TASK ORDER NUMBER: W81XWH-15-9-0001

MTEC RESEARCH PROJECT NUMBER: 2017-614-001

EGS NUMBER: 2 MT17002.15

TITLE: Pre-Clinical Assessment of Bioprinted Human Skin for Wound Healing and Skin Regeneration Research

PRINCIPAL INVESTIGATOR: Shay Soker

PERFORMING ORGANIZATION: Wake Forest University Health Sciences

CONTRACTING ORGANIZATION: Medical Technology Enterprise Consortium (MTEC)

REPORT DATE: 02/17/2021

TYPE OF REPORT: Final Report

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

REPORT DOCUMENTATION PAGE

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02/17/2021	Final Report	11/28/2017 – 02/25/2021

4. TITLE AND SUBTITLE
Pre- Clinical Assessment of Bioprinted Human Skin for Wound Healing and Skin Regeneration Research

5a. CONTRACT NUMBER
2017-614-001

5b. GRANT NUMBER
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5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER
MT17002.15

5e. TASK NUMBER
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5f. WORK UNIT NUMBER
N/A

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Wake Forest University Health Sciences
Medical Center Boulevard
Winston Salem, NC 27157-3972

8. PERFORMING ORGANIZATION REPORT NUMBER

MTEC-17-02-PA-15

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)

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

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

13. SUPPLEMENTARY NOTES

14. ABSTRACT
Burn injuries are major clinical and financial concerns for both the civilian and military populations. The current standard of care for permanent closure and wound coverage for full-thickness skin wounds is the use of skin autografts harvested from an uninjured donor site on the patient. However, in the case of extensive skin loss, the availability of donor sites for harvesting is limited. Tissue engineered skin substitutes are a promising alternative however, they have they do not stimulate full skin. In contrast, tissue bioprinting successfully closed large full-thickness wounds. A bioprinted full-thickness human skin construct, with stratified tri-layered structures containing epidermis, dermis and hypodermis skin will provide permanent wound closure in burn and trauma patients. It will lead to overall reduction in surgical procedures and hospital time.

15. SUBJECT TERMS

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

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Pre-Clinical Assessment of Bioprinted Human Skin for Wound Healing and Skin Regeneration Research

Research Project No. 2017-614-001

EGS# MT17002.15

Reporting Period: 11/28/2017 – 02/25/2021

MTEC Research Project Awardee

Shay Soker

Research Project Technical POC

Shay Soker

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Submitted: February 17, 2021

1. Project Status

   a. Accomplishments

   This may include completion of milestones, objectives, and/or tasks, regulatory approval received, publication of papers, presentations at conferences, filing of intellectual property, etc. for this project, followed by date in DD-MMM-YYYY. Write salient bullet points to highlight the requested information.

1. Milestones Completion

- Bioprint full thickness skin construct and mature in vitro (28/Feb/2019).
- Obtain samples from all groups (bioprinted skin, control hydrogel, and wound only) at all time points (30, 60 , 90 days) for histological and molecular analysis (27/May/ 2019).
- Analyze wound closure, cell viability and function of transplanted skin graft in mice after 30 days (28/Feb/2019).
- Analyze wound closure, cell viability and function and biomechanics of transplanted skin graft in mice after 60 days (27/March/2019).
- Analyze wound closure, cell viability and function and biomechanics of transplanted skin graft in mice after 90 days (27/May/ 2019).
- Analyze skin tissue maturation and integration of transplanted skin graft in mice after 30 days (28/Feb/2019).
- Analyze skin tissue maturation and integration of transplanted skin graft in mice after 60 days (27/March/2019).
- Analyze skin tissue maturation and integration of transplanted skin graft in mice after 90 days (27/May/ 2019).
- Prepare results and other data for a pre-IND meeting with the FDA (31/Dec/2020)

