ADAPT in SC: Determination of Inflammation Progression in Diabetic Chronic Wounds via PLA:MWCNT Nanocomposite Biosensor and Image Analysis

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Introduction: There are few reliable quantitative methods to clinically evaluate healing of diabetic chronic leg ulcers, a type of wound that affect 2% of the world population. When non-healing, the presence of complications such as infection may not be visible in the wound. The use of artificial intelligence (AI) through deep machine learning holds promise in augmenting clinical assessment, and advancements using artificial neural networks are emerging in wound healing science specific to detection of inflammation and infection. Here, the authors present a report of a protocol to determine the degree to which inflammatory biomarker concentrations, as measured by a novel nanofiber composite biosensor, can be combined with wound images to quantitatively study the progression of inflammation and healing towards forming a wound healing classification model ('healing' or 'non-healing) in patients with chronic diabetic wounds.

Methods: This report features data from 3 patients (part of the initial pilot group of 5 for a future larger study with 40 patients) recruited from a wound clinic in the Southeastern U.S. Inclusion criteria include 18 years of age and above, diabetic foot ulcer on the plantar surface with duration greater than 4 weeks and ulcer size greater than 2 X 2 cm (size requirement is due to dimension of the sensors), expected to receive wound care for at least weekly for 8 weeks with at least one clinic visit per week, intact skin sensory sensation around the peri-wound area, and able to provide written informed consent. Patient wounds were measured every other visit (every two weeks) via inflammatory biomarker testing using a novel nanocomposite biosensor and wounds were photographed and measured. The biosensors consisted of a single poly-ethylene film layer with screen printed silver electrode base layer. A conductive composite layer of poly-l-lactide (PLA) and multi-walled Carbon nanotubes (MWCNT) is then added via solution-blow-spinning. The conductive layers are functionalized with antibodies sensitive to TGF-β, TNF-α, and VEGF with a negative control. These growth factors serve as inflammatory biomarkers that characterize respective states of inflammation. The sensors are placed on the wound for a total of approximately 20 minutes (each inflammatory biomarker sensor is individually placed for 5 minutes plus a non-functionalized control sensor). During this time, a 1 $V_{pk\text{-}pk}$ sine wave will be applied across the interdigitated electrode array at a frequency sweep between 200 – 2000 Hz. The voltage will be supplied by a function generator and impedance spectra will be recorded at a 100 Hz sampling rate using an inductance, capacitance and resistance (LCR) meter. Characterization of the real and reactive components of the electrochemical impedance response can then be related to calibrated concentrations of the biomarkers. At each visit wounds are measured and photographed for analysis in ImageJ software.

Results and Conclusions: Across these first 3 patients, the real component of impedance (Z) has progressively increased as wounds have healed (as assessed by decreased wound area). However, in one patient in which the wound became progressively worse through the treatment, Z increased. The expression of particular inflammatory biomarkers also followed the same trend, where more expression of TNF-α increased as the wound shifted to a more anti-inflammatory state. Image data, while not yet enough for the machine learning implementation is showing strong positive correlations between wound closure (size decrease) and expression of TNF-α and VEGF, which are biomarkers more associated with progression toward healing and revascularization. The current results are aligned with our initial expectations of sensor behavior and correlation with wound healing. Continued analysis is expected to reveal ratios/relationships between TNF-α and TGF-β and the shift between pro-inflammatory and anti-inflammatory responses.

Sensory Augmentation to Improve Post-Stroke Standing Balance

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Introduction. Many people with chronic stroke (PwCS) exhibit deficits in standing balance, which are associated with a decreased quality of life and an increased risk of falls. Despite substantial work targeting this problem, no large-scale interventions have succeeded in reducing falls among PwCS. Development of a more effective method of improving post-stroke balance will likely require consideration of the underlying sensory deficits contributing to the mediolateral losses of balance in PwCS that often precede a fall. One sensation-focused approach to improving balance is sensory augmentation, in which artificial feedback provides the nervous system with information about the dynamic state of the body. However, sensory augmentation has thus far produced equivocal results in PwCS, likely due in part to inter- patient variability that reduces the effectiveness of a one-size-fits-all approach. The purpose of the current study was to investigate the effects of four distinct types of unisensory augmentation on balance performance in PwCS, as well as the effects of multisensory augmentation that is personalized to individual participants.

