrograph of ASCs= ×200.

We then optimized the dose and growth conditions. Hydrogels containing 50µl of Lipogems clusters, grown under Mesenpro growth media, exhibited cells sprouts in a linear phenotype with few closed networks. Tubular structures were seen in hydrogels with 100µl Lipogems, where the closed loop network started to appear. In the hydrogels with 250µl Lipogems, cell network were more robust with more prominent closed looped networks. Immunofluorescence staining with αSMA confirms the above observation and in addition shows the cells sprouts within the hydrogel are involved in formation of both tubular network-like structures as well as fibroblast like morphology (**Figure 6**).

Similar to human Lipogems porcine samples within PEG-PFP hydrogel showed cells sprouting out from the Lipogems into the hydrogel by day 3. Cells started to connect and form robust network over time (days 6 and 12). These network structures resembled to ASCs forming tubular network within the PEGylated-PFP hydrogels. In addition, the cryopreserved Lipogems exhibited tube formation resembling network observed in hydrogels with ASCs and freshly prepared and suspended Lipogems (**Figure 7**).

Effect of Lipogems+hydrogel formulation on full thickness wounds

Wound contraction

The overall contraction of wounds (day 60) treated with Lipogems±PEG-PFP hydrogels were significantly higher in comparison to the wound with intact subcutaneous adipose layer covered with

mSTSG. However, the no difference were found in rate of contraction in comparison to wounds excised to fascia and covered with mSTSG (**Figure 8**). A closer analysis indicated contraction rate observed in wounds treated with Lipogems alone, were slightly lower than the graft on to fascia. However, use of Lipogems showed significantly less contraction than the hydrogel treated group.
Note: We did not observe a significant difference in contraction between wound treated with 1 ml or 2 ml Lipogems±hydrogels. Therefore, we combined groups of different Lipogems concentrations with or without hydrogel into a single group analysis. Overall, the 'n' value per group increased.

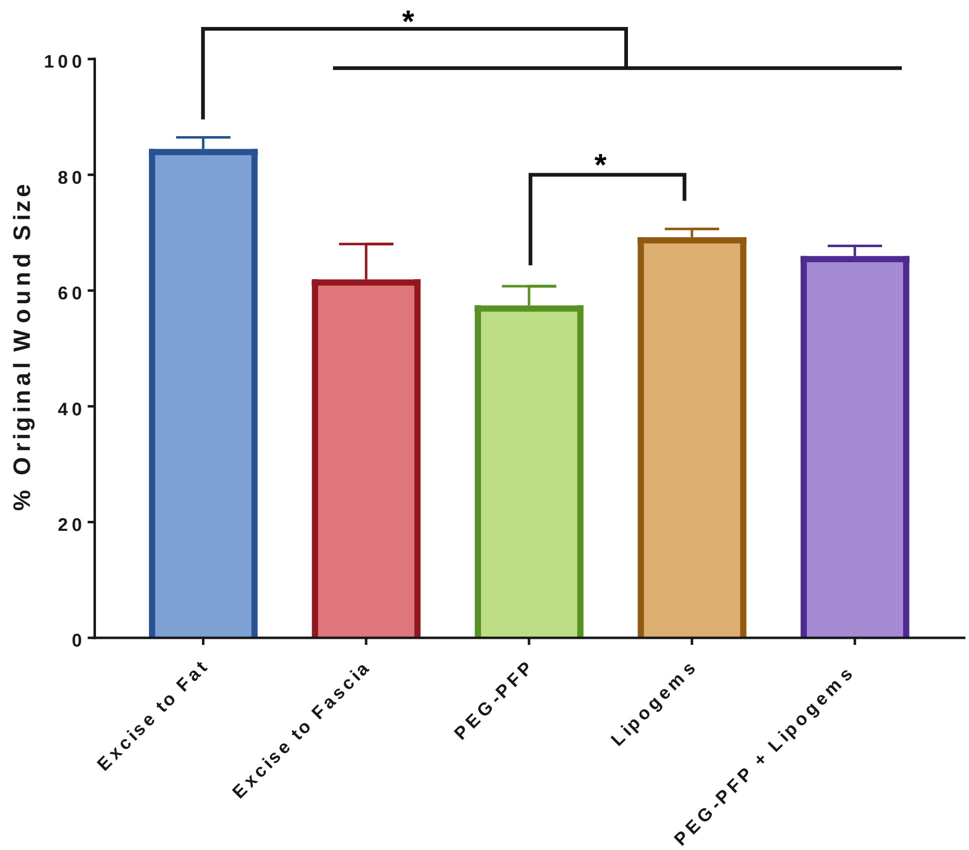


Figure 8: Percentage wound contraction observed for a period of 60 days (N=5).

Perfusion:
With the use of LSI we were able to capture the dynamics of blood perfusion over the course of healing. The flux was recorded at all the time points mentioned in figure 2. A characteristic panel of LSI produced live images are shown in **figure 9A**. The image color represent the blood flux distribution in a wound; low flux seen as blue, medium flux values are seen as green and high flux values are seen as orange/yellow and red. On day 7, all the wounds excised up to fascia exhibited high blood perfusion, regardless of the treatment groups. Wound excised up to fat and treated with mSTSG showed the least flux. On day 14, most of the wounds excised up to fascia and treated with Lipogems ± hydrogels started to resolve showing lower perfusion, whereas wounds treated with PEG-PFP hydrogel and mSTSG showed more visible flux, indicated by the presence of wide spread yellow – orange color pattern. Post day 14, all the wounds resolved with similar flux pattern. We further quantitated the fold change in blood perfusion for each region of interest within a wound. **Figure 9B** shows perfusion to be higher in wounds that were excised up to fascia and treated with Lipogems and PEG-PFP in comparison to wounds that had intact fat layer and treated with mSTSG. Of note, wound

excised to fascia but treated with Lipogems +PEG-PFP hydrogel were not significant from wounds excised to fat and treated with mSTSG, indicating the positive effect of PEG-PFP+Lipogems on wound perfusion.

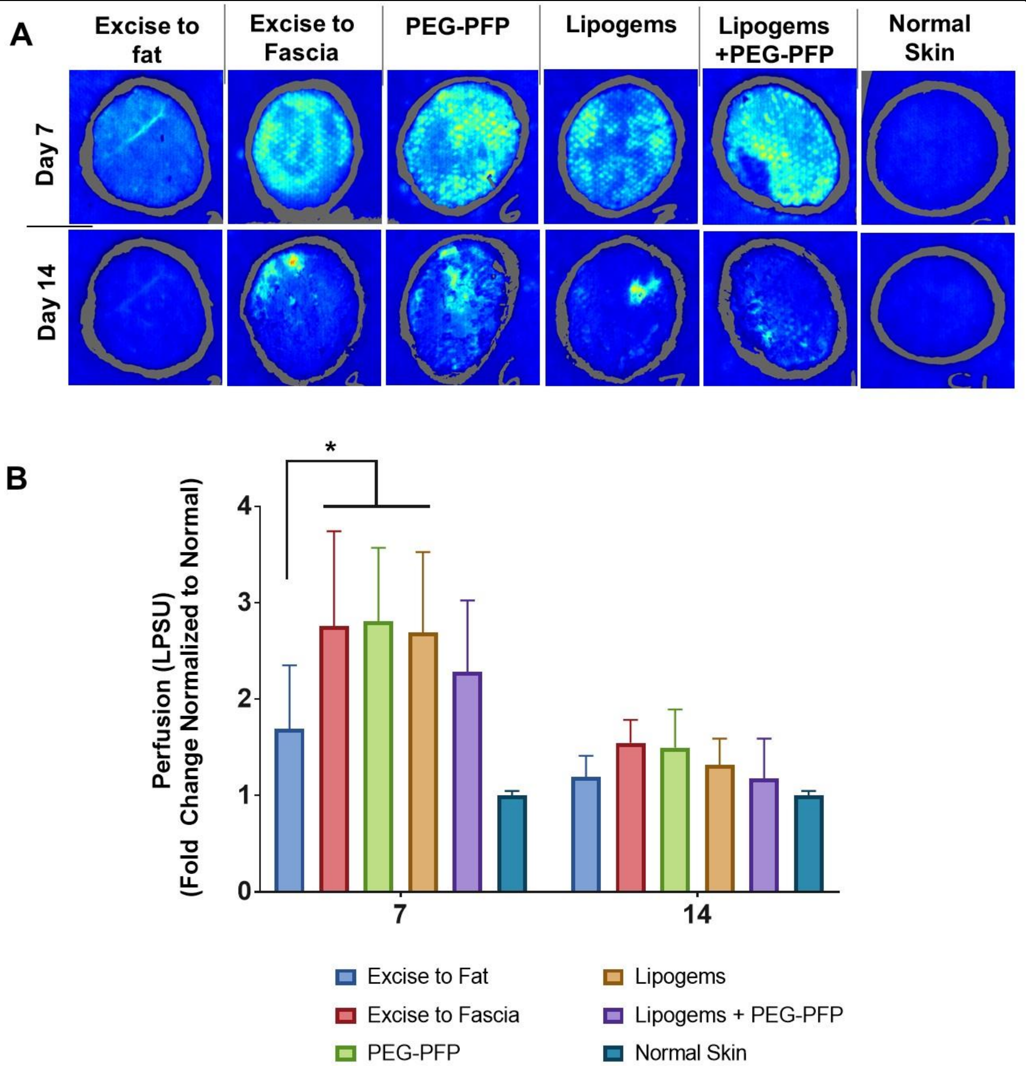


Figure 9: Laser speckle images of wound on day 7 and day 14 (A). The flux data with laser speckle perfusion unit showing the change in dynamics of wound perfusions from day 7 to day 14 (B).

