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Technical Support of Wound Healing Processes: Project Status

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Abstract: The optimized wound healing (OWID) project provides technical support of wound healing processes. Advanced biophysical treatment therapies using light (photobiomodulation), negative pressure wound therapy (NPWT), and electrical stimulation show biological effects. Specifically, a biphasic dose-response curve is observed where lower doses activate cells, while above a threshold, higher doses are inhibitory. However, no standard protocols and no multi-modal treatment studies determine specific therapy needs. The OWID project aims to develop a multi-modal treatment device and model-based therapy for individualized wound healing. This work presents the OWID project status. Currently, a photobiomodulation prototype delivers red, green, and blue light 'medicine' at prescribed therapeutic 'doses'. The calculation of incident light necessarily considers transmission properties of the intervening cell culture plate. Negative pressure wound therapy (NPWT) and electrical impedance tomography (EIT) hardware are being adapted for use in vitro. Development of mathematical models of wound healing and therapy control are supported by treatment experiment outcome measures conducted in a wounded 3D tissue model. Parameter sensitivity analysis conducted on an existing mathematical model of reepithelialization results in changing parameter values influencing cellular movement rates. Thus, the model is robust to fit model parameters to observed reepithelialization rates under treatment conditions impacting cellular activation, inhibition, and untreated controls. Developed image analysis techniques have not captured changes in wound area after photobiomodulation treatment experiments. Alternatively, EIT will be tested for wound area analysis. Additionally, live dyes will be introduced to non-invasively visualize the reepithelialization front on a smaller, cellular scale. Finally, an overall therapeutic feedback control model uses model reference adaptive control to incorporate the intrinsic biological reepithelialization mechanism, treatment loops, and treatment controller modulation at a wound state. Currently, the OWID project conducts photobiomodulation treatment experiments in vitro and has developed mathematical models. Future work includes the incorporation of multi-modal wound healing treatment experiments.

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1 Introduction

Chronic wounds represent control dysfunction of wound healing processes [1]. In 2012 in Germany, 1.04% of insured patients had a wound diagnosis (including leg ulcers and diabetic ulcers) and 0.43% received wound treatment [2]. Wounds have 786,407 prevalence and 196,602 incidence in the German population with an annual increasing frequency [2]. Thus, there remains a need for wound treatment interventions.

Treatment therapies using advanced biophysical modalities, such as light (photobiomodulation, PBM), negative pressure wound therapy (NPWT), and electrical stimulation (ES) show biological effects. Specifically, a biphasic dose-response curve is observed where lower doses activate cells, while above a threshold, higher doses are inhibitory [3]. However, no standard protocols and no multi-modal treatment studies determine specific therapy needs [4].

The optimized wound healing (OWID) project provides technical support of wound healing processes. The project aims to develop a multi-modal treatment device with model-based control for individualized wound healing therapy. A previous project summary was reported [5]. This work presents the current status of OWID project.

A PBM prototype delivers red, green, and blue light 'medicine' at prescribed 'dose' exposures to wounded in vitro tissues. The LED array configuration was determined by Light Tools (Synopsys) simulation to deliver uniform light to 2 tissue culture plates (each 3x4 tissue cups). A programmable Arduino operates a driver to modulate LED selection, light intensity, and exposure schedule [6]. The prototype electronics are shielded to fit into the humid incubator environment.

Wound area is a primary measurement of wound healing outcomes. Previous imaging systems were developed in agar [7], then improved for evaluation of the in vitro experimental system [8]. Current work develops an automated system and is applied to evaluate wound area in time [9].

Engineering modeling and control systems approaches are used to support optimization of wound healing treatment parameter selection and individualization of therapy protocols. Existing mechanochemical models of wound healing are robust to fit model parameters to observed healing rates under treatment conditions impacting cellular activation [10, 11]. However, there are no wound healing models incorporating treatment inputs and no models of wound healing therapy systems. The OWID project aims to support wound healing processes through engineering modeling of the wound healing system and control systems for personalized wound healing therapy systems [12, 13].

2 Methods

The OWID project aims to develop a multi-modal treatment device with model-based control for individualized wound healing therapy. Towards this aim, the OWID project is based on in vitro wound healing treatment experiments advancing over three stages. The inextricable components of the experimental systems are: 1) treatment devices, 2) the biological system, and 3) measurement systems.