Methodology. Thus far, 15 PwCS have participated in this study. We challenged participants' mediolateral standing balance by having them stand on a force platform that translated mediolaterally in an unpredictable pattern. The difficulty of these perturbations was standardized across participants to achieve a center of pressure (CoP) root-mean-square velocity of 20 mm/s, a risk factor for losses of balance and falls. In a subset of trials, participants were provided with sensory augmentation delivered in the form of vibration over one of four locations that can influence standing balance: lateral trunk; hip abductors; ankle invertors/evertors; foot sole. The intensity of the vibration scaled with the real-time CoP velocity using a standardized gain (maximum intensity when this velocity exceeded a 2 standard deviation threshold), providing users with sensory information about the dynamic state of their body through natural physiological pathways. Balance performance was quantified as the standard deviation of CoP displacement during the periods of platform translation.

In a subsequent session, multisensory augmentation was simultaneously delivered to all four stimulation locations. Rather than using a standardized gain, the gains for each stimulation location were allowed to vary, thus potentially accounting for interpatient variability in how sensory information is integrated into the perception of body dynamics. We used human-in-the-loop optimization (covariance matrix adaptation evolution strategy) methods to iteratively identify the combination of stimulation gains that minimized CoP displacement for each participant. Once a plateau in performance was reached, we compared balance performance with and without the identified optimal sensory augmentation parameters.

Results. The effects of standardized unisensory augmentation were highly variable across participants and stimulation locations. While the group average CoP displacement was slightly reduced while the stimulation was applied, only 40% of participants exhibited an improvement with at least a moderate effect size (Cohen's $d < -0.5$). In contrast, all participants tested thus far

exhibited a reduced CoP displacement when the personalized multisensory augmentation was applied (average Cohen's d = -0.6).

Conclusions. The present results support prior suggestions that a "one-size-fits-all" approach to sensory augmentation is unlikely to optimally improve balance performance for individual PwCS . Instead, personalizing the set of stimulation parameters to individual participants appears to hold promise for producing larger beneficial effects. Despite these positive early-stage results, our current optimization methods have notable limitations, including a lack of foundation in mechanistic models, an inability to improve efficiency by leveraging data from similar participants, and an inability to predict how users would respond to sensory augmentation parameters that were not directly tested. Future work will design and test a digital twin intended to overcome these barriers, which will subsequently be integrated into the sensory augmentation system to allow real-time adaptation to varying contexts.

Development of hPSC isogenic cardiac organoids as an implantable device

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Abstract: Implantable engineered tissues hold remarkable promise to augment and replace injured tissues and organs. To this end, human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs) have been used to fabricate tissue constructs to treat cardiovascular disease. We mimicked intramyocardial organization events in coronary vasculogenesis to fabricate human cardiac organoids through the self-assembly of hPSC-CMs, human cardiac fibroblasts (hcFBs), human umbilical vein endothelial cells (HUVECs), and human adipose-derived stem cells (hADSCs). Despite progress, the use of primary cells can lead to uncontrolled immunogenicity and result in immune-rejection post-implantation. To address this, our lab has established a collaboration with Dr. Sean Palecek at the University of Wisconsin-Madison to produce hPSC-CMs, hPSC-derived cardiac fibroblast (hPSC-cFBs), and hPSC-derived endoethelial cells (hPSC-ECs) from a single hPSC donor. To this end, we have successfully and reproducibly replicated the differentiation of hPSCs into hPSC-CMs, hPSC-cFbs, and hPSC-ECs from the 19- 9-11 human induced pluripotent stem cell (hiPSC) line. We have further successfully fabricated isogenic cardiac organoids from these 3 hPSC-derived cells. With an established cell seeding ratio (CMs:cFbs:ECs = 70:15:15), these cells reproducibly self-assemble into spherical microtissues. In addition, hPSC-ECs form lumen-like structures within the hPSC-derived organoids. These results laid the foundation to develop patient specific organoids for implantation studies.