REPORT DOCUMENTATION PAGE

Form Approved OMB NO. 0704-0188

1. REPORT DATE (DD-MM-YYYY)	2. REPORT TYPE	3. DATES COVERED (From - To)
23-03-2021	Final Report	8-Dec-2017 - 31-Dec-2020

4. TITLE AND SUBTITLE
Final Report: Nano- and Bio-Electronics: Semiconductor-enabled exploration of bioelectric properties of organelles

5a. CONTRACT NUMBER
W911NF-18-1-0042

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER
611102

6. AUTHORS

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS (ES)
U.S. Army Research Office
P.O. Box 12211
Research Triangle Park, NC 27709-2211

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

11. SPONSOR/MONITOR'S REPORT NUMBER(S)
71516-EL.14

12. DISTRIBUTION AVAILIBILITY STATEMENT
Approved for public release; distribution is unlimited.

13. SUPPLEMENTARY NOTES
The views, opinions and/or findings contained in this report are those of the author(s) and should not contrued as an official Department of the Army position, policy or decision, unless so designated by other documentation.

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	15. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Bozhi Tian
UU	UU	UU	UU		19b. TELEPHONE NUMBER 857-998-7984

RPPR Final Report
as of 30-Mar-2021

Agency Code:

Proposal Number: 71516EL **Agreement Number: W911NF-18-1-0042**
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Report Date: 31-Mar-2021 Date Received: 23-Mar-2021
Final Report for Period Beginning 08-Dec-2017 and Ending 31-Dec-2020
Title: Nano- and Bio-Electronics: Semiconductor-enabled exploration of bioelectric properties of organelles
Begin Performance Period: 08-Dec-2017 **End Performance Period:** 31-Dec-2020
Report Term: 0-Other

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Distribution Statement: 1-Approved for public release; distribution is unlimited.

STEM Degrees: 6 **STEM Participants:** 7

Major Goals: Intracellular signaling is the basis of the biological activity and cellular processes. Traditionally, our knowledge about intracellular dynamics has been limited to biochemical and transcriptional pathways. However, studies over the last several decades have demonstrated that single cells also use electrical and mechanical signals for processing intracellular information. These signals can be manifested as rapid voltage changes across intracellular membranes or as localized force generation within cytoskeleton network. Additionally, the intracellular electrical and mechanical properties and dynamics tend to be rather inhomogeneous. For instance, action potentials in nerve cells are typically initiated at the axon hillock and the resultant voltage pulse travels down the myelinated axon to the nerve terminal.

To understand and then modulate intracellular biophysical activity, tools that are minimally invasive, display high spatiotemporal resolution, and exhibit large signal-to-noise are necessary. Although optical methods have attempted to address these challenges for decades, their application has been limited to only a few areas; such as plasma membrane voltage recording, changes in intracellular ion concentrations, force mapping near focal adhesions, and optogenetic control of cell excitability. Implantable electronics have also been used in numerous cellular modulations. While these tools have been clinically beneficial in the intended patient populations, the fundamental mechanisms by which they are able to elicit therapeutic effects at the cellular and subcellular levels remain elusive.

Our proposed research aims to address these fundamental questions by investigating the bioelectric dynamics of individual organelles and their networks with nanoscale silicon-based electronic, optoelectronic and thermal devices, which would all yield changes of the bioelectric environment near single organelles.

To realize the central goal of the project, we have synthesized and characterized nanoscale semiconductor materials that are uniquely suited for modulating subcellular components. For direct intracellular interfaces, we have explored different surface chemistry for the nanostructures to promote their internalization and subsequent subcellular targeting. We have constructed semiconductor-based nanoscale electrical capacitor, photoelectrochemical cell, and heater as wireless, localized subcellular stimulators for single cells. We have used optical imaging and various biological assays to study how optical stimulations affect local bioelectric behavior of organelles.

Our work has addressed a few scientific questions in intracellular signaling, organelle dynamics, and spatiotemporal (up to nanometer spatial resolution and microsecond temporal resolution) organization of bioelectrical pathways; all being enabled by biocompatible semiconductor-based bioelectrical modulations. Additionally, the bioelectrical mechanisms being uncovered in this work will become powerful new “building blocks” for efforts in the emerging field of synthetic biology – representing a new physical tool for biologists and bioengineers. Finally, beyond gaining fundamental understanding, our work may enable new non-invasive treatment of certain diseases that can meet the Army need, such as large area wound healing, and traumatic brain

injury.

Accomplishments: Our team has taken several approaches for studying subcellular bioelectrical dynamics, ranging from laser-assisted semiconductor preparation to cellular composites construction, to biophysical studies across different length scales. The specific details are as follows.

(1) Photothermal modulation of cellular activities with porous silicon nanowires (SiNWs)
Engineered silicon-based materials can display photoelectric and photothermal responses under light illumination, which may lead to further innovations at the silicon-biology interfaces. Silicon nanowires have small radial dimensions, promising as highly localized cellular modulators, however, the single crystalline form typically has limited photothermal efficacy due to the poor light absorption and fast heat dissipation. In this work, we identified strategies to improve the photothermal response from silicon nanowires (SiNWs) by introducing nanoscale textures on the surface and in the bulk. The improved photothermal effect allows high-resolution extracellular modulation of calcium dynamics in a number of mammalian cells including glial cells, neurons, and cancer cells. The new materials may be broadly used in probing and modulating electrical and chemical signals at the sub-cellular length scale, currently a challenge in the field of electrophysiology and cellular engineering.

(2) Si nanowires and micropillars for oligodendrocyte-enabled neural interfaces
The development of functioning neuronal circuitry in the brain relies on the formation of a myelin sheath around the axons. The myelin sheath serves as an insulator, thus increasing the propagation velocity of the action potential through the axon. During the developmental stages of the brain, the myelination process is comprised of cascades of events that are governed by myelinating cells called oligodendrocytes. During the process, the oligodendrocytes perform axon selection for myelination and demyelination; this decision-making has been proposed to be dependent upon electrical activity. Although extensive efforts were made to investigate the role of intracellular calcium handling, the degree of which these transients are induced by the neuronal activity or independently by the oligodendrocytes is unclear. Our lab recently established a novel Si-based approach that will allow us to investigate this in a simple, temporarily and spatially controlled manner. We have designed two independent methodologies to investigate the effect of electrical activity that originates from the axon or the oligodendrocytes themselves.

(3) Intracellularly integrated Si-myofibroblast hybrid as a living bioelectric device
Recently, the Tian lab demonstrated that the localized and cell-specific photo-stimulation produced by this optoelectronic SiNW hybridization cell system enables intracellular interrogation with sub-micron resolution, and cell-specific interrogation in different heterogenous co-culture, namely cardiac and neuronal cells. We characterize the spontaneous SiNW internalization process in myofibroblasts (MFs), determine that the hybridized cells can undergo cell division, and confirm that the SiNWs remain internalized when the MFs are co-cultured with cardiomyocytes (CMs). We show that photo-stimulation of the internalized SiNWs can be utilized for local intracellular interrogation within the MFs at sub-micron resolution. We demonstrate the ability of the hybridized MFs to electrically couple with co-cultured CMs and report that photo-stimulation of SiNWs within an MF alters the electrical activity of co-cultured CMs.

