Imaging of Chronic Wounds For Point-of-Care Assessment of Wound infection

By

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Abstract

A chronic wound is medical condition where the wound fails to proceed through proper wound healing processes. Although many treatments exist for this condition, many of these treatments fail to properly rebuild the wounded tissue, increasing the risk of development of a chronic wound. In this following project, it was broken down into different project plans: (1) testing a novel, simple low-cost multispectral autofluorescence probe that could detect specific fluorescent characteristics of different biomarkers in the wound, (2) proposing a plan to understand the presence and role of *P. Aeruginosa* in the wound, and (3) presenting probiotics as an alternative treatment to chronic wound. All these routes represent the different methodologies and approaches that could arise ideas to tackle chronic wounds.

Acknowledgements

I would like to first thank my principal investigator, faculty mentor, and mentor, Dr. Manuela Martins-Green, for her unwavering support and guidance throughout my capstone project and undergraduate career. Although COVID-19 caused me to be away from campus and the lab, Dr. Manuela Martins-Green continued to adapt and found alternatives for my capstone project. I am thankful for the various opportunities Dr. Green has brought to me, and I am honored to have joined her lab and to be her student in the classroom and research settings. I would like to also thank graduate student, Jane Hannah Kim, for teaching me how to prepare the research mice and committing long hours to ensure I have the right skills to make my capstone a success. I am grateful to have worked with such a gifted and amazing graduate student during my time in this lab. Lastly, I want to thank my friends, my family, and God for their endless love and support throughout this journey. I will treasure the various connections and relationships I have made during my undergraduate years because they truly shaped me to become the person I am today.

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Introduction

Chronic wounds are defined as wounds, or skin barrier defects, that have not proceeded through the normal phases of wound healing, which include hemostasis, inflammation, proliferation, and remodeling (Frykberg and Banks; Eming et al.). Chronic wounds have been often characterized with bacterial infections, high oxidative stress, and inflammation that cause significant damage to the host tissue (Kim, Yang, et al.). Also, chronic wounds have been further linked to not only the previously mentioned characteristics but also various factors. Some of these factors include obesity, diabetes mellitus, vascular insufficiency, local-pressure effects, and advance age (Sen et al.; Eming et al.). All of these characteristics and factors represent the immense complexity of chronic wounds and the impairment of wound healing.

Although each chronic wound has its own unique origin, each chronic wound can be characterized into various groups: venous leg ulcers, diabetic foot ulcers, and pressure ulcers (Bjarnsholt et al.). Venous leg ulcers (VLUs) are caused by a malfunction of the venous valves – increasing pressure in the capillaries and edema (Bjarnsholt et al.). In the United States, there are approximately 600,000 who suffer through venous foot ulcers. Within the senior population, 1.69% of the US senior population suffer through venous ulcers. Consequently, the annual cost of treatment for venous ulcers in the US healthcare system has been estimated to be $2.5-3.5 billion annually (Sen et al.). Next, diabetic foot ulcers (DFUs) are caused by immense pressure upon the neurophatic and ischemic foot (Bjarnsholt et al.). It was reported that ulcers and other foot complications constituted about 20% of the 3 million hospitalizations every year. With over 23 million Americans being diabetic, DFU is becoming a major concern as the number of diabetics are expected to rise over the next 20 years not only in the United States but also around the world (Sen et al.). Lastly, pressure ulcers (PUs) are caused by repetitive pressure or load

upon skin and/or localized tissue (Sen et al.). These types of wounds are extremely deadly to those that are elderly, stroke victims, and immobilized patients. It has been estimated 7.4 million pressure ulcers has occurred around the world. In the United States, the cost of treatment for pressure ulcers has been $11 billion per year (Sen et al.). As a result, there is a need to understand the cause of wound healing impairment in chronic wound due to its immense burden to human life and the costs to various health systems.