2. Manuscripts

- Jorgensen A, Varkey M, Gorkun A, Clouse C, Xu L, Chou Z, Bennett J, Murphy SV, Molnar J, Lee SJ, Yoo J, Soker S, Atala A. Bioprinted Skin Recapitulates Normal Collagen Remodeling in Full-thickness Wounds. Tissue Eng Part A. (2020) 26(9-10):512-526.
- Jorgensen AM, Chou Z, Gillispie G, Lee SJ, Yoo JJ, Soker S, Atala A. Decellularized Skin Extracellular Matrix (dsECM) Improves the Physical and Biological Properties of Fibrinogen Hydrogel for Skin Bioprinting Applications. Nanomaterials (2020) 10(8):1484.
- Jorgensen A, Varkey M, Gorkun A, Clouse C, Xu L, Molnar J, Lee SJ, Yoo J, Soker S, Atala A. Bioprinted skin integrates into full-thickness porcine skin wound and forms a stratified epidermal barrier. (In preparation)
- Jorgensen A, Varkey M, Jeong C, Gorkun A, Clouse C, Xu L, Chou Z, Bennett J, Murphy SV, Molnar J, Lee SJ, Yoo J, Soker S, Atala A. Fabrication of a bioprinted trilayer skin contruct with vascularity, pigmentation, and hair follicles (In preparation)

3. Posters and Presentations

- Jorgensen A, Varkey M, Gorkun A, Clouse C, Xu L, Bennett J, Murphy SV, Molnar J, Lee SJ, Yoo J, Soker S, Atala A. Bioprinted Skin Recapitulates Normal Collagen Remodeling in Full-thickness Wounds. Tissue Engineering and Regenerative Medicine International Society-America Chapter Annual Meeting, 2019 (Oral Presentation)

- Jorgensen A, Varkey M, Gorkun A, Lee SJ, Yoo J, Soker S, Atala A. Bioprinted Human Skin Accelerates Scarless Wound Healing And Healthy Human-like Skin Formation. Biomedical Engineering Society, 2019 (Oral Presentation)

4. Research Awards

- **Jorgensen A.** TERMIS-AM Mary Ann Liebert, Inc Outstanding Student Award for the work titled "Bioprinted Skin Recapitulates Normal Collagen Remodeling in Full-thickness Wounds."
- **Jorgensen A.** NIH/NIAMS Fellowships: Musculoskeletal and Oral Sciences, Imaging, Surgery and Informatics F30AR074866 "Integration And Remodeling Of Bioprinted Skin In Full-Thickness Wound Healing"
- **Jorgensen A.** NIH/NIAMS supplement F30AR074866-01A1S1 "Characterization of an Ex Vivo Bioprinted Skin Model of Sulfur Mustard Injury"

b. Reportable Outcomes

This may include development of a product, prototype, new methodology, or any other similar items that have resulted from this research. Write salient bullet points to highlight the requested information. Please also include a cumulative chronological list of written publications in technical journals, papers, or other presentations at meetings, conferences, seminars, etc.; New discoveries, inventions, or patent disclosures, and specific applications.

- Bioprinted skin was implanted onto 2.5 cm × 2.5 cm full thickness wound in mice and follow up for up to 90 days (key timepoints – 21, 42, and 90 days).
- Bioprinted skin constructs, composed of 6 human cell types, generated each of the three skin layers, epidermis, dermis, and hypodermis.
- The bioprinted skin is easy to apply onto full thickness wounds, integrates, forms an epidermal barrier, and recapitulates normal collagen remodeling in full-thickness wounds in athymic mice.
- The remodeled skin is phenotypically similar to human skin.
- All bioprinted skin treated wounds closed by day 21, compared with open control wounds. Wound closure in bioprinted skin treated wounds was primarily due to epithelialization. In contrast, control hydrogel and untreated groups had sparse wound coverage and incomplete closure driven primarily by contraction.
- Picrosirius red staining confirmed a normal basket weave collagen organization in bioprinted skin-treated wounds compared with parallel collagen fibers in hydrogel only and untreated wounds.
- IHC staining at days 21 demonstrated the presence of human cells in the regenerated dermis, the formation of a stratified epidermis, dermal maturation, and blood vessel formation in bioprinted skin, none of which were present in control hydrogel treated wounds.
- IHC staining at days 42 and day 90 shows the formation of a stratified epidermis, dermal maturation, and blood vessel formation in bioprinted skin. Control hydrogel treated wounds showed similar results only at the 90 days point.
- RNA has been isolated from all samples at day 21, 42, and 90 to confirm histological findings as well as direct investigation of the mechanism driving accelerated wound healing an normal collagen remodeling.
- Human skin cell culture: Process Development for the isolation of fibroblasts, keratinocytes, adipocytes and endothelial continues to be optimized
- Hydrogel development for bioprinting including verification for GMP reagents and hydrogel preparation
- Bioprinting process development with GMP reagents