(4) Kinked Si nanowires for studying the rotational dynamics at the biointerfaces
The Tian lab reported the development of a widely applicable tool that measures the rolling motion of nanowires by tracking changes in the projected length of a short arm grown on the nanowire. We present a particle detection algorithm with sub-pixel resolution and image processing with principle component analysis that enables precise and automated detection of the nanowire. Furthermore, the versatility of these algorithms will allow them to be extended to processing other nanoscale objects with various geometries. In our proof of concept demonstration, we reported that the nanowires' rolling dynamics are significantly affected by their surroundings and showcase the probes' ability to reflect different cellular behaviors. Specifically, we tracked the rolling motion of the kinked nanowire by measuring the projected lengths of the nanowire arm on the microscope detection plane. While reminiscent of the bent microtubule, SiNWs provide better structural stability and tunable rigidity.

(5) Semiconducting polymer nanofibers as extracellular matrices for subcellular modulation
We recently evaluated the effect of photothermal stimulation from Extracellular matrices (ECM)-like P3HT nanofibers on the calcium loading in mitochondria and other subcellular structures. The process is likely enabled by the activation of TRPV1 ion channels. We discovered that the mitochondrial calcium loading happens on a time scale of minutes, whose dynamics becomes faster as optical stimulation power is progressively increased. In most cases, the mitochondrial loading is followed by the formation of apoptotic bodies. We also studied the Caspase signaling as a function of the stimulation power. This work enables us to quantify photothermal stimulation effects with an ECM-like semiconductor, providing new insights into the control of intracellular calcium and bioenergetics at

the organelle level.

(6) Silicon carbide-based nanostructures for optically enabled subcellular modulation
We demonstrated two- and three-dimensional (2D and 3D) laser patterning of SiC using polydimethylsiloxane (PDMS) as a precursor. Laser ablation creates a dense SiC layer, which is connected to the insulating PDMS matrix by a loose graphite network. By controlling lasing conditions such as power and scan speed, we produce composites with surface, trench, and cut-through geometries. The laser-assisted process incorporates nitrogen as an n-type dopant in SiC. Together with the graphite network, the SiC in the flexible composite shows pseudocapacitive electrochemical behavior and photoelectrochemical activity. We studied the calcium signals of individual cells in an ensemble of smooth muscle cells following photoelectrochemical stimulation from cell-interfaced SiC. We analyzed the calcium signals of individual cells in the region of stimulation and classified the cells as stationary or oscillatory. These perturbations in oscillatory responses are consistent with experimental and theoretical studies of the behavior of smooth muscle following an increase in inositol triphosphate (IP3) levels.

(7) Theoretical studies of the intracellular photothermal laser heating of biocompatible nanoparticles
Recently, Stroscio and Dutta used the dual-phase lag (DPL) heat transport model which considers nanoscale heating effects neglected by the frequently used Fourier's Law and introduces imperfect thermal interfacial contacts at the nanostructure/cytosol boundaries to accurately model heat transport between a photothermally excited nanostructure to the surrounding intracellular environment. After verification of our model, Stroscio and Dutta then proceed to model the laser heating of Au nanoparticles (NPs), Si NPs, and SiC NPs in the cytosol with various lasers for optimization of the problem based on the desired temperature around the nanostructure in time and space. These results also provide insights on the proper selection of material and laser parameters based off the desired outcome. We believe this model is generally applicable for future biomedical and bioengineering research into nanoscale bio-interfaces.

Training Opportunities: Several Tian lab members have been funded on this project. The researchers' development has been enhanced through a program of structured mentoring activities. The goal of the mentoring program is to provide the skills, knowledge, and experience to prepare the researchers to excel in their career path. Specific elements of the mentoring plan will include:

- Seminars and workshops on how to write competitive proposals, offered by the Chemistry Department or Materials Research Science and Engineering Center (MRSEC) at the University of Chicago.
- Participation in seminars and workshops on teaching and learning, conducted by the Center for Teaching and Learning at the University of Chicago (http://teaching.uchicago.edu/).
- Co-teaching a graduate course (i.e., Materials Chemistry-II, CHEM 391) where written feedback from the students has been provided.
- Training and mentoring in the responsible conduct of research, offered by the PI's annual research meetings. The meeting has been following the online course at citiprogram.org. The topics include research misconduct, data management and sharing, publication practices and responsible authorship, peer review, mentor and trainee responsibilities, conflict of interest, and collaborative research. In particular, the exercises in the online course serve as a starting point to discuss proper research conduct as related to the projects in this proposal.
- Travels to conferences, i.e., 2018, 2019, and 2020 fall Materials Research Society Annual Meeting, where the researchers (e.g., Youjin Lee and Yin Fang) presented a poster and gave a talk at the conference.
- Participation in workshops related to career development such as how to apply for a faculty position, career paths outside of academia, tips for negotiating salary and start-up funds, how to plan an independent research agenda. The workshops have been offered by the Physical Sciences Division at the University of Chicago.
- Participation in the PI's weekly research group meetings, in which the researchers have been presenting their research regularly; feedback and coaching were given to help them develop communication and presentation skills.

