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Predictive Chronic Wound Monitoring Protocol for Healing Assessment via Intelligent Biosensing Technology and Machine Learning

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ABSTRACT

Objective: There are few reliable quantitative methods to clinically evaluate healing of 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 promises 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 proof-of-concept protocol to determine the degree to which inflammatory biomarker concentrations, as measured by a novel nanofiber composite biosensor, can be combined with thermal and visible light photographic wound images to form a wound healing classification ('healing' or 'non-healing) in patients with chronic venous leg ulcers.

Methods: The protocol recommends that the chronic wounds of 10 patients receiving care in an outpatient wound clinic will be investigated in this longitudinal observational study during weekly clinical, imaging and biosensing wound assessments conducted over 4 continuous weeks of care. Through deep-machine learning, predictive models will be generated connecting sensor data and thermal image data as inputs for wound healing (or non-healing) classification with respect to the Bates-Jensen wound assessment criteria. Three models (image data only, sensor data only, and fusion of both image and sensor data) will be generated and corroborated with clinical assessment findings to determine whether the models accurately reflect wound healing status. The protocol was approved by the institution's review board.

Abbreviations: AI: Artificial Intelligence; BWAT: Bates-Jensen Wound Assessment Tool; CNN: Convolutional Neural Network; eTO: Ethylene Oxide; FN: False Negative; FP: False Positive; GCP: Good Clinical Practices; ICH: International Conference of Harmonization; LOD: Limit Of Detection; LSTM: Long Short-Term Memory; MANOVA: Multivariate Analysis Of Variance; ML: Machine Learning; NRS: Numerical Rating Scale; PLA: MWCNT: Porous Polylactide Multiwall Carbon Nanotube; RED Cap: Research Electronic Data Capture; RNN: Recurrent Neural Network; SBS: PLA:MWCNT: Styrene-Butadiene-Styrene Porous Polylactide Multiwall Carbon Nanotube; SVM: Support Vector Machine; TGF-β: Transforming Growth Factor – Beta; TL: Transfer Learning; TN: True Negative; TNF-α: Tissue Necrosis Factor – Alpha; TP: True Positive; VEGF: Vascular Endothelial Growth Factor

Introduction

During the past five years, the application of artificial intelligence (AI) through machine learning (ML) to advance wound care science has grown rapidly for both the assessment of wounds and the development of new treatment models of care for patients with chronic wounds [1-3]. Chronic or slow-to-heal wounds effect 9-12 million individuals each year, costing the U.S. healthcare system over $32 billion [4]. Chronic wounds are particularly challenging for clinicians because this patient population often presents with multiple comorbidities such as obesity, diabetes mellitus, renal disease, neuropathy, and vascular disease that increase the risk for ulcer complications including infection, leading to a state of continual inflammation that negatively influences healing [5]. Regardless of wound types such as venous leg or diabetic foot ulcers, most chronic wounds are treated by primary care providers and when treatment does not produce healing, patients are typically referred to specialized outpatient clinics where available. These clinical visits are a premium resource for patients; and, although governmental health care and private insurance in the U.S. generally covers a maximum of two visits per patient per week, most patients typically physically interact with a wound care specialist or home healthcare provider only once per week due to barriers to access such as lack of transportation or job-related responsibilities, even when complications arise [6].

Methods to augment clinical care are needed because infrequent access to wound care providers can place particularly high demands on patients to be adherent with wound self-care instructions. This is especially true when complications are present, which may require multi-faceted therapy and/or frequent dressing changes and application of topical and/or oral agents. Self-managing these additional treatment demands at home can pose a major challenge for many patients who may lack adequate functional dexterity, mobility, or environmental and financial means to care for their wounds. Additionally, many patients are unable to adequately assess when these additional treatment modalities are no longer needed or useful due to lack of knowledge of signs and symptoms of healing or non-healing. These challenges present a clinical opportunity for ongoing wound monitoring using currently available "high-tech" approaches in the patient's home and clinical care settings. Specifically, the quantitative classification of wound status and determination of the current stage of inflammation as related to clinical signs such as unusual redness or hyper granulation tissue readily discernible through visible inspection or imaging [7]. The prediction of wound healing outcomes such as wound contracture and epithelization by utilizing AI through convolutional neural networks (CNN) is an emerging classification technique that could potentially augment clinical wound assessment, enhance patient self-care, and reduce the need for additional and often prolonged wound visits [2].