c. Progress Detail

Describe each Statement of Work (SOW) task or logical segment of work on which effort was expended during this quarterly reporting period only. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved or problems encountered. A succinct description of the methodology used shall be provided.

- Full thickness wounds were created with a uniform size and depth. Representative images of wounds and treatments. Overall, both the bioprinted skin and hydrogel only constructs were able to be administered by a single surgeon with minimal preparation steps.
- Digital photos were taken for each of the time-points and compiled for each of the wounds. Gross morphology demonstrated that bioprinted skin treated wounds had complete closure at day 21, compared with open wounds in all other treatment groups. Epithelialization in the bioprinted skin treated group was confirmed through hematoxylin and eosin staining, which demonstrated complete wound closure and a stratified epithelium. Alternatively, the hydrogel only and untreated wounds only had sparse coverage and incomplete epithelialization.
- For each time point, the percentage of wound area present relative to the original wound area was measured to describe wound closure. By day 7, wounds treated with bioprinted skin had a significantly greater percentage of total wound closure compared with the hydrogel only and wound only controls (91.0% ± 7.3 vs. 23.5% ± 18.0, 19.9% ± 4.9, respectively. $p<0.0001$). This trend continued at day 14, with bioprinted skin treated wounds all achieving complete closure (100% ± 0.0 vs. 64.1% ± 5.0, 64.8% ± 13.9, respectively. $p<0.0001$). By Day 21, all wounds were above 70% total wound closure. 100% of the bioprinted skin treated wounds had closed by day 21, compared with open wounds and incomplete closure in all untreated wounds and all wounds treated with the hydrogel only.
- For each time-point, the outer visible wound border relative to the original wound area was expressed as a percentage to quantify the wound contracture. All three groups showed consistent contraction up to 21 days, at which point there was no significant difference in contraction between bioprinted skin, control hydrogel, wound only groups (49.6% ± 20.3, 53.8% ± 9.7, 54.5% ± 12.4, respectively. $p>0.05$).
- Epithelialization was measured at all time-points with epithelium defined visually by the pink/white color and presence of a matte appearing epithelial coating distinct from the wound area. The area within the contracted outer wound borders of epithelialization was measured, and the area of wound subtracted. By day 7, wounds treated with bioprinted skin had significantly greater percentage wound closure due to epithelialization compared with the hydrogel only and wound only controls (83.6% ± 13.2 vs. 1.5% ± 3.0, 4.6% ± 9.3, respectively. $p<0.0001$). This trend continued at day 14 (69.5% ± 22.7 vs. 24.0% ± 10.1, 17.0% ± 8.2, respectively. $p<0.001$). By Day 21, epithelialization had contributed to over 30% of wound closure for all wounds, with a greater percentage of wound closure due to epithelialization on average in the bioprinted skin group compared with hydrogel and wound only controls (50.4% ± 20.3 vs. 34.6% ± 13.3, 31.1% ± 7.8, respectively. $p>0.05$).
- To ensure that the epithelialization seen in the wound images correlated with true epidermal barrier formation, samples of the wound area were histologically processed and stained with hematoxylin and eosin. The hydrogel only and untreated wounds had sparse coverage and incomplete epithelialization only, compared with a thick, stratified epidermis in wounds treated with bioprinted skin. In comparing the wound histology showed that the bioprinted skin group had the most robust epidermal covering at the wound center, compared with granulation tissue in the control hydrogel and wound only groups. All groups had increased cellularity compared with normal tissue controls, suggesting increased inflammatory cells in the healing wound area. The bioprinted skin treated wounds had an immature appearing epidermis that laced rete-peg protrusions into the dermis with some stratification. Control hydrogel treated wounds had areas of maturing epidermis, but the wound center had no identifiable