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Results Dissemination: We have used several ways to disseminate our results to the communities of interest. First and foremost, over past three years of support, the PI had 26 on-site visits to Universities and 6 virtual conferences and symposiums, and discussed some of these results.
Second, the supported personnel have traveled to and presented the research in multiple national or international conferences.
Third, we have 13 papers published, 1 in submission. They are:
1. W. Troy, B. Z. Tian, M. Dutta, and M. Stroscio, Intraellular photothermal laser heating of biocompatible nanoparticles for biomedical and bioengineering applications. Manuscript submitted.
2. Y. Fang, H. Rotenberg, A. Prominski, L. Meng, H. A. Ledesma, Y. Lv, J. Yue, E. Schaumann, J. Jeong, N. Yamamoto, Y. Jiang, B. Elbaz, W. Wei, B. Z. Tian, Micelle-enabled self-assembly of hierarchical and flexible micro-supercapacitor for bioelectric modulation." Nature Nanotechnology, 2021, 16, 206-213.
3. V. Nair, J. Yi, D. Isheim, M. Rotenberg, L. Meng, F. Shi, X. Chen, X. Gao, A. Prominski, Y. Jiang, J. Yue, C. T. Gallagher, D. N. Seidman, and B. Z. Tian, Laser writing of nitrogen-doped silicon carbide for biological modulation. Science Advances, 2020, 6 (34), eaaz2743.
4. G. Shreya, Y. H. Chen, A. George, M. Dutta, and M. A. Stroscio, Fluorescence resonant energy transfer-based quantum dot sensor for the detection for calcium ions. Frontiers in Chemistry, 2020, 8, 594.
5. Y. Fang, L. Y. Meng, A. Prominski, E. N. Schaumann, M. Seebald, B. Z. Tian, Recent advances in bioelectronics chemistry. Chemical Society Reviews, 2020, 49, 7978-8035.
6. Y. V. Lee, D. Wu, Y. Fang, Y. Peng, and B. Z. Tian, Tracking longitudinal rotation of silicon nanowires for biointerfaces. Nano Letters, 2020, 20, 3852-3857.
7. M. Y. Rotenberg, B. Elbaz, V. Nair, E. N. Schaumann, N. Yamamoto, N. Sarma, L. Matino, F. Santoro, B. Z. Tian, Silicon nanowires for intracellular optical interrogation with subcellular resolution. Nano Letters, 2020, 20, 1226-1232.
8. M. Y. Rotenberg, N. Yamamoto, E. N. Schaumann, L. Matino, F. Santoro, B. Z. Tian, Living myofibroblast-silicon composites for probing electrical coupling in cardiac systems. Proc. Natl. Acad. Sci. USA, 2019, 116, 22531-22539.
9. H. Acaron Ledesma, X. Li, J. Carvalho-de-Souza, W. Wei, F. Bezanilla, B. Z. Tian, An atlas of nano-enabled neural interfaces. Nature Nanotechnology, 2019, 14, 645-657.
10. B. Z. Tian, C. M. Lieber, Nanowired bioelectric interfaces. Chemical Reviews, 2019, 119, 15, 9136-9152.
11. Y. W. Jiang, R. Parameswaran, X. Li, J. L. Carvalo-de-Souza, X. Gao, L. Meng, F. Bezanilla, G. M. G. Shepherd, B. Z. Tian, Nongenetic optical neuromodulation with silicon-based materials. Nature Protocols, 2019, 14, 1339–1376.
12. Y. V. Lee, B. Z. Tian, Learning from solar energy conversion: Biointerfaces for artificial photosynthesis and biological modulation. Nano Letters, 2019, 19, 2189-2197.
13. Y. W. Jiang, B. Z. Tian, Inorganic semiconductor biointerfaces. Nature Reviews Materials, 2018, 3, 473-490.
14. R. Parameswaran, K. Koehler, M. Rotenberg, M. Burke, J. Kim, K.-Y. Jeong, B. Hissa, M. Paul, K. Moreno, N. Sarma, T. Hayes, E. Sudzilovsky, H.-G. Park, B. Z. Tian, Optical stimulation of cardiac cells with a polymer-supported silicon nanowire matrix. PNAS, 2018, 116, 413-421.
15. Y. Fang, Y. W. Jiang, H. A. Ledesma, J. Yi, X. Gao, D. E. Weiss, F. Shi, B. Z. Tian, Texturing silicon nanowires for highly localized optical modulation of cellular dynamics. Nano Letters, 2018, 18, 4487-4492.

Honors and Awards: Bozhi Tian received an honor from the Chemical Society Reviews: 2020 Emerging Investigators.
Menahem Rotenberg received a Kharasch Postdoctoral Award from the University of Chicago.
Youjin Lee received a Windt Travel Award from the University of Chicago.
Bozhi Tian received the runner-up of Science & PINS Prize for neuromodulation.
Youjin Lee, received the Martha and Joseph Chenicek Graduate fellowship.

Protocol Activity Status:

Technology Transfer: Nothing to Report

PARTICIPANTS:

Participant Type: Other Professional
Participant: CLEMENTENE CLAYTON
Person Months Worked: 2.00 **Funding Support:**
Project Contribution:

International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Undergraduate Student
Participant: CHARLES GALLAGHER
Person Months Worked: 2.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Graduate Student (research assistant)
Participant: KELLIANN KOEHLER, KELLIANN
Person Months Worked: 3.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Graduate Student (research assistant)
Participant: YOUJIN LEE
Person Months Worked: 9.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Graduate Student (research assistant)
Participant: VISHNU NAIR
Person Months Worked: 13.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Graduate Student (research assistant)
Participant: ANDREW PHILLIPS
Person Months Worked: 5.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Postdoctoral (scholar, fellow or other postdoctoral position)
Participant: MENAHEM ROTENBERG
Person Months Worked: 4.00 **Funding Support:**

Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Graduate Student (research assistant)
Participant: ERIK SCHAUMANN
Person Months Worked: 3.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: PD/PI
Participant: Bozhi Tian
Person Months Worked: 6.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Undergraduate Student
Participant: HERBERT WANG
Person Months Worked: 2.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

ARTICLES:

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as of 30-Mar-2021

Publication Type: Journal Article Peer Reviewed: Y **Publication Status:** 1-Published
Journal: Nano Letters
Publication Identifier Type: DOI Publication Identifier: 10.1021/acs.nanolett.8b01626
Volume: 18 Issue: 7 First Page #: 4487
Date Submitted: 10/5/18 12:00AM Date Published: 6/1/18 5:00AM
Publication Location:
Article Title: Texturing Silicon Nanowires for Highly Localized Optical Modulation of Cellular Dynamics
Authors: Yin Fang, Yuanwen Jiang, Hector Acaron Ledesma, Jaeseok Yi, Xiang Gao, Dara E. Weiss, Fengyuan S
Keywords: biointerface; calcium imaging; membrane depolarization; metal-assisted chemical etching; photothermal; porous materials; Silicon nanowire; vapor?liquid?solid growth; wireless cellular modulation
Abstract: Engineered silicon-based materials can display photoelectric and photothermal responses under light illumination, which may lead to further innovations at the silicon–biology interfaces. Silicon nanowires have small radial dimensions, promising as highly localized cellular modulators, however the single crystalline form typically has limited photothermal efficacy due to the poor light absorption and fast heat dissipation. In this work, we report strategies to improve the photothermal response from silicon nanowires by introducing nanoscale textures on the surface and in the bulk. We next demonstrate high-resolution extracellular modulation of calcium dynamics in a number of mammalian cells including glial cells, neurons, and cancer cells. The new materials may be broadly used in probing and modulating electrical and chemical signals at the subcellular length scale, which is currently a challenge in the field of electrophysiology or cellular engineering.

Acknowledged Federal Support: **Y**

Nano- and Bio-Electronics: Semiconductor-enabled exploration of bioelectric properties of organelles

Contract Number: W911NF1810042

Final progress report

Submitted to

ARO, USA

Attention: Dr. Albena Ivanisevic

Research Area: the Electronics Division

By

PI: Bozhi Tian

Department of Chemistry, the James Franck Institute, and the Institute for Biophysical Dynamics

The University of Chicago,

929 E 57th Street, IL 60637

Sub-award PI: Michael Stroscio, University of Illinois at Chicago

Sub-award Co-PI: Mitra Dutta, University of Illinois at Chicago

A. What were the major goals and objectives of the project?

Intracellular signaling is the basis of the biological activity and cellular processes. Traditionally, our knowledge about intracellular dynamics has been limited to biochemical and transcriptional pathways. However, studies over the last several decades have demonstrated that single cells also use electrical and mechanical signals for processing intracellular information. These signals can be manifested as rapid voltage changes across intracellular membranes or as localized force generation within cytoskeleton network. Additionally, the intracellular electrical and mechanical properties and dynamics tend to be rather inhomogeneous. For instance, action potentials in nerve cells are typically initiated at the axon hillock and the resultant voltage pulse travels down the myelinated axon to the nerve terminal.