Long-term healing outcome:

DermaLab Combo was used to assess the melanin (pigmentation) and erythema (redness or vascularity) at day 60. Five individual regions of interest within a wound and the normal skin area were measured. **Figure 10A** shows the melanin content measure on terminal day (D60). The melanin content within all the wounds were significantly higher to the normal skin, indicating the wound remodeling is still in progress. However, there was no difference in pigment content between the treatment group, regardless of the treatment type, the wounds with intact fat and covered with mSTSG. Similar to melanin, erythema was measured using the vascularity measure probe to assess the erythema within the wound on day 60. **Figure 10B** shows the erythema measured on terminal day (D60). The erythema in the wounds excised to fascia and covered with mSTSG were significantly higher than the normal skin. All of the treatment groups other than Lipogems+PEG-PFP did not show a significant erythema value, indicating synergistic treatment with hydrogel and Lipogems to reduce wound erythema. In addition there was not significant difference in erythema content in Lipogems+PEG-PFP treated wounds to wounds excised to fat and covered with mSTSG and also the normal skin.

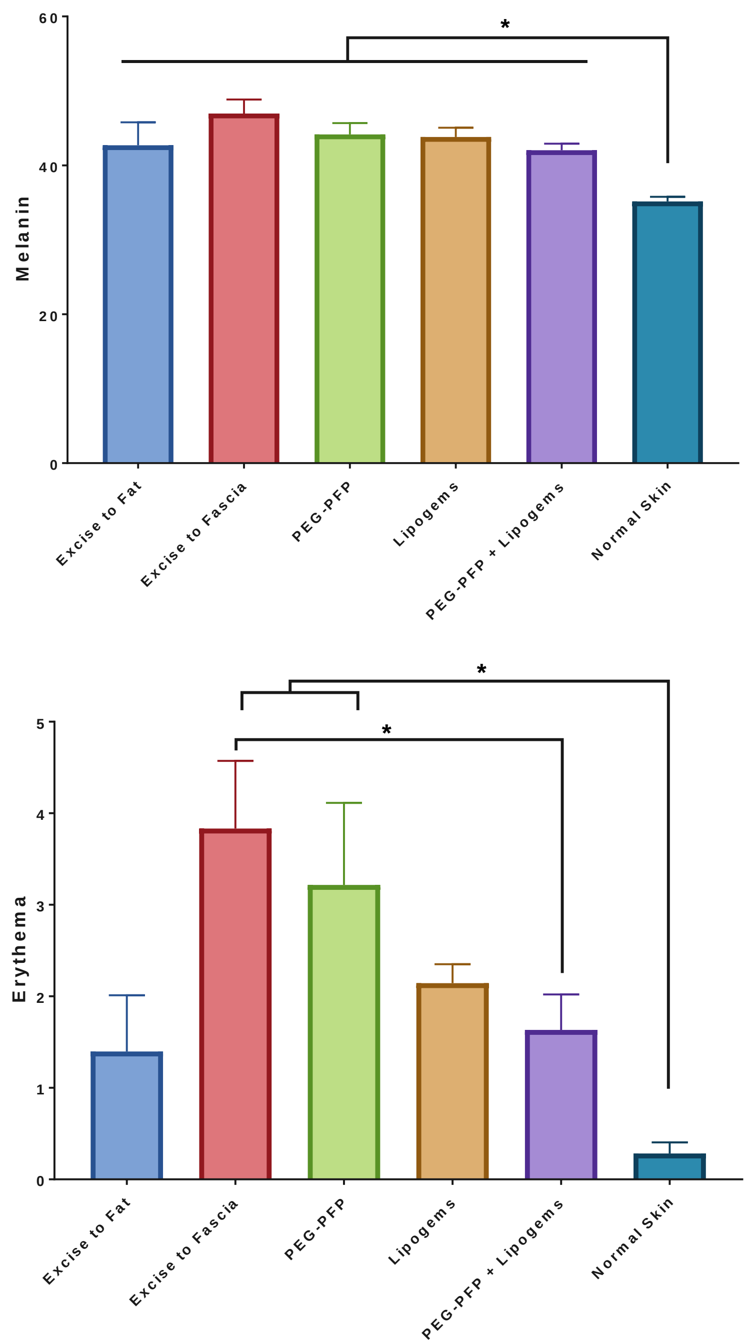


Figure 10: Pigmentation and vascularity of wounds treated with Lipogems±PEG-PFP hydrogels measured using DermaLab Combo. Graph shows values of melanin (A) and erythema (B) to represent pigmentation and vascularity respectively.

Histology:

The MTS histological images showed presence of thicker granulation tissue on day 28 than the wound with intact subcutaneous adipose layer covered with mSTSG, indicating active remodeling. It is interesting to note the wounds excised to fascia and treated with mSTSG were still showing areas with discontinuous epidermis. On day 60, all the treatment groups and the wound with mSTSG alone exhibited complete closure (**Figure 11**). Still, the dermal layer were thicker in treatment groups (Lipogems±PEG-PFP hydrogels) in comparison to the wound with intact subcutaneous adipose layer covered with mSTSG. Overall, there were no graft losses in the groups treated with hydrogel alone and hydrogel+ 1 ml and 2 ml Lipogems (the histological panel show representative images captured with 2 ml Lipogems±PEG-PFP hydrogel).

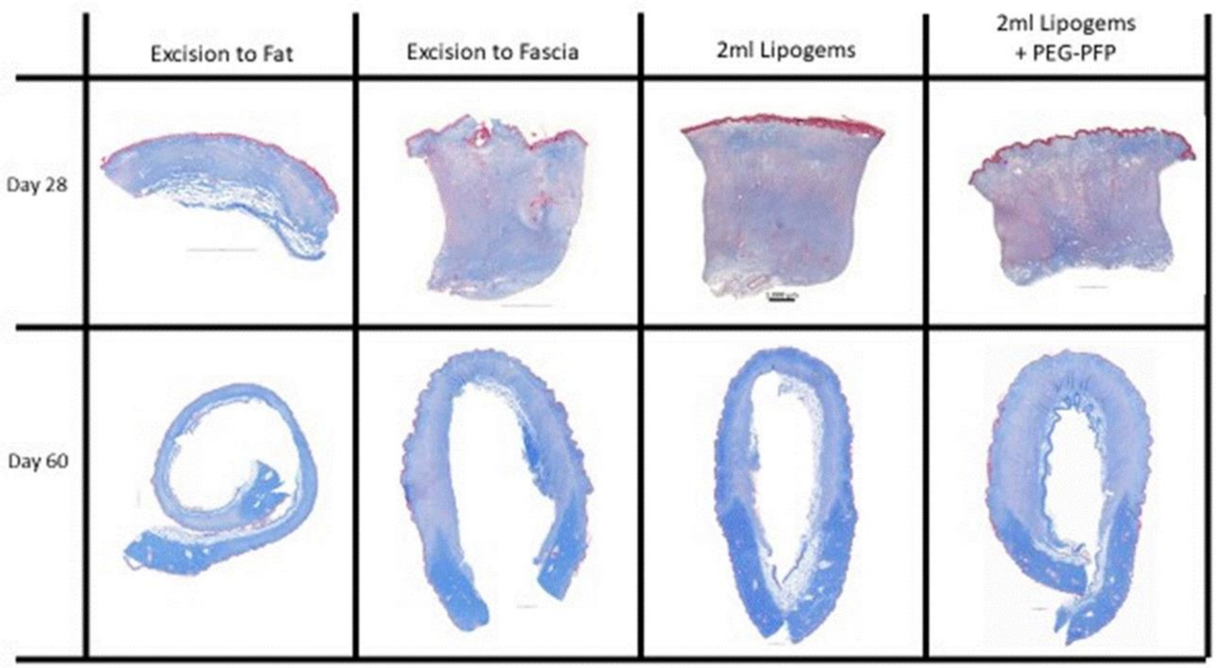


Figure 11: MTS stained sections of tissue biopsies collected from wound treated with 2 ml Lipogems±PEG-PFP hydrogels

Effect of Lipogems+hydrogel formulation on full thickness porcine burn wounds

***Photo documentation*:** Digital images captured during days of revisit were documented to visualize the wound closure and the contraction over entire period of study (**Figure 12**).