The response to this need has been through animal models. Understanding chronic wounds through humans is extremely difficult. Oftentimes, patients arrive with these wounds in their advanced state, so it becomes difficult to analyze these wounds to understand the initial development of chronic wounds. Instead, animal models are used to study the process of chronic wound development from initiation to fully developed chronicity (Dhall, D. Do, et al.). In the animal models of venous hypertension, there have been efforts to understand the mechanisms of how venous hypertension stimulate inflammation and cause changes within skin and venous valves to get a better understanding of VLUs. One model is the mesenteric venule occlusion rodent model. The mesentery was exposed and analyzed the fluid pressure going through. A mesentery is a fold membrane that attaches the small intestine to the abdominal wall. It proved insightful in gaining more understanding about the impact of venous hypertension. However, its applicability to human valves was questioned due to the anatomy of a rodent and a human are quite different (Bergan et al.). Next, in the animal models of DFUs, there have been many models proposed. One model is the NONcNZ010 mouse model. This model utilizes a new polygenic strain of Type 2 diabetes. It argued this new strain of Type 2 diabetes is more applicable to the human type 2 diabetes. Although the study's efforts of being more applicable, the various mouse wound models failed to close (Fang et al.). Lastly, in the animal models of

PUs, there have been models proposed. One model is the rat magnet ischemia-reperfusion model. In this model, there is a steel plate underneath the skin, and a magnet is applied to induce the pressure wound to occur. This model has been successful in replicating some certain features of human pressure ulcers like reduced blood flow, hypoxia, and immune cell influx. However, this model neglects the microbiota that exists within the wound (Nunan et al.). Clearly, there have been many efforts in attempt to understand chronic wounds, but these models fail in their applicability to human chronic wounds and their ability to mimic observed characteristics in human chronic wounds (Salcido et al.; Nunan et al.).

Although many models have been developed, many have failed to become chronic nor did they develop biofilm like found in human chronic wounds. These various models fall short in replicating the various conditions and characteristics found in actual human chronic wounds. Our laboratory group has a chronic wound mice model that has significant characteristics of human chronic wound. It has high levels of OS, remains chronic for long periods of time, and forms natural-occurring biofilm on the wounds, which contain bacterial species commonly found in human chronic wounds. This model mouse is what I will be using as I progress through the various applications outlined in this project.

Part I: Imaging Chronic Wounds for Point-of-Care Assessment of Wound Infection (UCI-UCR collaboration)

Background

Over 75% of modern war wounds have been caused by explosive-related injuries (Eardley et al.). These complex traumatic injuries suffered by military personnel are the leading causes of morbidity and mortality (Akers et al.). Despite efforts of debridement and antibiotic

treatment towards these wound injuries, wound infection remains the main complication for these type of battlefield injuries, which often pose a risk of health and function to these injured military personnel (Eardley et al.). In addition, these persistent wound infections can result in amputations, extended periods of rehabilitations, and high utilization of hospital resources (Akers et al.).

One of the first steps of wound infection is contamination. Contamination describes all wounds acquire micro-organisms, and if the wounds have a favorable environment, the point when these microbial species begin to proliferate within the wound bed. As infection continues, these microbials begin to form biofilm. It has been acknowledged: when biofilms are present in chronic wounds, the infected wounds become resistant against antimicrobial and antibiotic treatments (Haesler et al.). Consequently, bacterial biofilms are involved in 78.2% of chronic wounds (Gajula et al.). Typically, once biofilm has been created, the bacteria community in the biofilm begin to mutate to obtain antibiotic resistance, which make various antibiotics at various concentrations ineffective for treatment. Also, mechanical debridement, the scraping of biofilms from wounds, becomes ineffective because it is difficult to completely remove all the biofilm once it has been sheared into pieces (Yan and Bassler). For those reasons, the capability of detecting this contamination of microbials during the early stages of wound infection is crucial to ensure good clinical outcomes. As the wound infection continues towards chronicity, the wound's condition worsens through changes within the microbiome and tissues which further imped the healing process (Mustoe et al.).

