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TITLE: Strain-Programmable Bioadhesive Patch for Accelerated Healing of Diabetic Ulcer

PRINCIPAL INVESTIGATOR: Xuanhe Zhao, PhD

CONTRACTING ORGANIZATION: Massachusetts Institute of Technology

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Xuanhe Zhao, PhD

E-Mail: zhaox@mit.edu

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

We have accomplished all tasks that were proposed for months 1-12. More specifically, in collaboration with Veves team, my team designed and validated the strain-programmable patch (Subtask 1), carried out theoretical analysis and experimental validation the patch (Subtask 2), and characterized the patch (Subtask 3). Furthermore, we assisted Veves team to complete all proposed animal studies that evaluated the in vivo biocompatibility of the patch (Subtask 4). Our collaborative results were very positive and resulted in a publication in a journal of major impact (Theocharidis et all. Strain-Programmed Patch for Diabetic Wound Healing. *Nature Biomedical Engineering,* https://doi.org/10.1038/s41551-022-00905-2). We also achieved all proposed milestones.

15. SUBJECT TERMS

NONE LISTED

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TABLE OF CONTENTS

Page

The objective of this proposal is to develop a novel strain-programmed bioadhesive patch as a potential therapeutic solution for enhanced wound healing in diabetic foot ulcers (DFU). Our proposed strain-programmed bioadhesive patch offers a unique and novel treatment for diabetic wound healing by synergistically combining 1) instant yet robust adhesion on wet wounded tissues and 2) fully programmable mechanical contraction of wounds, potentially offering an unprecedented platform that can fulfill the unmet clinical needs to improve impaired diabetic wound healing. We hypothesized that a tissue adhesive biomaterial capable of providing programmed mechanical contractions can facilitate healing of DFU by addressing this mechanical imbalance. The first aim focuses on the development of a strain-programmed bioadhesive patch to control mechanical contraction around wound and the second aim on the optimization of the strain-programmed bioadhesive patch for DFU treatment. The third aim involved the pre-clinical validation of the strain-programmed bioadhesive patch for DFU treatment via *in vivo* and *ex vivo* models.

Diabetes, impaired wound healing, strain-programmed patch, rapid robust adhesion, controlled mechanical contraction, theoretical and numerical models, *ex vivo* human skin culture, *in vivo* diabetic mice and porcine skin, and diabetic humanized mouse skin, wound healing models.

What were the major goals of the project?

The goal of this grant is to develop a novel strain-programmed bioadhesive patch to achieve improved diabetic wound healing by programming mechanical contractions of wounds. We envision that the proposed platform can serve as a promising therapeutic solution for diabetes patients with DFU and other chronic diabetic wounds.

What was accomplished under these goals?

The accomplished work is indicated in the SOW below.

Specific Aim 1: Development of a strain-programmed bioadhesive patch to control mechanical contraction around wound.	**Timeline**	**Site 1 (Zhao, MIT)**	**Site 2 (Veves, BIDMC)**
Major Task 1	Months		
Subtask 1: Validation of the strain-programmed bioadhesive patch design.	1-12	Material preparation & fabrication **(accomplished)**	Clinical input on patch design **(accomplished)**
Subtask 2: Theoretical analysis and experimental validation the bioadhesive patch.	1-12	Theoretical & experimental validation of patch **(accomplished)**	
Subtask 3: Characterization of strain-programmed bioadhesive patch.	1-12	Mechanical characterization of patch **(accomplished)**	
Subtask 4: *In vivo* biocompatibility evaluation.	6-24	Patch preparation **(in progress)**	Animal study **(in progress)**
Milestone(s) Achieved			
#1: Local IACUC Approval			IACUC approval from BIDMC and MIT
#2: ACURO Approval			ACURO approval from USAMRDC ORP
Specific Aim 2: Optimization of the strain-programmed bioadhesive patch for DFU.			
Major Task 2			
Subtask 1: Biomechanical characterization of diabetes skin.	12-18	Biomechanical characterization **(in progress)**	Skin sample supply & data analysis **(in progress)**
Subtask 2: Modeling and optimization of programming mechanical contraction of wound.	18-30	Finite-element modeling & optimization **(in progress)**	Biomechanical analysis & input **(in progress)**
Subtask 3: Validation and optimization of the strain-programmed bioadhesive patch based on the mechanical models.	18-30	Patch optimization & mechanical modeling **(in progress)**	Clinical input on validation & optimization **(in progress)**
Milestone(s) Achieved			