Convolutional Neural Networks

In technical terms, a CNN is a type of neural network that employs ML algorithms to process various types of data and draw conclusions and has been shown to achieve high accuracy in recognizing objects [8]. A CNN consists of multiple convolutional layers, the main building blocks to which filters are applied, each responsible for combining model inputs (e.g., images and sensor data) to create a map from which to extract relevant features or components/relationships of the model inputs. These inputs are then utilized in the final layer for classification or prediction of an output value such as healing or non-healing based on a learned mathematical relationship. CNNs are advantageous as there is no need to manually label features since the relevant classification or prediction features are learned automatically at each layer of the network. As it relates to the medical field, CNNs have been used to classify tumors, [9] analyze x-ray images, [10,11] and have been shown to be accurate in wound characteristic analysis [12-14]. Shenoy, et al. [13] used a CNN to classify and determine classification accuracy (%) of post-surgical wounds and wound characteristics via smartphone images; four categories included drainage (72%), fibrinous exudate (83%), granulation tissue (85%), and surgical site infection (84%). Closed wound images were also analyzed for the presence and accuracy of detecting presence of staples (95%), steri-strips, (97%) or sutures (85%) [13].

In another example, Wang, et al. [14] used a CNN to automatically segment wound images for computation of wound closure over multiple weeks of healing. In the Wang study, the wound image features extracted by CNN were then used by a support vector machine (SVM) which is a ML model that uses input features to create a mathematical vector (hyperplane) or algorithm to classify whether the wound was infected or not infected, achieving an accuracy classification of 95.7%. The CNN was also used by Nejati, et al. [15] in fine-grained tissue analysis of chronic wounds in which a feature extraction algorithm and a SVM was used to assess the accuracy of classifying wound characteristics into seven distinct tissue types including necrotic (91%), healthy granulation (83%), slough (81%), infected (96%), unhealthy granulation (82%), hyper granulation (94%) and epithelialization (78%) with an overall 86% accuracy of classifying wound characteristics. Based on these previous studies we posit that is possible to use CNNs for classification of the wound status to augment clinical assessment data. Additionally, the wound images taken with a wound camera as input into the CNN and the associated output classification or "prediction" can be explored as a benchmark for wound healing status.

Biosensing

In addition to wound imaging, biosensing technology has been

developed to detect the presence of wound contaminants and biomarkers that allow for the characterization of features analysis, for example, of pyocyanin, an important signaling molecule in the quorum sensing cascade for Pseudomonas aeruginosa, one of the most prevalent wound bacteria leading to infection and amputation [10]. Previous work includes active efforts in thermal image acquisition and machine learning-based image analysis in the biomedical context and the use of a real-time, intelligent image acquisition, processing, and analysis platform for cell culture images that can quickly be adapted to wound images [9].

Objective

The purpose of this pilot study is to establish proof-of-concept for this diagnostic AI-based approach that links clinically validated wound image data and quantitative inflammatory biomarkers to the healing state of chronic leg ulcers. This study uses deep-ML approaches to distinguish connections or predictive measures between these two data types. This combination has the potential to provide objective data through continuous remote monitoring of the wound, similar to the protocol by Zoppo and colleagues using remote monitoring through a telehealth platform [8] to alert patients about wound complications and to communicate critical information to clinicians. The long-term objective is to improve subjective wound assessment by providing a more "precise" parameter for healing status. The wound state of 10 patients with chronic wounds will be classified by evaluating thermal image and biochemical sensor data specific to the wound bioburden collected during 4 consecutive weekly wound clinic visits. These data will enable the development and evaluation of the accuracy of three ML models in wound classification tasks (healing or non-healing) by testing two assumptions:

1. The sensor-only model will be at least as accurate as the thermal image-only model
2. A fusion model (integration of data layered from both sources) will be more accurate than the image-only model or the sensor-only model.

This work will lay the foundation for the further refinement and development of a valid, quantitative, and predictive biomarker-based sensor that can be combined with real-time image analysis to improve the impact and efficiency of clinical visits. Potentially relevant outcomes would be preventing and/or decreasing wound complications, reducing major healthcare costs from complications (such as infection or amputation) and improving the quality of care and quality of life for patients suffering with chronic wounds.