epidermis in most samples. Untreated wounds also appeared to have inconsistent coverage, with a thinner epidermis in the areas of coverage. The dermis of healthy skin consisted of large organized fibers that were light pink in color with only minimal disorganized, thin, purple fibers and hair follicles in mouse skin. Bioprinted skin treated wounds also had light pink organized fibers in the dermis as well visible capillaries. The control hydrogel group has less organized pink fibers in the dermis, while untreated wounds did not show organization of the dermal fibers and consisted completely of thin, purple unorganized fibers and a thick dermis.

- Masson's trichrome staining was performed to assess the keratin and collagen composition of the wounds. Trichrome staining allowed the identification of multiple ECM components simultaneously. Trichrome stains collagen blue, keratin red, cytoplasm pink, and nuclei brown. Healthy skin had intense collagen staining (blue) in the dermis, and intense keratin staining (red) in the epidermis. High cellularity was visible in the epidermis and dermis (brown nuclei). The bioprinted skin treated wounds also had intense keratin staining (red) with moderate collagen staining (blue), particularly deeper in the dermis, and high cellularity in the epidermis and dermis. Hydrogel treated wounds had faint keratin staining (red) with mostly cytosol staining instead of keratin (pink vs. red), slight collagen staining (blue), and high cellularity only in the dermis. Untreated wound tissues showed highly disorganized and only faint collagen staining in the dermis (blue) with a disorganized epidermal layer with high cellularity (brown nuclei) and cytosol staining instead of keratin (pink vs. red).
- Picrosirius red staining differentiates between immature and mature collagen when viewed under polarized light. Immature unorganized collagen stains green/yellow (Collagen Type III) and mature bundled and organized collagen fibers stain orange/red (Collagen Type I). Furthermore, collagen fiber orientation can be described as basket weave (normal, healing) or parallel (scarred, fibrotic). Healthy skin shows a strong staining of both read and orange, indicating an organized network of larger mature collagen fibers with some slightly smaller immature collagen fibers intermixed in a basket weave orientation. The bioprinted skin treated wounds was mostly composed of orange collagen fibers with both strong red and yellow staining, intermixed in a basket weave orientation, indicating normal healing. Alternatively, the control hydrogel group had long, thick, collagen fibers which were mostly orange and red. The collagen fibers were oriented in parallel, indicating fibrosis. This general pattern was also seen in the wound only group, with most collagen fibers also aligned in parallel.
- Multiple methods were used to analyze collagen fiber color, alignment, length and width to confirm the gross histological findings described above. First, we measured differences in collagen fiber maturity using a custom MATLAB code to measure the average percentage of each collagen fiber color in each treatment group**.** Bioprinted skin treated wounds had significantly less mature, red fibers than both mouse and human skin (34% ± 14.8 vs. 91% ± 9.0 and 67% ± 25.0, respectively; $p<0.0001$). Alternatively, bioprinted skin was composed primarily of orange fibers (53.24 ± 10.3), and was most similar to control hydrogel treated wounds. Untreated wounds were most similar to mouse skin, both in terms of the number of red fibers (57% ± 40.3 vs. 67% ± 25.0) and orange fibers (34% ± 30.3 vs. 24% ± 15.0). Next, CurveAlign was used to determine the coefficient of alignment of collagen fibers in each treatment group. Control hydrogel and wound only treated wounds both had highly aligned collagen fibers (0.78 ± 0.10 vs. 0.79 ± 0.10, respectively; $p>0.05$), typical of a parallel scar formation. Alternatively, normal human and mouse skin were both had similarly unaligned collagen fibers (0.29 ± 0.12 vs. 0.34 ± 0.20, respectively; $p>0.05$), typical of normal basket weave collagen fiber alignment. We found that Bioprinted skin had less aligned collagen fibers than the control hydrogel and wound only controls (0.52 ± 0.19 vs. 0.78 ± 0.10 and 0.79 ± 0.10, respectively; $p<0.001$), representing a more normal, basket weave collagen extracellular matrix. However, bioprinted skin treated wounds had greater alignment that normal human and mouse skin (0.52 vs. 0.29 ± 0.12 and 0.34 ± 0.20, respectively; $p<0.01$), suggesting that ECM remodeling was not yet complete at 21 days in this model.

