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Abstract

Non-healing wounds represent a substantial medical burden with few effective treatments available. To address this challenge, we developed a novel epidermal wound healing model using suction blisters in healthy volunteers. This model allowed for the comprehensive assessment of wound healing dynamics and the evaluation of INM-755, a topical cream containing cannabinol, as a potential therapeutic agent.

Two clinical studies were conducted: an observational study and an interventional study. In both studies, healthy volunteers underwent a suction blister procedure on their lower back, creating open epidermal wounds. Wound healing parameters were assessed using advanced imaging systems. Skin barrier function and perfusion were evaluated through trans epidermal water loss (TEWL) and dynamic optical coherence tomography (D-OCT), respectively.

The observational study demonstrated the successful and reproducible Induction of blisters and the removal of epidermal sheet, enabling quantifiable measurements of wound healing parameters over time. Re-epithelialization was observed, revealing recovery of skin barrier function and perfusion. In the interventional study, differences of treatments over time were quantified using the above-described techniques.

Despite differences from disease-specific blistering, our developed model provides a valuable platform for studying wound healing mechanisms and assessing novel therapeutic interventions. The sensitivity to treatment effects demonstrated in our study underscores the potential utility of this model in early-phase clinical drug development programs targeting wound healing disorders.

1. INTRODUCTION

Cutaneous wound healing is a complex process divided into four main phases: hemostasis, inflammation, proliferation, and remodeling (Guo & Dipietro, 2010). In chronic, non-healing wounds, one or more of the four phases of wound healing is delayed or disturbed. Most commonly this impairment is present in the inflammation phase, in which persistent inflammatory activity induced by infection or re-injury interferes with healing of the wound (Gonzalez et al., 2016; Velnar et al., 2009).

Current treatment options for chronic non-healing wounds are limited and mainly focused on wound care and patient-reported symptoms, e.g. itch, highlighting the high medical need in the field of chronic non-healing wounds (Han & Ceilley, 2017; Hou et al., 2021) However, developing novel therapies for non-healing chronic wounds is challenging, from both a discovery and a clinical development perspective. Current treatment strategies focus on gene and cell therapies albeit still in early stage (Eming et al., 2007; Prodinger et al., 2019; Shaabani et al., 2022). Wounds can be highly heterogeneous with a high variability in severity and symptoms among patients, and research on pathophysiology shows that many interconnected pathways are involved.

Testing new treatment strategies is challenging due to the lack of sensitive objective biomarkers that can I) quantify the characteristics of wounds and II) are able to detect changes after treatment. Both aspects are needed for clinical trials. The absence of a robust human wound healing model suitable for early-phase clinical studies is one reason for the restricted number of clinical trials addressing an evidence-based approach for treating wounds (Brölmann et al., 2013; Pastar et al., 2018). Most wound models are transferred from pre-clinical mouse models to humans and primarily focus on excisional, scratch, or burn wounds (Masson-Meyers et al., 2020). However, the process of epidermal wound healing most likely differs from wound healing of deeper and/or differently induced wounds, making the previously developed models likely unsuitable for clinical trials. Previously, we studied normal wound healing in healthy volunteers and established a key set of biomarkers for clinical trials. We developed a model in

which normal wound healing of a full-thickness skin biopsy was extensively characterized using objective imaging techniques combined with molecular readouts in re-excised biopsies. The results of this study showed that with these techniques, it is possible to thoroughly follow the process of normal wound healing, making it a suitable model for early-phase clinical drug development programs targeting wound healing (Ten Voorde et al., 2023). To date, no epidermal wound healing model exists with full separation of the epidermis. Despite the use of suction blistering in several clinical studies to investigate various components of wound healing, these suction blister models only included one or two fragmented objective readouts, e.g. isolated transepidermal water loss assessment, but never employed a full comprehensive characterization looking at perfusion, wound parameters, barrier function, and immunological readouts (Wilhelm et al., 2017a).

A suction blister model to characterize wound healing would be beneficial for therapeutic agents addressing bullous diseases, such as epidermolysis bullosa (EB). INM-755 represents such a novel therapeutic agent – a topical cream containing cannabinol (CBN). Pre-clinical studies demonstrated the capacity of INM-755 to reduce the expression of matrix metalloproteinase-9 (MMP-9) and interleukin-8 (IL-8), factors that are typically elevated in blisters of epidermolysis bullosa (EB) patients and are believed to play a role in blister formation in this patient population (Lettner et al., 2013; Prodinger et al., 2019a).

In the current studies, we aimed to develop a novel epidermal wound healing model suitable for the full characterization of epidermal wound healing and for testing the efficacy of novel therapeutics in healthy volunteers. Specifically, the objectives were I) to develop an epidermal wound healing model based on suction blisters; II) to extensively characterize epidermal wound healing using the developed model in healthy volunteers, and III) to test the effects of INM-755, a novel therapeutic agent for epidermal wounds, using the developed model in healthy volunteers.

2. MATERIALS AND METHODS

Two clinical studies were performed. The first study was a prospective observational study in healthy volunteers to characterize time-dependent epidermal wound healing after a suction blister-induced wound (NL71806.056.19). The second study was a randomized, double-blind, vehicle-controlled interventional Phase I study in healthy volunteers to study the effects of INM-755 on epidermal wound healing in the model as developed in the first study (NL72831.056.20). Both studies were performed at the Centre for Human Drug Research, Leiden, The Netherlands and lasted from February 2020 to March 2020 and from July 2020 to September 2020, respectively, with the Declaration of Helsinki as the guiding principle. Ethical study approval was received from the independent Medical Review and Ethics Committee 'Medische Ethische Toetsingscommissie van de Stichting Beoordeling Ethiek Biomedisch Onderzoek' (Assen, the Netherlands) prior to the start of the clinical phase for each study. Subjects gave written informed consent before participation in the study after receiving oral and written information.