At the first project stage, a single device inputs treatment to a wounded in vitro tissue and the outcome biological responses are used to support development of wound healing mathematical models. The second stage incorporates sensors to design a therapy feedback control system. The third stage combines multi-modal treatment therapies. The three OWID project stages are presented in Table 1.

To date, six PBM treatment experiments have been conducted in vitro (Table 2). Treatment experiments are conducted on an in vitro tissue model [14, 15]. The general experimental workflow starts with a matured in vitro tissue [14, 15] that is wounded using a 2 mm biopsy punch [7], then tissue images are taken of treated and untreated control groups throughout the experiment duration [7–9] to calculate wound area, along with histology sections and biomarkers [14, 15]. Table 2 shows

Tab. 1: OWID project stages

	Prototype	System	Analysis
1	PBM, NPWT, ES	In vitro	Model
2	PBM, NPWT, ES	In vitro + Sensors	Model + Control
3	PBM + NPWT + ES	In vitro + Sensors	Model + Control

Tab. 2: OWID experimental conditions

Experiment	PBM Intensity	In vitro	Wounding
1	Minimum	Design 1	Full thickness
2	Untreated	Design 1	Full thickness
3	Maximum	Design 2	Full thickness
4	Maximum	Design 2	Unwounded
5	Maximum	Design 3	Epidermal
6	Maximum + Middle	Design 3	Epidermal

changing conditions of treatment levels (maximum, middle, minimum, and untreated), 3 different in vitro tissue designs, and wounding depths (full thickness, unwounded, and epidermal) throughout the course of the OWID project.

All prototype treatment devices (PBM, NPWT, and ES) must be adapted for use in the in vitro experimental setting, such as shielding from incubator humidity and fit relative to the 2 cell culture plates (24 tissue targets). At stages 1 and 2, treatments are single, independent modalities where light, pressure, or electrical 'medicine' are delivered to the tissue targets according to a prescribed protocol 'dose'. Only during stage 3 are all modality treatments combined.

The primary outcome measure in wound healing treatment studies is wound area. Wound area measurements are made in time, showing closure relative to an initial wound size (100%) and comparing wound healing rates between treated wounds and untreated control wounds [7–9]. Biomarkers are secondary measures of wound healing, evaluated by tissue histology and chemical assays [14, 15].

Two types of sensors are used in the project. Stage 1 uses imaging and biomarker measurements systems, where these measures are used to develop mathematical models of dose-response relationships. Stage 2 employs real-time, non-invasive sensors of wound closure for development of a therapeutic feedback-control system.

The main outcome, at the OWID project first stage, is a mathematical model of the biological system treatment dose-response relationship. The second stage monitors wound closure to modulate treatment in a feedback therapy system. Finally, the third stage combines multi-modal treatment and sensors into a full model-based feedback control therapy system.

The PBM prototype was measured in a lighting laboratory to determine the light incident to the tissue surface. The prototype light was measured at the 24 (tissue equivalent) position targets across red, blue, and green LEDs set at 100%, 50%, and 10% light intensity. In addition, the light transmission properties of the polystyrene cell culture plate cover were determined in an integrating sphere using a SunLike LED (Seoul Semiconductor) light source chosen for its full coverage of the visible spectral range with and without the material sample. The percentage light transmission per wavelength was then used to adjust the light measurements in order to estimate the effective light irradiance reaching the tissue surface.

Algorithms were developed to automatically evaluate wound area in camera and microscope images [9]. Then, a method comparison was conducted between the algorithm output and ImageJ. Finally, the wound area results of a treatment experiment were evaluated in time.

An existing reepithelialization model of wound healing was reproduced and a parameter sensitivity analysis was conducted on two unreported parameter values [16]. Model simulation outcomes were evaluated in terms of cell movement and time to wound closure. In addition, closed-loop feedback control models of the therapy system were introduced [17].

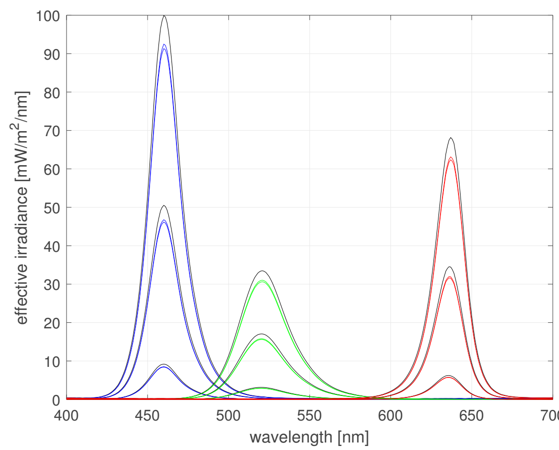

Fig. 1: Spectral curves of the PBM device at 100%, 50%, and 10% intensity settings. The measures are from a single position of 24 total tissue target positions. The original values are dotted lines, while the effective incident light spectrums are in color.