Histology:

α-smooth muscle actin staining: In order to understand the remodeling of the wound after application of Lipogems, and Lipogems+hydrogels, biopsies taken on days (0,7, 14, 21, 28, 60 and 90) were sectioned and immune stained with α-smooth muscle actin (α-SMA). The α-SMA antibody targets actin filaments surround the mature blood vessels and the contractile myofibroblasts. When mSTSG were placed onto fat (positive control), myofibroblasts were observed in all wounds by day 7 and started to decrease by Day 28 (**Figure 13**). Then by day 60, α-SMA decreased, staining mainly the actin around blood vessels and sparsely the myofibroblasts, indicting resolving dermal wound bed slowly returning approaching normal dermal architecture. The mSTSG placed onto fascia (negative control), exhibited strong expression of α-SMA, with significant actin-stained myofibroblasts on day 21. The dermal myofibroblasts exhibited expression of actin fiber along with mature blood vessels on day 28. Day 60 biopsy sections still showed large number of myofibroblasts with less mature collagen (counterstain). These results shows aberrant dermal remodeling in the wounds grafted on fascia with no hypodermal layer. PEG-PFP hydrogel treated wounds less infiltrated with actin myofilaments by day 28 and were seem to have less positively stained for actin by day 60 in comparison to the negative control. The Lipogems treated wounds showed evidence of lesser amount of myofibroblasts by day 28 in comparison to negative control. However, their expression subsided drastically in comparison to the negative controls (Day 60 magnified α-SMA images). In addition, the Lipogems± PFP gels showed better remodel by day 60 (MTS Stained sections). Within the Lipogems groups, there did not show vast difference in actin staining. Collective, our observations shows that grafts on fascia tend to tether to the remodeling dermis with observable stress fibers (actin stained myofibroblasts) which may contribute to more contraction in comparison to positive control and the wounds reconstructed with Lipogems.

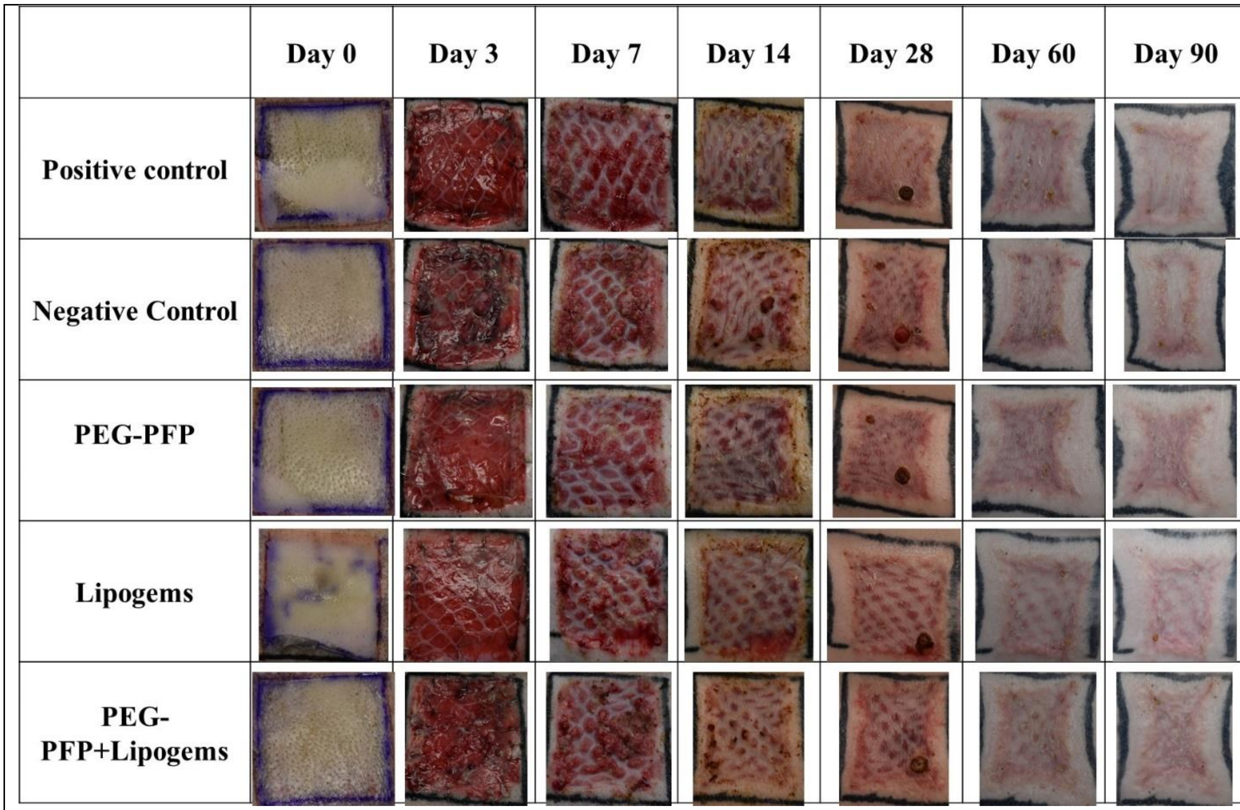


Figure 12: Representative photo images of burn wounds debrided and treated with Lipogems± PEG-PFP hydrogels. Positive control – Burn wounds debrided up to fat and negative control: wounds debrided up to fascia. All the wounds were grafted with 1:4 ratio meshed split thickness skin grafts.

Then by day 60, α-SMA decreased, staining mainly the actin around blood vessels and sparsely the myofibroblasts, indicting resolving dermal wound bed slowly returning approaching normal dermal architecture. The mSTSG placed onto fascia (negative control), exhibited strong expression of α-SMA, with significant actin-stained myofibroblasts on day 21. The dermal myofibroblasts exhibited expression of actin fiber along with mature blood vessels on day 28. Day 60 biopsy sections still showed large number of myofibroblasts with less mature collagen (counterstain). These results shows aberrant dermal remodeling in the wounds grafted on fascia with no hypodermal layer. PEG-PFP hydrogel treated wounds less infiltrated with actin myofilaments by day 28 and were seem to have less positively stained for actin by day 60 in comparison to the negative control. The Lipogems treated wounds showed evidence of lesser amount of myofibroblasts by day 28 in comparison to negative control. However, their expression subsided drastically in comparison to the negative controls (Day

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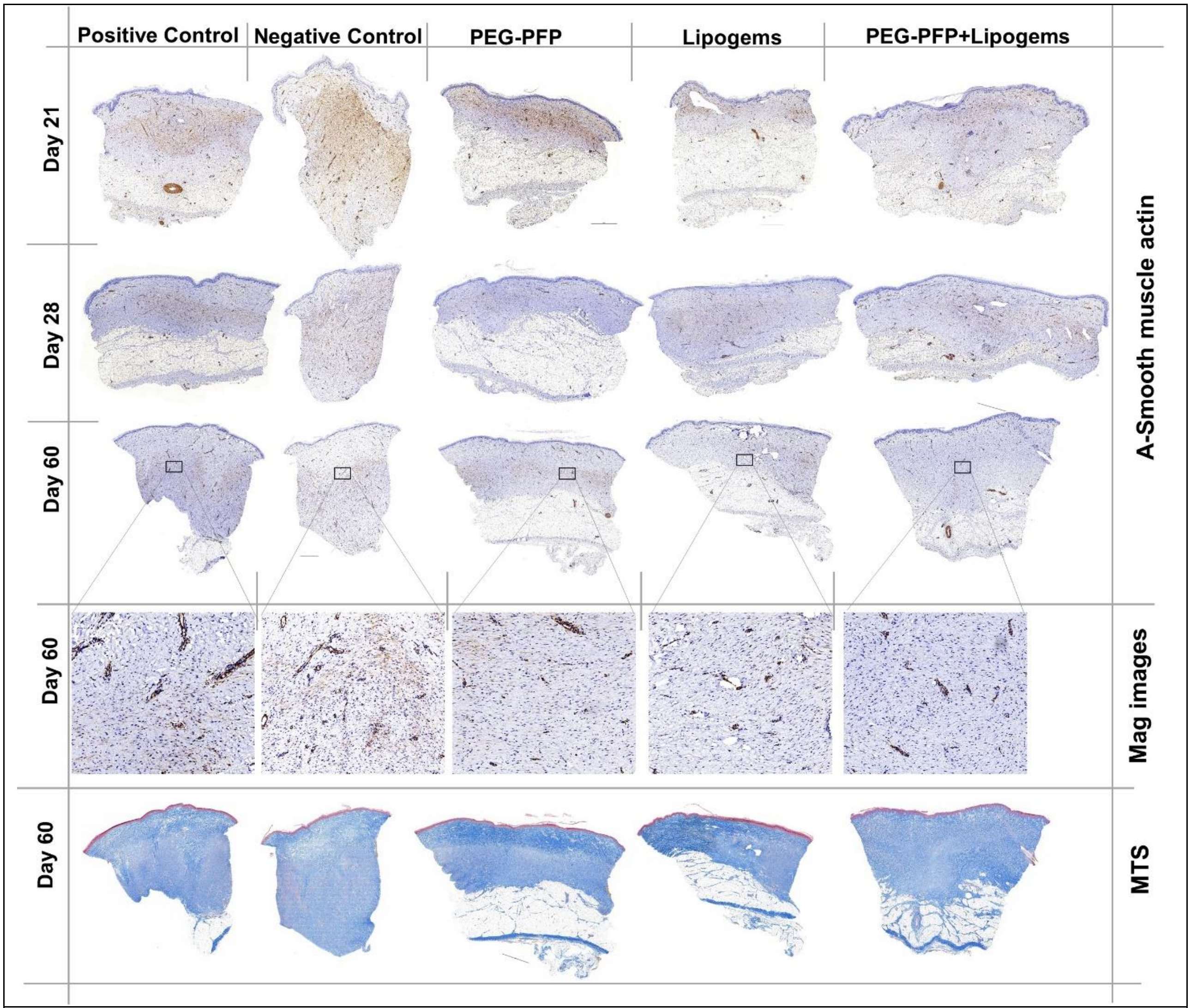

