Form B: IGEM-HERC Full Proposal Cover Sheet

Idaho State Board of Education

PROPOSAL NUMBER: (to be assigned by HERC)	TOTAL AMOUNT REQUESTED: $165,600.00

Proposal Track (select one):

- Proof of Concept
- Initial Startup ✓
- Innovation

TITLE OF PROPOSED PROJECT:

A microneedle-based transcutaneous *Staphylococcus aureus* vaccine

SPECIFIC PROJECT FOCUS:

The bacterium *Staphylococcus aureus* is a top priority human pathogen that causes sepsis, or bloodstream infections, and is commonly antibiotic-resistant. Despite improved control measures and many vaccine attempts over decades, there is no licensed human vaccine, and *S. aureus* continues to cause significant morbidity and mortality worldwide. This project proposes a collaborative and innovative approach to an *S. aureus* vaccine that can be delivered to the skin to induce local and systemic immunity, and to protect at-risk populations from invasive disease. *We hypothesize that the transcutaneous delivery of a bivalent, enterotoxin-based, S. aureus vaccine (IsdA+ClfA-CTA2/B) will induce antigen-specific immune responses and protect mice against systemic disease*. We propose feasibility studies to construct and characterize a microneedle skin patch containing the *IsdA+ClfA*-CTA2/B vaccine, and assess the immunogenicity and efficacy of this vaccine in preclinical animal studies. Specifically, we will: *1)* construct *S. aureus IsdA+ClfA*-CTA_2/B containing dissolvable microneedles and determine antigen deposition ex vivo, using pig skin, *2)* comprehensively assess the immunogenicity of *IsdA+ClfA*-CTA2/B after transcutaneous delivery in mice, and *3)* determine the efficacy of transcutaneous *IsdA+ClfA*-CTA2/B to protect against *S. aureus* systemic infection in mice. The proposed studies are based upon previous advancements in *S. aureus* vaccine development, but will uniquely incorporate an enterotoxin adjuvant to promote transcutaneous delivery and complex B and T cell immunity. Expected results will establish the feasibility of delivery using a skin patch and the efficacy of this vaccine platform to protect against *S. aureus* disease. Developing an effective *S. aureus* vaccine will significantly impact human health and promote the advancement of related vaccine technologies. Outcomes will support current and future STTR/SBIR funding, and technology transfer to Pentamer Biologics, LLC., a newly established small business located in Idaho.

PROJECT START DATE: July 1, 2024	PROJECT END DATE: June 30, 2025

NAME OF INSTITUTION:	DEPARTMENT:
Boise State University	Biological Science, and Mechanical and Biomedical Engineering

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	NAME:	TITLE:	SIGNATURE:
PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR	Juliette Tinker	Professor	Juliette Tinker
CO-PRINCIPAL INVESTIGATOR	Sophia Theodossiou	Assistant Professor	Sophia K Theodossiou Sophia K Theodossiou (Mar 12, 2024 13:57 MDT) Sophia K Theodossiou

NAME OF PARTNERING COMPANY: Pentamer Biologics, LLC	COMPANY REPRESENTATIVE NAME: Bryan Allinson
SIGNATURE: Bryan Allinson Bryan Allinson (Mar 12, 2024 13:59 EDT)	

Authorized Organizational Representative	NAME: Ella Christiansen	SIGNATURE:

I. Primary Idaho public institution. Boise State University

II. Project title. A microneedle-based transcutaneous *Staphylococcus aureus* vaccine

III. Principal investigator and project-related credentials. Dr. Juliette Tinker is a Professor in the Department of Biological Sciences and the Biomolecular Graduate Program at Boise State University. Dr. Tinker has a Ph.D. in Microbiology and background in pathogenic bacteriology and vaccine development. Since joining Boise State in 2005, her research has focused on bacterial enterotoxins as mucosal vaccine adjuvants. She has published studies on cholera toxin (CT) that characterize its structure/function, technologies for manipulation and purification, and ability to modulate the immune system. Dr. Tinker has been supported by the University of Washington ITHS and the Department of Defense for immunogenicity studies on an enterotoxin-based mucosal *Staphylococcus aureus* vaccine in mice, and also by the USDA to assess vaccine efficacy in cows to prevent mastitis. This research has been collaborative with the University of Idaho, Washington State University and local veterinarians and farmers. Currently Dr. Tinker is supported by a USDA STTR Phase I grant in partnership with a start-up company, Pentamer Biologics. A recent NSF ART (TRANSFORM) seed grant is also supporting Dr. Tinker's vaccine research.

IV. Key personnel and project-related credentials. The Co-P.I., Dr. Sophia Theodossiou, is an Assistant Professor in the Department of Mechanical and Biomedical Engineering (MBE) at Boise State. Dr. Theodossiou has a Ph.D. in Biological Engineering and an interdisciplinary background in tissue engineering, mechanobiology, developmental biology, and biomaterials fabrication. She is uniquely qualified to contribute to the proposed microneedle project. Dr. Theodossiou joined the MBE Department in the fall of 2022. Prior to this, she completed a post-doc with a focus on fabricating mechanically dynamic (i.e. stiffening and softening) biomaterials for understanding how mechanical signals impact musculoskeletal cell development, differentiation, and disease. During this time, she was awarded 2 prestigious NIH fellowships, and began to work with silk microneedles for transcutaneous drug delivery. Dr. Theodossiou is currently a P.I. and Research Project Lead for a Matrix Biology NIH COBRE. She has considerable biomaterials expertise that will be essential for the success of microneedle construction and characterization, as described in Objective 1.

V. Project objectives and total amount requested.
Our long-term goal is to develop a vaccine to reduce or eliminate *S. aureus* invasive disease. *We hypothesize that the transcutaneous delivery of a bivalent S. aureus vaccine (IsdA+ClfA-CTA2/B) will induce antigen-specific immune responses and protect against systemic disease*. For this project, we will construct sugar and silk-based dissolvable microneedles (dMN) containing the *IsdA+ClfA*-CTA2/B vaccine and assess the immunogenicity and protective efficacy of this vaccine in mice. We propose the following three objectives:

A. Objectives

Objective 1: Determine the stability and antigen-delivery capacity of *S. aureus IsdA+ClfA*-CTA2/B dissolvable microneedles. We have previously constructed protein fusions, or chimeras, of the *S. aureus* adhesins, IsdA and ClfA to cholera toxin (*IsdA+ClfA*-CTA2/B). We have also previously constructed silk and sucrose-based dMN and tested them for mechanical stability. We will incorporate *IsdA+ClfA*-CTA2/B into these dMN and assess the stability and capacity of both types of microneedle patches to support antigen deposition, ex vivo, using pig skin. *The milestone of successful IsdA+ClfA-CTA2/B dMN construction will be measured quantitatively as antigen deposition ex vivo determined by fluorescent immunohistochemistry.*

Objective 2: Assess *IsdA+ClfA*-CTA2/B immunogenicity after transcutaneous delivery in mice. To establish vaccine feasibility, we will conduct a pre-clinical trial of dMN containing *IsdA+ClfA*-

CTA2/B in mice to determine antigen-specific B and T cell responses. *The milestone of successful IsdA+ClfA-CTA2/B immunogenicity will be measured quantitatively by immune analysis after vaccination in mice.*
Objective 3: Determine the efficacy of transcutaneous *IsdA+ClfA*-CTA2/B to protect against *S. aureus* systemic infection in mice. We will use a mouse systemic *S. aureus* challenge model to assess the protective efficacy of dMN containing *IsdA+ClfA*-CTA2/B. *The milestone of protective efficacy will be determined by bacterial enumeration in the spleen and kidneys after vaccination and challenge in mice.*

It is expected that outcomes will produce a dissolvable microneedle patch prototype that can be used for transcutaneous vaccination, and provide essential evidence of *IsdA+ClfA*-CTA2/B safety and efficacy in a relevant preclinical model.

B. **Total direct costs requested**: $165,600.00

VI. Resource commitment.

The proposed studies will be performed at Boise State in the Departments of Biological Sciences and Mechanical and Biomedical Engineering. The scientific environment at Boise State is conducive to performing high-level research, and the University's Blueprint for Success includes a commitment to innovation, advancing research and fostering partnerships. Faculty are highly collaborative and participate in NIH-funded, statewide COBRE and INBRE grants that also support the Biomolecular Research Center and stipends for undergraduate researchers. Faculty from multiple departments also participate graduate programs, such as Biomolecular Sciences (BMOL) and Biomedical Engineering (BME). The university-wide NSF ART (TRANSFORM) grant, that started in the spring of 2024, has a specific focus on translational research. The P.I. and Co-P.I. have large laboratory spaces, significant support for budget development and grants management, and material support that includes maintenance of core facilities and instrumentation. Other accomodations that demonstrate University commitment include:
Teaching: A typical research active teaching load in Biological Science consists of 9 credit hours/year. Teaching is supported with graduate teaching assistants (MS, or PhD), who help to run laboratory sections and grade assignments and exams.
Travel: The Department of Biological Sciences typically covers the cost for each faculty member to attend one scientific conference each year, and BMOL will pay for travel for each student. Faculty and students may apply for travel awards through the College of Arts and Sciences.
Graduate Students: Faculty in BMOL and Biological Sciences are provided 3-year graduate teaching assistantships to support Ph.D. and MS students. BMOL supports students for an additional year as a research assistant. Teaching assistantships cover tuition, fees and a stipend.