3 Results

To date, the OWID project is at the first stage of development. A photobiomodulation treatment device prototype has been developed [6], measured, and used in six in vitro wound healing treatment experiments (unpublished results). NPWT and electrical impedance tomography (EIT) hardware are being adapted for use in vitro. Data collection includes wound area images [7–9], histology, and biomarkers of a wounded in vitro tissue [14, 15]. Existing mathematical models of wound healing have been reproduced undergone parameter sensitivity analysis [10, 11, 16] and a control system has been conceptualized for the therapy system [12, 13, 17].

Figure 1 shows the effective irradiance at a single tissue target position at device inputs of 100%, 50% and 10% intensity settings. The spectral curves show maximum device inputs (100% intensity setting) of blue (460 nm), green (520 nm), and red (636 nm) light peaks at 91, 30, and 62 mW/m^2/nm, respectively. Importantly, the spectral curves shown are adjusted from the original measured values (dotted lines), so the light transmission properties of the intervening polystyrene in vitro tissue plate cover is taken into account. Thus, the plot shows the effective light incident to a single tissue target surface.

Both algorithms for camera and microscope images are useful to calculate wound area [9]. Observed variability can be attributed to the imaging systems conditions. The experimental results show no change in wound area over time.

A reepithelialization model was successfully reproduced and a parameter sensitivity analysis was performed [16]. Increasing the cohesion coefficient parameter impacted individual cell movement and the resulting intracellular distribution. Increasing the adhesion coefficient parameter increased the collective cell migration rate to wound closure. Closed-loop feedback control of the wound healing therapy system was introduced as a treatment loop, intrinsic feedback of the biological system, and model reference adaptive control (as example) of the wound healing therapy feedback loop [17].

4 Discussion

The OWID project is at the first stage of development with advances in preparation for the project second stage. OWID is dedicated to independent measurement and reporting of treatment parameters, development of wound healing and therapy system modelling and control, and incorporation of multimodal therapeutics. Device adaptation of treatment systems and sensors to the in vitro system remains ongoing.

The effective incident light of a developed photobiomodulation prototype has been determined. Few PBM experiments studies measure and/or report the light treatment parameters and exposure protocol as well as the necessary consideration of light transmission properties of intervening materials. These efforts highlight the need for Physics expertise and resources in photobiomodulation research. Future work includes the full description of the prototype light treatment parameter space and protocols, which together describe the operational capacity of the PBM prototype dosages. New and different exposure protocols (Table 2) of ongoing treatment experiments support development of a dose-response model of the in vitro systems and validation of the biphasic dose-response curve to guide therapy modulation of wound healing processes.

Automated systems were developed to calculate wound area. Initial results are shown from microscope images and wound area in time [9]. Observations that wound area does not change in both treated and untreated groups may be due to the imaging system scale. Wound area reduction was assumed to be visible on a tissue level, where changes may be on a cellular scale. Future work incorporates live dyes to visualize keratinocytes as the migrating epithelial front during reepithelialization processes and for modelling.

The OWID project is unique in applying engineering modeling and control systems to wound healing systems. Ongoing research includes the development of mathematical models of wound healing validated by observed dose-response relationships of the OWID in vitro biological system. The current experimental methodological approach is to treat at 'operational maximum' and then reduce to find a threshold dose of activating and inhibiting dose- response. These models can be supplemented by existing dose-response relationships published in the scientific literature, specifically in similar systems reporting negative, nil, and positive outcomes at prescribed treatment protocols. Stage 2 of the OWID project is currently active in preparing non-invasive, real-time monitoring systems for adaptation for use in vitro.

5 Conclusion

The OWID project is at the first stage of development with advances in preparation for the project second stage. The effective incident light delivered to tissue targets by a PBM prototype has been determined. The automated image analysis system must be adjusted to visualize migrating keratinocytes on the reepithelialization front. Biological dose-response models and therapy feedback-control models are being developed for technical support of wound healing processes.

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