Figure 13: MTS stained sections of tissue biopsies collected from wound treated with 2 ml Lipogems±PEG-PFP hydrogels

Long term histological analysis:Masson's trichrome staining (MTS): The MTS histological images on day 60 corroborates with results observed with α-SMA stained section. Day 60 MTS stained negative controls exhibiting the wound debrided till fascia and then treated with mSTSG were undergoing active remodeling with aberrant collagen bundles directly tethered to the fascial layer of the wound bed. This was further confirmed on day 90 using MTS (**Figure 14**) and α-SMA stained sections (**Figure 15**), where the negative controls exhibited drastically contracted wound bed in comparison to all other groups. The positive controls with mSTSGs grafted on an intact/viable hypodermis were less contracted inundated with resolving dermal layer with observable amount of remodeling nascent collagen. Through PFP hydrogel treated wounds contracted more in comparison to positive controls, they were observed to be less contracted than the negative controls. Wounds treated with Lipogems, a thin layers of hypodermis was observable indicating reconstruction of wounds with hypodermal layer to have induced hypodermal regeneration. In groups treated with Lipogems and PFP hydrogels a pronounced increase in hypodermal line thickness can be observed (**Figure 16**). We speculate PFP, a vasculogenic adjunct, may have helped maintain viability of wound bed, which in turn may have resulted in better hypodermal regeneration. Collective these results indicates, early fat reconstruction to have positive effect on long term hypodermal regeneration with less contraction.

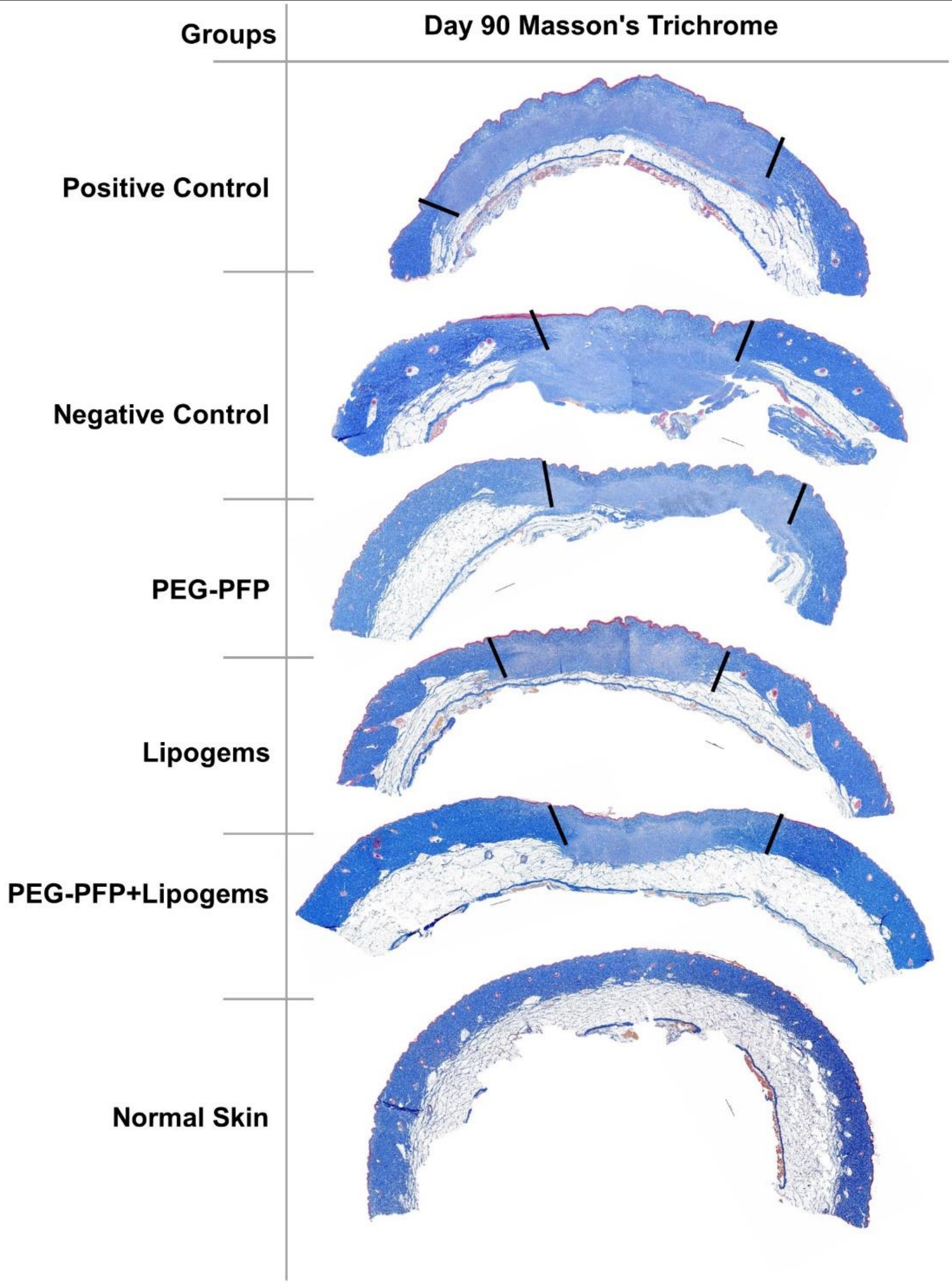

Figure 14: Masson's Trichrome Stained Day 90 tissue strips. Dark lines indicates area of wound bed with remodeled dermal and hypodermal layers.

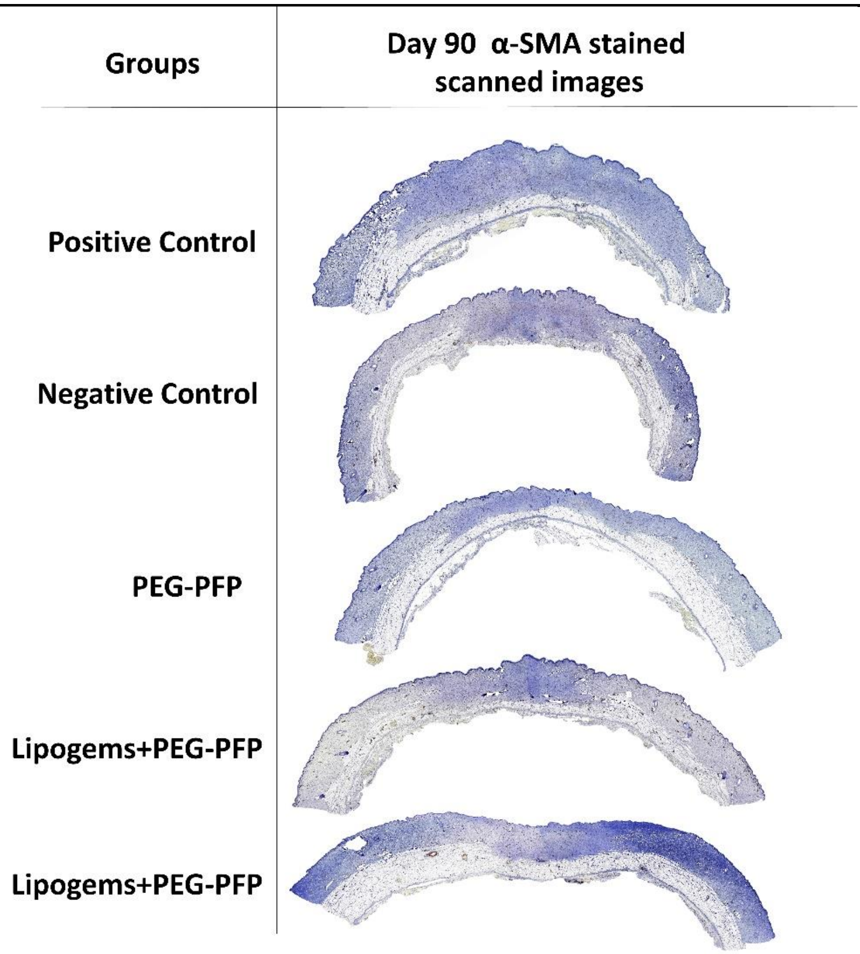

Figure 15: scanned images of alpha smooth muscle actin (α-SMA) stained sections of tissue biopsies collected from burn wounds treated with PEG-PFP hydrogels and Lipogems±PEG-PFP hydrogels.