#1: Biomechanical characterization protocol establishment		Biomechanical characterization Protocol **(accomplished)**	Biomechanical characterization Protocol **(accomplished)**
#2: Finite-element modeling platform establishment		Finite-element modeling **(in progress)**	
Specific Aim 3: Pre-clinical validation of the strain-programmed bioadhesive patch for DFU treatment via in vivo and ex vivo models.			
Major Task 3			
Subtask 1: Assessment of patch in the *in vivo* diabetic mice model including the humanized skin-graft diabetic mice model.	18-36	Patch preparation **(In Progress)**	Animal study **(In Progress)**
Subtask 2: Assessment of patch in the *in vivo* diabetic pig model.	24-38	Patch preparation **(In Progress)**	Animal study **(In Progress)**
Subtask 3: Assessment of patch in *ex vivo* human skin model.	30-48	Patch preparation **(In Progress)**	Animal study **(In Progress)**
Milestone(s) Achieved			
#1: Local IACUC approval			IACUC approval from BIDMC and MIT
#2: ACURO Approval			ACURO approval from USAMRDC ORP
#3: USAMRDC HRPO Approval			USAMRDC HRPO approval from BIDMC

The accomplished work was recently published in the paper that is mentioned under the Products section. Figures that are mentioned in this report refer to the Figures of the published paper.

Major Task 1.

Subtask 1. Validation of the strain- programmed bioadhesive patch design (months 1-12).

In Subtask 1, the Zhao Lab first fabricated the strain-programmed bioadhesive patch following the design illustrated in **Figure 1** in the published paper. The detailed materials and procedures for fabricating the strain-programmed bioadhesive patch are described in detail in the **Methods** section (**Materials** and **Preparation of**

the strain-programmed patch subsections). Notably, the strain-programmed patch integrates the advantages of both tough adhesion on skins and programmable contracting strains of the wound.

The Zhao Lab next validated the proposed strain-programmable capability (**Fig. 2a-c** in the published paper) of the bioadhesive patch. The experimental results in **Fig. 2c** and **Extended Data Fig. 2** show that the strain-programmable bioadhesive patch can apply programmed strain and stress on the skin it adheres to. The stress level ranges from 0kPa to 200kPa (**Fig. 2c** and **Extended Data Fig. 2**) suitable for contracting the wound.

The Veves Lab personnel discussed in detail the proposed work with the Zhao Lab and provided appropriate clinical input on patch design.

Subtask 2. Theoretical analysis and experimental validation the bioadhesive patch (months 1-12).

In Subtask 2, the Zhao Lab carried out theoretical analysis and experimental validation of the bioadhesive patch. The Zhao Lab first carried out finite-element theoretical analysis of the strain programmable patch adhered on the wound. In **Fig. 3a, b, d, e** and **f**, the Zhao Lab provided the results of the finite-element theoretical analysis. One important insight from the theoretical analysis is that a sufficiently high strain (e.g., pre-stretch ratio=1.5) can dramatically reduce the hoop stress around the edge of the wound (**Fig. 3e**). The Zhao Lab then validated the theoretical analysis with experimental results in **Fig. 3c, g-i**. It can be seen that the programmed strain in the patch can reduce the area of the wound (shrink the wound) and alleviate the stresses around the edge of the wound, validating the theoretical analysis.

Subtask 3: Characterization of strain-programmed bioadhesive patch (months 1-12).

In Subtask 3, the Zhao Lab characterized the mechanical properties of the strain-programmed bioadhesive patch. In **Fig. 2d-f**, the Zhao Lab reported the interfacial toughness, shear strength and wound closure strength of the patch, respectively. The Zhao Lab further compared the mechanical performances of the patch with many commercially-available counterparts. As shown in **Fig. 2d-f**, the interfacial toughness, shear strength and wound closure strength of the patch are much higher than the values of the commercially-available counterparts.

Subtask 4: *In vivo* biocompatibility evaluation (months 6-24).