VII. Specific project plan and timeline.

A. Background and significance

Adjuvants are compounds that are often required in vaccines to stimulate immunity when the vaccine is not made from a live or attenuated organism. There is an urgent need to develop novel adjuvants that can induce immune responses from surfaces, such as the skin, nose and gastrointestinal tract (*reviewed in* [1]). Studies have established that bacterial enterotoxins, such as CT and *E. coli* heat-labile toxin (LT) are among the most effective adjuvants for mucosal and skin vaccination [2-7] . Native CT is especially active at these surfaces, however, it is also toxic. Chimeric fusions, or chimeras, contain the non-toxic domains of CT (CTA2/B) combined with an antigen to form a holotoxin-like molecule [8,9]. CTA2/B chimeras possess unique advantages over other enterotoxin adjuvants as vaccine platforms, these include: *a stable structure, direct attachment to the antigen, and simple purification*. Evidence suggests that the CTA2/B structure is beneficial for antigen uptake into dendritic cells and immune induction [10-16]. The non-toxic CT binding subunit (CTB), which

is the adjuvant component of chimeras, has long been used experimentally, and is a component of the human oral *V. cholerae* vaccine (WC-rBS, Dukoral®). Dukoral® is licensed in over 60 countries and has a strong safety record [17,18]. CTB also has a record of production scale-up [19,20]. We have previously constructed CTA2/B chimeras for use as a vaccine to prevent *S. aureus* disease [13,21,22]. The production and holotoxin-like structure of CTA2/B chimeras is shown in Figure 1. These and related molecules are strong immunomodulators, and have been shown to enhance antigen presentation, upregulate immune cell surface molecules, and stimulate B cell isotype switching [23-25]. CTB can also induce multilayered T-cell immunity, such as: Th1, Th2, and Th17- type CD4+ T cell responses [26-30]. Immunizing through the skin with CTB specifically enhances both local and systemic immunity [5,31].

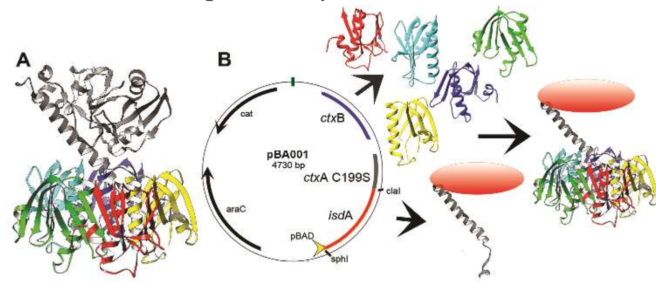


Figure 1. Design and production of the *S. aureus IsdA*-CTA2/B chimeric vaccine. **A)** ribbon diagram of *Vibrio cholerae* cholera toxin, **B)** expression of the *IsdA*-CTA2/B chimera from *E.coli* +pBA001 (pARLDR19).

Each year, 1.7 million U.S. adults develop hospital-acquired sepsis, and nearly 270,000 die as a result [32]. *S. aureus* is a leading cause of this inflammatory condition, and it often occurs because of bloodstream, or systemic, invasion of the bacteria [33]. *S. aureus* is also commonly antibiotic-resistant, produces many virulence factors, and has a complex interaction with the human host. Clinical outcomes can range from skin or wound infections to severe invasive systemic disease. Antibiotic-resistant strains of *S. aureus* are dangerous and costly; causing 19,000 deaths per year and costing up to $40,000 per hospital stay [34,35]. An estimated 80% of *S. aureus* surgical site infections, that often lead to sepsis, arise from endogenous, or commensal, bacteria [36]. Patients entering the hospital for major surgery or dialysis are often tested for *S. aureus* and pre-treated with mupirocin ointment to decolonize nasal passages. While this practice reduces bacterial shedding and the risk of systemic disease, it does not prevent recolonization, and can have negative effects on patient microbiota and exacerbate resistance [37]. *A vaccine that can reduce or eliminate colonization would prevent disease and reduce use of antibiotics: constituting a significant improvement over current clinical practice*. Complex local and systemic immunity, including Th1, Th2 and Th17, is required to reduce *S. aureus* colonization and prevent sepsis [38,39]. CTB can activate local immune cells and induce systemic B and T cell immunity to co-administered antigens delivered to the skin [30,40,41]. We propose that *IsdA+ClfA*-CTA2/B can be delivered to key skin immune cells using a dMN patch. The advantages of this vaccine platform as an *S. aureus* vaccine include: *1)* the potential to induce both local and systemic immunity, *2)* the induction of complex B and T cell immunity, and *3)* a flexible vector for the addition of multiple antigens or serotypes.

B. Preliminary studies

We have constructed multiple chimeras using a vector expressing the CTA2 and CTB peptides (pARLDR19, Figure 1) [42-46]. The *IsdA*-CTA2/B chimera was purified from *E. coli* using D-galactose chromatography. This construct is stable after lyophilization, re-hydration and re-purification (Figure 2). We determined the immunogenicity of *IsdA*-CTA2/B in mice after intranasal immunization and showed induction of systemic Th2-type specific responses. Mucosal IgA was significantly higher when antigen and adjuvant were combined as a chimera (Figure 3) [13,43]. We have also determined the immunogenicity and protection against *S. aureus* in bovines using a mix of clumping factor A (ClfA) and IsdA chimeras (*IsdA+ClfA*-CTA2/B) [21,22]. The vaccine was delivered intranasally and was well-tolerated and safe. Analysis of milk and serum indicated significant antigen-specific antibody, and mixed Th2/Th1 cellular responses. After vaccination and intramammary challenge, all animals shed bacteria, however, vaccinated animals shed less overall,

and the somatic cell count (SCC) of the milk was significantly lower in vaccinated animals. Although the vaccine did not prevent infection, the outcome of lower SCC indicates reduced microbial load and improved milk quality. A second challenge study, supported by a USDA Phase I STTR, is currently underway, and will determine efficacy after intramammary vaccination. A family of patents: *Cholera toxin chimera and its use as a Staph vaccine. U.S. issued 8,834,898 (12/16/11), 8,911,748 (5/17/13), 9,943,582 (8/11/14), 10,576,140(7/3/19)* and *10,383,933 (8/20/19)* cover the *IsdA+ClfA*-CTA2/B vaccine, with a licensing agreement to Pentamer Biologics, LLC.

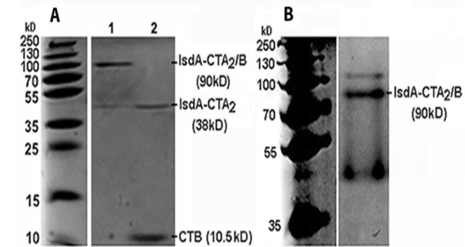


Figure 2. Purification and stability of the *S. aureus IsdA*-CTA_2/B chimera. **A)** SDS-PAGE of D-galactose affinity purified *IsdA*-CTA_2/B: *1)* native and *2)* denatured, and **B)** SDS-PAGE of *IsdA*-CTA_2/B after lyophilization in 20% sucrose and re-purification (native).

C. Research design and methods

Objective 1: Determine the stability and antigen-delivery capacity of *S. aureus IsdA+ClfA*-CTA2/B dissolvable microneedles (dMN). **Hypothesis and rationale:** *We hypothesize that the IsdA+ClfA-CTA2/B vaccine can be incorporated into sugar and silk dMN to deliver antigen transcutaneously.* Microneedle patches are arrays of micrometer-sized needles designed to pierce the stratum corneum, or outer layer, of the epidermis to deliver antigen to immune cells in the underlying dermis. Microneedles can be solid and coated with antigen or made of dissolvable materials containing antigen. This route of vaccine delivery is well-studied with recent advances (*reviewed in* [47,48]). As shown in Figure 4, we have constructed dMN in collaboration with the Idaho Microfabrication Lab. For preparation of a micromold made of polymethylsiloxane (PDMS), a resin stamp was first constructed using stereolithography (SLA) on a MLSA 3D printer [49]. Resulting molds contained ≈10,000 needles of 0.01-0.02μL volume (100-200μL total) in a 10 x 10mm patch (Figure 4B). dMN were made from molds using a two-step drying process [50]. The filling solution of 5% sucrose/1% carboxymethyl cellulose (CMC) was cast into molds under vacuum and dried for 24 hours, with resulting needles having a length of over 400μm (Figure 4D) [51]. Dr. Theodossiou has experience with the construction of silk MN (Figure 4E). Silk is also a biocompatible material that is very stable, can be dissolvable and can be modified for antigen incorporation [52,53]. The stability, flexibility and immune targeting of CTA2/B chimeras make them ideal for incorporation into dMN for transcutaneous vaccine delivery. To establish feasibility, we will assess antigen deposition ex vivo using pig ears. **Experimental design and methods:** Induction from pARLDR19 results in the co-expression of antigen-CTA_2 and CTB (Figure 1). Chimeras will be expressed in the endotoxin-modified *E. coli* strain ClearColi® (Lucigen), isolated and purified using D-galactose resin (ThermoFisher) as described [13]. We will confirm purification and A2/B structure using SDS-PAGE and mass spectrometry. Needles will be prepared as above, but also with 8-10% silk fibroin, and also with a modification to the stamp/mold to create a 350μm needle for delivery to the mouse dermis [54]. 50μg of *IsdA+ClfA*-CTA2/B per 200μL patch will be mixed with filling solution and dried. dMN will be mounted for scanning electron microscopy to assess shape, sharpness, and length after storage under different conditions. After preparation, dMN will be dissolved in buffer to determine protein concentration and stability of CTA2/B. Antigen