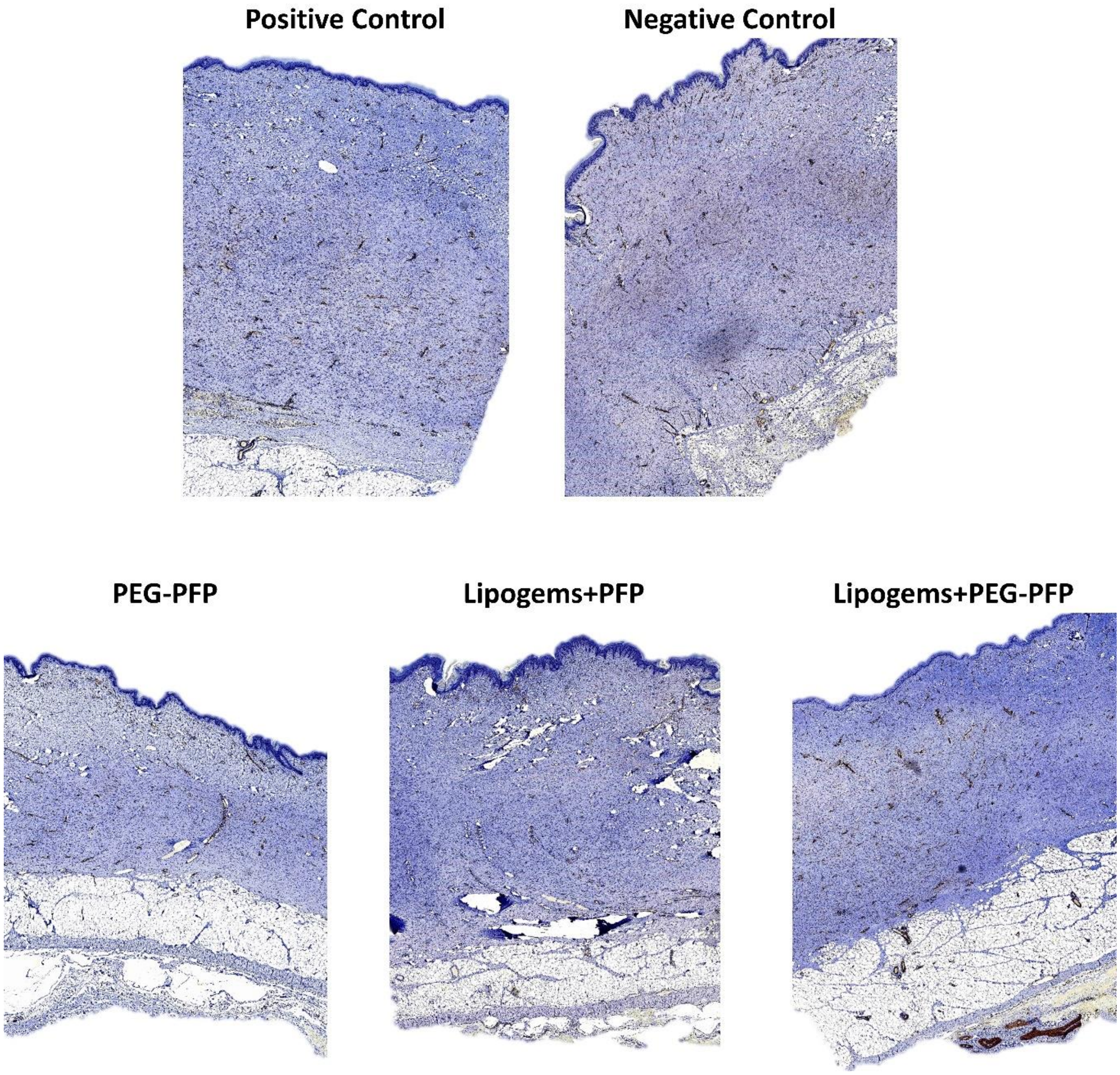

Figure 16: Magnified images of alpha smooth muscle actin (α-SMA) stained sections of tissue biopsies collected from burn wounds treated with PEG-PFP hydrogels and Lipogems±PEG-PFP hydrogels.

KEY FINDINGS OR ACCOMPLISHMENTS: ***(consolidated - all three specific Aims):***

Discussion: We were able to successfully isolate microfragmented adipose tissue (Lipogems), from both human and porcine adipose tissue and were able to successfully optimize a method to derive porcine Lipogems clusters of desired size range, similar to the range suggested for human Lipogems by the manufacturer of the kit. ASCs from the Lipogems showed spindle shaped morphology typically exhibited by ASCs, enzymatically isolated from the adipose tissue. In addition, the P1 cells retained cytosolic neutral lipid, indicating the preadipocytic origination. Further, Lipogems clusters yielded close to similar number of cells isolated form minced adipose tissue.

We then prepared different type of Lipogems-hydrogel formulations using, collagen, PEG-PFP and FPEG hydrogels. The results shows PEF-PFP hydrogels to provide a more conducive microenvironment for the cells to grow out of Lipogems cluster which were able to proliferate and differentiate. Fewer numbers of cells were found with FPEG hydrogels in comparison to PEG-PFP hydrogels. Of all the hydrogels used in the study, collagen medium had very few cell grown out from the Lipogems cluster, suggesting Lipogems to favor hydrogels with fibrin frame-work, corroborating to our finding that PEG-PFP hydrogel promotes human ASCs to form tubular like networks. However, further optimization, including collagen concentration and dose of Lipogems may be results in cell sprouting out from the Lipogems cluster into collagen hydrogels.

To this end, based on our current observation with PEG-PFP hydrogels to be most favored by the cells with Lipogems we further investigated and optimized human Lipogems and the growth conditions. There exists a Lipogems cluster dose response and culture condition influence on the cells sprouting out from the Lipogems into the hydrogel space. Cells within hydrogel supplemented with Mesenpro growth media showed a proportional increase in closed loop tubular structure to the volume of Lipogems embedded. We speculate that growth conditions may influence cells sprouting from Lipogems and phenotype changes within the hydrogel space. In addition, the immunohistochemical staining with αSMA, a marker usually found in smooth muscle cells around blood vessels and myofibroblast, clearly showed cells sprouted formed more number of stable tubular networks in hydrogels supplemented with Mesenpro media. These observations prompt us to choose an appropriate growth media and dose for further experimental analysis. Finally, we tested porcine Lipogems cluster at a1:10 mixture ratios with PEG-PFP volume ration were found to form a robust network within the hydrogel similar to porcine ASCs in PEG-PFP hydrogels. More importantly, cell from frozen stocks of Lipogems were still able to exhibit similar characteristic features of fresh Lipogems clusters; forming tubular phenotype. We are further analyzing the Lipogems-hydrogel samples to identify immunophenotype of various cells types within the hydrogels and identify secreted growth factors from the cells sprouting from Lipogems into the hydrogels.

Based on the *in vitro* results, we identified Lipogems cluster volume and the best performing hydrogel to deliver the Lipogems. For the *in vivo* studies, three different dose of Lipogems were selected and delivered using PEG-PFP hydrogels. The primary reason for this approach is optimizing a formulation and establishing an *in vivo* treatment protocol. In particular porcine model, which are considered closest surrogate to human skin wound healing, still have different hypodermis with a dense fibrous architecture. We optimized isolation of Lipogems from porcine adipose tissue from the excised skin and combined with autologous PFP to treat full thickness excision wounds. Full-thickness excision wounds treated with 1 ml and 2 ml Lipogems±PEG-PFP hydrogels did not show graft failure, and the applied Lipogems integrated within the wound with minimal rejection, consistent with the previous treatments. Contraction measurement of wound treated with Lipogems (1 ml, and 2 ml) ±PEG-PFP hydrogel formulations did not significantly impact reduction in contraction, though not significant, however there was positive trend in the contraction with Lipogems+2 ml hydrogels.

The quality and long term effect of Lipogems±PEG-PFP on the wound healing was monitored using non-invasive techniques. Early assessment (Day 7) all the wounds excised up to fascia shows high blood perfusion, regardless of the treatment groups. This may be attributed to high capillary plexus formed during this initial phase of healing (**10, 11**). However wound excised to fascia but treated with Lipogems +PEG-PFP hydrogel were not significant from wounds excised to fat and treated with mSTSG, indicating the positive effect of PEG-PFP+Lipogmes on wound perfusion. Two weeks post treatment, blood flow measurement of all the wounds, regardless of treatment group, regressed and did not show any significant difference. These results indicate early blood flow flux may be an indicator of how well a wound may progress to complete healing, i.e., regression of perfusion comparable to wound with intact fat layer may proceed with normal progression.