To understand and then modulate intracellular biophysical activity, tools that are minimally invasive, display high spatiotemporal resolution, and exhibit large signal-to-noise are necessary. Although optical methods have attempted to address these challenges for decades, their application has been limited to only a few areas; such as plasma membrane voltage recording, changes in intracellular ion concentrations, force mapping near focal adhesions, and optogenetic control of cell excitability. Implantable electronics have also been used in numerous cellular modulations. While these tools have been clinically beneficial in the intended patient populations, the fundamental mechanisms by which they are able to elicit therapeutic effects at the cellular and subcellular levels remain elusive.

Our proposed research aims to address these fundamental questions by investigating the bioelectric dynamics of individual organelles and their networks with nanoscale silicon-based electronic, optoelectronic and thermal devices, which would all yield changes of the bioelectric environment near single organelles.

To realize the central goal of the project, we have synthesized and characterized nanoscale semiconductor materials that are uniquely suited for modulating subcellular components. For direct intracellular interfaces, we have explored different surface chemistry for the nanostructures to promote their internalization and subsequent subcellular targeting. We have constructed semiconductor-based nanoscale electrical capacitor, photoelectrochemical cell, and heater as wireless, localized subcellular stimulators for single cells. We have used optical imaging and various biological assays to study how optical stimulations affect local bioelectric behavior of organelles.

Our work has addressed a few scientific questions in intracellular signaling, organelle dynamics, and spatiotemporal (up to nanometer spatial resolution and microsecond temporal resolution) organization of bioelectrical pathways; all being enabled by biocompatible semiconductor-based bioelectrical modulations. Additionally, the bioelectrical mechanisms being uncovered in this work will become powerful new “building blocks” for efforts in the emerging field of synthetic biology – representing a new physical tool for biologists and bioengineers. Finally, beyond gaining fundamental understanding, our work may enable new non-invasive treatment of certain diseases that can meet the Army need, such as large area wound healing, and traumatic brain injury.

B. A description of what was accomplished under the goals during the total award period.

Our team has taken several approaches for studying subcellular bioelectrical dynamics, ranging from laser-assisted semiconductor preparation, to cellular composites construction, to biophysical studies across different length scales. The specific details are as follows.

(1) Photothermal modulation of cellular activities with porous silicon nanowires (SiNWs)

Engineered silicon-based materials can display photoelectric and photothermal responses under light illumination, which may lead to further innovations at the silicon-biology interfaces. Silicon nanowires have small radial dimensions, promising as highly localized cellular modulators, however the single crystalline form typically has limited photothermal efficacy due to the poor light absorption and fast heat dissipation. In this work, we identified strategies to improve the photothermal response from silicon nanowires (SiNWs) by introducing nanoscale textures on the surface and in the bulk. The improved photothermal effect allows high-resolution extracellular modulation of calcium dynamics in a number of mammalian cells including glial cells, neurons and cancer cells. The new materials may be broadly used in probing and modulating electrical and chemical signals at the sub-cellular length scale, currently a challenge in the field of electrophysiology and cellular engineering.

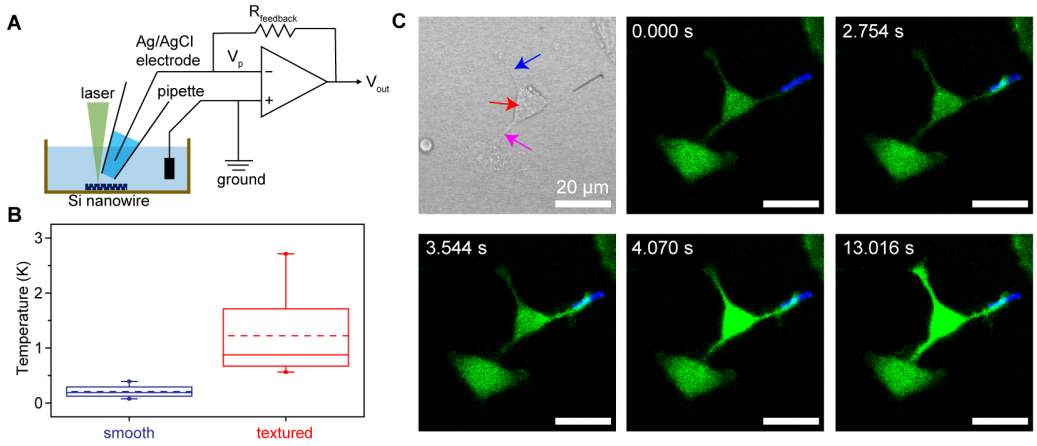

Fig. 1. Porous SiNW for cellular modulation. (**A**) Photothermal measurement setup. (**B**) Photothermal response. (**C**) Cellular modulation.

To confirm the potential use of textured i-SiNWs for freestanding cellular modulation, we first assessed the physicochemical responses of these SiNWs to laser illumination in saline. Individual photo-responses can then be extrapolated from all the measurements and compared between textured and smooth nanowires (**Figs. 1A, 1B**). From the recorded ionic current dynamics from both textured and smooth SiNWs, the fitted intercept values of the $\Delta I_{light}(t)$-I_0 curves stay nearly zero throughout the entire illumination period, indicating minimal contributions from the photoelectric effect of i-SiNWs. On average, the textured i-SiNWs demonstrate larger photothermal responses (~ 2 °C) than the smooth i-SiNWs (~ 0.2 °C) over the 1-ms illumination. The porosity in the textured SiNWs likely contributes to the enhanced thermal output, due to reduced thermal conductivity, heat capacity and enhanced light absorption.

Laser illumination of a textured i-SiNW in close contact with a glial cell protrusion elicits rapid calcium surges initiated at the stimulation site, likely related to a series of coupled biophysical processes (**Fig. 1C**). The locally generated calcium concentration gradient can drive the propagation of the signal

inside the cell by going through the cell body and then to other branches of protrusions (**Fig. 1C**). Besides glial cells, SiNWs can also interface with DRG neurons for direct neuromodulations. Upon laser illumination of the Si/neuron junction, calcium dynamics can be evoked in a similar manner through either voltage or mechano-sensitive calcium channels expressed on the neuron membrane.

(2) Si nanowires and micropillars for oligodencyte-enabled neural interfaces

The development of functioning neuronal circuitry in the brain relies on the formation of myelin sheath around the axons. The myelin sheath serves as an insulator, thus increasing the propagation velocity of the action potential through the axon. During the developmental stages of the brain, the myelination process is comprised of cascades of events that are governed by myelinating cells called oligodendrocytes. During the process, the oligodendrocytes perform axon selection for myelination and demyelination; this decision-making has been proposed to be dependent upon electrical activity. Although extensive efforts were made to investigate the roll of intracellular calcium handling, the degree of which these transients are induced by the neuronal activity or independently by the oligodendrocytes are unclear. Our lab recently established a novel Si-based approach that will allow us to investigate this in a simple, temporarily and spatially controlled manner. We have designed two independent methodologies to investigate the effect of electrical activity that originates from the axon or the oligodendrocytes themselves.