To improve these clinical outcomes, better tools are needed for clinicians to quickly diagnose and treat these wound infections before they reach chronicity. Current methods of diagnosis are quite extensive that cause delay in treatment. These methods include physical

sampling of wound site and sending these samples to off-site specialist. A tool that would map the infection site would greatly expedite the diagnosis process and treatment process to prevent these wounds to becoming chronic (Wilder-Smith et al.).

In this project, Dr. Wilder-Smith and her team from UCI medical center collaborated with my PI, Dr. Manuela Martins-Green, to fulfill this need of efficient diagnosis tools for chronic wounds. Dr. Wilder-Smith has a novel auto-fluorescence probe that would map these wounds to provide information about the infection state of the wound. My PI, Dr. Manuela Martins-Green, would provide the wounds to sample on for the probe. My role in this project was to collect images using the probe over a duration of 3 months. This was my project before COVID-19 hit.

Methods

In this chronic wound infection model, I, with the help and supervision of a graduate student, would induce high levels of oxidative stress into mouse wound tissue immediately after wounding by using a one-time treatment with inhibitors specific to the antioxidant enzymes catalase and glutathione peroxidase (GPx). These wounds with high levels of oxidative stress develop biofilm naturally, become acute within 4 days, fully chronic within 20 days after treatment, and can remain open for more than 60 days (Dhall, D. C. Do, et al.; Kim, Yang, et al.).

These mice were kept at the University of California, Riverside vivarium. Whenever mice met the satisfied age and size, they were subject through the wounding process. The mice were anesthetized with a single intraperitoneal injection of ketamine (80 mg/kg body weight)/xylazine (16 mg/kg body weight). Full thickness 7 mm punch wounds (excision of the skin and the underlying panniculus carnosus) were made on the back of the mice using a 7 mm

biopsy punch (Acuderm, Inc). Twenty minutes prior to wounding, mice were treated once intraperitoneally (IP) with 3-amino-1,2,4-triazole (ATZ) (Aldrich Chemistry; St. Louis, MO) at 1 g/kg body weight, an inhibitor for catalase. Immediately after wounding, they were treated once topically with the inhibitor for GPx, mercaptosuccinic acid (MSA), (Sigma Lifesciences; St. Louis, MO) at 150 mg/kg body weight. Next, the wounds were covered with Tegaderm (3 M; St. Paul, MN) to prevent contamination and were kept covered for the duration of the experiments. For the controls, these mice experienced the same process except had PBS instead of the antioxidant inhibitors (Dhall, D. C. Do, et al.; Kim, Yang, et al.).

After preparing the mice, I would remove the Tegaderm from the wound for imaging, and this process was outlined at a certain set of days after wounding. To take the pictures, the Tegaderm, covering the wound, needs to be removed. After the wound, the camera needs to be hovered above the wound and a steady hand is required. The camera would take a series of pictures, so the probe needs to be held still for about a few minutes. Without this steady hand, the quality of the pictures may decrease and risk unreadable data. After pictures are collected, these pictures are stored in a specific folder on the camera until further use. Also, after imaging, I would collect wound samples using a sterile swab, which was stored away for future reference at -80 °C freezers. After these images were obtained, these pictures were sent to Dr. Wilder-Smith and her team for analysis.

Implications/Future-Plans

The probe functions by emitting different colors at the wound. The purpose of these different colors is to excite the chromophores from specific components in the biofilm to later emit a fluorescence. Chromophores that emit fluorescence are classified as fluorophores. Each fluorophore has a distinct absorbance and emission wavelength. These various fluorescence