The biocompatibility of the patch was studied and confirmed by the Zhao lab at MIT. The Veves lab discussed the experiments with Zhao lab and provided appropriate feedback. In **Extended Data Fig. 4**, the Zhao and Veves Labs reported the in vivo biocompatibility of the patch and compared the biocompatibility of the patch with commercially available counterparts. The biocompatibility of the patch and inflammation level of the patch are similar to those of commercially available counterparts (**Extended Data Fig. 4**).

Major Task 2.

Subtask 1: Biomechanical characterization of diabetes skin (12-18 months).

The Zhao Lab provided the strain programmable patch, participated in the design of the studies and provided appropriate feedback. The Veves Lab provided the images of representative human diabetic foot ulcers (**Figures 3g and 3j** in the published paper). The Veves Lab observed that the strain programmable patch can indeed shrink the size of the wound in the diabetes skin (**Figures 3c** in the published paper)

Subtask 2: Modeling and optimization of programming mechanical contraction of wound (18-30 months).

The Zhao and Veves Labs are currently modelling and optimizing the mechanical contraction of wound.

Subtask 3: Validation and optimization of the strain-programmed bioadhesive patch based on the mechanical models (18-30 months).

The Zhao and Veves Labs are currently optimizing the strain-programmed bioadhesive patch based on the mechanical models we developed (**Figure 3** in the published paper).

Major Task 3.

Subtask 1: Assessment of patch in the *in vivo* diabetic mice model including the humanized skin-graft diabetic mice.

The Veves Lab first evaluated the efficiency of the strain-programmed patch in 12-week old diabetic db/db mice that have impaired diabetic wound healing and heal mostly by re-epithelialization and less by contraction, which is similar to human to skin would healing. Two wounds were created at the dorsum of each mouse. Application of the strain-programmed patch onto 6 mm dorsal excisional wounds (n=48 wounds) resulted in markedly improved wound closure re-epithelialization and area of the migrating hyperproliferative neo-

epidermis, at both 5 and 10 days post-injury when compared to application of no strain patches (n=47 wounds) or no patches but application of only Tegaderm (n=44 wounds) (**Figure 4** of published paper). In addition, the strain-programmed patch-treated wounds exhibit enhanced vascularization and increased the number of αSMA^+ cells, in all probability myofibroblasts at Day 10 postwounding.

As an additional preclinical animal model with high translational relevance, we transplant human skin onto the backs of athymic nude mice for in vivo humanized mouse wound healing (**Extended Data Fig. 10a** of published paper). After 5 weeks of transplant engraftment period, we administered streptozotocin to induce diabetes. Following 7 weeks of confirmed hyperglycemia, the human skin grafts were wounded with a 3 mm punch biopsy and either no strain (n=4 mice) patch or strain-programmed patch (n=4 mice) was applied for 5 days. The strain-programmed patch-treated wounds exhibit acceleration in wound closure and re-epithelialization by Day 5(**Extended Data Fig. 10b-d** of published paper). The gene expression of multiple human growth factors and regenerative markers, namely *VEGFA*, *FGF2*, *EGF*, *EGR1* and *MFGE8*, was also enriched for the strain-programmed patch treatment group, in agreement with the porcine wound healing model results (**Supplementary Fig. 23**, published paper).

The Zhao Lab provided the strain-programmable patches in the Subtask, participated in the study design and discussion, received feedback from the Veves Lab, and further optimized the patches.

Subtask 2: Assessment of patch in the *in vivo* diabetic pig model.

To further verify the strain-programmed patch's efficacy, the Veves Lab selected a porcine wound healing model that most closely mirrors the human wound healing process. Diabetes was induced in two Yucatan mini-pigs through Alloxan injection. After 20 weeks of diabetic state, nine square full-thickness wounds (2.25 cm2 wound area) was created on each side of the dorsum (**Figure 8a** of published paper). We identified that the strain-programmed patch effectively provided wound contraction as well as removal of hoop stress concentration at the wound edge for porcine skin (**Supplementary Fig. 20d,e**, published paper). Furthermore, an additional finite-element analysis showed that the strain-programmed patch can favorably modulate skin wounds with diverse geometries including square wounds adopted in the porcine model (**Supplementary Fig. 21**, published paper).