Finally, we further analyzed the picrosirius red images by quantifying the collagen fiber properties using CT-FIRE, a program designed by the Laboratory for Optical and Computational Instrumentation at the University of Wisconsin, which quantifies parameters of fiber length and width. Briefly, Fibers are isolated from images by identifying edges (curvelet transform [CT]) and fiber extraction (FIRE) algorithm and connecting those edges to segment total fibers. These segmented fibers are then analyzed to generate histograms of fiber parameters, such as angle, width, length, and straightness. When fiber length was measured, the control hydrogel and untreated controls had similarly long fibers (68.0 ± 45.9 vs. 67.2 ± 43.4, $p>0.05$), typical of scar formation. Alternatively, bioprinted skin treated wounds had an average collagen fibers length that was significantly shorter, and was most similar to normal human skin (55.9 ± 27.8 vs. 56.3 ± 28.3, $p>0.05$), and was also shorter than mouse skin collagen fibers (55.9 ± 27.8 vs. 58.9 ± 32.23, $p<0.0001$). These findings suggest that bioprinted skin promoted generation of collagen fibers of normal length for human skin. Finally, fiber width measurements showed that the bioprinted skin treated wounds were narrower than normal human, mouse, and untreated wound skin (6.9 ± 1.5 vs. 7.4 ± 1.5, 7.5 ± 1.7, 7.3 ± 1.6, respectively; $p<0.0001$). The fibers of bioprinted skin treated wounds were wider than control hydrogel treated wounds (6.9 ± 1.5 vs. 4.1 ±1.7), suggesting greater maturity towards normal extracellular matrix formation.

- PanCytokeratin and Lamin A+C stained sections were used to evaluate and compare the epidermal structure and human cell integration. PanCytokeratin stains epidermal keratinocytes while lamin A+C is a human cell specific nuclear stain. In human skin, the stratified epidermis stained positively for PanCytokeratin (green) and highlighted the rete-peg protrusions into the dermis. Lamin A+C staining was positive throughout the sample, with the highest concentration cells in the epidermis, and moderate cellularity in the dermis. The bioprinted skin treated wounds also stained positively for PanCytokeratin, but no rete-pegs were present in the wound area. In addition, Lamin A+C stained positively, confirming the integration of human cells into the wound area. However, Lamin A+C staining was only present in the dermis. Finally, control hydrogel treated wounds had only sparse pancytokeratin staining and no positive signal for Lamin A+C.
- Mel5 and Pan Cytokeratin stained sections were used to evaluate and compare melanin production and melanocyte integration in relation to the epidermis. In human skin, Mel5 staining was found sparsely in the epidermis and scattered throughout the dermis. Similarly, Mel 5 was found just below the epidermis and randomly throughout the dermis in the bioprinted skin treated group. Only faint staining for pan cytokeratin was present in the hydrogel only group, with no signal present for mel5.
- Adiponectin and vimentin stained sections were used to evaluate and compare human dermal fibroblast and pre-adipocyte integration. In human skin, vimentin stained positively in a thin dermal area below the epidermis while adiponectin stained a large hypodermal area. In wounds treated with bioprinted skin the entire dermal area stained positively for vimentin, but no adiponectin staining was present. The high number of human fibroblasts present in the dermis likely guided the normal collagen deposition described above. In the control hydrogel treated group there was only sparse staining for adiponectin, and no staining for the highly human specific vimentin antibody.
- ZO-1 and Pan Cytokeratin stained sections were used to evaluate the presence of tight junctions in relation to the epidermis. We found that ZO-1 staining highlighted blood vessel lumens on our stained samples. In human skin, ZO-1 staining was most pronounce deep in the hypodermal region. Alternatively, ZO-1 stained both in the superficial and deep in the dermis, with pronounced lumen in both areas. The control hydrogel treated wounds also had positive staining of ZO-1 in both the superficial and deep dermis, however, fewer blood vessel lumens were present.
- Vital information is gained from analyzing individual components of wound healing parameters such as wound closure, contraction and epithelialization. However, a combined analysis of the wound during healing can better illustrate the overall wound healing quality and the relative contributions of each