signals from these various components of biofilm are the main indicators to determine infectivity because there are structural changes that are occurring. The rise and disappearance of certain molecules within the wound are serving as markers to assist in the indication. Pictures, Fig 1 and Fig 2, of this functionality is placed in the picture section of this paper. In the picture, it shows the different colors being emitted to the different chromophores to induce a florescence from them. The specific type of components and how these signals determine infectivity are unknown because Dr. Wilder-Smith algorithms and parameters were never described to me. These pictures were meant to show how the camera was analyzing the wounds between the nonchronic and chronic wound samples. Despite this setback, the overall functionality of the probe was successful. As described by Dr. Wilder-Smith, the probe was able to map out wound margins and dimensions, inflammation, and swelling. Future talks are currently taking place between my PI, Dr. Green, and Dr. Wilder-Smith to perform a continuation of this project. Instead of monitoring biofilm, the purpose of the next phase would be to monitor the rise of different bacterial species through fluorescence.

Part II: Analysis of *Pseudomonas aeruginosa* Oxidative Stress Defense Genes in a Novel Chronic Wound Model

Background

Pseudomonas aeruginosa (*PA*) is a biofilm-forming bacterial species that is commonly found in human chronic wounds. Biofilm is composed of protein, polysaccharides, and DNA that enhances the colonization of bacteria on a biotic surface. These biofilm communities promote several beneficial features to assist bacteria to quickly adapt and survive in even the harshest environment. These biofilms are typically found on chronic wounds and make healing chronic wounds difficult (Costerton et al.; dos Santos et al.; Nunan et al.; Roy et al.; Seth et al.;

Trøstrup et al.). It was reported chronic wounds have a mortality rate of 50% in the first 3 years, but this rises to 70% after 5 years (Sen). As a result, it is urgent to understand the cellular and molecular mechanism of chronic wounds.

One way to stimulate biofilm is oxidative stress (OS) (Kim, Yang, et al.). Oxidative stress is simply a build-up of oxygen radicals, like superoxide anion and hydrogen peroxide. At normal levels, these radicals help fight off invading pathogens; however, at excessive levels, these radicals become toxic to cells. *PA* can survive in these harsh conditions because of unique OS defense enzymes: superoxide dismutase (SOD), catalase (Kat), and alkyl hydroxy peroxidase (Ahp). SOD converts the superoxide anion into hydrogen peroxide. Kat and Ahp then converts hydrogen peroxide into water (Dhall, D. Do, et al.; Dhall, D. C. Do, et al.; Kim, Yang, et al.; Ochsner et al.). *PA's* persistence in these harsh environments has been shown in one of our laboratory group's latest papers. It showed as oxidative stress was increased, *PA* soon became the dominant strain in the microbiome of the wound (Kim, Yang, et al.). Understanding how *PA* survives will give insight about microbial infection in chronic wounds and can lead to the identification of biomarkers that may prove beneficial during the development of treatments.

Since this was a proposal, I proposed the question: How does *Pseudomonas aeruginosa* respond to OS in a novel chronic wound mouse model? I hypothesized that SOD, Kat, and Ahp will be highly expressed in the chronic wound model. The approach to test my hypothesis is by analyzing the various gene expressions of SOD, Kat, and Ahp in our laboratory's chronic wound mice model. In this project, I, with the help of my PI Dr. Manuela Martins-Green, formed a proposal to plan for my involvements in the lab if, at the time, COVID would be resolved. Despite this plan not occurring due to the extensive period of COVID-19, I would like to show my efforts during this time.

Methods

The following describes the methods I would be doing if not canceled by COVID-19.

All the mice will initially receive the same treatment: shaving of the hair on its back, applying *PA* isolated from known chronic mice from previous experiments before wounding, and finally, creating a wound on the exposed surface of the mice. The wounding process is the same as described in the methods section in Part I, so for specifics, please refer to part I. For the control group, mice will receive injections of PBS to create a non-chronic wound. For the experiment group, mice will receive injections of known inhibitors of antioxidants enzymes, catalase and glutathione peroxidase (GPx). From both mice groups, biofilm will be collected at 12^{th} and 18^{th} hour after wounding and analyzed to investigate activation of *sodA* and *sodB* (SOD), *katA, katB,* and *katE* (Kat), and *ahpC* and *ahpB* (Ahp) as it goes through the central dogma.