Clinically, severe wounds, like burns, are susceptible to either hyper or hypopigmentation and increased erythema (**12**). Therefore, it is important to assess these parameters to demonstrate the effect of treatments used in long-term healing outcome. Data generated from the DermaLab probes showed significantly higher melanin content within all the wounds in comparison to the normal skin, indicating the wound are hyperpigmented and are still in remodeling phase. However, the erythema values were lower in the wounds treated with Lipogems+PEG-PFP, which did not show a significant increase in comparison wounds excised to fat and covered with mSTSG and also the normal skin. These findings suggest the application of PEG-PFP+Lipogems may have a positive effect on long-term scar outcome. Moreover, a recent study shows burn wound in a Red Duroc porcine wound model with pronounced erythema, pigmentation and contraction, in comparison to scars from excision wounds (**13**). Our next step was to use porcine burn wound model to assess the effect of plasma hydrogels and Lipogems on long-term healing.

Finally, we evaluated PE-PFP hydroge±PEG-PFP in a porcine full thickness wound model.
We prepared allogenic porcine Lipogems and used for treating burn wounds.The Lipogems±PEG-PFG hydrogel can be successfully delivered to the burn wound without graft loss. The Lipogems formulation exhibited better remodeling with active regeneration of subcutaneous hypodermis and the Lipogems+PEG-PFP treatment showed positive effect on long-term healing outcome with resolving dermal layer with less myofibroblasts.

KEY FINDINGS:

- Optimized porcine Lipogems isolation protocol to obtain size range of Lipogems clusters similar to human Lipogems.
- Determined ASCs obtained from Lipogems clusters and minced adipose tissue to yield cell in close proximal numbers.
- Prepared Lipogems-hydrogels – collagen, PEG-PFP and FPEG and identified most favorable hydrogel media for the cells to sprout from the Lipogems cluster. We observed Lipogems to favor PEG-PFP hydrogel than the FPEG and collagen hydrogels.
- Optimized volume of human and porcine Lipogems and the media conditions to grow ASCs, which is Mesenpro basal media.
- Cells from porcine Lipogems exhibited morphological characteristics and tube forming ability with PEG-PFP hydrogels, similar to the human Lipogems clusters. Further, cell from frozen stocks of Lipogems were still able to exhibit similar characteristic features of fresh Lipogems clusters.
- Autologous Lipogems can be isolated and used as a bed-side treatment option full thickness wounds for treatment of full thickness skin wounds and formulated with autologous platelet free blood plasma hydrogels.
- The Lipogems±PEG-PFG hydrogel can be successfully delivered to the excision wound without graft loss. The Lipogems formulation exhibited contraction trending positively towards positive control, i.e., wound with intact subcutaneous adipose layer covered with mSTSG.
- The Lipogems+PEG-PFP treatment showed positive effect on long-term healing outcome, specifically erythema.
- Prepared allogenic porcine Lipogems and used for the current burn wound healing studies.
- The Lipogems±PEG-PFG hydrogel can be successfully delivered to the burn wound without graft loss. The Lipogems formulation exhibited better remodeling with active regeneration of subcutaneous hypodermis..
- The Lipogems+PEG-PFP treatment showed positive effect on long-term healing outcome with resolving dermal layer with less myofibroblasts.

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

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. 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 "Not applicable" or "Nothing to report."

1. We were able to train two technical support staffs, Ms. Michelle Holik and Ms. Lucy Shafer in performing porcine burn wound protocol, skin harvest and grafting. Various non invasive imaging techniques and data analysis..
2. PI of this project (Dr. Natesan) and staff scientist (Dr. Stone) were able to self-train and develop formulations combining Lipogems and biomaterials in-house, perform pre-clinical studies.

How were the results disseminated to communities of interest?

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. Also, if applicable, you may simply state, "See publications and/or presentations." If there is nothing significant to report during this reporting period, state "Nothing to report."

Presentations at international and nation conferences (listed below)

Plans for the next reporting period to accomplish the goals

Describe briefly what you plan to do during the next reporting period to accomplish the remaining goals and objectives. If this is the final technical report, state "Work is completed."

Work completed

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?

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

The observation made from this study that stem cells can be non-enzymatically sourced from adipose tissue and used in 3-D hydrogel scaffold to derive similar function of an isolated ASC population, has provided a new, and less-manipulative method to use stem cells for further wound healing application. The results may be more favorable, because of fewer hurdles for future FDA approval.

What was the impact on other disciplines?

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

- Isolation and storage of adipose derived stem cells involved several different steps, time and labor. Lipogems can impact the process of stem cell storage and use, since the clusters can be easily isolated by a service provider (e.g., research nurse), stored and retrieved and still possess similar potentials of enzymatically isolated stem cells.
- The entire process of Lipogems isolation can be carried out in a clinical suite, therefore requiring sophisticated laboratory space and equipment to isolate stem cells.
- Use of Lipogems may improve functional recovery and cosmesis of burn wound patients

What was the impact on technology transfer?

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including: 1) transfer of results to entities in government or industry; 2) instances where the research has led to the initiation of a start-up company; or 3) adoption of new practices, etc. If there is nothing significant to report during this reporting period, state "Nothing to report."

LIPOGEMS, USA, has an established CRADA with USAISR, wherein they provide Lipogems isolation kits. Upon mutual agreement and interest, Lipogems-hydrogel technology can be transferred in future to the company for further product development and approval

What was the impact on society beyond science and technology?

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: 1) improving public knowledge, attitudes, skills, and abilities; 2) changing behavior, practices, decision making, policies (including regulatory policies), or social actions; 3) improving social, economic, civic, or environmental conditions, etc. If there is nothing significant to report during this reporting period, state "Nothing to report."

Lipogems beyond burn wound treatment can have a significant impact on plastic and cosmetic reconstructive surgeries. In addition, newer avenues, such as treatments degenerative fat diseases can be benefited through this technology.

5. CHANGES/PROBLEMS

The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Contracting/Grants Officer whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information.

Changes in approach and reasons for change

Describe any changes in approach during the reporting period and reasons for these changes. If there is nothing significant to report during this reporting period, state "Nothing to report."

Nothing toreport.

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. Also report any actual or anticipated financial or contracting matters in addition to technical challenges that may impact the project's performance or progress. If there is nothing significant to report during this reporting period, state "Nothing to report."

1. *There was a delay in start of the specific aim 3 studies due to delayed ACURO approval*
2. *There was delay in procurement of pigs (number 3 and 4) due to unforeseen delivery delays, which eventually pushed back completion of all 6 porcine experiments proposed.*
3. *Manuscript pending submission due to COVID-19 pandemic-compilation. Since the project involved robust data sets from both in vivo, specific Aim (N=8) and specific Aim 3 (N=6) and in vitro studies, co-ordination with project associated personnel and data-reach were difficult. The manuscript is currently drafted and expected to be completed by June 2020 and submitted for publication*

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

There are no significant changes in expenditure, however a no cost extension was approved. We anticipate to complete the pending manuscripts for peer-review by June 2020.

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 approval dates, including continuing reviews or renewals, and/or amendments for animal use and/or human subject research protocol(s) for each regulatory reviews, i.e. the Institutional Animal Care and Use Committee (IACUC) and the DoD Animal Care and Use Review Office (ACURO), and the Institutional Review Board (IRB) and the DoD Human Research Protection Office (HRPO).

Significant changes in use or care of human subjects: Nothing to report

Not Applicable

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. Follow the format:

1. Authors names in format of Last Name First Initial followed by comma for each additional names (use et al if more than 10 authors). Title of paper. Journal Name Abbreviated Form. Year Month Date; Volume (Issue#):page numbers. doi: #. PubMed PMID: #; PubMed Central PMCID: #
   a. List publication type (e.g. original manuscript, review, abstract, etc.)
   b. State publication status (e.g. submitted, under review, accepted, or published)
   c. Reference which specific aim (e.g. Directly related to SOW, specific aim 1)
   d. State award funding acknowledgement (e.g. DoD funding acknowledged)

1. Larson DA, Stone R2nd, Clay NE, Holik M, Wall JT, Christy RJ, Natesan S. Treatment of Full-Thickness Wounds with Microfragmented Adipose Tissue and plasma based hydrogels, ***Manuscript in Preparation***, *to be submitted June 2020*

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.
Follow the format:

1. Authors names in format of Last Name First Initial followed by comma for each additional names (use et al if more than 10 authors). Chapter title if applicable. Title of the book. Edition. Publication. Publication date. (Chapter, page info if applicable). ISBN/doi: / PMID.
   a. List publication type (e.g. book, dissertation, conference proceedings, supplemental, etc.)
   b. State publication status (e.g. submitted, under review, accepted, or published)
   c. Reference which specific aim (e.g. Directly related to SOW, specific aim 1)
   d. State award funding acknowledgement (e.g. DoD funding acknowledged)

1. Shanmugasundaram Natesan, Randolph Stone II, Rodney K Chan, Robert J Christy; Editor: Xiao-Dong Chen Chapter 8 - Mesenchymal Stem Cell–Based Therapies for Repair and Regeneration of Skin Wounds A Roadmap to Non-Hematopoietic Stem Cell-based Therapeutics, From the Bench to the Clinic; 2019, Pages 173-222, *Academic Press*.