parameter. The best performing treatments were categorized as having the smallest wound area, the least contraction, and the most epithelialization the worst performing treatments had the opposing characteristics. Bioprinted skin treated wounds had the smallest wound area as the only group to have complete wound closure at the close of the study, the greatest amount of epithelialization, and the least contraction. The hydrogel and wound-only groups had small open wounds at the end of the study, with less wound closure due to epithelialization, and greater than half of wound closure due to contraction. The control hydrogel group performed slightly better in all categories than untreated wounds. Based on the combined analysis, the order of best performance was 1) bioprinted skin, 2) hydrogel only control, and 3) wound only with standard bandaging.

- Human skin tissue and cell processing:
    - Cell Isolation: Process Development for the isolation of fibroblasts, keratinocytes, adipocytes and endothelial continues to be optimized:
        - Isolation of Human Pre-Adipocytes and Microvascular Endothelial Cells from the Stromal Vascular Fraction (SVF) of Lipoaspirate
            - Endothelial cell fraction was isolated by using CD31 and CD144 magnetic microbeads
            - SVF cells were 58.7% positive for adiponectin and 2.2% positive for HLA-DR at P1
        - Isolation of Human Keratinocytes, Fibroblasts, and Endothelial Cells from Foreskin Biopsy
            - Endothelial cells were isolated using CD31 and CD144 magnetic microbeads
- Hydrogel Development:
    - Testing sterilization of hydrogel components
    - Bracketing optimal time to prepare hydrogel
    - Functional verification for GMP reagents
- Bioprinting:
    - Replication of research protocol with GMP reagents
    - Technical Study: comparison of the efficacy of aprotinin versus tranexamic acid in producing a stable bioprinted fibrin hydrogel

2. Problems / Issues

Provide a description of problems or issues that impeded performance or progress of this project along with corrective action taken. This may include administrative, technical, and/or logistical issues.

- NONE

3. Financial Health

Comment on the financial health of the study. Was the study financially on track during for completion as proposed within the period of performance? If not, describe the cause(s), whether this had a short-term or long- term impact. Provide amount expended cumulatively. State if there was any major equipment procured, sub-award implemented, and/or travel conducted.

- NOTHING TO REPORT

4. Personnel Effort

Provide names of current staff along with their roles and percent effort of each on this project. Add additional rows if necessary to list the complete team. If there is more than one project on this award, breakdown according to each project (one table per project).

Personnel	Role	Percent Effort
Shay Soker	PI	10%

5. Protocol and Activity Status

For awards involving the use of human subjects, use of human cadavers, and/or use of animal subjects, prepare a summary in accordance with the following subsections. For all other awards, including those involving the use of human anatomical substances (such as tissue or cells or identifiable private information), mark as directed below.

a. Human Use Regulatory Protocols

TOTAL PROTOCOLS: State the total number of human use protocols required to complete this project (e.g., 5 human subject research protocols will be required to complete the Statement of Work."). If not applicable, write "No human subjects research will be performed to complete the Statement of Work."

No human subject research will be performed to complete the Statement of Work.

PROTOCOLS: List all human use protocols to be performed to complete the project, an include approved target number for clinical significance, followed by type of submission and type of approval with associated dates, and performance status for each.

Protocol: IRB00051592

Title: Discarded Skin Tissue for Use in Laboratory Cell Processing and Scaffold Development

Target required for clinical significance: 500

Target approved for clinical significance: 500

Protocol [HRPO Assigned Number]: MT17002.15

Title: Discarded Skin Tissue for Use in Laboratory Cell Processing and Scaffold Development

Target required for clinical significance: 500

Target approved for clinical significance: 500

Submitted to and Approved by:

Provide bullet point list of protocol development, submission, amendments, and approvals (include IRB in addition to HRPO).