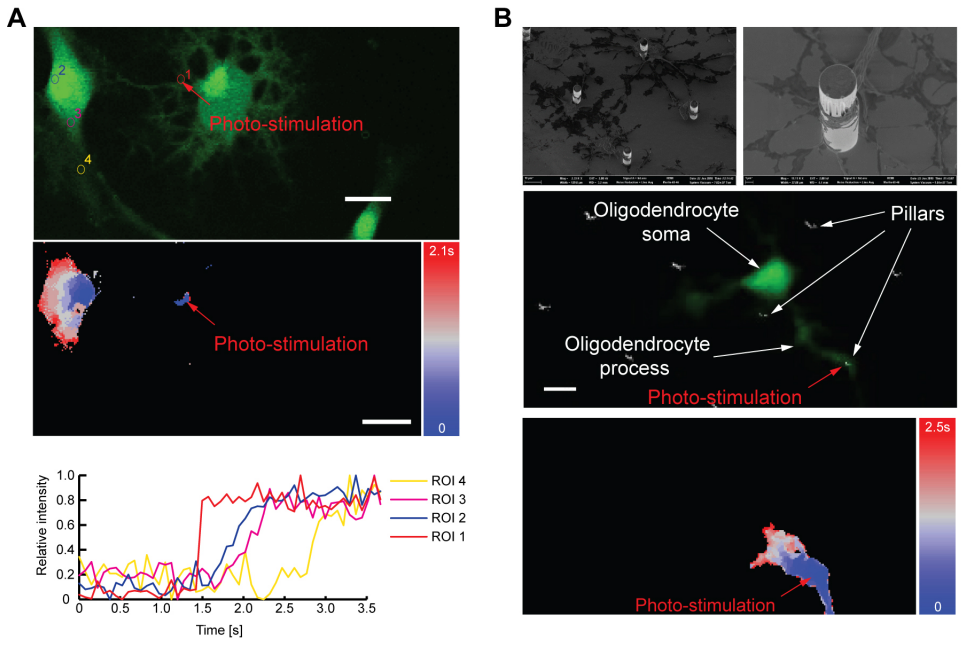

Fig. 2. Intracellular (**A**) and extracellular (**B**) interfaces between Si and oligodendrocytes. Optical stimulation through Si can elicit responses in oligodendrocytes and neurons.

We isolated oligodendrocyte progenitor cells (OPCs) and expanded them *in vitro*. During the expansion, we seeded Si nanowires that were spontaneously internalized into the OPCs. Then the OPCs were seeded on a culture of DRG neurons and were differentiated into oligodendrocytes. After three days, we used the pre-internalized Si nanowires to perform local, cell specific stimulation of the

oligodendrocytes. Optical mapping of the calcium flux generated by this stimulation shows a propagating wave originating from the oligodendrocyte soma and propagating to the DRG neuron (**Fig. 2**).

Additionally, to investigate the effect of axonal electrical activity on myelination, we fabricated a Si micropillar array that interacted directly with oligodendrocytes (**Fig. 2**). We used p-type doped Si that allows for spatially-controlled photoelectrochemical stimulation. The oligodendrocytes interacted with the micropillars and different stages of pillar myelinations were observed. We are currently investigating whether these pillars can be used as artificial axons with photo-modulated electrical activity for myelination studies.

(3) Intracellularly integrated Si-myofibroblast hybrid as a living bioelectric device

Intracellular bioelectric interrogation requires the undisruptive introduction of an electrical probe into the cell cytosol. Micropipette electrodes, currently the most widely used tool for intracellular investigations, cannot be used for prolonged times due to mechanical instabilities and the cytosol dilution effect. Nano-electrode arrays and field effect transistors, powerful new tools for intracellular multiplexed measurements, are still substrate-bound and therefore cannot be applied long term in motile biological systems neither. Optogenetics presents unique modulation capabilities, allowing optical control at the cellular scale and eliminating the need for direct contact between the light stimulation device and the cell. However, the need for genetic modifications limits the translational applications of optogenetics, especially to nonhuman primates and other human-relevant models. Moreover, optogenetic methods for electrical interrogation are currently limited to perturbations of light-activated, spatially distributed ion channels. These techniques therefore have limited 3D spatial resolution, and cannot presently be used for intracellular stimulation.

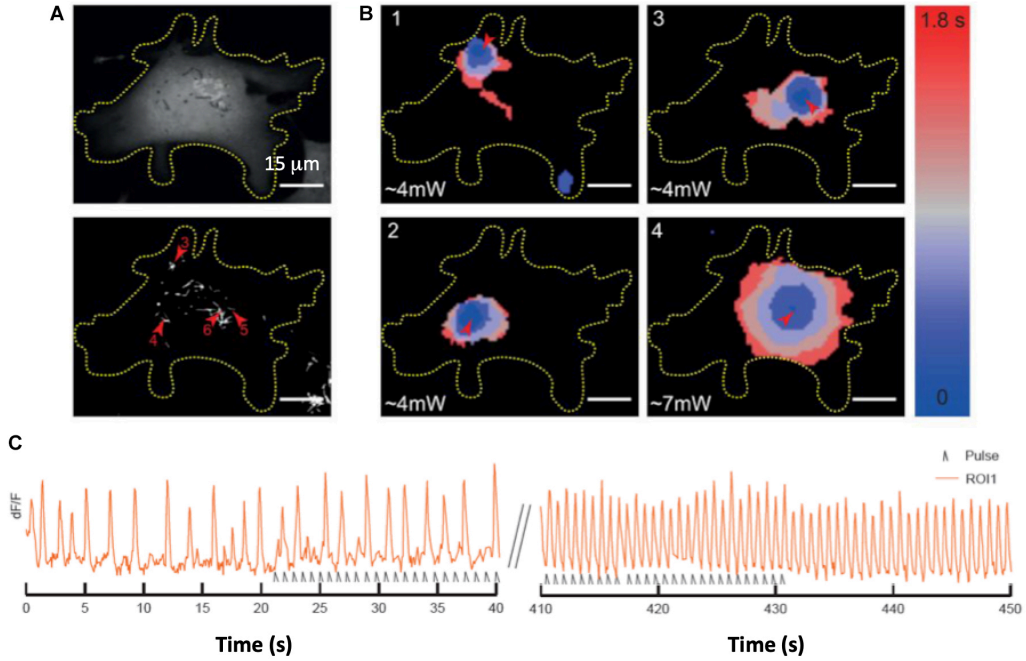

Fig. 3. Silicon nanowire-integrated fibroblasts serve as a biophysical tool to probe the subcellular signal propagation in cardiac modulation. (**A**) Calcium and dark field optical microscope images showing the MF-Si composite. The red arrows mark the intracellular Si for local photo-stimulation. (**B**) Dynamics of

calcium propagation upon photo-stimulation at positions 1-4. (**C**) Calcium imaging traces recorded from CMs, showing the effect of photostimulation from MF-Si composite.

Silicon nanowires (SiNWs) offer local optical bioelectric modulation via photo-electrochemical or photothermal mechanisms. Extracellularly, light-directed stimulation of SiNWs has been used to modulate electrical signals in excitable cells such as neurons and cardiomyocytes (CMs). However, SiNWs are also spontaneously internalized by many cell types, leading us to postulate that they may be pre-hybridized with cells to serve as a non-genetic, intracellular, optoelectronic living system. Photo-stimulation using such a system relies on co-localizing high intensity focused light and a SiNW, which also allows for sub-micron spatial resolution in two and three dimensions. In co-culture, the light reflecting properties of SiNWs allow for the identification of the SiNW-containing cells without need for fluorescent genetic labeling.