Method

Study Design

A total of 10 patients will be recruited from a wound clinic in the Southeastern U.S. Inclusion criteria include 18 years of age and above, venous leg ulcer of greater than 4 weeks duration, ulcer size greater than 4 X 4 cm (1.6 X 1.6 inches) (size requirement is due to dimension of the sensors), expected to receive wound care for at least weekly for 4 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. Exclusion criteria include ulcer currently being treated for infection or is symptomatic for infection including odor, excessive exudate, purulence, pain, and/or a beefy red wound bed. Participants will participate over 4 weeks, in which they will return to the clinic weekly for dressing changes and wound treatment according to the standard of care. All necessary equipment will be supplied to the clinic.

At each clinic visit, the wound will be cleansed, debrided (if necessary), visually assessed and wound scored using the Bates-Jensen Wound Assessment Tool (BWAT) scale, and imaged three times with the thermal camera by the research assistant. Thermal images will be captured with the participant in a reclined position after 15 minutes of acclimatization to ambient conditions. After acclimatization, four sensors (one for each individual biomarker and one control) will be placed on the wound for a total of approximately 30 minutes (each inflammatory biomarker sensor is individually placed for 7 minutes plus a non-functionalized control sensor). During this time, a 1 Vpk-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. After all data are recorded, the wound will be treated and re-dressed per clinical protocol by wound clinic personnel. (Figure 1) provides a CONSORT diagram that depict patients flow throughout the study.