The central dogma illustrates how the information in genes encode for proteins. The first step in the central dogma is transcription. Transcription is the process in which DNA is made into mRNA by copying DNA into RNA. To quantify mRNA levels, mRNA will be extracted using our laboratory's standard protocol and purified to ensure the sample is of high quality. After preparing the sample, we will use Reverse-Transcriptase Quantitative Polymerase Chain Reaction, or RT-qPCR, to convert mRNA to cDNA; to amplify this cDNA with specific primers to the genes of SOD, Kat, and Ahp; and to quantify the cDNA to measure the starting amount of mRNA.

The second step in the central dogma is translation. Translation is the process of converting mRNA into proteins by proteins by correlating triplets of nucleic acids to specific

amino acids. To quantify protein levels, proteins will be used be extracted, purified, and ran on an SDS page to separate the proteins based on molecular weight. Antibodies of SOD, Kat, and Ahp will be used to measure specific antigen-antibody interaction from the complex mixture of proteins on a membrane. In addition, the activity of these enzyme will be analyzed through enzyme activity assays because the production of proteins does not guarantee that the proteins are active and functioning. These various assays will help verify the extracted and purified proteins are active and functioning.

All of the data produced will undergo statistical analysis. We will be using a two-tail t-test. The data will be evaluated as a ratio between the experimental and control groups to determine if upregulation or downregulation occurs.

Expected Results/Implications

Chronic wounds are a serious and growing health concern. Approximately 6.5 million patients suffering from chronic wound infections in the US, costing the healthcare system $25 billion. If left untreated, statistics suggest an increase of 125 million chronic wound cases by 2050, 20 million of which will require an amputation (Dhall, D. C. Do, et al.; Sen et al.). It's evident as these cases continue to grow so does the need for understanding the underlying mechanisms of chronic wounds. This project is a step forward towards this need. By utilizing a novel wound mouse model, it provides unique *in vivo* approach on how *PA* can colonize and survive in a host under oxidative stress. These findings would not only useful insight about microbial colonization and survivability but also provide an innovative way to study different aspects of chronic wounds through this mice model.

From these experiments, I predict the transcription of these genes will be higher in the experimental group than the control. Also, I predict the translation of these genes will be higher in the experimental group and the control. Finally, I predict the enzyme activity will be higher in the experimental group and the control. I suspect this because in our laboratory's microbiome study, we observe *PA* to be the dominant microorganism in the biofilm of our chronic wound model, implying *PA* is surviving in the OS conditions (Kim, Yang, et al.). In addition, a cell culture study found increased genetic activation of these enzymes, and it found mutated versions of *PA*, missing some Kat and Ahp genes, to be negatively impacted by oxidative stress (Ochsner et al.). Therefore, all of these align on supporting my hypothesis because *PA* needs to survive in the environment to stimulate biofilm production in the host, so SOD, Kat, and Ahp need to be activated.

Lastly, the findings from this project will be used to help prepare and plan future project plans addressing the other stages for *PA* stages of biofilm development. Also, it will be sent out to our laboratory collaborators and serve as a reference for their similar experiments to gain a better perspective on the cellular and molecular mechanisms that are occurring with *PA* in chronic wounds.

Setbacks

However, during the planning phase of this proposal, there were some setbacks. During the equipment search, I faced some struggles finding commercially available antibodies for the western blot. There were some antibodies for the target proteins, but these proteins were targeted for mammalian cells not *P. aeruginosa*. These antibodies from other animals would not be viable because my proteins were from bacteria, so the western blot would find nothing. Furthermore, genetic analysis would be required to continue. Also, I struggled to find some

enzyme assays for my target genes. As I researched, I encountered the same problem as with the antibodies: the enzyme assays were intended for mammalian cells. If ever this proposal gets used again, these several challenges need to be resolved before any experiments can take place.