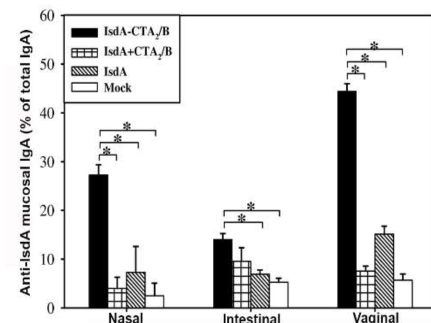
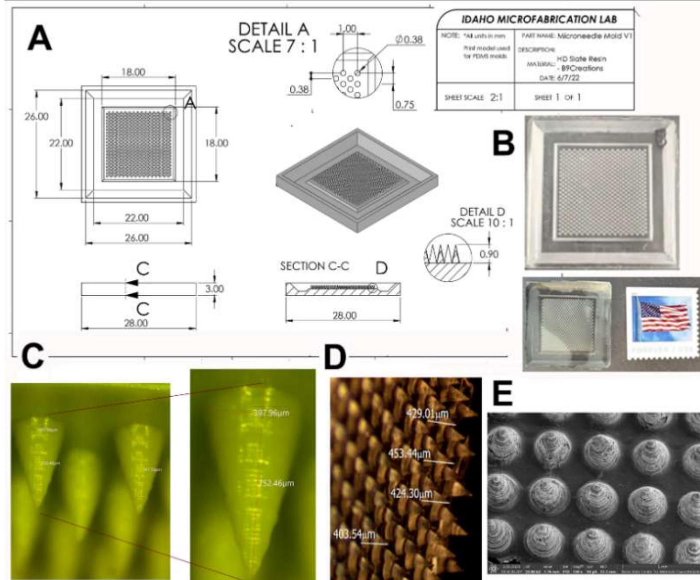


Figure 3. Mucosal response to IsdA-CTA_2/B in mice. Percent IsdA-specific IgA of total in day 45 nasal (1:2), intestinal (1:8), and vaginal (1:16) wash pooled by immunization group (n = 6). Significance ($p < 0.05$ *).

deposition will be assessed with 450µm needles ex vivo using pig ears. Pig ears will be obtained from local butchers and shaved prior to manual application of dMN. Initially patches will be left on skin for different time points up to 2 hours to determine optimal dissolution time [55]. Experimental patch groups will be: *1)* filling solution (FS), *2)* FS + IsdA + ClfA antigens (recombinant, purified as described below), and *3)* FS + *IsdA+ClfA*-CTA2/B. A no-microneedle control will consist of 50µg of *IsdA+ClfA*-CTA2/B directly applied to ear skin. After dMN removal, skin appearance will be assessed by staining with tissue dye and light microscopy. Removed needles will be assayed for protein concentration and composition. Antigen deposition will be determined by preparing thin sections of ear skin for immune staining with anti-CTB (Sigma-Aldrich), anti-ClfA (Cusabio, Houston, TX) anti-CD11c (Thermo-Fisher), and examination by confocal microscopy (Boise State BRC). Assays will be performed in duplicate for an estimated 8 dMN patches. **Expected data and analysis**: Data from this study will include microscopic images and electron micrographs to assess size, structure and reproducibility of dMN. Force and puncture testing will also be performed to determine dMN stability and sharpness. Incorporation of *IsdA+ClfA*-CTA2/B into stable dMN will be determined by protein analysis of dissolved patches. Immuno-histochemistry of pig ear skin will provide evidence of antigen deposition into dermal layers near dendric cells (anti-CD11c), and will be determined quantitatively by fluorescence microscopy. *Objective 1 is expected to occur within months 1-4 of the project period.*

Figure 4. Construction of MN. **A)** design of 3D printed stamp and resulting **B)** PDMS micromold from the Idaho Microfabrication Lab. Microscopic images and dimensions of **C)** 3D printed stamp and **D)** resulting sucrose/CMC microneedles. **E)** electron micrograph of silk microneedles from Theodossiou Lab (Boise State).



<u>Objective 2. Assess IsdA+ClfA-CTA2/B immunogenicity after transcutaneous delivery in mice.</u>
Hypothesis and rationale: *We hypothesize that the transcutaneous delivery of IsdA+ClfA-CTA2/B will induce antigen-specific responses and these responses will be superior to those induced by antigen alone.* We will deliver *IsdA+ClfA*-CTA2/B to the dermis using dMN and comprehensively assess antigen-specific immune responses. **Experimental design and methods:** We will purify the *S. aureus IsdA*-CTA2/B and *ClfA*-CTA2/B chimeras, as described above. Tagged (6XHIS) recombinant IsdA and ClfA antigens will be purified, as described [21]. Vaccines will be dialyzed into 1X PBS + 5% glycerol, cultured for sterility, and tested for endotoxin levels (Pierce LAL kit, Thermo-Fisher). Groups of BALB/c mice (age 6-8 weeks) will be screened for *S. aureus* by culture of nasopharyngeal swabs and feces [56]. Based on our previous immune analysis in mice with an expected group difference of 2.3% of proliferating CD3+ cells isolated from the spleen (1% s.d.), a sample size of 7 animals per group will provide a power of 0.976 [13,57]. We will increase to a sample size of 8 to ensure equal numbers of male and female animals. As shown in Figure 5, animals will be randomized to receive 50µg of vaccine or antigen/adjuvant control by transcutaneous immunization (TCI) on days 1 and 21, and euthanized on day 28. Dosage/timing is based on previous studies [21,46]. For TCI, 50µg of *IsdA+ClfA*-CTA2 (25 µg of each chimera) or control in a silk or

sugar dMN patch will be delivered by manual application to the shaved back of an anesthetized mouse and left on skin for 15 minutes, as described [58]. A second dose of equal concentration will occur on day 21 in a different location on the back. Feces will be collected on days 1, 14, 21 and 28 for mucosal antibody isolation and analysis by ELISA using HIS-IsdA/HIS-ClfA, as described [21,46]. Blood will be collected by tail vein on day 14 and serum diluted in protease inhibitor (IB, 1X Halt Protease Inhibitor, Thermo Fisher) for ELISA. On day 28, animals will be euthanized and blood collected for ELISA. A patch of skin from each vaccination site will be excised and stored in media prior to treatment with collagenase and DNase for single cell suspension. Skin patches will also be frozen and sectioned for immunohistochemistry, using anti-DC (CD11c), anti-CTB and anti-ClfA antibody, as above for pig ears. Spleens will be isolated for culture of splenocytes. Splenocytes will stimulated for six days with purified HIS-IsdA/ClfA as described [21,46]. Following stimulation, supernatants will be collected for analysis of Th1/Th2/Th17 cytokines by ELISA (R&D Systems, Minneapolis, MN). Spleen cells will be assessed for viability and phenotyped using flow-cytometry and anti-CD3, CD4, and CD8. For skin cells, we will perform antigen-stimulation and immunophenotyping by flow cytometry to identify antigen-specific tissue-resident T cells ($T_{rm;}$ CD4 and CD69). Proposed immune analysis is shown in Table 1.

Figure 5. Vaccination schedule for immunogenicity trial, Objective 2.

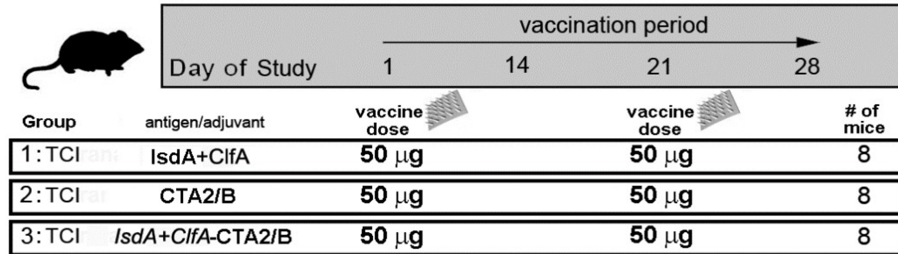


Table 1. Proposed Immune Analysis, Objective 2.

Sample	Analysis	Antigen/antibodies	Method
serum	antigen-specific antibody	IsdA, ClfA; anti-IgG	ELISA (d 14, 28)
feces	antigen-specific antibody	IsdA, ClfA; anti-IgA	ELISA (d 1, 14, 21, 28)
spleen	T cell proliferation, cytokine assay	CD3, CD4, CD8, and IL-17A, IFN-GMA, IL-4	flow cytometry, ELISA (d 28)
skin tissue	T_{RM} proliferation, antigen deposition	CD3, CD4, CD69, and anti-CT, anti-ClfA	flow cytometry, histology (d 28)

Expected data and analysis: Quantitative data will include antigen-specific ELISA, cytokine analysis, and immunophenotyping by flow cytometry. IgG endpoint titers and antigen-specific IgA/total IgA ratios will be calculated after background is subtracted. Antigen deposition will be assessed in skin quantitatively using immunohistochemistry. Immune analysis, including: ELISA, flow cytometry and confocal microscopy will be performed by the post-doctoral fellow in collaboration with graduate students and the Boise State Imaging and Flow Cytometry cores. *Objective 2 is expected to occur within months 5-8 of the project period.*