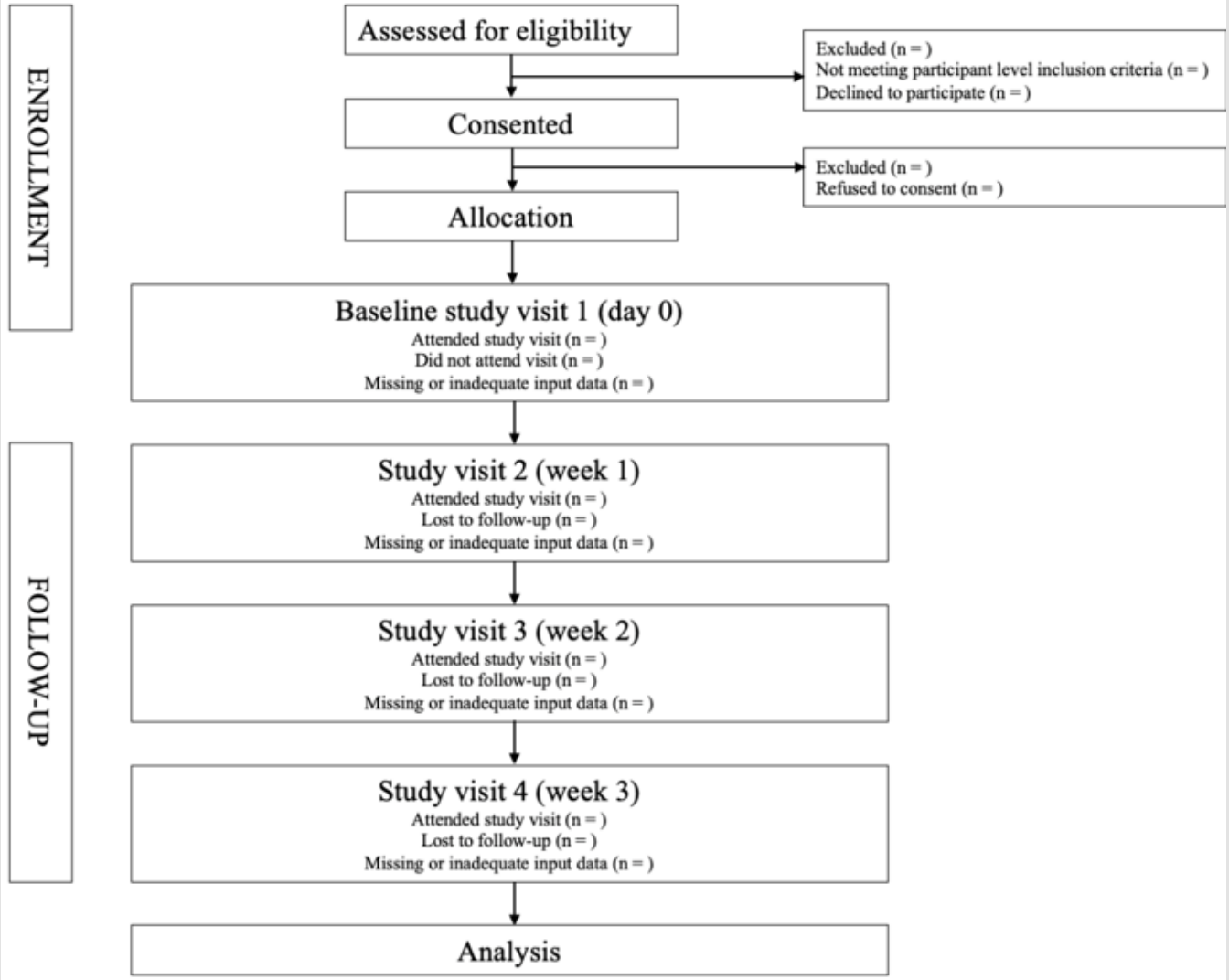

Figure 1: Study CONSORT flow diagram.

Thermal Imaging

Thermal images of the wound will be captured with the Wound Vision Scout camera (Wound Vision, Indianapolis, IN), an FDA-approved visual and thermal imaging device that has been found to produce reliable and reproducible digital and long-wave infrared images of radiation emitted by the wound [16]. In a study conducted by Langemo and Spahn of a long-wave infrared device to identify the accuracy of relative tissue temperature variations of the body surface and underlying tissue, findings showed the intraclass correlation coefficient was 0.94, indicating excellent reliability [17]. It is well established that higher or lower temperature differences over time can suggest presence or absence of inflammation, infection or underlying tissue perfusion [17]. Images will be analyzed for wound temperature and characterized for the distal and proximal wound edges and medial, lateral, and center aspects of the wound bed. The mean wound temperature and difference between the wound and control temperatures will also be calculated.

Standardized Clinical Assessment

Wound status will be measured with the BWAT (used with permission) [24]. The BWAT, a 13-item evidence-based assessment tool to monitor wound characteristics and healing progress was selected because it has been used in numerous studies of chronic wound healing including lower extremity and foot ulcers and pressure injuries and provides a detailed score of several wound characteristics including color, odor and necrosis, indicative of non-healing complications such as infection. This instrument also includes 2 items related to wound size. Each item is scored from 1-5 on a modified Likert scale where 1 indicates least severe and 5 indicates most severe and are summed for a maximum total score of 65. Wound status is scored on a continuum where higher

total scores indicate more severe wound degeneration and lower indicate wound regeneration and tissue health. The instrument has interrater reliability reported to be 0.78 - 0.92 [19,24]. The score from this clinical assessment will be compared with the final model output to determine correlations with healing status. The progression (or regression) of scores over the course of the 4-week study and the wound size will be used to determine the overall classification of 'non-healing' or 'healing' (i.e., a score reduction of 40% [20] after four weeks).

Biosensor Device

The biosensor device [21,22] (Figure 2) consists of a conductive nanofiber (black portion in contact with wound) composite, the silver portion on the top is where the sensor gets connected to the electrical signal measuring device (LCR meter, potentiostat, etc.) and the darker black portion in the middle is an insulation layer. The sensor is embedded in the composite and functionalized (antibodies added) to provide a proportional electrochemical response to changes in biomarker concentration similar to devices published in the literature [18,23]. The biosensor consists of a combination of biocompatible elements, including a polyester base layer, silver ink and MWCNT both having been demonstrated as safe in short topical exposures such as presented here (except for individuals with silver allergies), and the poly-l-lactide polymer base for conductive nanofibers. We developed the nanofiber composite biosensor to provide quantitative assessment of the following inflammatory markers related to various stages of wound healing: transforming growth factor – beta (TGF-β), tissue necrosis factor – alpha (TNF-α), and vascular endothelial growth factor (VEGF). TNF-alpha, commonly associated with the pro-inflammatory M1 macrophage phenotype, will be measured against TGF-beta (anti-inflammatory, associated with proliferation phase of wound healing and M2 macrophage phenotype) and VEGF (associated with angiogenesis).