Part III: Probiotic Bacteria in Wound Healing

Background

The human skin is the largest organ that serves as the first line of defense against the external environment. The skin is inhabited by a complex and diverse community of microbes that can benefit or harm the host. This community of microbes living on the skin is often termed as a skin microbiota. The composition of these microbiotas is often quite diverse and vary between person to person due to different individual and environmental factors (Kim, Ruegger, et al.; Fijan et al.; Knackstedt et al.). As a result, the composition of these microbiotas is never static but dynamic. These various fluctuations in the composition can lead to disease states on the skin level, like chronic wounds. It has been observed the healing process of chronic wounds is delayed due to the prevalent presence of pathogenic bacteria taking advantage of the available nutrients and contributing damage within the host tissue (Kim, Ruegger, et al.; Fijan et al.). For these various reasons, for wound healing to resume within chronic wounds, beneficial bacteria need to be promoted, while pathogenic bacteria need to decrease within the composition of the skin microbiota (Knackstedt et al.).

The traditional approach has been to reduce the microbial load within these infected chronic wounds. Methods that have been used include saline irrigation, debridement of necrotic tissue, and the use of antibiotics (Fijan et al.; Murphy and Evans). Despite these strict protocols to wound care, there is a growing concern about the increase of antibiotic resistance

microorganisms against antimicrobial drugs. While there is a debate on the pros and cons of the usage of antibiotics, this complete dependence of antibiotics would inevitably develop more antibiotic resistance microbial strains that would prove to be more challenging to fight against in the future. Therefore, the use of alternatives is imperative (Fijan et al.; Nakamura and Daya).

One of these possible alternatives is probiotics. Probiotics are live bacteria and yeasts that are beneficial for the human body. This specific category of microorganisms has been found to bring a positive effect on host health and skin healing. Although the exact mechanism of action by these probiotics are still under investigation, studies suggest the mechanism of action is multifaceted (Walker; Mohammedsaeed et al.; Fijan et al.). Probiotics have been found to interfere with the growth and quorum sensing system of pathogens and to promote wound healing at the epidermis and dermis level (Fijan et al.). Also, probiotics were shown to produce EPS for immunostimulatory activity, regulate antimicrobial peptides, and augment cell proliferation angiogenesis. As a result, probiotics remains a worthy alternative but requires further study on the mechanisms of action and to reverify the discrepancies being seen in clinical studies (Fijan et al.).

In this project, I performed a small search on some probiotic bacteria strains that promote wound healing. Through this search, I highlighted some probiotic bacterial strains that have showed wound healing or any antagonism towards biofilm-forming bacteria strains. I hoped these various strains can be used as a reference for future project plans with my PI's diabetic mouse model.

Methods

For this search, I mainly relied on Google Scholar to find these various studies, and the various search terms were probiotics, chronic wounds, wound healing, and bacterial strains. As instructed by my PI, I focused mainly on the probiotic bacterial strains and neglected the probiotic yeast strain studies.

Findings

Lactobacillus plantarum

A lactic acid bacterium that is a common microbe in many foods like diary, meat, and fish. This lactic acid bacterium is being used for its medical properties which include wound healing (Halper et al.). Studies utilizing *lactobacillus plantarum* focused on testing the probiotic's antipathogenic properties and wound healing capabilities. Ramos and his team found *L. plantarum* to inhibit metabolically active *Pseudomonas aeruginosa (PA)* and the production of various *PA* virulence factors, which include elastase, pyocyanin, and rhamnolipids, in *PA* cultures mixed with various *L. planetarium* supernatants (Ramos et al.). Valdez and his team found similar results in their *in-vitro* studies as well. Also, Valdez and his team utilized a burn wound model. With their burn wound model, Valdez and his team found enhanced phagocytosis of *PA*, improved tissue repair, and a decrease of apoptosis (Valdéz et al.). Satish and her team also utilized a burn wound model and found the pathogenicity of *PA* to be reduced. In their burn wound model, Satish and her team found the reduced severity and length in burn infection and reduced scarring in the model (Satish et al.). All of these studies found *L. plantarum* as a potential therapeutic agent for wound infection and an antipathogenic agent in these wound infections (Valdéz et al.; Ramos et al.; Satish et al.).