Objective 3. Determine efficacy of transcutaneous *IsdA+ClfA*-CTA2/B to protect against *S. aureus* systemic infection in mice. **Hypothesis and rationale:** *We hypothesize that the transcutaneous delivery of IsdA+ClfA-CTA$_2$/B will induce responses that are protective against systemic disease in mice and superior to antigen alone.* To determine feasibility, we will assess vaccine efficacy against *S. aureus* challenge using a systemic challenge model. *S. aureus* mouse models are well established and can be improved by the use of human-adapted strains and determination of previous exposure [59,60]. In addition, studies assessing CT, CTB or CTA2/B vaccine adjuvanticity have largely been performed in mice. **Experimental design and methods:** We will determine the protective efficacy

of TCI with *IsdA+ClfA*-CTA2/B using an intraperitoneal systemic challenge [61]. The well-characterized human isolate CA-MRSA USA300 (LAC), which has a high level of identity to vaccine IsdA (99%) and ClfA (98%) sequences, will be used for challenge. The trial schedule will be performed as shown in Figure 6. Based on an expected mean difference of 2 $\log_{10}$ CFU/mL of kidney tissue at 24hrs post-*S. aureus* sepsis (1.1 $\log_{10}$ s.d.) between TCI of *IsdA+ClfA*-CTA2/B and CTA2/B control, a sample size of 9 animals per group will provide a power of 0.951 [57]. A sample size of 8 or less per group has been reported as significant [61,62]. Mice will be vaccinated, as in Objective 2, on days 1 and 21 by TCI with 50µg of *IsdA+ClfA*-CTA2/B. Animals will be challenged by intraperitoneal injection with $5x10^7$ CFU/100µL of *S. aureus* on day 35. It is expected that animals will develop abscess lesions in the peritoneal as well as systemic spread of bacteria to the spleen and kidneys within 24 hours. Upon infection, animals will be monitored for signs of systemic disease by assessing body weight, condition of fur, eyes and posture, motility and respiration, and scored as described (disease activity index, DAI)[63]. After 24 hours on day 36, animals will be sacrificed, spleen and kidneys will be harvested for CFU determination on mannitol salt agar (MSA) and blood agar [61]. Blood will also be collected for antigen-specific ELISA, performed as above. **Expected data and analysis***:* Data from this aim will consist of the quantitative determination of bacterial colonies from mouse spleen and kidneys. Groups will be compared directly for statistical significance in averages of bacterial numbers as determined by CFU. Antibody responses from day 36 will also support immune analysis from Objective 2. *Objective 3 is expected to occur within months 9-12 of the project period.*

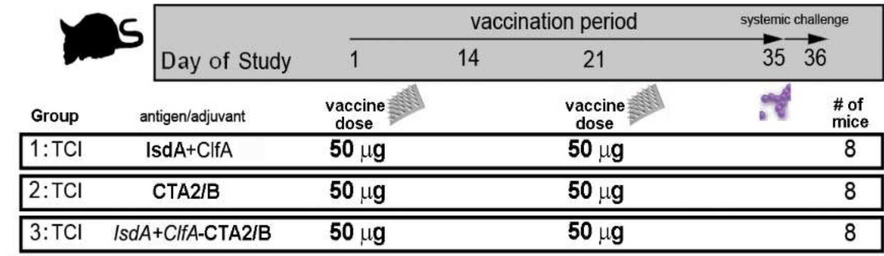


Figure 6. Vaccination schedule for challenge trial, Objective 3.

D. Alternative strategies and expected outcomes.

The P.I. and Co-P.I. have previously constructed sugar and silk-based microneedles. However, if necessary because the vaccine-incorporated dMN from Objective 1 are not available, direct TCI will be performed in Objectives 2 and 3 by preparing 100µL of *IsdA+ClfA*-CTA2/B in sterile buffer and placing directly on the shaved abdomen for 20 minutes, as described [7,64]. This approach will provide evidence of transdermal immunogenicity and efficacy without dependence on Objective 1. Additional immune analysis post-vaccination may also include the identification of tissue-resident and memory T-cell populations using CD3, CD69 and CD103 antibodies. The function of antibodies collected from serum and feces may be determined by opsonophagocytic killing assays (OPK), as described [21,60]. It is expected that this project will: *1) produce a dMN patch prototype that can deliver antigen to the dermal layer*, and *2) provide preclinical evidence of IsdA+ClfA-CTA2/B immunogenicity and efficacy after transdermal delivery*. The combined experience of the research team and anticipated expertise of the post-doctoral fellow (immunology and animal studies) are expected to strongly support these outcomes.

VIII. Potential economic impact.

There is no licensed human *S. aureus* vaccine and current practice is dependent upon the use of antibiotics. A vaccine that can protect against invasive disease would significantly reduce human morbidity and mortality, improve hospital care and reduce healthcare costs. Studies indicate that an *S. aureus* vaccine that targets hemodialysis patients and neonates would be highly cost-effective across a range of efficacies and costs [65,66]. The market for sepsis treatments and therapeutics is

estimated to be $3.4 billion, and expected to grow to $7.5 billion by 2030 [67]. The technology of dMN may have additional economic benefits: this route of delivery is more accessible, improves the patient/provider experience and reduces vaccine hesitancy. The market for vaccine adjuvants that can support novel delivery routes is growing along with the global vaccine adjuvant market, which is expected to be $1.2 billion by 2028 [68]. Pentamer Biologics, LLC., is our commercialization partner, and a new start-up company located in Idaho. This company is focused on the goal of developing a vaccine to prevent *S. aureus* disease. Currently, Pentamer Biologics consists of the CEO (Bryan Allinson) and Lead Research Scientist (Elise Overgaard, Ph.D.), and is supported by a USDA STTR Phase I with Boise State (Tinker) to assess the efficacy of a bovine *S. aureus* vaccine to prevent mastitis. The development of an effective mastitis vaccine will have significant statewide and national impacts on the dairy industry. Importantly, the lessons learned from vaccinating cows will contribute to our understanding of a human *S. aureus* vaccine, and vice-versa. Pentamer Biologics has a licensing agreement in place for *IsdA+ClfA*-CTA2/B, and the success of this industry partner will provide opportunities for high-tech workers in Idaho, while supporting regional agriculture, biomedicine and biotechnology. As shown in Figure 7, the timeline is long for the traditional licensure of human vaccines [69]. The *IsdA+ClfA*-CTA2/B vaccine has been tested in preclinical studies, and produced at lab-scale quantities, and is at TRL 4 for human licensure. For veterinary use, this vaccine has been tested in the end-user animal and is at TRL 5-6 with a significantly shorter expected timeline to licensure [70].

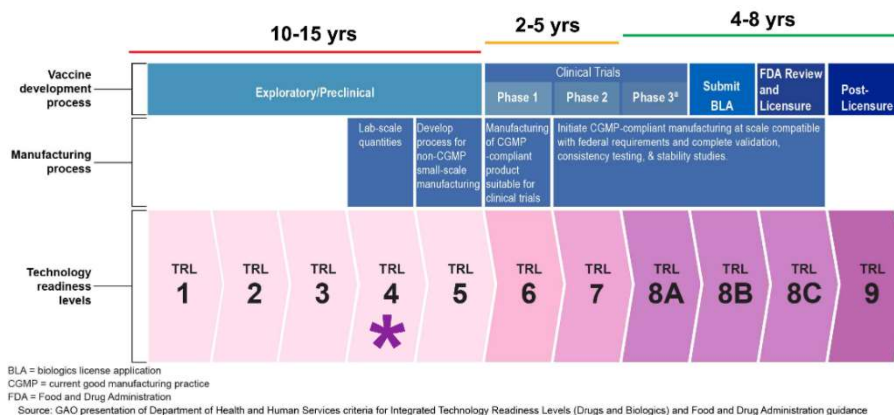


Figure 7. Department of Health and Human Services' Integrated Technology Readiness Level (TRL) Scale and the Traditional Vaccine Development and Manufacturing Processes. The *IsdA+ClfA*-CTA2/B vaccine is at TRL 4 for human use * (69).

IX. Criteria for measuring success.

Objective 1: The milestone of successful IsdA+ClfA-CTA2/B TCI delivery using dMN will be measured quantitatively by fluorescent immunohistochemistry of antigen deposition ex vivo. Success will be determined by an outcome of significant IsdA and ClfA antigen deposition into the dermal layer in pig ear skin, using *IsdA+ClfA*-CTA2/B loaded dMN, over no dMN controls.

Objective 2: The milestone of successful IsdA+ClfA-CTA2/B immunogenicity will be measured quantitatively by immune analysis after vaccination in mice. Success will be determined by an outcome of significant IsdA and ClfA antibody and/or T-cell response in *IsdA+ClfA*-CTA2/B vaccinated animals over adjuvant alone and no adjuvant controls.

Objective 3: The milestone of protective efficacy will be determined by bacterial enumeration after vaccination and challenge in mice. Success will be determined by an outcome of significant reduction of bacterial burden in the spleen and/or kidneys on day 36 of *IsdA+ClfA*-CTA2/B vaccinated and infected animals over those of adjuvant alone and no adjuvant controls.

X. Anticipated development challenges and barriers.
The barriers to the advancement along the TRL scale to TRL5 and toward human clinical trials are; *1)* optimization of vaccine composition for broader protection against multiple *S. aureus* serotypes, *2)* scaling up of vaccine production to meet current Good Manufacturing Process (cGMP) guidelines to support USDA/FDA approval, and *3)* successful preclinical testing that identifies an optimal vaccine dosage and schedule using multiple disease models. Because the IsdA and ClfA chimeras are easily purified, stable, and already tested in animals, we propose initial construction of dMN using these antigens. However, we are actively pursuing the incorporation of additional antigens and it is expected that these will be available for future proposals. We are currently working to overcome the barrier of production scale-up with support from the recent NSF ART (Boise State TRANSFORM) seed project. A postdoctoral fellow will be supported for one year on this grant with the goals of identifying optimal host and vector systems for vaccine production, and developing culture and fermentation protocols for scale-up. This researcher will also attend training in vaccine licensure and cGMP processes to promote a transition to industry. The animal studies proposed here in Objectives 2 and 3 are designed as small feasibility studies to be performed within the proposed timeline, however they will provide essential evidence for moving forward. Future preclinical studies will increase group numbers to compare different adjuvants, dosages, schedules and disease models, and will be designed to produce a product suitable to enter human clinical trials.