Recently, the Tian lab demonstrated that the localized and cell-specific photo-stimulation produced by this optoelectronic SiNW hybridization cell system enables intracellular interrogation with sub-micron resolution, and cell specific interrogation in different heterogenous co-culture, namely cardiac and neuronal cells (**Fig. 3**). We characterize the spontaneous SiNW internalization process in myofibroblasts (MFs), determine that the hybridized cells can undergo cell division, and confirm that the SiNWs remain internalized when the MFs are co-cultured with cardiomyocytes (CMs). We show that photo-stimulation of the internalized SiNWs (**Fig. 3A**) can be utilized for local intracellular interrogation within the MFs at sub-micron resolution (**Fig. 3B**). We demonstrate the ability of the hybridized MFs to electrically couple with co-cultured CMs and report that photo-stimulation of SiNWs within an MF alters the electrical activity of co-cultured CMs (**Fig. 3C**).

(4) Kinked Si nanowires for studying the rotational dynamics at the biointerfaces

Si nanowires can form both extracellular and intracellular biointerfaces (**Fig. 4A**). The rolling motion (i.e. longitudinal rotation) of nanomaterials may serve as a proxy to probe local microscopic environments. Furthermore, nanoscale rotations in biological systems are common but difficult to measure.

Recently, the Tian lab reported the development of a widely applicable tool that measures rolling motion of nanowires by tracking changes in the projected length of a short arm grown on the nanowire. We present a particle detection algorithm with sub-pixel resolution and image processing with principle component analysis that enables precise and automated detection of the nanowire. Furthermore, the versatility of these algorithms will allow them to be extended to processing other nanoscale objects with various geometries. In our proof of concept demonstration, we reported that the nanowires' rolling dynamics are significantly affected by their surroundings and showcase the probes' ability to reflect different cellular behaviors. Specifically, we tracked the rolling motion of the kinked nanowire by measuring the projected lengths of the nanowire arm on the microscope detection plane (**Fig. 4B**). While reminiscent of the bent microtubule, SiNWs provides better structural stability and tunable rigidity. Given their high scattering efficiency, SiNWs can be seen in low magnification without fluorescence staining using dark/bright field microscopy thus preserving fluorescence spectrum for orthogonal measurements. In addition, they are synthesized in one-step *via* the vapor-liquid-solid growth mechanism using a chemical vapor deposition system to display a wide range of lengths, morphologies, and thicknesses, from a few nanometers to a few micrometers. Most importantly, the option to have a narrow (i.e. small thickness) nanowire significantly expands researchers' toolset because it makes the probe sensitive to small mechanical perturbations as shown in the nanowire's cellular uptake and intercellular force study.

Our results showed that a non-interacting nanowire undergoes fast and mostly sub-diffusive rotation, while a cell-interacting nanowire exhibits slow sub-diffusive random rotation when it is fully

encompassed by the cell and super-diffusive unidirectional rotation when the cell actively contracts/expands or initially contacts the nanowire. Our method can be used to yield insights into nano-mechanics in various biophysical and synthetic processes.

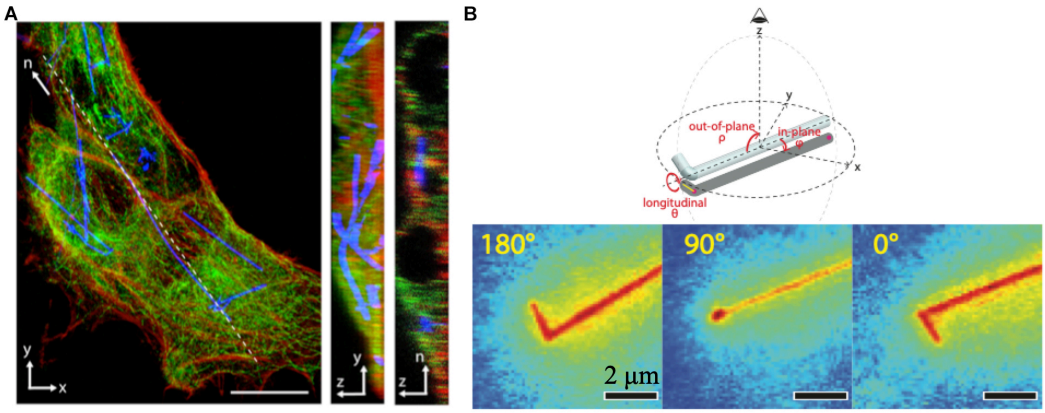

Fig. 4. Kinked silicon nanowires can be used as a probe for extra- and intracellular rotational dynamics studies. (**A**) Confocal fluorescence images showing an intracellular cell/SiNW interface. SiNWs are show in blue. Actin and microtubules are in red and green, respectively. (**B**) By tracking the projected length of the kink, we can study the extra- and intracellular rotation dynamics of the nanowire.

(5) Semiconducting polymer nanofibers as extracellular matrices for subcellular modulation

Extracellular matrices (ECM) can provide cells with structural and biochemical support. Cells interface with ECM through focal adhesions that enable force transduction from the ECM to the cytoskeletal systems and other organelles. Organic semiconductors are promising optically active biomaterials as they are soft, biocompatible and can display direct bandgap. One candidate that has been demonstrated to be useful in bioelectronics is poly-(3-hexyl thiophene), known as P3HT. Tian lab recently synthesized P3HT nanofibers through solution phase process and showed that they have ECM-like mechanical properties and morphology.

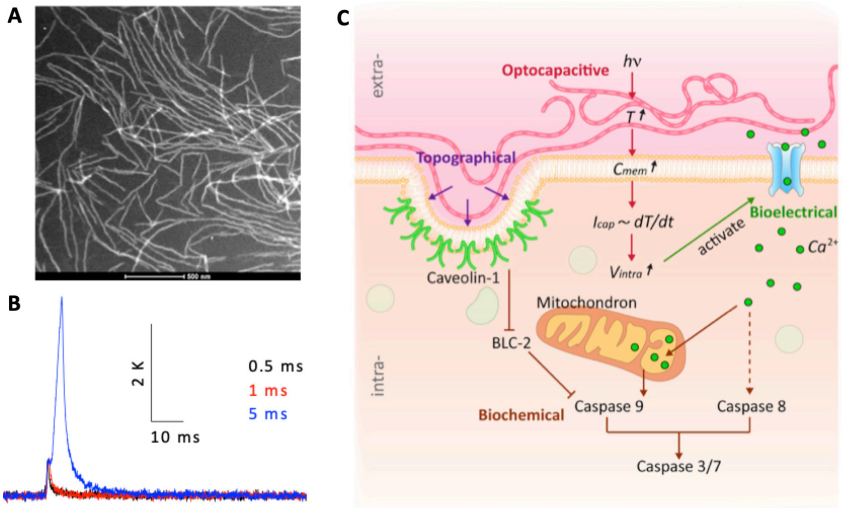

Fig. 5. ECM-like P3HT nanofibers for optical modulation of intracellular activities. (**A**) Scanning transmission electron microscopy images of the P3HT nanofibers. (B) Photothermal recording from the

surface of the P3HT nanofibers. (**C**) Local heating from P3HT triggers TRPV1 ion channel activation, which subsequently induces calcium loading into mitochondria and focal adhesion disassembly. These events yield a series of *Caspase*-based enzymes (*i.e.*, *Caspase* 8, *Caspase* 9 and *Caspase* 3/7) and cell death.