Lactobacillus brevis

A lactic acid bacterium that was extracted from traditional dairy products of Iran. *L. brevis* was chosen because of its high exopolysaccharide (EPS) production and mucoid coloniese production. In a study performed by Nasrabadi and his team, *L. brevis'* cutaneous wound healing properties were testing by applying *L. brevis* to wounds on rats and observing the effects over a set amount of time. After the experimental period, they found the wound diameter in the experimental group to have the smaller wound diameter compared to the control group that had a slightly larger wound diameter. Also, through histological examination, they found increased number of fibroblasts and decreased number of neutrophils in the experimental group. The study indicated the potential of *L. brevis* but pointed out the need of further studies to confirm their findings and to determine the specific mechanism of *L. brevis* (Nasrabadi and Ebrahimi).

Setbacks/Implications

It has been estimated 78.2% of human chronic wound are associated with biofilm (Gajula et al.). Within biofilm, there have been 20 genera of bacteria and 60 different subtypes. If these microbials persist within the wound, these strains can mutate to be resistant against antimicrobial and antibiotic treatments (Haesler et al.). When biofilm reach state, antibiotic treatment, regardless of dose and concentration, seem to be ineffective. Thus, antibiotic treatment may cause more trouble than good by giving rise to more antibiotic resistant strains (Haesler et al.). Therefore, antagonisms against these biofilm-forming bacteria and pathogenic bacteria need to be further studied.

Topical probiotic bacteria strains have shown to be potential therapeutic agents in wound healing. In terms of those highlighted, they were able to reduce pathogenicity of biofilm forming bacteria, reduce virulence factors, reduce wound diameter size, and promote healing. However, the amount of literature on the impact of probiotic bacteria strains on wound healing has been

scarce, and even the studies highlighted point to this need. More studies are needed to not only expand the probiotics under question but also to verify existing studies to establish consistency among current data. Through these strains, I hope to demonstrate the potential of probiotics in wound healing and inspire future prospective wound healing studies utilizing probiotics, which would prove instrumental on providing more knowledge chronic wounds.