XI. Budget (see Form D).

XII. Budget justification.

LINE ITEM REQUEST	JUSTIFICATION	TOTAL REQUEST
Personnel		
Post-doctoral fellow (TBD)	Salary ($67,100 + fringe) for 1 year for a Ph.D - level immunologist to direct the project	$97,200.00
PI (Tinker)	Summer salary ($9,300 + fringe) for 0.75 mths for the P.I.	$12,300.00
Co-PI (Theodossiou)	Summer salary ($7,900 + fringe) for 0.75 mths for the Co-P.I.	$10,700.00
MS student BMOL (TBD)	Summer salary ($6,700 + fringe) for a MS student in the Biomolecular Graduate Program (BMOL)	$7,400.00
MS student BME (TBD)	Summer salary ($6,700 + fringe) for an MS student in the Mechanical and Biomedical Engineering program (BME)	$7,400.00
Travel		
Vaccines Summit, Boston, MA 2025	Travel for post-doctoral fellow to one vaccine conference	$2,000.00
ASM Microbe, Los Angeles, CA 2025	Travel for post-doctoral fellow to annual ASM conference	$1,500.00
Other Direct Costs		
Materials and supplies	Supply funds for the P.I's and Co-P.I.'s laboratory for vaccine purification and microneedle construction, as described in Objective 1. These include: supplies for bacterial growth and culture, vaccine and ELISA antigen purification, PDMS, resins, and silk and sucrose for microneedles. Animal study supplies and immunoassay antibodies and reagents, flow-cytometry antibodies, qPCR supplies, tissue culture media and supplies for MALT and PBMC preparation, as described in Objectives 2-3, will also be purchased with these funds.	$16,000.00
Publication charges	Funds are requested to support publication in one high-quality vaccine journal, such as *NPJ Vaccines*.	$1,500
Recharge center service	Use of recharge centers will include the Biomolecular Research Center for confocal microscopy (Objective 1), and the Center for Materials Characterization for microneedle construction and characterization (Objective 1), and the Boise State Vivarium and Flow Cytometry core for animal studies (Objective 2-3).	$4,000

1 set of 4 pipettes	New laboratory pipettes for use by the post-doctoral fellow (estimated).	$2,000.00
Vacuum oven	Small programmable vacuum oven for microneedles	$2,000.00
GRA Health Insurance	Three months health insurance coverage - 2 graduate students	$1,600.00
Total Request		**$165,600.00**

XIII. Project management.

Juliette Tinker, Ph.D., Principal Investigator (effort = 0.75 summer month). Dr. Tinker will participate in all phases of the project, including; daily execution and troubleshooting of experimentation, mentoring of the post-doc and students, writing of manuscripts, preparation of reports and adjustment experimentation as needed to support goals. The P.I. will also work across campus with the Co-P.I. to support dMN production and to ensure that studies comply with Boise State Biosafety (IRB), laboratory safety and animal care (IACUC) guidelines.

Sophia Theodossiou, Ph.D. Co-PI (effort = 0.75 summer month). Dr. Theodossiou will work with the post-doctoral fellow and graduate students to construct sucrose/CMC microneedles, and silk microneedle patches, incorporating the CTA2/B vaccine. Dr. Theodossiou will also work with the Idaho Microfabrication Lab and Center for Materials Characterization to determine microneedle structure and stability, as described in Objective 1, and will help prepare all manuscripts.

Project timeline and management

Objective	Supervisor	Location	Period (months) 1-2	3-4	5-6	7-8	9-10	11-12
Obj. 1: *dMN construction/ antigen deposition*	Theodossiou/ Tinker	Engineering/ Biology	X	X				
Obj. 2: *Mouse immunogenicity*	Tinker	Biology			X	X		
Obj. 3: *Mouse challenge*	Tinker	Biology					X	X

XIV. Additional institutional and other sector support.

This project is supported by the commercialization partner, Pentamer Biologics, LLC (Bryan Allinson, CEO Halo Bioventures, see letter of support). Pentamer Biologics has a current Facilities Use Agreement with Boise State for use of limited laboratory space and equipment within the Tinker laboratory. This project is also strongly supported by Boise State in the form of staff, facilities and equipment as detailed below (Appendix A). The Boise State office of Technology Transfer also provides continual support, which includes: assistance with the analysis of invention disclosures, securing of five US patents, support assessing the commercial value and marketing of current innovations, assisting with start-up development, and negotiating license agreements.

XV. Future funding.

Through current USDA Phase I funding, the P.I. and Pentamer Biologics have been provided a mentor through the LARTA institute (https://larta.org/) to support market analysis and business development for a Phase II SBIR/STTR that will be submitted in the spring of 2025. Pentamer Biologics has also applied for supplemental STTR funding through Innovate Alabama (https://innovatealabama.org/programs/supplemental-grant-program/) to support animal model development in collaboration with the Southern Research Institute. The P.I. and a graduate student (Haley Bridgewater) have completed the regional I-Corps program in August of 2023, and will apply for a national team in the summer of 2024. The P.I. has submitted two NIH STTR grants with the focus of a human *S. aureus* vaccine based upon the CTA2/B platform. These grants have scored well (36 and 38) but have not been funded. It is expected that successful outcomes from this proposed project will strongly support a successful NIH STTR/SBIR in 2025-2026 in collaboration with Pentamer Biologics.

FORM D: IGEM-HERC Full Proposal Budget Sheet

Track (select one): *Initial Startup*
PI First & Last Name: Juliette Tinker
Project Title: A microneedle-based transcutaneous *Staphylococcus aureus* vaccine
Milestone description (if applicabl Enter milestone number and/or brief description here

Insert more rows in each section, as needed. *Copy/paste cell formulas, as needed.* *See cell notes for additional information.*
Do not remove or hide rows. *Shaded areas have preset formulas.*

Personnel

Name	FTE (opt)	Months	Base Salary	Salary Request	Fringe Rate	Other Ben Rat	Fringe Request	Total
Post doctoral fellow (TBD- 1 year)		12	$67,100.00	$67,100.00	0.4496		$30,100.00	**$97,200.00**
PI (Tinker) summer (0.75 mth)		0.75	$9,300.00	$9,300.00	0.329		$3,000.00	**$12,300.00**
Co-PI (Theodossiou) summer (0.75 mth		0.75	$7,900.00	$7,900.00	0.3508		$2,800.00	**$10,700.00**
MS student BMOL		3	$6,700.00	$6,700.00	0.1		$700.00	**$7,400.00**
MS Student BME		3	$6,700.00	$6,700.00	0.1		$700.00	**$7,400.00**
		1	$0.00	$0.00			$0.00	**$0.00**
								$135,000.00

Equipment

Item Description	Units	Unit Cost	Total
			$0.00
			$0.00
			$0.00
			$0.00
			$0.00
			$0.00
			$0.00

Travel

Tentative Date(s)	# persons	Total days	Transit cost/ person	Lodging/ day	Meal per diem	Registration	Total
Oct-25	1	3	$700.00	$200.00	$75.00	$475.00	**$2,000.00**
26-May	1	5	$300.00	$150.00	$80.00	$50	**$1,500.00**
							$0.00
							$0.00
							$0.00
							$0.00
							$3,500.00

Participant Support

Description	# persons	Cost/ Stipend	Total
			$0.00
			$0.00
			$0.00
			$0.00
			$0.00
			$0.00

Other Direct Costs

Item	Units	Cost	Total
Materials/ Supplies	1	$16,000.00	**$16,000.00**
Publication Charges	1	$1,500.00	**$1,500.00**
Consultants (add consultant travel here)			**$0.00**
Computer Services			**$0.00**
Subcontract 1			**$0.00**
Subcontracts 2			**$0.00**
Other (list specifics if over $1,000) - Set of pipettmen & vacuum oven	2	$2,000.00	**$4,000.00**
Recharge Center Service	1	$4,000.00	**$4,000.00**
Other - GRA Health Insurance	2	$800.00	**$1,600.00**
Other			**$0.00**
			$27,100.00
TOTAL DIRECT COST REQUEST			**$165,600.00**

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Appendices

Appendix A: Facilities and equipment.

Laboratory

The P.I.'s (J. Tinker) laboratory consists of spacious 1000 sq. ft. on the second floor of the Science Building Department of Biological Sciences, Boise State University. This is a well-equipped laboratory space with room for 12 individual workstations, a separated space for Biosafety Level 2 work, and a dark room area for fluorecence microscopy. Machine-shop services are available on the first floor for experimental equipment, and Facilities and Operations are available on campus for general maintenance and repairs.

Office

200 sq. ft. of office space is available for use by the P.I. In addition, the Biological Sciences department has three administrative assistants to aid in budget preparation and accounting, track personnel files and order office supplies and equipment.

Animal

Animal use is proposed in Objectives 2 and 3 and is essential for a vaccine pre-clinical phase. The animal facilities and expertise located at Boise State University is continually expanding and strongly support the proposed studies. The Biomedical Research Vivarium, located on campus and opened in 2015, provides capacity for 2,000 cages and up to 10,000 rodents when at maximum capacity. The facility includes six holding rooms, three procedure rooms, a surgical suite, and separate areas for rack and cage washing, bottle filling, necropsy, storage, quarantine and showers. Space is dedicated for approved BSL-2 studies, and is also available for a proposed expansion of this vivarium. A full time Animal Coordinator (Bev Montegomery) and Animal Research Compliance Officer (Chelsea Garrison) are available for highly qualified research support in mice as well as ruminants. The P.I. has had Boise State and Boise VA IACUC approval for previous murine models, and has also performed large animal studies on commercial farms approved by IACUC, the Idaho State Veterinarian and the USDA (Transport of Experimental Biologic, CFR 103.3). IACUC approval will be obtained through Boise State prior to studies.