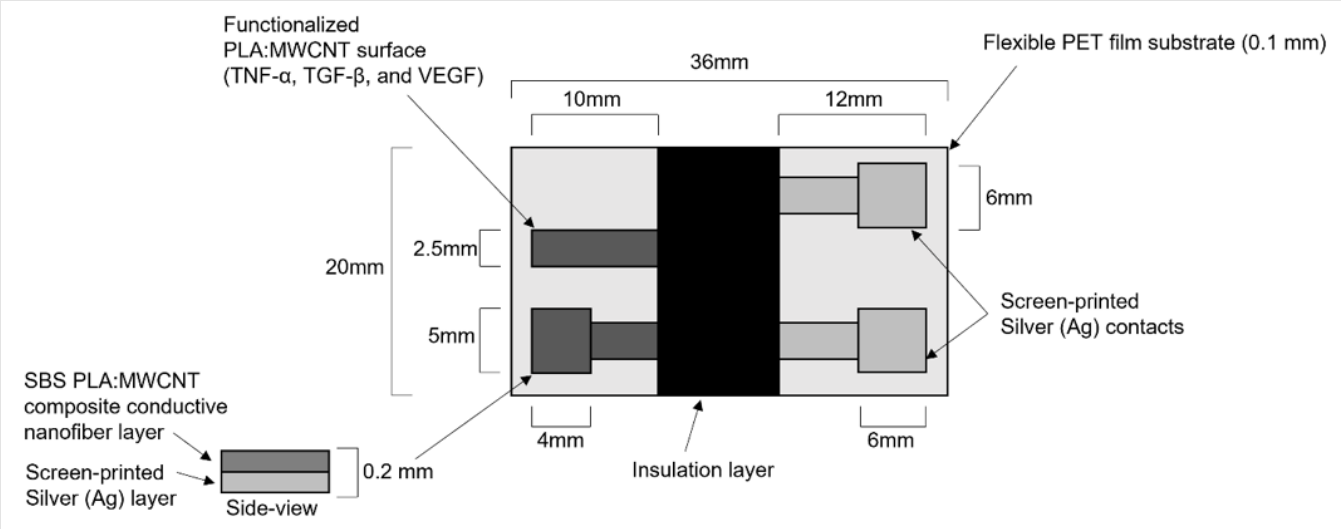

Figure 2: Flexible nanofiber composite biosensor on polyethylene backing.

Each biomarker (TGF-beta, TNF-alpha, and VEGF) measurement will be normalized using a control (non-functionalized) sensor that will characterize the general electrochemical impedance response to the wound bed. Taken together, adding biosensor data and ML image analysis to classification modeling provides a major advantage in the ability to leverage information from the wound that is not readily available through visible inspection (i.e., image-based approaches or human evaluation in-clinic). In this way a more objective and quantitative approach to classification that advances the state-of-art and enables further validation and prediction of AI-based wound healing models will use biomarkers already accepted as indicators of progression of wound healing.

Classification

These data will be used to implement a CNN that evaluates a sequence of images. This approach builds on the classic implementation of CNNs that classify single images by adding a time sequence component to capture wound changes as an input into the classification decision of the model. By exploring longitudinal sequencing, we posit that the deep ML model will be able to account for the change in wound features (size, color) across multiple images. This approach is similar to the work of other researchers [14] in which a series of extracted features from multiple images over time was evaluated to estimate the healing state for the wound. However, instead of estimating the date in which the wound will be

healed, our model will classify the wound as 'healing' (versus 'non-healing') by calculating changes in surface area and temperature from thermal images and analyzing the clinical assessment via the BWAT score. The ML classification will be accomplished by using the later layers from a previously trained CNN model (i.e., Alex Net and ImageNet) as a high-level feature extraction algorithm. These pre-trained models will readily extract basic features from the image, such as wound edges and background color, so that the newly constructed CNN will only focus on features specific to wound analysis.