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, including international, national, local societies, military meetings, etc. Use an asterisk (*) if presentation produced a manuscript. Follow the format:

1. Authors names in format of Last Name First Initial followed by comma for each additional names (use et al if more than 10 authors), Speaker*. Month. Year. Title. Name of Meeting, Location of Meeting
    a. List presentation type (e.g. invited, talk, poster, or government review, etc.)
    b. State presentation status (e.g. submitted, under review, accepted, or presented)
    c. Reference which specific aim (e.g. Directly related to SOW, specific aim 1)
    d. State award funding acknowledgement (e.g. DoD funding acknowledged)

1. Randolph Stone II, David Larson, John Wall, Nicole L Wrice, Kyle Florell, Robert Christy, Shanmugasundaram Natesan*; Treatment of Full Thickness Wounds with Microfragmented Adipose Tissue and Plasma-Based Hydrogels Improves Healing Outcome; International Society for Burn Injuries, New Delhi, 03 DEC 2018, Poster; acknowledgement of federal support under this award: Yes
2. Randolph Stone II, David Larson, John Wall, Nicole L Wrice, Kyle Florell, Robert Christy, Shanmugasundaram Natesan*; Treatment of Full Thickness Wounds with Microfragmented Adipose Tissue and Plasma-Based Hydrogels Improves Healing Outcome; International Society for Burn Injuries, New Delhi, 03 DEC 2018, Poster; acknowledgement of federal support under this award: Yes
3. Treatment of Full-Thickness Wounds with Microfragmented Adipose Tissue and plasma based hydrogels. Natesan S, Larson DA, Stone R2nd, Wall JT, Christy RJ. 2019 Military Health System Research Symposium
   Poster; acknowledgement of federal support under this award: Yes

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. In addition to a description of the technologies or techniques, describe how they will be shared.

1. Lipogems-hydrogel technology has potentials to be transferred in future. Pre-clinical data in following year will enable us to generate results to evaluate the efficacy of the formulation.
2. The current indications for Lipogems use is in reconstructive surgeries. Dialogues with clinicians may be initiated to use this technology to isolated adipose tissue fragments for burn wound healing application.

Inventions, patent applications, and/or licenses

Identify inventions, patent applications with date, and/or licenses that have resulted from the research. State whether an application is provisional or non-provisional and indicate the application number.

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. Follow the format:

1. Inventors names in format of Last Name First Initial followed by comma for each additional names (use et al if more than 10 authors). Year. Title. US Patent No.
    a. List patent type (e.g. provisional, international, etc.)
    b. State patent status (e.g. filed, issued)
    c. Reference which specific aim (e.g. Directly related to SOW, specific aim 1)
    d. Anything else (e.g. filed before award)

"Nothing to report."

a.

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: 1) data or databases; 2) biospecimen collections; 3) audio or video products; 4) software; 5) models; 6) educational aids or curricula; 7) instruments or equipment; 8) research material (e.g., Germplasm, cell lines, DNA probes, animal models); 9) clinical interventions; 10) new business creation; etc.

Biospecimen: Human Lipogems were isolated and cryopreserved. This can be used for future in vitro experiment purposes.
Model: A porcine excision and burn wound healing model to reconstruct subcutaneous hypodermis is established.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Report PDs/PIs and for 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."

Name: ***Shanmugasundaram Natesan****, PhD*
Project Role: *PI*
Researcher Identifier (e.g. ORCID ID): orcid.org/0000-0003-4213-3111
Nearest person month worked: *18*
Contribution to Project: *Dr. Natesan was responsible for overall experimental design, involved in optimizing the process of isolating Lipogems from porcine adipose tissue and oversee progress of this proposal. Also, Dr. Natesan was responsible for the submission of animal and IRB protocols for ACRUO and HRPO approval*

Name: ***Robert J Christy****, PhD*
Project Role: *Co-PI*
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: *10*

Contribution to Project: *Dr. Christy was responsible for overall experimental design, administrative oversight, and coordinated with Dr. Natesan for the submission of animal and IRB protocols for ACRUO and HRPO approval*

Name: ***Randolph Stone II**, PhD*
Project Role: *Postdoctoral research associate and AI*
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: *20*
Contribution to Project: *Dr. Stone was key in optimizing the size range of Lipogems clusters from porcine adipose tissue. He conducted in vitro studies using Lipogems-PEGylated PFP hydrogel formulations.*

Name: ***Ms. Nicole Wrice**, MS*
Project Role: *Research Technician*
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: *3*
Contribution to Project: *Ms. Wrice isolated stem cells from Lipogems and fresh adipose tissue and determined growth conditions for ASCs.*

Name: ***Mr. David Larson**, MS*
Project Role: *Research Technician*
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: *21*
Contribution to Project: *Mr. Larson isolated helped in Lipogems isolation, in vitro culture, and data analysis.*

Name: ***Mr. Sergio Garcia**, BS*
Project Role: *Research Technician*
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: *12*
Contribution to Project: *Mr. Garcia performed animal studies, maintenance of medical records and every day animal care.*

Name: ***Ms. Michelle Holik**, BS*
Project Role: *Research Technician*
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: *4*
Contribution to Project: *Ms. Holik was a support staff in the animal studies, she was capturing noninvasive images during the experimental revisits.*

Name: ***Ms. Lucy Shaffer**, BS*
Project Role: *Research Technician*
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: *4*
Contribution to Project: *Ms. Shaffer was responsible for biopsy specimen section and staining. She also performed histological slide scanning and data analysis.*

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 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?

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.
Follow the format:

Organization Name:
Location of Organization: (if foreign location list country)
Partner's Contribution to the Project: (identify one or more, e.g. 1) financial support; 2) in-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff); 3) facilities (e.g., project staff use the partner's facilities for project activities); 4) collaboration (e.g., partner's staff work with project staff on the project); 5) personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); etc.

LIPOGEMS, USA, in-kind provided Lipogems isolation kits

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS

For collaborative awards, independent reports are required from both the Initiating PI and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site.

QUAD CHART

Insert an updated Quad Chart as one of the appendices (best to convert this technical report in Word file and the quad chart in PowerPoint file into separate PDF files, and then assemble into a single PDF document for submission via eBRAP).

REFERENCES:

1. http://ameriburn.org/who-we-are/media/burn-incidence-fact-sheet/; ABA burn incidence fact sheet.
2. Statistics, N. C. f. H., National Ambulatory Medical Care Survey: 2015 State and National Summary Tables. 2015.
3. Statistics, N. C. f. H., National Hospital Ambulatory Medical Care Survey: 2015 Emergency Department Summary Tables. 2015.
4. Kapur SK, Dos-Anjos Vilaboa S, Llull R, Katz AJ. 2015. Adipose tissue and stem/progenitor cells: discovery and development. Clin Plast Surg. 42(2):155-67.
5. Statistics, N. C. f. H., National Hospital Ambulatory Medical Care Survey: 2015 Emergency Department Summary Tables. 2015.
6. Song G, Jia J, Ma Y, Shi W, Wang F, Li P, Gao C, Zuo H, Fan C, Xin N, Wu Q, Shao Y. 2015. Experience and efficacy of surgery for retaining viable subcutaneous tissue in extensive full-thickness burns. Burns. S0305-4179(15)00184-9.
7. Coccè V, Brini A, Giannì AB, Sordi V, Berenzi A, Alessandri G, Tremolada C, Versari S, Bosetto A, Pessina A. A Nonenzymatic and Automated Closed-Cycle Process for the Isolation of Mesenchymal Stromal Cells in Drug Delivery Applications. Stem Cells Int. 2018;2018:4098140.
8. Bianchi F, Maioli M, Leonardi E, Olivi E, Pasquinelli G, Valente S, Mendez AJ, Ricordi C, Raffaini M, Tremolada C, Ventura C. A new nonenzymatic method and device to obtain a fat tissue derivative highly enriched in pericyte-like elements by mild mechanical forces from human lipoaspirates. Cell Transplant. 2013;22(11):2063-77.
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10. Rittié L. Cellular mechanisms of skin repair in humans and other mammals. J Cell Commun Signal. 2016;10(2):103-20.
11. Tonnessen MG, Feng X, Clark RAF. Angiogenesis in Wound Healing. Journal of Investigative Dermatology Symposium Proceedings, 2000; 5:40-46.
12. Goel A, Post-burn scars and scar contractures. Indian J Plast Surg. 2010;43(Suppl):S63-71.
13. Blackstone BN, Kim JY, McFarland KL, Sen CK, Supp DM, Bailey JK, Powell HM. Scar formation following excisional and burn injuries in a red Duroc pig model. Wound Repair Regen. 2017;25(4):618-31.