The Boise State Biomolecular Research Center

The BRC is a state-of-the-art laboratory facility with equipment and expertise for use by faculty and outside interests (for details on instrumentation see equipment). The facility houses seven main services:

1) Molecular and Cellular Imaging Laboratory
2) Molecular Interactions Laboratory
3) Mass Spectrometry and Proteomics Laboratory
4) Cell and Tissue Culture Laboratory
5) Histology Laboratory
6) Bioinformatics Core
7) Flow cytometry Core Facility

The proposed project will utilize protein sequence analysis (Objective 1) from the Mass Spectrometry and Proteomics Laboratory, histology and tissue culture (Objectives 1 and 2) in the Cell and Tissue Culture Laboratory, flow cytometry (Objective 2) and statistical and bioinformatics (Objectives 2 and 3) expertise from the Bioinformatics Core.

These are further described below:

Mass Spectrometry and Proteomics Research Laboratory: The BRC operates a 950 sq. ft laboratory dedicated to mass spectrometry and biomolecular interaction analysis. The laboratory provides facilities to isolate proteins and prepare samples for mass spectrometry analysis. The BRC currently

has three mass spectrometers; a Bruker Daltonics MaXis Quadrupole Time-of-Flight, a Thermo Scientific Velos Pro Dual-Pressure Linear Ion Trap, and a Matrix Assisted Laser Desorption/Ionization (MALDI) spectrometer. This mass spectrometer is coupled with a Dionex Ultimate 3000 HPLC system and a captive electrospray source. In combination with software tools, including Bruker Compass Data Analysis, SmartFormula, ProteinScape, Mascot protein search engine, and Profile Analysis, we use this LC-MS system in small molecule identification, metabolomics analysis and protein characterization. The Thermo Scientific Velos Pro Linear Ion Trap Mass Spectrometer offers Trap-HCD (Higher-Energy Collisional Dissociation) combined with CID (Collision-Induced Dissociation), and PQD (Pulsed-Q Dissociation) for proteomic analysis. A nano liquid chromatographic system is coupled to the mass spectrometer through a nanoelectrospray source for protein characterization.

Cell and Tissue Culture Laboratory: The BRC provides 600 sq. ft laboratory space for tissue dissection, explant production and maintenance of experimental samples in a controlled environment. Three laminar flow biosafety cabinets and four CO_2 incubators are available. Equipment includes a four vessel Rotating Wall Vessel (RWV) Bioreactor for 3-D cultures. Refrigerators and freezers (-20°C, -86°C, and liquid nitrogen), and centrifuges are available. The Countess® II FL Automated Cell Counter is a benchtop cell assay platform equipped with optics and image analysis software for rapid assessment of cells in suspension. With bright-field and two optional fluorescence channels—researchers can count cells, monitor fluorescent protein expression, and measure cell viability. The BioFlux Inverted microscope/microfluidic flow chamber is available for analysis of cell interactions under flow. The BioFlux system includes high speed capabilities based on automated experimental control, multiplexing up to 96 simultaneous experiments, and sophisticated data analysis software simplifying and accelerating complex functional assays.

Histology Laboratory: Histology equipment includes standard microtome and cryotomes, slide staining station, and Olympus BX60 Fluorescence microscope. Services include paraffin embedding (Lecia TP1020/Leica EG1150 embedding equipment), paraffin sectioning (Leica RM2235 Microtome), frozen sectioning (Leica CM1950 Cryostat), vibratome sectioning (Lecia Vibratome VT1000) and staining (Leica Autostainer XL).

Bioinformatics Core: The Center of Excellence in Biomedical Research and the Biomolecular Research Center offer data science services; including data analysis, biostatistical and bioinformatics support. Available support is determined on a case-by-case basis and cost of service is available by quote after consultation. Statistical services are offered with the help of an in-house biostatistician, Laura Bond (llbond@boisestate.edu).

Boise State Flow Cytometry Core Facility: Flow cytometry will be used in proposed Objective 2 for immune analysis of mouse samples. The Flow Cytometry Core Facility (FCCF) was created to aid researchers and graduate students in implementing flow cytometry into their experimentation. The facility's services are available primarily to the Boise State University and Treasure Valley communities. The FCCF is equipped with a state of the art four-laser BD INFLUX cell sorter. This instrument supports four-way sorting, plate sorting for single cell isolation, 9-color analysis, and can operate at up to 200,000 events per second. The BD INFLUX at BSU is also equipped with a small particle detector allowing particle detection near 200 nm. The BD Biosciences Accuri™ C6 is equipped with a blue and a red laser, two light scatter detectors, and four fluorescence detectors with optical filters optimized for the detection of fluorochromes such as FITC, PE, PerCP, and APC.

The College of Engineering

The Boise State College of Engineering houses significant expertise and available resources to complete the proposed development and testing of vaccine microneedles. Resources are divided into 6 main laboratories or centers (for details on instrumentation see equipment):

1) Advanced nanomaterials and manufacturing laboratory

2) Transport characterization laboratory
3) Center for materials characterization
4) Surface science laboratory
5) Idaho microfabrication laboratory
6) Northwest tissue mechanics laboratory

Equipment that is available in the College of Engineering and related to this project:

Advanced Nanomaterials and Manufacturing Laboratory

Axon MultiClamp 700B patch clamp amplifier on a vibration isolation table
Axon Digidata 1550 low-noise data acquisition system
pClamp 10 electrophyisology data acquisition and analysis software
Custom built quartz tube variable pressure chemical vapor deposition system with 4 inlet gases and up to two solid-source precursors.
QSonica Q125 probe-tip ultra-sonicator
Branson 2800 variable temperature ultra-sonicator
Mettler Toledo Analytical Balance
Thermo Scientific Legend Micro 21 Microcentrifuge
Think Planetary Centrifuge
Thermo Scientific Heratherm Programmable Gravity Convection Oven
Heraeus Megafuge 8 with TX-150 Cell Cult Pkg (8 x 50 ml)
MTI 2” Quartz Tube furnace with inert gas inlet and vacuum compatible
Agilent and Varian Eclipse Fluorescence Spectrophotometers
Agilent Cary 5000 UV-Vis-nIR spectrophotometer
COMSOL Multiphysics FEM software

Boise State Center for Materials Characterization

JEOL JEM-2100 HR Analytical Transmission Electron Microscope
Hitachi S-3400N-II Analytical Scanning Electron Microsope
Bruker AXS D8 Discover X-Ray Diffractometer
Rigaku Miniflex 600 Benchtop X-Ray Diffractometer
Leica DM6000 M Materials Microscope

Idaho Microfabrication Laboratory

<u>Characterization and Metrology</u>

J.A. Woollam M-2000 Spectroscopic Ellipsometer
Nanometrics NanoSpec 212
Bruker Dektax XT-A Stylus Profilometer
Wyko/Veeco NT1100 Optical Profiler
Superior Electronics Automatic 4-Point Probe

<u>Deposition</u>

AJA Orion 5 Sputtering Machine
Torr CrC-150 Benchtop Sputterer
CHA 600 Thermal Evaporator

<u>Plasma Etch</u>

Oxford PlasmaLab 180 ICP with Bosch Etch (fluorine-based)
Branson 3000 Series RIE/Asher
Veeco ME-1001 Ion Mill
Surface Chemistry/Wet Processing
JST Manufacturing Acid Station with RCA clean
JST Manufacturing General Base Station

JST Manufacturing Solvent Processing Station
SemiTool ST-460 Spin Rinse Dryer
Photolithography
Quintel Q-4000 Contact Aligner
OAI 5000 Contact Aligner
CEE Model 200X-F Spin Coater (Integrated with JST Solvent Processing Station)
Thermal Processing
MiniBrute MB-80 Thermal Oxidation/Diffusion Furnace
Modular Process Technology RTP-600s Rapid Thermal Annealer
Blue Electric SV-57A Vacuum Oven
Programmable Hot Plates (Integrated With Each of Three JST Chemical Processing Stations)
Systems Integration 7200-1453 Hot Plate
Back-End Processing
WestBond Model 7476 Wire Bonding System
K&S Model 4526 Wedge Bonding System
ADT982-6 Wafer Dicer
Materials Printing
Dimatix 2800 Inkjet Materials Printer
Optomec 200 Aerosol Jet Printer
Xenon Sinteron2000 Photonic Sintering

Appendix B: Biographical sketches. (see attached)

Appendix C: Senior personnel. a post-doctoral fellow with a Ph.D. in microbiology, immunology, vaccine development, or a related field, will be recruited to direct the proposed animal studies in Objectives 2 and 3. This will specifically include animal study coordination, immunization, sample collection, immune analysis and data analysis. This scientist will also support the Co-PI and engineering graduate student to complete Objective 1, and will be encouraged to partake in workforce training and entrepreneurship offered through I-Corp and NSF TRANSFORM.

Appendix D: Other. (see attached letters)

*NAME Juliette K. Tinker

*Required fields

ORCID ID (Optional)

*POSITION TITLE Professor

*PRIMARY ORGANIZATION & LOCATION Boise State University, Boise Idaho

***PROFESSIONAL PREPARATION - (see PAPPG Chapter II.D.2.h.i.a.3)**

PREVIOUS ORGANIZATION(S) & LOCATION(S)	DEGREE (if applicable)	RECEIPT DATE* (MM/YYYY)	FIELD OF STUDY
University of Colorado	Post-doctoral	12/2004	Microbiology
University of Iowa	Ph.D.	12/2000	Microbiology
Washington University	B.A.	05/1994	English/Biology

Note - For Fellowship applicants only, please include the start date of the Fellowship.