We recently evaluated the effect of photothermal stimulation from ECM-like P3HT nanofibers (**Figs**. **5A** and **5B**) on the calcium loading in mitochondria and other subcellular structures (**Fig. 3**). The process is likely enabled by the activation of TRPV1 ion channels (**Fig. 5C**). We discovered that the mitochondrial calcium loading happens on a time scale of minutes, whose dynamics becomes faster as optical stimulation power is progressively increased. In most cases the mitochondrial loading is followed by the formation of apoptotic bodies. We also studied the *Caspase* signaling as a function of the stimulation power. This work enables us to quantify photothermal stimulation effects with an ECM-like semiconductor, providing new insights into the control of intracellular calcium and bioenergetics at the organelle level.

(6) Silicon carbide-based nanostructures for optically enabled subcellular modulation

Laser-assisted processes are used in material synthesis due to their ease of use, low cost, and unique capacity to generate complex phases. Laser-produced composites can potentially expand the available design principles for materials and devices aimed at biological sensing or modulation. Recently, graphene/graphite-based conducting materials produced by laser writing were used for electrochemical sensing of metabolites in sweat, and further research efforts toward laser-assisted synthesis of conductors for biosensing are anticipated. Recently, we developed a method of using laser-assisted process to prepare silicon carbide (SiC) from elastomer substrates for biomodulation. SiC is much more stable under physiology conditions than Si, suggesting its usage in long term biointerface studies.

We demonstrated two- and three-dimensional (2D and 3D) laser patterning of SiC using polydimethyl siloxane (PDMS) as a precursor. Laser ablation creates a dense SiC layer, which is connected to the insulating PDMS matrix by a loose graphite network. By controlling lasing conditions such as power and scan speed, we produce composites with surface, trench and cut-through geometries. The laser-assisted process incorporates nitrogen as an n-type dopant in SiC. Together with the graphite network, the SiC in the flexible composite shows pseudocapacitive electrochemical behavior and photoelectrochemical activity (**Figs. 6A** and **6B**). Surface functionalization of SiC with manganese dioxide (MnO_2) further enhances the photoelectrochemical activity of the composite (**Fig. 6C**). Finally, we used these SiC-based devices to modulate activity in isolated hearts and cultured cells (**Fig. 6**). Our work suggests that laser writing can efficiently produce flexible and multifunctional semiconductor/elastomer composites for biointerface studies.

More specifically, we studied the calcium signals of individual cells in an ensemble of smooth muscle cells following photoelectrochemical stimulation from cell-interfaced SiC (**Figs. 6D-6F**). We analyzed the calcium signals of individual cells in the region of stimulation and classified the cells as stationary or oscillatory (**Fig. 6E**). These perturbations in oscillatory responses are consistent with experimental and theoretical studies of the behavior of smooth muscle following an increase in inositol triphosphate (IP3) levels.

To demonstrate the potential of integrating laser-produced SiC into a cell culture platform, we stimulated a sheet of smooth muscle cells using a printed trench-like SiC device (**Fig. 6F**) or freestanding 3C-SiC particles. Our results revealed an overall increase in calcium levels and its propagation into adjacent cells as a calcium wave. We also studied the correlation between calcium signals from individual smooth muscle cells in the ensemble.

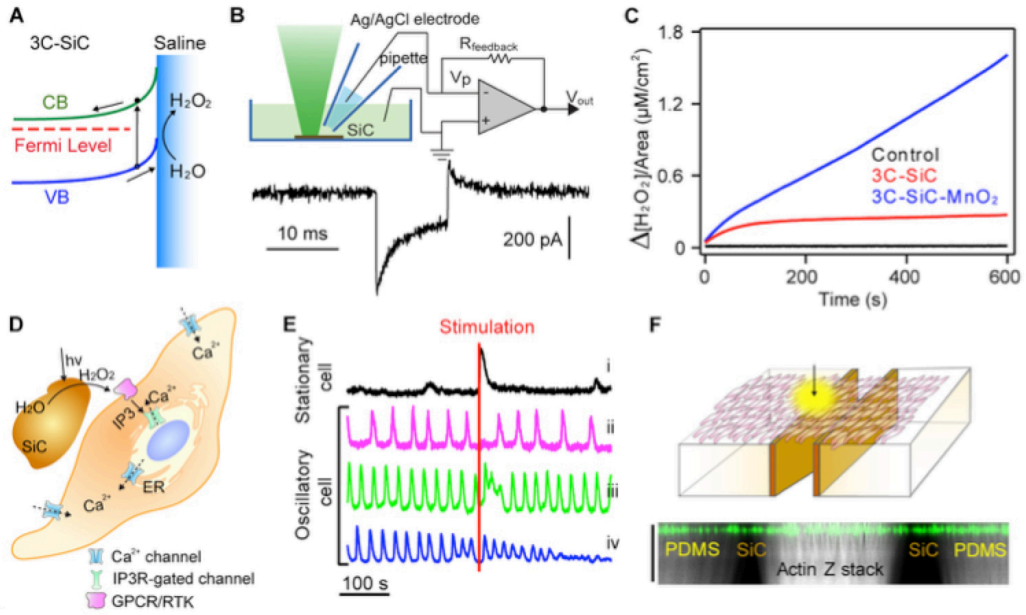

Fig. 6. SiC nanostructures for photoelectrochemical modulation of cells. (**A**) Schematics at the SiC/water interface. (**B**) Photoelectrochemical measurements. (**C**) MnO_2 can enhance the photoelectrochemical output. (**D**) Schematics for the biointerface, showing the light-generated H_2O_2 production. (**E**) Different calcium oscillations upon photostimulation at the SiC/cell interfaces. (**F**) Photostimulation can control smooth muscle bioelectrical activities over a device surface.

(7) Theoretical studies of the intracellular photothermal laser heating of biocompatible nanoparticles

The photothermal heating of nanostructures via lasers has been shown to be able to cause localized temperature increases inside of cells that are able to cause a range of effects and phenomena. These effects range from killing tumors, causing cell blebbing, affecting the permeability and fluidity of the cell membrane, and manipulating intracellular cytoskeletal structures, calcium concentrations, and protein dynamics. Many of these phenomena appear to be associated with the observation that cellular function is very sensitive to temperature changes.