- The protocol was approved by HRPO on 28-JAN-2019.

STATUS: Provide bullet point list of performance and/or progress status relating to the above protocol and discuss recruitment number, enrollment number, drop outs, disqualified, etc. Discuss

any administrative, technical, or logistical issues that may impact performance or progress of the study (e.g. slow enrollment, large dropouts, or adverse events) for the above HPRO approved protocol.

The protocol is active.

b. Use of Human Cadavers for RDT&E, Education or Training

"Cadaver" is defined as a deceased person or portion thereof, and is synonymous with the terms "human cadaver" and "post-mortem human subject" or "PMHS." The term includes organs, tissue, eyes, bones, arteries or other specimens obtained from an individual upon or after death. The term "cadaver" does not include portions of an individual person, such as organs, tissue or blood, that were removed while the individual was alive (for example, if a living person donated tissue for use in future research protocols, that tissue is not considered a "cadaver" under this policy, regardless of whether the donor is living or deceased at the time of tissue use).

No RDT&E, education or training activities involving human cadavers will be performed to complete the Statement of Work (SOW)

TOTAL ACTIVITIES: State the total number of RDT&E, education or training activities that involved cadavers. If not applicable, write "No RDT&E, education or training activities involving human cadavers will be performed to complete the Statement of Work (SOW)."

ACTIVITIES: Provide the following information in a bulleted list for all RDT&E, education or training activities involving human cadavers conducted or supported during the quarter:

- Title of the RDT&E, education or training activity
- SOW task/aim associated with the activity
- Date the activity was conducted
- Identification of the organization's responsible individual (e.g., PI or individual primarily responsible for the activity's conduct)
- Brief description of the use(s) of cadavers in the activity and the total number of cadavers used during the reporting period
- Brief description of the Department of Army organization's involvement in the activity
- Status of document submission and approvals
- Problems encountered in the procurement, inventory, use, storage, transfer, transportation and disposition of cadavers used for RDT&E, education or training. Examples of problems include but are not limited to: loss of confidentiality of cadaveric donors, breach of security, significant deviation from the approved protocol, failure to comply with state laws and/or institutional policies and public relations issues.

c. Animal Use Regulatory Protocols

TOTAL PROTOCOLS: State the total number of animal use protocols required to complete this project (e.g., 2 animal use research protocols will be required to complete the Statement of Work.). If not applicable, write "No animal use research will be performed to complete the Statement of Work."

PROTOCOLS: List all animal use protocols to be performed to complete the project, include approved target number for statistical significance, followed by type of submission and type of approval with associated dates, and performance status for each.

IACUC Protocol #A18-065 has been approved for the use of mice in this study.
Target required for statistical significance: 200
Target approved for statistical significance: 200

Protocol [ACURO Assigned Number]: MT17002.15
Title: Optimization of Bioengineered Skin
Target required for statistical significance: 200
Target approved for statistical significance: 200

Submitted to and Approved by:
Provide bullet point list of protocol development, submission, amendments, and approvals (include IACUC in addition to ACURO).

- IACUC protocol approved 6-6-2018
- ACURO protocol approved 10-12-2018

STATUS: Provide bullet point list of performance and/or progress status relating to the above protocol and discuss any administrative, technical, or logistical issues that may impact performance or progress of the study (e.g. animal use protocol need revision to minimize animal suffering, animal protocol modification to include additional staff) for the above ACURO approved protocol.