This process greatly reduces the number of images needed for model training, and in turn gives this study a feasible scope with respect to numbers of patients and required clinical interactions. This technique is a form of transfer learning [21] and was deployed by researchers [13,15] due to the limited number of wound images that could be collected and used in clinical image analyses. As presented, n=10 participants will produce 120 thermal images and 120 counter light images (3 images per visit, 4 weekly visits, 10 patients). These 120 images will be combined with the 30 minutes of time-series data (4 sets per patient) to comprise the training set, which is also supplemented with open-source images using the transfer learning approach. Tools such as Efficient Net have successfully been used to pre-train models for image classification tasks using small datasets. We will employ these tools, and others in the Python Keras library to successfully implement the deep learning approach, despite the small dataset for this proof-of-concept work. Based on this transfer learning technique, for each image given as input the pre-trained CNN will extract a feature vector; in other words, if n=X -images are provided as input, then an equal number of feature vectors will be generated. These feature vectors will then be passed to a SVM that can be used to separate data into various classes. Thermal images (and the associated extracted features) will then be classified into 'healing' or 'non-healing' based on the BWAT score that serves as an alternative to traditional expert labelling used in supervised ML approaches.

To summarize this approach, the CNN-SVM classifies a wound thermal image as healing or non-healing through extraction of basic features via pre-trained CNN. The model will be trained to recognize healing through patterns observed in input image data and BWAT scores. The inclusion of biosensor data as a time series requires the use of a different model. The CNN approach can also be used for feature extraction from large sets of sensor data, but the later layers will then be fed sensor data in sequential order to a recurrent neural network (RNN), specifically a long short-term memory (LSTM) neural network, that performs the healing or non-healing classification task. An LSTM neural network is a type of RNN consisting of a chain of repeating units that enable learning from time series input (i.e., sequences of data).Ohura [21] Unlike traditional RNNs, LSTM networks can learn long-term dependencies. For this classification model, the CNN-SVM architecture exhibited in previous research Shenoy [13,15] serves as a foundation for the CNN-LSTM deep ML approach to chronic wound diagnostics. This pilot study introduces an advancement in current approaches by incorporating as additional input, biosensor time series data quantifying the levels of inflammatory biomarkers including transforming growth factor – beta (TGF-β), tissue necrosis factor – alpha (TNF-α), and vascular endothelial growth factor (VEGF), known to be associated with wound healing (Figure 3).

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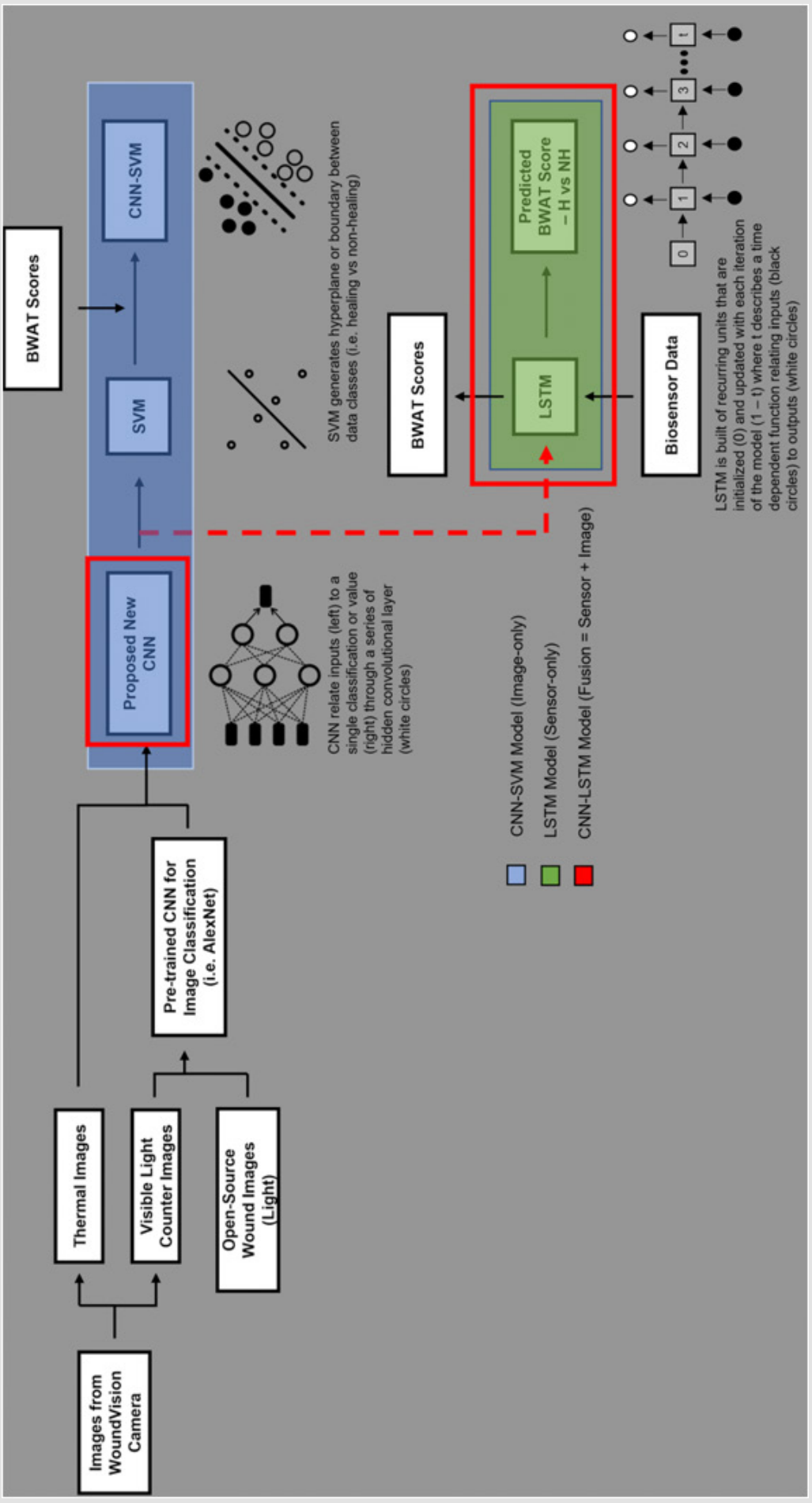

