**Project Title:**
Advanced Method for Testing Biocompatibility of Skin Wearable Biosensor System

**Team Members:**
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**Statement of Project Goals:**
- Development of advanced methods to screen biocompatibility of component materials for skin-wearable ASSIST platforms;
- Discovery of innovative and inexpensive non-animal *in vitro* assays to evaluate the biocompatibility of the skin-attachable platforms;
- Performance of three biological tests developed for skin-wearable platforms based on FDA Blue Book Memorandum #G95-1: cytotoxicity, skin irritation and sensitization.
- Development a biocompatibility survey and safety datasheet for materials present in wearable platforms generated by ASSIST.

**Project's Role in Support of the Strategic Plan:**
The purpose of this project is performing biocompatibility testing to ensure that the fitness of a skin-wearable sensor systems developed in ASSIST could be used on human without potentially harmful biological risks arising from the use of sensors. The FDA G95-1 and ISO 10993-1 standards serve as a framework for selecting tests to evaluate biocompatibility of these platforms. There is a risk in testing the finished device without developing data on component materials. If a negative result occurs, it can be difficult to track down the component that is causing the problem. Screening device component materials minimizes this risk.

We have 3 major roles to support ASSIST NERC goals:
- Assemble vendor data and initiate material characterization and analysis of devices’ components
- Conduct inexpensive non-animal *in vitro* studies to provide an additional screen for material safety
- Conduct confirmatory *in vivo* assessments for pre-clinical phases on finished device for safety evaluation

Our 3-step support will insure that a redesign of ASSIST sensor systems due to biocompatibility test failures will not be necessary

**Discussion of Fundamental Research, Educational, or Technology Advancement Barriers and the Methodologies Used to Address Them:**
Although FDA G95-1 and ISO 10993-1 provided frameworks for selecting tests to evaluate biocompatibility of skin-wearable platforms, the lack of appropriate test methodology for the specific types of novel engineered materials being developed in ASSIST testbeds is a fundamental barrier to collection of the safety and efficacy data that will be required for human clinical trials.
We are using the combinations of both \textit{in vitro} and \textit{in vivo} methods to evaluate the safety of skin-wearable sensor systems. We are also developing new and more appropriate methods for evaluating the materials and ultimately, the whole testbed/platform.

\textbf{Foreign Collaborations:} N/A

\textbf{Achievements in Year 3 and Previous Years:}
We have developed analytical and biological methods and provided protocols used for screening the biocompatibility of candidate materials in ASSIST platforms, including:

\begin{itemize}
  \item Evaluation of a silver nanowire biosensor system: the system developed in Yong Zhu’s group at NCSU is to be placed on the skin where embedded electrodes can detect muscle activity and hydration levels, such ECG and EMG monitoring. We have collected biosafety data and created a data file for skin-attached materials. In addition, material characterization was performed with LC-MS and SEM. Moreover, we performed multiple biological evaluation procedures (cytotoxicity and irritation) on these sensor materials. Preliminary results obtained for human fibroblast cells indicate cytotoxicity for high concentrations of Ag nanowires. Experiments are currently underway to correlate the sheet resistance of these electrodes with cytotoxicity.
  \item Biocompatibility assessment of a flexible textile dry electrode used for long term ECG monitoring: These electrodes employ printed Ag/AgCl electrodes explored in Jesse Jur’s group at NCSU. Cytotoxicity testing has been performed on a single cell line. We will rerun using a second cell-line. Extraction of leachable materials that could compromise device safety has been performed. Characterization of extractable material, bulk material and surface has been characterized subsequent to biological testing.
  \item Discovery of inexpensive non-animal \textit{in vitro} studies: We have developed a new 3-D skin model assay for cytotoxicity and irritation.
  \item Development of biocompatibility datasheet for materials present in ASSIST wearable platforms: we generated a survey and are preparing a safety datasheet, and systematically investigating the biocompatibility of the materials used in wearable biosensors.
\end{itemize}

\textbf{Summary of other relevant work being conducted within and outside of the ERC and how this project is different:}

\textbf{Plans for the Next Year:}

Based on the results of the survey and the discussions that followed, we have identified two new materials of highest priority for biocompatibility testing. These include

\begin{enumerate}
  \item Graphene-doped PDMS currently considered both at FIU (El-Zahab) and NCSU (Jur) as a heat spreader under thermoelectric devices.
  \item Hydrogels used by the Orlin/Velev group for hydration sensing.
\end{enumerate}

In our opinion, employing both \textit{in vitro} and \textit{in vivo} testing to evaluate the safety of skin-wearable sensor systems will provide more reliable data on materials safety. Next year, we will differentiate our approach from academic and industry efforts in this area below:
Discovery of new in vitro assays to measure skin sensitization while minimizing the use of in vivo animal models

Skin sensitizers are substances able to produce an allergic response after contacting skin. Currently, FDA and ISO standards use in vivo animal models to evaluate potential skin sensitization. Alternative non-animal related in vitro methods have been demanded by animal welfare advocates and there has been EU legislation requiring use of these methods. Significant investments have been made in the development of suitable in vitro test methods. We will develop novel in vitro assays to measure skin sensitization.

Combination of in vitro assays with in vivo animal and human models for pre-clinical phases on a finished device for safety evaluation

As a part of the regulatory clearance process, FDA and ISO both require that manufacturers conduct adequate safety testing data for finished devices through pre-clinical and clinical phases. Besides screening component materials used on skin-wearable sensors in vitro, we will also evaluate dermal cytotoxicity, irritation and sensitization in vivo for wearable devices generated by ASSIST technology. We will cooperate with our Medical Director, David Peden, to obtain biocompatibility evaluation data on human-skin to finished devices generated by ASSIST.

**Expected Milestones and Deliverables for the Project:**
- Benchmarking of biocompatibility of materials used in ASSIST platform materials to existing currently used materials for skin-wearable devices.
- Evaluation of dermal cytotoxicity, irritation and sensitization in vitro for ASSIST platform materials.
- Novel biocompatibility test methodology for wearable devices generated by ASSIST technology.
- List of biocompatible safety datesheets for materials present in wearable platforms.

**Member Company Benefits:**
Provided biocompatibility evaluation for the materials used in ASSIST platform. It is required by FDA that the potential manufacturers should conduct adequate safety testing data for finished devices through pre-clinical and clinical phases. Besides screening component materials used on skin-wearable sensors in vitro, our technology will also provide methods to evaluate dermal cytotoxicity, irritation and sensitization in vivo for the finished wearable devices generated by ASSIST technology.

**Commercialization Impacts or Course Implementation Information:** N/A