***APPOINTMENTS AND POSITIONS - (see PAPPG Chapter II.D.2.h.i.a.4)**

Start Date - End Date	Appointment or Position Title, Organization, and Location
2019-present	Professor, Department of Biological Sciences, Boise State University, Boise, ID
2012-2019	Associate Professor, Department of Biological Sciences, Boise State University, Boise, ID
2005-2012	Assistant Professor, Department of Biological Sciences, Boise State University, Boise, ID

***PRODUCTS - (see PAPPG Chapter II.D.2.h.i.a.5) Products Most Closely Related to the Proposed Project**

1. Tinker, J.K. Cholera toxin chimera and its use as a Staph vaccine. U.S. issued patents 8,834,898 (12/16/11), 8,911,748 (5/17/13), 9943582 (8/11/14), 10,383,933 (3/2/18), and 10,576,140 (3/3/20).

2. Alabdullah, H., Overgaard, E., Scarbrough, D., Williams, J.E., Mohammad Mousa, O., Dunn, G., Bond, L., McGuire, M.A., and J.K. Tinker. 2020. Evaluation of the efficacy of a cholera toxin-based Staphylococcus aureus vaccine against bovine intramammary challenge. Vaccines. Dec 24;9(1):E6.doi: 10.3390/vaccines9010006.

3.Oxford JT, Cornell KA, Romero JJ, Smith DB, Yarnell TL, Wood RM, Jorcyk CL, Lujan TJ, Albig AR, Mitchell KA, McDougal OM, Fologea D, Estrada D, Tinker JK, Nagarajan R, Warner DL, Rohn TT, Browning J, Beard RS Jr, Warner LR, Morrison BE, Fitzpatrick CK, Uzer G, Bond L, Frahs SM, Keller-Peck C, Pu X, Woodbury LG, Turner MW. Center of Biomedical Research Excellence in Matrix Biology: Building Research Infrastructure, Supporting Young Researchers, and Fostering Collaboration. Int J Mol Sci. 2020 Mar 20;21(6):2141. doi: 10.3390/ijms21062141.

4. Misra, N, Wines, T.F., Knopp, C.L. Hermann, R., Bond, L., Mitchell, B., McGuire, M. and J.K. Tinker. 2018. Immunogenicity of a Staphylococcus aureus-cholera toxin A2/B vaccine for bovine mastitis. Vaccine 36(24):3513-3521. doi:10.1016/j.vaccine.2018.04.067

Other Significant Products, Whether or Not Related to the Proposed Project (see PAPPG Chapter II.D.2.h.i.a.5)

1. Overgaard, E., Morris, B., Mohammad Mousa, O., Price, E., Rodriguez, A., Cufurovic, L., Beard, R. and J.K. Tinker. 2021. Cellular Activity of Salmonella Typhimurium ArtAB Toxin and Its Receptor-Binding Subunit. Toxins. 2021 Aug 27;13(9):599. doi: 10.3390/toxins13090599

2. Misra, N., Pu, S., Holt, D., M. McGuire and J.K. Tinker. 2018. Immunoproteomics to identify Staphylococcus aureus antigens expressed in bovine milk during mastitis. J. Dairy Science 101(7) 6296-6309. doi: 10.3168/jds.2017-14040.

3. Misra, N., Wines, T.F., Knopp, C.L., McGuire, M.A., and J.K. Tinker. 2017. Expression and sequence variation of iron-regulated surface protein A from bovine isolates of Staphylococcus aureus. FEMS Micro Lett 364(9): fnx082. doi: 10.1093/femsle/fnx082.

4. Tinker, J.K., Yan, J., Knipple, Panayiotou, P. and K. Cornell. 2014. Immunogenicity of a West Nile virus DIII-cholera toxin A2/B chimera after intranasal delivery. Toxins 6(4): 1397-141.doi: 10.3390/toxins6041397.

5. Tinker, J.K., Davis, C.T., and B.M. Arlian. 2010. Purification and characterization of Yersinia enterocolitica and Yersinia pestis LcrV-cholera toxin A2/B chimeras. Protein Expr Purif. 74(1):16-23. doi: 10.1016/j.pep.2010.04.021.

***Synergistic Activities - (see PAPPG Chapter II.D.2.h.(i)(a)(6))**

1. Technology transfer and research in vaccinology: I hold five patents for Staphylococcus vaccine development and was awarded an Idaho Innovation Award, Early-Stage Innovation in 2012. I have participated in training programs in technology transfer (BSU Ignite, 2022; ASCEND Hub SBIR/STTR Accelerator,2021, 2022; NIAID Application Assistance Program 2023; NSF Building Bridges workshop, 2023; I-Corp Regional Program, 2023) and acted as a consultant for industry (ERAD Therapeutics, 2019; Advarra Research Compliance Solutions, 2019-2020, Merck Animal Health, 2024), and I am co-founder of Pentamer Biologics, LLC, and P.D. of a USDA STTR that was awarded in 2023 (#2023-00817) to this start-up company. Lastly, I have recently been awarded a seed grant from the Boise State NSF ART (TRANSFORM) project that will provide funding for improved vaccine production methods and workforce training in licensure and GMP.
2. University curriculum development and graduate coordinator: I have served as Chair of the Biology Undergraduate Curriculum Committee (2014-2019) and member of the Biomolecular Ph.D. Steering committee (2012-2014, 2019-present). I attended the PULSE Northwest Educators conference on Biology curriculum (Seattle, WA) and helped to guide major undergraduate curriculum reform within the Department in 2018. Currently I am on the Biology Graduate committee and will take over as graduate coordinator in the summer of 2024.
3. Service-learning and community outreach in vaccines: I have served as a member of the Central District Health Immunization Advisory Board, in Boise (2010-2018) and regularly incorporate service-learning in coursework with support from my graduate students to reduce vaccine misinformation, including: volunteering in K-12 schools, vaccine clinics and social media outreach.
4. Research internships and fellowships: I teach a Vertically Integrated Project course in Vaccinology and average 7-10 undergraduate researchers in my laboratory, including over 20 summer interns since 2005. These students use bioinformatics to identify vaccine antigens and then clone and purify these antigens.

***Certification**:

When the individual signs the certification on behalf of themselves, they are certifying that the information is current, accurate, and complete. This includes, but is not limited to, information related to domestic and foreign appointments and positions. Misrepresentations and/or omissions may be subject to prosecution and liability pursuant to, but not limited to, 18 U.S.C. §§287, 1001, 1031 and 31 U.S.C. §§3729-3733 and 3802.

Signature
(Please type out full name): Juliette K. Tinker Date: 03/06/2024

IDENTIFYING INFORMATION:

NAME: Theodossiou, Sophia K

ORCID iD: https://orcid.org/0000-0003-2116-1279

POSITION TITLE: Assistant Professor

PRIMARY ORGANIZATION AND LOCATION: Boise State University, Boise, Idaho, United States

Professional Preparation:

ORGANIZATION AND LOCATION	DEGREE (if applicable)	RECEIPT DATE	FIELD OF STUDY
Tufts University, Medford, Massachusetts, United States	Postdoctoral Fellow	09/2020 - 07/2022	Biomedical Engineering
Brigham & Women's Hospital, Boston, Massachusetts, United States	Postdoctoral Fellow	09/2020 - 08/2021	Biomedical Engineering and Developmental Biology
University of Idaho, Moscow, Idaho, United States	PHD	08/2020	Bioengineering
Northwestern University , Evanston, Illinois, United States	BA	06/2012	Human Biology and Anthropology

Appointments and Positions

2022 - present Assistant Professor, Boise State University, Boise, Idaho, United States

Products

Products Most Closely Related to the Proposed Project

1. Jones CL, Penney BT, Theodossiou SK. Engineering Cell-ECM-Material Interactions for Musculoskeletal Regeneration. Bioengineering (Basel). 2023 Apr 7;10(4) PubMed Central PMCID: PMC10135874.
2. Foster O, Shaidani S, Theodossiou SK, Falcucci T, Hiscox D, Smiley BM, Romano C, Kaplan DL. Sudan Black B Pretreatment to Suppress Autofluorescence in Silk Fibroin Scaffolds. ACS Biomater Sci Eng. 2023 Jun 12;9(6):3193-3205. PubMed PMID: 37171982.
3. Conley Natividad G, Theodossiou SK, Schiele NR, Murdoch GK, Tsamis A, Tanner B, Potirniche G, Mortazavi M, Vorp DA, Martin BA. Ex-vivo quantification of ovine pia arachnoid complex biomechanical properties under uniaxial tension. Fluids Barriers CNS. 2020 Nov 12;17(1):68. PubMed Central PMCID: PMC7664091.
4. Schneider KH, Goldberg BJ, Hasturk O, Mu X, Dötzlhofer M, Eder G, Theodossiou S, Pichelkastner L, Riess P, Rohringer S, Kiss H, Teuschl-Woller AH, Fitzpatrick V, Enayati M, Podesser BK, Bergmeister H, Kaplan DL. Silk fibroin, gelatin, and human placenta extracellular matrix-based composite hydrogels for 3D bioprinting and soft tissue engineering. Biomater Res. 2023 Nov 17;27(1):117. PubMed Central PMCID: PMC10656895.
5. Pancheri NM, Daw JT, Ditton D, Schiele NR, Birks S, Uzer G, Jones CL, Penney BT, Theodossiou SK. The LINC complex regulates Achilles tendon elastic modulus, Achilles and tail tendon collagen crimp, and Achilles and tail tendon lateral expansion during early postnatal

development. bioRxiv. 2023 Nov 13; PubMed Central PMCID: PMC10680625.