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: ABSTRACT ACCEPTED FOR PRESENTATION

Treatment of Full Thickness Wounds with Microfragmented Adipose Tissue and Plasma-Based Hydrogels Improves Healing Outcome

Randolph Stone II, David Larson, John Wall, Nicole L Wrice, Kyle Florell, Robert Christy, Shanmugasundaram Natesan
US Army Institute of Surgical Research, JBSA Fort Sam Houston, TX

Background: Full thickness skin wounds, such as third degree burns, often suffers severe damage including the subcutaneous adipose tissue. Most of the surgical interventions involves autograft or allograft application and does not replace adipose tissue; often leading to wound contraction and scarring. We hypothesize that early reconstruction of hypodermis using purified microfragmented adipose tissue called "Lipogems" and blood plasma-based hydrogels will improve angiogenesis, epithelialization, healing, and scar appearance. In this study we have used porcine full-thickness excision wounds as a screening model to test our hypothesis.

Methods: Lipogems were obtained using a point-of-care non-enzymatic mechanical shearing method. Lipogems-plasma hydrogels were prepared by suspending Lipogems (10-25% v/v) in a polyethylene glycol (PEG)-platelet free plasma (PEG-PFP) liquid mixture followed by cross-linking with thrombin. Morphology of cells sprouting from Lipogems within the PEG-PFP hydrogel was observed for 2 weeks, *in vitro*, using light microscopy. Frozen sections of the Lipogems-gel were cut and stained using alpha smooth muscle actin (αSMA) antibody to observe the cytoskeletal morphology. *In viv*o studies were carried out using porcine full-thickness 6 cm diameter excisional wound model, created on the dorsum of three anesthetized Yorkshire pigs. The efficiency of porcine Lipogems-PEG-PFP hydrogels to improve healing outcomes, such as, contraction, and scarring, was then assessed using paraffin sections stained with Masson's trichrome stain.

Results: Light microscopic images of cells growing out from Lipogems within the PEG-PFP hydrogel formed tubular networks, similar to adipose-derived stem cells and immunofluorescence staining with αSMA confirmed the observation of cells sprouts within the hydrogel are involved in formation of tubular network-like structures. Porcine full-thickness excision wounds treated with Lipogems-PEG-PFP hydrogels as an adjunct with meshed split-thickness skin graft (mSTSG) showed successful graft take assessed on day 7. Histological images analyzed on days 14, 28, 60 and 90 days showed the wounds treated with Lipogems±PEGylated PFP hydrogels started to exhibit a decreasing trend in contraction by day 90.

Conclusions: Results indicate that mechanically processed adipose tissue retains viability and essential factors necessary for active healing to take place. Further, Lipogems-PEG-PFP treatment could reduce scarring and avoid the necessity of multiple revision surgeries.

Applicability of Research to Practice: The Lipogems-PEG-PFP hydrogels described in this study provides a 'point-of-care' treatment option to mitigate scarring that may be translated to burn wounds as well for burn wounds. The entire process of Lipogems isolation and hydrogel preparation can be carried out in a clinical suite; therefore, requiring less sophisticated laboratory space and equipment to isolate and deliver stem cells.

Theme: Burn Reconstruction, Scar Management and Rehabilitation.

<u>APPENDIX 2</u>: Book Chapter

Shanmugasundaram Natesan, Randolph Stone II, Rodney K Chan, Robert J Christy; Editor: Xiao-Dong Chen Chapter 8 - Mesenchymal Stem Cell–Based Therapies for Repair and Regeneration of Skin Wounds A Roadmap to Non-Hematopoietic Stem Cell-basesd Therapeutics, From the Bench to the Clinic; 2019, Pages 173-222, *Academic Press*.

Abstract
Human body has the natural ability to self-defend an injury and restore homeostasis. Recent ground-breaking research in the field of cell biology has opened the gateway of "Stem Cells" and added them to the armory to ensue tissue repair and restoration. In particular, mesenchymal stem cell (MSCs) are the most exuberant cell type, integrated within various tissues spaces, and mediate a balanced response to tissue injury. Researchers, across multidisciplinary fields have harnessed the beneficial uses of MSCs across different disciplines of medicine. This chapter particularly provides an insight on the role of different MSCs in repair of skin wounds.

Micro-fragmented Adipose Tissue and Blood Plasma-based Hydrogels for Treatment of Combat Associated Burn Injuries

MB150163

W81XWH-16-2-0063

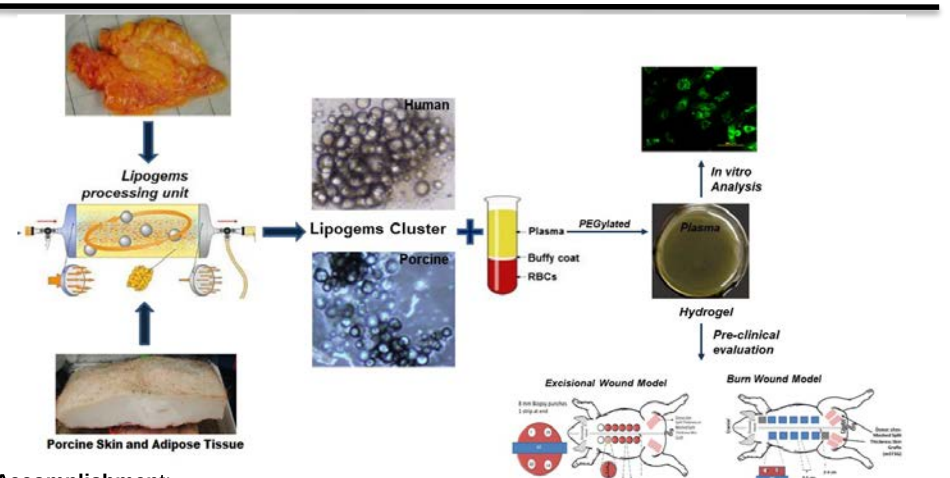
PIs: Shanmugasundaram Natesan   Org: Metis Foundation | USAISR   Award Amount: $494,266.85

Study Specific Research Aims

- Optimize and characterize micro-fragmented adipose tissue (Lipogems) – hydrogel formulations *in vitro*.
- Screen and evaluate optimized Lipogems-hydrogel based formulations in a 30 cm^2 porcine full-thickness acute excision wound model for improved graft take, vascularization, and wound healing.
- Evaluate the optimal Lipogems-hydrogel formulation using a 30 cm^2 full-thickness porcine burn wound model to assess healing and scarring.

Approach

We propose to improve acute burn wound healing using purified micro-fragmented adipose tissue and hydrogel based formulations. The proposed approach provides burn surgeons an active platform for a successful grafting protocol that will improve burn wound repair through enhanced wound vascularization, graft take, and reduced scarring.



Accomplishment:

- Isolated microfragmented adipose tissue (Lipogems) from subcutaneous fat and formulated with hydrogel based biomaterials.
- The hydrogel-Lipogems formulation was delivered successfully to reconstruct burn wounds.
- Overall Lipogems and was able to improve overall scar outcome.

Timeline and Cost

Activities	FY 16	FY 17	FY 18
Optimization and characterization of Lipogems and hydrogel based formulations			
Screening and evaluation of Lipogems in full-thickness (30cm^2 wounds)porcine wound model			
Evaluation of optimized Lipogems-plasma hydrogel formulations in a full-thickness burn wound model (30cm^2 wounds).			
Estimated Budget ($K)	$171K	$156K	$167K
Actual Budget ($K)			

Updated: 2-July-2020

Goals/Milestones:

CY16 Goal – Optimization and characterization of Lipogems and hydrogel based formulations.

☑ Optimize and characterize Lipogems-hydrogels formulations.

CY17 Goals – Screening and evaluation of Lipogems in full thickness porcine excision wound model

☑ Establish excision wound animal protocol to evaluate Lipogems and Lipogems-hydrogel formulation.

☑ Identify effective dose of Lipogems and Lipogems-hydrogels.

☑ **Determine efficacy of cryopreserved vs. freshly isolated Lipogems .**

CY18 Goal – Evaluate the effect of Lipogems on full-thickness burn wounds

☑ Determine feasibility of isolating Lipogems from debrided hypodermis.

☑ Efficacy of debrided fat Lipogems vs. freshly isolated/cryopreserved Lipogems in a full thickness burn wound.

☑ Efficacy of optimized Lipogems and Lipogems-hydrogels dose on graft take and healing.

☑ Determine efficacy of optimized Lipogems and Lipogems-hydrogels dose on contraction and scarring.

Budget Expenditure to Date

Projected Expenditure from September 30, 2016 – May 28, 2020: $494,266.85

Actual Expenditure: $494,266.85