Pre-Clinical Assessment of Bioprinted Human Skin for Wound Healing and Skin Regeneration Research
Research Project No. 2017-614-001
EGS# MT17002.15
Reporting Period: 11/28/2017 – 02/25/2021

MTEC Research Project Awardee
Shay Soker

Research Project Technical POC
Shay Soker
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Submitted: February 17, 2020

1. CURRENT STAFF

Personnel	*% of Effort on project*
Shay Soker: Principal Investigator	10%

2. CURRENT EXPENDITURES

A. Cost Reimbursable Contracts

Contract Expenditures	Current QTR Expenditures	*Cumulative To Date Expenditures*
Labor (Personnel and Fringe)	$	$142,215
Supplies/Materials	$	$ 51,303
Travel	$	$
Equipment	$	$
Subcontractors and Consultants	$	$
Other Direct Costs	$	$ 30
Indirect Costs	$	$106,451
Total	**$**	**$299,999**

B. Fixed Priced Contracts: n/a

C. Cost Share Contributions:

Funding Source (Cash)	This Period	Cumulative to Date
Cash	$0.00	$0.00
Labor Dollars	$10,505.99	$41,740.15
Indirect Labor Rates (Fringe Benefits)	$1,470.84	$5,739.21
Travel	$0.00	$650.34
General & Administrative Services	$0.00	$0.00
Equipment (New)	$0.00	$0.00
Material	$5,723.41	$101,599.15
Other Direct Costs	$0.00	$271.15
Other * *(Overhead)*	$6,641.59	$22,500.00
Sub-Total	$24,341.83	$172,500.00
Funding Source (In-Kind)	**This Period**	**Cumulative to Date**
Use of Existing Equipment (Estimated fair market value)	$0.00	$0.00

Use of Existing Software (Estimated fair market value)	$0.00	$0.00
Intellectual Property (Estimated fair market Value)	$0.00	$0.00
Space (Land or buildings)	$0.00	$0.00
Sub-Total	$24,341.83	$172,500.00
Cost Share Total	$24,341.83	$172,500.00

3. STATUS OF MILESTONES

MTEC Milestone Number	Milestone Description	Due Date	% Completed this Reporting Period	Cumulative % Complete
	Task 1: Bioprinted skin viability, anatomical integrity and cellular function in a skin graft model			
1.1	Bioprint full thickness skin construct and mature in vitro	02/28/2019		100%
1.2	Analyze wound closure, cell viability and function and biomechanics of transplanted skin graft after 30 days	02/28/2019		100%
1.3	Analyze wound closure, cell viability and function and biomechanics of transplanted skin graft after 60 days	03/27/2019		100%
1.4	Analyze wound closure, cell viability and function and biomechanics of transplanted skin graft after 90 days	05/27/2019		100%
	Task 2: Integration and remodeling of bioprinted skin graft in mice			
2.1	Analyze skin tissue maturation and integration of transplanted skin graft after 30 days	02/28/2019		100%
2.2	Analyze skin tissue maturation and integration of transplanted skin graft after 60 days	03/27/2019		100%
2.3	Analyze skin tissue maturation and integration of transplanted skin graft after 90 days	05/27/2019		100%
	Task 3: Prepare for a pre-IND meeting with FDA			
3.1	Prepare results and other data for a pre-IND meeting with the FDA	05/27/2019	2%	100%
4	Q1 Report	05/15/2018		100%
5	Q2 Report	12/03/2018		100%
6	Q3 Report	12/03/2018		100%
7	Q4 Report	01/25/2019		100%
8	Q5 Report	04/25/2019		100%

9	Q6 Report	07/25/2019		100%
10	Q7 Report	08/14/2019		100%
	NCE: Cost Share Contribution			
11	Skin cell isolation for GMP production (Keratinocytes, Fibroblasts, Adipocytes, Endothelial)	10/31/2020		100%
12	Skin cell expansion optimization for GMP production	10/31/2020		0%
13	Cryopreservation studies for GMP production	10/31/2020		0%
14	Initiate optimization of bioprinting and construct maturation for GMP production	10/31/2020		100%
15	Preliminary bioprinted construct characterization for GMP production and product release	12/31/2020		100%
16	Preliminary bioprinted construct stability testing	12/31/2020		0%
17	Q8 Report	01/25/2020		100%
18	Q9 Report	04/25/2020		100%
19	Q10 Report	07/25/2020		100%
20	Q11 Report	10/25/20	100%	100%
21	Final Report	02/25/21	100%	100%

4. DEVIATION FROM PROJECT PLAN

None