Figure 3: Flow diagram of classification model.

Data Analytic Processes

To determine feasibility of the ML approach, three different models with different input values will be implemented and evaluated with the same output classification of 'healing' or 'non-healing'. The first model (baseline method) will consist of the series of thermal images as input, the second model will take as input a series of sensor readings for biomarker concentration from the biosensor device, and the last model will take both image and sensor data as input.

To develop the models, two major processes will include

1. A training set consisting of the input values of image and biosensor data and the output label (BWAT score of wound state along continuum of healing over 4 weeks) and

2. Evaluation of model accuracy. To accomplish the training, a multiphase approach was developed for determining wound healing that consisted of an advancement on the CNN-SVM architecture:

- Step one will utilize transfer learning to associate thermal images (these provide thermal information at respective locations within the wound bed) and visible light images with the wound state. Thermal and light wound images, the later collected from open-source wound image databases [25,26] and the counter image produced by the Wound Vision Scout camera, will be used to establish a training set for the CNN. Because there are far fewer thermal images than light images, a transfer learning approach will first be employed in which a CNN model is trained using light wound images against BWAT wound status scores to determine the appropriate category to which an image belongs. After training the CNN model, the later layers from the model will be removed and replaced with an SVM. The model's knowledge on light wound images and any respective correlations with the BWAT wound scores will be transferred to thermal images with the same BWAT scores. A pre-trained CNN for basic image segmentation will be applied to the thermal images and extracted features will be inputs into the SVM and will be used to make the healing and non-healing classifications.

- In step two, image classification will be associated with biosensor readings to generate corresponding BWAT wound states. Because the sensor readings were collected at the same time as the thermal images, it can be assumed that the sensor readings taken at this time will be related to the BWAT classifications generated by SVM from step one. Using this assumption, a RNN will be trained to predict (output) sensor-derived wound state classifications from taking inputs as the TGF-beta, TNF-alpha, and VEGF sensor readings and the extracted features from the thermal images given the shared time scale. Specifically, the LSTM neural network will be used to combine image-based wound state labels and real-time sensor data. The LSTM is uniquely equipped to handle time-series data, [25-27] but the combination of images to classification is a novel advancement in the field.

This sub-objective will be separated into two LSTMs, with one including both biosensor data and images as input (fusion model) and one including biosensor data as inputs (biosensor model). In the second phase, a set of model accuracy experiments will be conducted to evaluate the assumptions that

a) A model that uses biosensing data as input will be as accurate, if not more accurate, than a model that uses only the image data; additionally,

b) A model that fuses both the image and biosensing data as input will be more accurate than a model that uses only images or only biosensing data. In testing these assumptions, a set of experiments will be conducted in which each model (CNN-SVM image, LSTM biosensor, LSTM fusion) will be evaluated using the same collected validation data which is made up of wound characteristics (size, depth, edges, etc.) that form an overall BWAT score wound state based on the image, biosensing, or combination data presented. This will serve as the ground truth to which the model's output classification will be compared.