List of Figures

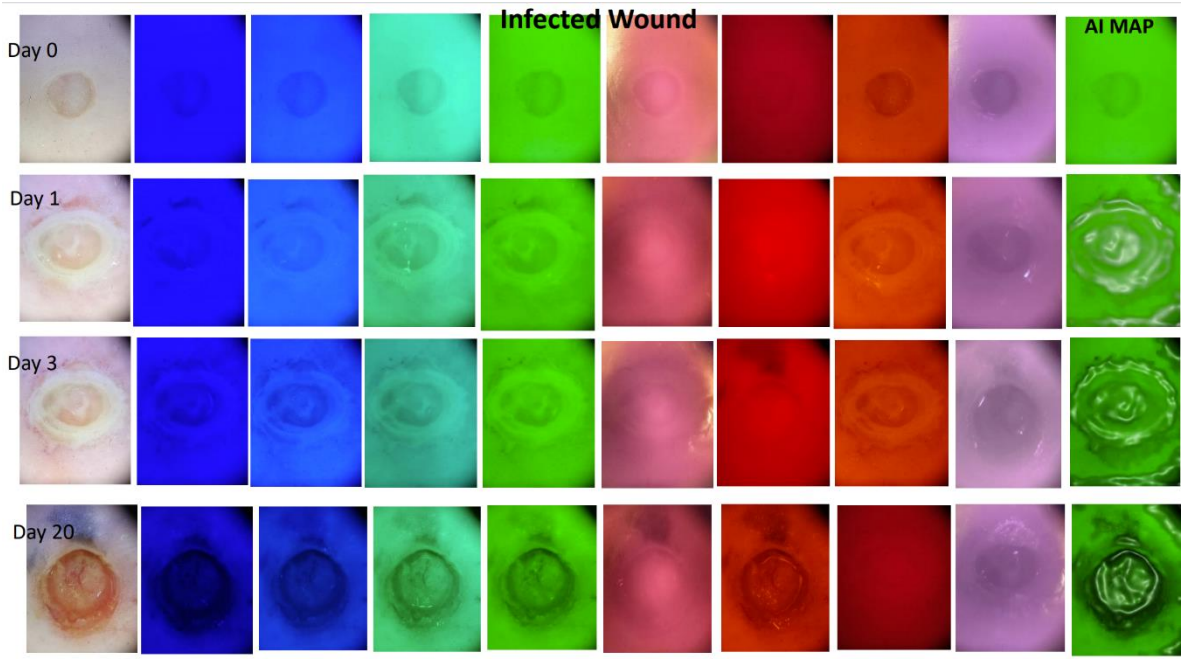

Fig 1: Each color represents a specific wavelength to excite specific components within the biofilm. The parameters and algorithm to decipher these various signals are unknown.

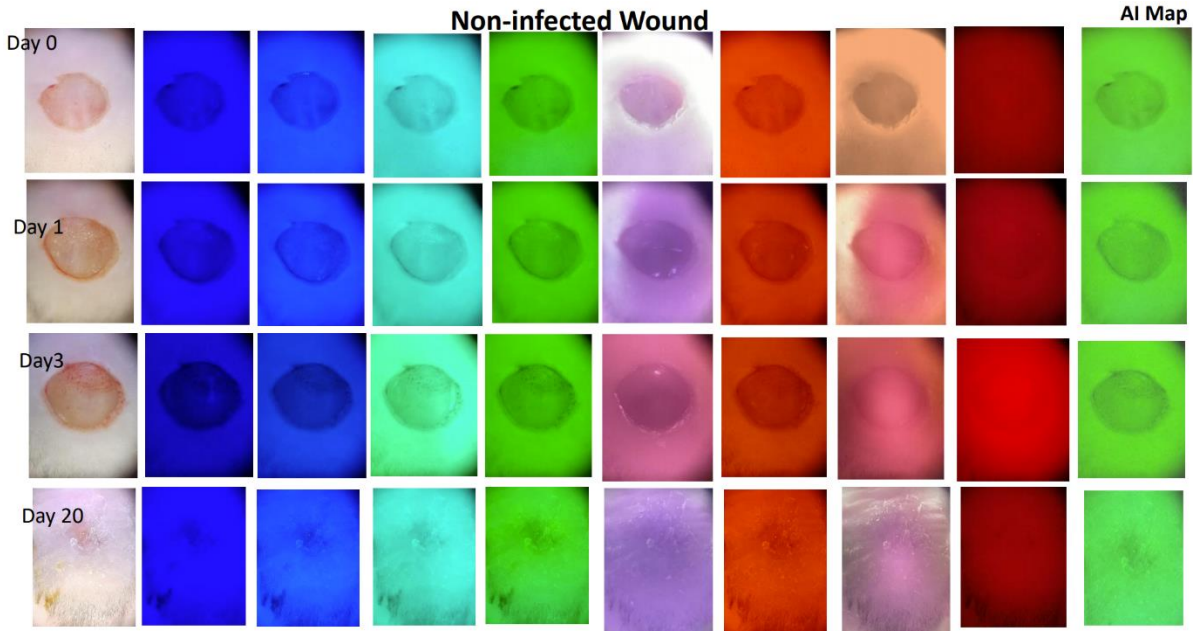

Fig 2: Each color represents a specific wavelength to excite specific components within the biofilm. The parameters and algorithm to decipher these various signals are unknown.

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