The strain-programmed patch-treated wounds showed expedited wound closure at both examined time points (**Figures 8c–e,h,** published paper). On day 7, the strain-programmed patch-treated wounds exhibit increased

% re-epithelialization (**Figure 8f**, published paper) and area of the migrating hyperproliferative neo-epidermis (**Figure 8g**, published paper). By day 14, all wounds are almost fully re-epithelialized but only the strain-programmed patch treatment leads to thicker epidermis (**Figure 8i**, published paper) with complete stratification and enhanced formation of numerous rete ridge structures (**Figure 8j**, published paper), which are strong indications of the skin attaining its pre-injury form.

The Zhao Lab provided the strain-programmable patches in the Subtask, participated in the study design and discussion, received feedback from the Veves Lab, and further optimized the patches.

Subtask 3: Assessment of patch in *ex vivo* human skin model.

The Veves Lab assessed the wound healing efficacy of the strain-programmed patch on panniculectomy-derived discarded human skin that was kept in cell culture conditions. We inflicted 6 mm punch biopsy wounds and monitored healing over 4 days. When compared to the application of no strain patch (n=19) and Tegaderm (n=16), the strain-programmed patch (n=17) improved wound healing and increased collagen production, which was measured by employing Masson's trichrome staining (**Figure 7**, published paper).

The Zhao Lab provided the strain-programmable patches in the Subtask, participated in the study design and discussion, received feedback from the Veves Lab, and further optimized the patches.

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

The project has been instrumental in the professional development of researchers working in my lab at MIT. More specifically, Dr. Jingjing Wu, a postdoctoral associate, gained considerable experience by participating in all activities, including planning of activities, study design, data collection, analysis and interpretation and participating in the writing the first draft of the paper. In addition, Ms. Sarah Wu, Mr. Chonghe Wang, and Mr. Chase Michael Hartquist, PhD students, were trained in the patch design, fabrication, characterization, and validation.

How were the results disseminated to communities of interest?

We believe that the publication of the obtained data in a prominent scientific journal is one of the best ways at this point to disseminate our results in the scientific community. We have published the results in *Nature*

Biomedical Engineering (https://doi.org/10.1038/s41551-022-00905-2), one of the most prominent journals in this field.

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

The next reporting period we plan to perform all tasks at all included in our SOW. More specifically:

Major Task 1: We have accomplished Subtasks 1-3 as planned. The Veves and Zhao Labs have tested the *in vivo* biocompatibility of existing patches (**Extended Data Fig. 4**) in Subtask 4. The Veves and Zhao Labs will further test the *in vivo* biocompatibility of the optimized patches in Subtask 4.

Major Task 2: The Veves and Zhao Labs will further characterize the mechanical properties of diabetes skin in Subtask 1. In Subtask 2, the Zhao Lab will further optimize the programming mechanical contraction of wound based on models we developed. In Subtask 3, the Zhao Lab will further validate and optimize the strain-programmed bioadhesive patch based on the mechanical models we developed. The Veves Lab will work closely with Dr. Zhao's team at MIT and provide all required input to complete all remaining elements in Subtasks 2 and 3.

Major Task 3: *Subtask 1.* The Veves Lab will investigate the mechanisms of action based on RNA seq and immunohistology studies that involve humanized mice. The Zhao Lab will provide the strain-programmable patches, participate in the study design and discussion, receive feedback from the Veves Lab, and further optimize the patches.

Subtask 2. The Veves Lab will perform additional pig studies and we will investigate the efficacy and mechanism of action of the strained patch at two different time points, which correlate with either the early inflammatory phase (Day 3 postwounding) of wound healing or the late proliferation/early remodeling phase (Day 28 postwounding).

The Veves Lab also plan to initiate extensive analysis of the mechanism of action that will involve single cell transcriptomics and, if needed, proteomics. These studies will provide valuable information regarding the response of various cell types to the applied intervention and can guide us to further develop and improve efficacy of our product.

The Zhao Lab will provide the strain-programmable patches, participate in the study design and discussion, receive feedback from the Veves Lab, and further optimize the patches.