Data Management

This study will use Research Electronic Data Capture (RED Cap) for direct data entry and management. RED Cap is a software toolset and workflow methodology for the electronic collection and management of research and clinical trials data. RED Cap provides secure, web-based, flexible applications, including real-time validation rules with automated data type and range checks at the time of data entry. Exports are made available for several statistical packages including SPSS, SAS, SATA, R and Microsoft Excel.

Statistical Analysis

For this study, the independent variables are the three models, and the dependent variables are the accuracy evaluation metrics listed below. A multivariate analysis of variance (MANOVA) test will be used to determine whether there is a significant difference (P value < 0.05) between the models and their performance on the validation dataset as it relates to each of the accuracy metrics. Given the relatively small sample size, statistical power may not be achieved for the MANOVA test depending on the variance between participants and weekly visits. Any limitations in the statistical approach will be properly reported during dissemination of pilot-study results. Descriptive statistics will be used to characterize the population and determine relationships between models and

BWAT score classifications. This analysis will prove or refute the two assumptions.

- True Positive (TP), the number of times the model is correct in classifying the wound along the BWAT score.
- True Negative (TN), the number of times the model is correct in classifying the wound as not belonging to a particular BWAT score.
- False Positive (FP), the number of times the model is incorrect in classifying the wound along the BWAT score.
- False Negative (FN), the number of times the model is incorrect in classifying the wound as not belonging to a particular BWAT score.
- Precision, the number of correct actions made (TP) divided by the number of all actions made (TP + FP).
- Recall, the number of correct actions made (TP) divided by the number of instances where an action existed (TP + FN).
- F-Measure, the balance between precision and recall.

Ethical Approval

This protocol (#Pro00100687) was approved by the Medical University of South Carolina Institutional Review Board on 6/29/2020, conforms to the Declaration of Helsinki and will be conducted in compliance with Good Clinical Practices (GCP) of the International Conference of Harmonization (ICH). Participants will provide written informed consent for their study participation and receive $50 per study visit, total $200 (USD) for completion of all 4 visits. The study is expected to commence January 2023.

Data Safety and Monitoring

The attending wound physician will serve as the study's medical safety monitor. Participants will be asked to rate their level of pain on a numerical rating scale (NRS) of 0-10 from their wound before, during, and after operation of the biosensor on their wound bed. Study procedures will be stopped during biosensor operation if wound pain levels become elevated. Patient wounds will also be clinically assessed for any deleterious adverse events that may remove them from study participation, such as: excessive bleeding or severe wound deterioration as noted on the BWAT score. Participants will also be asked to report any suspected or noted adverse events that may occur outside of the study visit. The study team will confer with the study physician on the study's risk assessment profile and study continuance.

Conclusion

Chronic wounds often require months or, in some cases, years to heal, frequently develop complications, and require multiple treatment modalities and specialty wound care. Clinical assessment methods that provide quantitative data such as the use of biosensors to augment wound inspection could potentially revolutionize current strategies for detecting complications such as wound infection. The recently developed biosensing device using artificial intelligence will test a proof-of-concept of input from three deep-ML models that include individual and combined data from thermal images and the biosensor on healing and non-healing classification in a patient suffering with chronic wounds such as venous leg ulcers. The ultimate goal is to fill the gap in the need for point-of-care wound monitoring using novel technology to combat complications in this highly challenging patient population.

Main Take Away Points

1. Chronic wounds are often fraught with complications such as prolonged inflammation that lead to poor healing.
2. Artificial intelligence developed through machine learning models using convolutional neural networks have the potential to advance wound science for the detection and prevention of wound complications during healing.
3. Biosensing devices that monitor the wound during healing and utilize artificial intelligence can augment clinical assessment and enhance wound care.

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Author Contributions

All authors conceived the presented study idea and drafted the manuscript with critical content and/or editorial contributions. All authors discussed the final draft of the manuscript and granted approval of the version to be published.

Conflict of Interest Statement

The authors declare no conflict of interest.

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