Other Significant Products, Whether or Not Related to the Proposed Project

1. Theodossiou SK, Pancheri NM, Martes AC, Bozeman AL, Brumley MR, Raveling AR, Courtright JM, Schiele NR. Neonatal Spinal Cord Transection Decreases Hindlimb Weight-Bearing and Affects Formation of Achilles and Tail Tendons. J Biomech Eng. 2021 Jun 1;143(6) PubMed Central PMCID: PMC8114905.
2. Theodossiou SK, Murray JB, Hold LA, Courtright JM, Carper AM, Schiele NR. Akt signaling is activated by TGFβ2 and impacts tenogenic induction of mesenchymal stem cells. Stem Cell Res Ther. 2021 Jan 26;12(1):88. PubMed Central PMCID: PMC7836508.
3. Theodossiou SK, Murray JB, Schiele NR. Cell-cell junctions in developing and adult tendons. Tissue Barriers. 2020;8(1):1695491. PubMed Central PMCID: PMC7063869.
4. Raveling AR, Theodossiou SK, Schiele NR. A 3D printed mechanical bioreactor for investigating mechanobiology and soft tissue mechanics. MethodsX. 2018;5:924-932. PubMed Central PMCID: PMC6111048.
5. Giduthuri AT, Theodossiou SK, Schiele NR, Srivastava SK. Dielectrophoretic Characterization of Tenogenically Differentiating Mesenchymal Stem Cells. Biosensors (Basel). 2021 Feb 16;11(2) PubMed Central PMCID: PMC7919818.

Synergistic Activities

1. National Institutes of Health (NIH) T32 Organ Design and Engineering Training (ODET) Program Fellow: As a postdoctoral fellow, I was awarded the NIH ODET T32 training grant from 2020-2022. I participated in a program co-hosted by the Harvard Stem Cell Institute and the Brigham & Women's Hospital in Boston, MA, to learn how to use stem cells and developmental cues to regenerate complex organ and tissue structure and function. This program allowed me to work closely with leaders in the organ design and organoid fields at Harvard University, Boston University, and Tufts University, and learn skills critical to the proposed project's success. I maintain professional contacts at the program who will be invaluable in guiding the proposed work and serving as collaborators on projects and grants. My unique support network is ideally suited to our proposed EFRI activities and will help us achieve our predicted outcomes.
2. Orthopaedic Research Society (ORS) Tendon Research Section Membership Committee Member and Social Media Co-Chair: I currently serve as a standing member of the ORS Tendon Section Membership committee, and am also the social media co-chair. This activity connects me to hundreds of researchers working on muscle-related projects, as well as to labs that work in the organoid space. Beyond the networking opportunities, this position allows me to help other scientists, particulalry junior faculty members, find their research communities. I will use my experience as an ORS Tendon Section Membership Committee Member to share our workshops and other project deliverables and outcomes with broad and diverse scientists looking to expand their research portfolio and professional network.
3. Founding Faculty Advisor of Boise State University's Biomedical Engineering Society (BMES) Student-Chatper: Shortly after I began my job at BSU, I founded the student-chapter of the Biomedical Engineerign Society (BMES). BMES is a national organization of biomedical engineers that seeks to improve human health and wellbeing. The annual BMES conference is

extremely student- and trainee-friendly, and many of my foundational experineces that drew me to and kept me in engineering happened in connection with BMES. The BSU Biomedical Engineering (BME) minor is a new program housed within the Mechanical and Biomedical Engineering (MBE) department, and is the only true biomedical engineering undergraduate program in the state of Idaho. It is criticial for Idaho's students to be connected to the thriving - and growing - biomedical engineering community, in order to secure jobs, internships, and graduate school admissions. As of September 2023, the BSU BMES student chapter has 22 members (12 URM, 15 women, 20 first generation) at the undergraduate and graduate level. We have applied for and received funding from the student activities board, and this funding is allowing one of our graduate members to travel to the annual conference in October 2023 to network with potential employers.

4. Mentoring of Undergraduate and Graduate Students: I am deeply commited and invested in mentoring the next generation of scientists. I demonstrate this commitment by consistently participating in various programs to build an inclusive lab, and holistically assessing students who wish to join my lab for research. I consider my lab a "teaching lab," where the only required qualifications for a paid trial position are motivation, enthusiasm, and a willingness to learn. I am building an inclusive culture while improving my culturally-aware mentoring skills. Since starting at Boise State University, I have mentored 3 graduate students (3 first generation and low socioeconomic status, 1 URM) and 8 undergraduate students (4 URM, 7 first generation, 7 women). I also frequently have visiting doctoral, medical, and masters students from other labs on campus utilizing my lab and working with me to learn various biomaterials fabrication and cell culture techniques.

Certification:

When the individual signs the certification on behalf of themselves, they are certifying that the information is current, accurate, and complete. This includes, but is not limited to, information related to domestic and foreign appointments and positions. Misrepresentations and/or omissions may be subject to prosecution and liability pursuant to, but not limited to, 18 U.S.C. §§ 287, 1001, 1031 and 31 U.S.C. §§ 3729-3733 and 3802.

Certified by Theodossiou, Sophia K in SciENcv on 2024-02-14 15:40:15

Halo Bioventures | 195 Vista Oak Drive, Longwood FL 32779 USA

February 20, 2024

Higher Education Research Council
Idaho Global Entrepreneurial Mission Initiative – IGEM-HERC

Dear Idaho State Board of Education Research Council,

This letter is to indicate my support for Dr. Juliette Tinker's IGEM-HERC application titled: "*A microneedle-based transcutaneous Staphylococcus aureus vaccine*". I am founder and CEO of Halo Bioventures. Halo Bioventures is a full-service ventures and consulting firm that supports new and emerging biotechnologies. We expand access to strategic non-dilutive and investor/dilutive funding streams, and help develop early stage concepts for translation to market. We also provide strategic direction and leadership to navigate complex business situations, technology challenges, and regulatory hurdles. We have a proven track record of supporting university-backed technologies, and have been working with Dr. Juliette Tinker at Boise State since 2020 on the translation of a *Staphylococcus aureus* vaccine. During this time, we have founded a start-up company, Pentamer Biologics, LLC., and have successfully received USDA STTR Phase I funding in 2023 to support a vaccine clinical trial in dairy cows. This trial will assess the efficacy of Dr. Tinker's vaccine against *S. aureus* infection in the udder after intramammary vaccination. If there is evidence of protection from this trial, it will strongly support expansion of field trials and vaccine licensure through a Phase II application in 2025.

We are enthusiastic about this research, and in support of this proposed IGEM-HERC project that will promote translation of this vaccine to Pentamer Biologics. The proposed activities of; development of sugar and silk microneedles containing the vaccine and mouse pre-clinical studies to assess immunogenicity and efficacy against systemic disease will expand the characterization of this vaccine and potential use of it for use in humans. These studies will generate essential data to support NIH STTR/SBIR applications.

I would be pleased to be involved in the proposed project by continuing to support Pentamer Biologics, and the innovative *S. aureus* vaccine. In addition, we will work to identify private and public matching funds to multiply the effect of the Halo Bioventures investment and bridge the gap to commercialization for this research. I look forward to collaborating on this exciting project, and helping to bring innovative new use-inspired technologies to Southwest Idaho.

Sincerely,

Bryan Allinson
Founder and CEO
Halo Bioventures | Pentamer Biologics

BOISE STATE UNIVERSITY

COLLEGE OF ENGINEERING

Mechanical and Biomedical Engineering

February 21, 2024

Higher Education Research Council

Idaho Global Entrepreneurial Mission Initiative – IGEM-HERC

Dear Idaho State Board of Education Research Council,

This letter is to indicate my support for Dr. Juliette Tinker's IGEM-HERC application titled: "*A microneedle-based transcutaneous Staphylococcus aureus vaccine*". I am an Assistant Professor in the department of Mechanical and Biomedical Engineering and have a Ph.D. in Biological Engineering from the University of Idaho. My research is focused on the mechanical and biochemical signals that support musculoskeletal tissue formation during normal development, and using developmentally-inspired cues to regenerate tissues after injury or disease. As a post-doctoral fellow in the Tufts University Department of Biomedical Engineering in Boston, Massachusetts I learned how to exploit natural protein polymers, including silk, to develop high performance biomaterials. This included the construction of silk microneedles for transcutaneous drug or vaccine delivery. We have continued to develop these microneedles using 3D printing at Boise State. I am happy to provide expertise and equipment, in collaboration with the Idaho Microfabrication Laboratory and the Boise State Center for Materials Characterization, for the construction and characterization of silk and sugar-based microneedles, as described in Objective 1. Our preliminary evidence indicates that we can consistently produce stable silk microneedles at low, medium, and high molecular weights and with varying silk contents (7% to 10%) and hydrophobicities that are optimized for protein delivery and controlled microneedle degradation. Additionally, these microneedles have the capacity to stabilize, maintain sterility of, and deliver a variety of purified proteins over controllable timeframes for use as transcutaneous vaccines.

I am in full support of these efforts and look forward to working with you on this exciting project.

Sincerely,

Sophia Theodossiou, Ph.D.
Assistant Professor
Mechanical and Biomedical Engineering
College of Engineering
Boise State University
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SophiaTheodossiou@boisestate.edu