4. IMPACT

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

We believe that the obtained data so far has the potential to have a considerable impact in the field of impaired diabetic wound healing in specific and chronic wound in general. More specifically, the project can provide alternative novel therapeutic approaches for the management of diabetic foot ulceration. In addition, it contains very innovative research design, which includes *ex vivo* human skin cultures, humanized mice and state-of-the-art transcriptomics that can be adapted by other researchers will conduct research that will investigate additional potential therapeutic approaches.

What was the impact on other disciplines?

We believe that the patch, or alterations of it, along with the research design we developed, can be employed for the management of father chronic or acute wounds.

What was the impact on technology transfer?

A patent titled '*Shape Memory Adhesive Materials for Diabetic Wound Healing'* has been already submitted.

Discussions about licensing the patent to startup companies are currently under discussion.

What was the impact on society beyond science and technology?

Nothing to Report

5. CHANGES/PROBLEMS

Changes in approach and reasons for change

There is no change in the approach of research at MIT.

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

We do not anticipate any problems or delays in the next reporting period.

Changes that had the significant impact on expenditures

We do not anticipate any impact on the budget of the study and the expenditures.

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

There is no change in this aspect at MIT.

6. PRODUCTS

Publications, conference papers, and presentations

Theocharidis G[#], Hyunwoo Yuk H[#], Roh H, Wang L, Mezghani M, Wu J, Kafanas A, Contreras M, Sumpio B, Li Z, Wang E, Chen L, Fei Guo C, Jayaswal N, Katopodi XL, Kalavros N, Nabzdyk CS, Vlachos IS, Veves A*, Zhao X*. A strain-programmed patch for the healing of diabetic wounds. Nature Biomedical Engineering, https://doi.org/10.1038/s41551-022-00905-2 (2022). *[#]: These authors contributed equally, *:These authors jointly supervised this work*

Inventions, patent applications, and/or licenses

Shape Memory Adhesive Materials for Diabetic Wound Healing, U.S. Application No. 63/148,901.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Jingjing Wu
Project Role:	Postdoctoral associate
Researcher Identifier (ORCID ID):	
Nearest person month worked:	6
Contribution to Project:	Participate in all activities, including planning of activities, study design, data collection, analysis and interpretation and participating in the writing the first draft of the paper.
Funding Support:	MIT Institute of Soldier Nanotechnology

Name:	Sarah Wu
Project Role:	Graduate student
Researcher Identifier (ORCID ID):	
Nearest person month worked:	6
Contribution to Project:	Participate in patch design, fabrication, characterization, and validation.

Funding Support:	NSF graduate fellowship

Name:	Chase Michael Hartquist
Project Role:	Graduate student
Researcher Identifier (ORCID ID):	
Nearest person month worked:	6
Contribution to Project:	Participate in patch design, fabrication, characterization, and validation.
Funding Support:	NSF graduate fellowship

Name:	Chonghe Wang
Project Role:	Graduate student
Researcher Identifier (ORCID ID):	
Nearest person month worked:	12
Contribution to Project:	Participate in patch design, fabrication, characterization, and validation.
Funding Support:	

Name:	Bolei Deng
Project Role:	Postdoc associate
Researcher Identifier (ORCID ID):	
Nearest person month worked:	3
Contribution to Project:	Participate in patch design, fabrication, characterization, and validation.
Funding Support:	

Name:	Shucong Li
Project Role:	Postdoc associate
Researcher Identifier (ORCID ID):	
Nearest person month worked:	2

Contribution to Project:	Participate in patch design, fabrication, characterization, and validation.
Funding Support:	

Name:	Hyunwoo Yuk
Project Role:	Research Scientist
Researcher Identifier (ORCID ID):	
Nearest person month worked:	3
Contribution to Project:	Participate in patch design, fabrication, characterization, and validation.
Funding Support:	

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

No changes. Professor Xuanhe Zhao is a co-founder and on the board of directors of Sanaheal Inc, a startup company that is translating bioadhesive technology. The start-up and the board service do not affect Professor Zhao's effort as a PI for this research project.

What other organizations were involved as partners?

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

Collaborative awards

Independent reports are provided

9. APPENDICES

a. Paper published at *Nature Biomedical Engineering*

b. U.S. Patent Application No. 63/148,901.