Recently, Stroscio and Dutta used the dual phase lag (DPL) heat transport model which considers nanoscale heating effects neglected by the frequently used Fourier's Law and introduce imperfect thermal interfacial contacts at the nanostructure/cytosol boundaries to accurately model heat transport between a photothermally excited nanostructure to the surrounding intracellular environment. After verification of our model, Stroscio and Dutta then proceed to model the laser heating of Au nanoparticles (NPs), Si NPs, and SiC NPs in cytosol with various lasers for optimization of the problem based on the desired temperature around the nanostructure in time and space. These results also provide insights on the proper selection of material and laser parameters based off the desired outcome. We believe this model is generally applicable for future biomedical and bioengineering research into nanoscale bio interfaces.

C. What opportunities for training and professional development did the project provide?

Several Tian lab members have been funded on this project. The researchers' development has been enhanced through a program of structured mentoring activities. The goal of the mentoring program is to

provide the skills, knowledge and experience to prepare the researchers to excel in their career path. Specific elements of the mentoring plan will include:

- Seminars and workshops on how to write competitive proposals, offered by Chemistry Department or Materials Research Science and Engineering Center (MRSEC) at the University of Chicago.
- Participation in seminars and workshops on teaching and learning, conducted by the Center for Teaching and Learning at the University of Chicago (http://teaching.uchicago.edu/).
- Co-teaching a graduate course (i.e., Materials Chemistry-II, CHEM 391) where written feedback from the students have been provided.
- Training and mentoring in the responsible conduct of research, offered by the PI's annual research meetings. The meeting has been following the online course at citiprogram.org. The topics include research misconduct, data management and sharing, publication practices and responsible authorship, peer review, mentor and trainee responsibilities, conflict of interest, and collaborative research. In particular, the exercises in the online course serve as a starting point to discuss proper research conduct as related to the projects in this proposal.
- Travels to conferences, i.e., 2018, 2019 and 2020 fall Materials Research Society Annual meeting, where the researchers (*e.g.*, Youjin Lee and Yin Fang) presented a poster and gave a talk at the conference.
- Participation in workshops related to career development such as how to apply for a faculty position, career paths outside of academia, tips for negotiating salary and start-up funds, how to plan an independent research agenda. The workshops have been offered by the Physical Sciences Division at the University of Chicago.
- Participation in the PI's weekly research group meetings, in which the researchers have been presenting their research regularly; feedback and coaching were given to help them develop communication and presentation skills.

D. Results Dissemination

We have used several ways to disseminate our results to the communities of interest.

First and foremost, over past three years of support, the PI had 26 on-site visits to Universities and 6 virtual conferences and symposiums, and discussed some of these results.

Second, the supported personnel have traveled to and presented the research in multiple national or international conferences.

Third, we have 13 papers published, 1 in submission. They are:

1. W. Troy, B. Z. Tian, M. Dutta, and M. Stroscio, Intraellular photothermal laser heating of biocompatible nanoparticles for biomedical and bioengineering applications. Manuscript submitted.
2. Y. Fang, H. Rotenberg, A. Prominski, L. Meng, H. A. Ledesma, Y. Lv, J. Yue, E. Schaumann, J. Jeong, N. Yamamoto, Y. Jiang, B. Elbaz, W. Wei, B. Z. Tian, Micelle-enabled self-assembly of hierarchical and flexible micro-supercapacitor for bioelectric modulation." ***Nature Nanotechnology***, 2021, 16, 206-213.
3. V. Nair, J. Yi, D. Isheim, M. Rotenberg, L. Meng, F. Shi, X. Chen, X. Gao, A. Prominski, Y. Jiang, J. Yue, C. T. Gallagher, D. N. Seidman, and B. Z. Tian, Laser writing of nitrogen-doped silicon carbide for biological modulation. ***Science Advances***, 2020, 6 (34), eaaz2743.

4. G. Shreya, Y. H. Chen, A. George, M. Dutta, and M. A. Stroscio, Fluorescence resonant energy transfer-based quantum dot sensor for the detection for calcium ions. ***Frontiers in Chemistry***, 2020, 8, 594.

5. Y. Fang, L. Y. Meng, A. Prominski, E. N. Schaumann, M. Seebald, B. Z. Tian, Recent advances in bioelectronics chemistry. ***Chemical Society Reviews***, 2020, 49, 7978-8035.

6. Y. V. Lee, D. Wu, Y. Fang, Y. Peng, and B. Z. Tian, Tracking longitudinal rotation of silicon nanowires for biointerfaces. ***Nano Letters***, 2020, 20, 3852-3857.

7. M. Y. Rotenberg, B. Elbaz, V. Nair, E. N. Schaumann, N. Yamamoto, N. Sarma, L. Matino, F. Santoro, B. Z. Tian, Silicon nanowires for intracellular optical interrogation with subcellular resolution. ***Nano Letters***, 2020, 20, 1226-1232.

8. M. Y. Rotenberg, N. Yamamoto, E. N. Schaumann, L. Matino, F. Santoro, B. Z. Tian, Living myofibroblast-silicon composites for probing electrical coupling in cardiac systems. ***Proc. Natl. Acad. Sci. USA***, 2019, 116, 22531-22539.

9. H. Acaron Ledesma, X. Li, J. Carvalho-de-Souza, W. Wei, F. Bezanilla, B. Z. Tian, An atlas of nano-enabled neural interfaces. ***Nature Nanotechnology***, 2019, 14, 645-657.

10. B. Z. Tian, C. M. Lieber, Nanowired bioelectric interfaces. ***Chemical Reviews***, 2019, 119, 15, 9136-9152.

11. Y. W. Jiang, R. Parameswaran, X. Li, J. L. Carvalo-de-Souza, X. Gao, L. Meng, F. Bezanilla, G. M. G. Shepherd, B. Z. Tian, Nongenetic optical neuromodulation with silicon-based materials. ***Nature Protocols***, 2019, 14, 1339–1376.

12. Y. V. Lee, B. Z. Tian, Learning from solar energy conversion: Biointerfaces for artificial photosynthesis and biological modulation. ***Nano Letters***, 2019, 19, 2189-2197.

13. Y. W. Jiang, B. Z. Tian, Inorganic semiconductor biointerfaces. ***Nature Reviews Materials***, 2018, 3, 473-490.

14. R. Parameswaran, K. Koehler, M. Rotenberg, M. Burke, J. Kim, K.-Y. Jeong, B. Hissa, M. Paul, K. Moreno, N. Sarma, T. Hayes, E. Sudzilovsky, H.-G. Park, B. Z. Tian, Optical stimulation of cardiac cells with a polymer-supported silicon nanowire matrix. ***PNAS***, 2018, 116, 413-421.

15. Y. Fang, Y. W. Jiang, H. A. Ledesma, J. Yi, X. Gao, D. E. Weiss, F. Shi, B. Z. Tian, Texturing silicon nanowires for highly localized optical modulation of cellular dynamics. ***Nano Letters***, 2018, 18, 4487-4492.

E. Honors or awards received under this project in this reporting period?

Bozhi Tian received an honor from the Chemical Society Reviews: 2020 Emerging Investigators.

Menahem Rotenberg received a Kharasch Postdoctoral Award from the University of Chicago.

Youjin Lee received a Windt Travel Award from the University of Chicago.

Bozhi Tian received the runner-up of Science & PINS Prize for neuromodulation.

Youjin Lee, received the Martha and Joseph Chenicek Graduate fellowship.