2.1 SUBJECTS AND STUDY DESIGN

Twelve (observational study) and eight (intervention study) healthy non-smoking male or female volunteers were included, aged between 18 and 45, with a Fitzpatrick skin type of I-III, and a BMI ranging from 18-30 kg/m^2. Individuals were excluded from the study if they had a history of pathological scar formation or clinically significant skin conditions requiring immunosuppressive/immunomodulatory medication. Participants' overall health status was evaluated through physical examination, ECG, vital signs, and blood analysis.

In the observational study, subjects underwent the suction blister procedure on Day 0. One blister was created on each subject's lower back. The blister roof, i.e. epidermal sheet, was harvested after blister

formation to create an open, non-bleeding epidermal wound. Initially, the wound was covered with non-adhesive gauze dressing which was renewed daily. After Day 6, the wound was left uncovered.

In the interventional study, at baseline four blisters were created on each subject's lower back, spaced at least 5 cm apart. The blister roof was harvested after blister formation to create an open epidermal wound. Each subject received four treatments randomly assigned to the four blister locations: high concentration INM-755 cream (HD), low concentration INM-755 cream (LD), a matching vehicle, or no treatment. Assignment was blinded for treated blisters (INM-755 and vehicle). The assigned treatment per blister was applied in excess (approximately 75 mg/cm^2) by a dedicated blinded physician for 14 consecutive days and the treated wounds were afterwards covered with a semi-adhesive Mepitel® dressing. To ensure optimal condition for drug uptake, a non-adhesive gauze dressing was applied over the Mepitel® dressing. The untreated blister, as a control for wound healing, was covered with the same type of non-adhesive gauze dressing.

Pharmacodynamic assessments comprised clinical imaging, biophysical assessments, clinical scores, and molecular readouts, and were performed on days 0, 1, 3, 6, 9, and 12 for the observational study and daily from Day 0 up to Day 21 for the interventional study.

2.2 TREATMENT

INM-755 is a topical cream containing CBN, a weak agonist for the Cannabinoid-1 and Cannabinoid-2 receptors. Pre-clinical studies with INM-755 showed reduced expression of MMP-9 and IL-8 after challenging incubated cells with tumor necrosis factor α and interferon ɣ. MMP-9 and IL-8 are known to be upregulated in blisters of EB patients and are suspected to contribute to blister formation. Additionally, after treatment with INM-755, an upregulation in basal keratin 15 (K15) was observed. K15 might substitute basal keratin 14 (K14) in forming a construct with keratin 5, which could lead to strengthening of the skin in EB patients with a K14 mutation (data on file). In addition to the high concentration INM-

755 cream, low concentration INM-755 cream, a matching vehicle, or no treatment were assigned to the blister locations.

2.3 SKIN SUCTION BLISTER PROCEDURE

Suction blisters were induced using the NP-4 suction blister device (Electric Diversities in Maryland, USA) following Standard Operating Procedures. The device generated an approximately 10mm diameter blister in 79 to 154 minutes by applying under pressure (up to 8 inHg). After the blister was formed, the roof was punctured with a needle to aspirate the fluid. The blister roof, or epidermal sheet, was removed with scissors. The wound diameter was measured using a standardized caliper.

2.4 OUTCOME ASSESSMENTS

2.4.1 CHARACTERISATION OF EPIDERMAL WOUND HEALING

2.4.1.1 Wound healing parameters

Clinical images to evaluate dimensional wound healing parameters (e.g. wound surface, diameter, volume) were taken of the suction blister-induced wound study using a 3D stereo camera system (LifeViz® QuantifiCare, Valbonne, France). Analysis of the data was performed according to the CHDR standard procedure (Rijsbergen et al., 2019). Additionally, epidermal wound healing was qualitatively evaluated using dynamic-optical coherence tomography (D-OCT) from Michelson diagnostics (VivoSight OCT, Kent, UK).

2.4.1.2 Skin barrier function

Trans epidermal water loss (TEWL) was used to determine the barrier function of the skin. Before the measurement began, the subjects underwent a 15-minute acclimatization process to the room. Subsequently, a probe was affixed to the wound to establish a 7 mm closed chamber, after which the measurement procedure was initiated as described previously (Ten Voorde et al., 2023). The disparities

in humidity between the TEWL chamber and the skin causes the movement of water molecules, which are detected by the sensors within the chamber over time. The measurement was sustained for either 90 seconds or until the steady-state flux was attained.

2.4.1.3 Skin perfusion

Cutaneous microcirculation was measured using D-OCT from Michelson diagnostics (VivoSight OCT, Kent, UK). The procedure was performed at baseline and during each visit throughout the trial, following the protocols described previously (Jacobse et al., 2021). A 6mm probe was placed directly over the inner wound. 120 consecutive scans were taken, each with a depth of up to 1.5 mm, in about 20 seconds. The cutaneous microcirculation was calculated by determining the average speckle signal that returned from a depth of 0.1 mm to 0.35 mm to reduce artifacts and noise signals.

2.4.2 EVALUATION OF INM-755 EFFECTS

2.4.2.1 Skin erythema

Multispectral imaging (Antera 3D, Miravex, Dublin, Ireland, was used to quantify skin's erythema in the interventional study as described previously (Saghari et al., 2021). By creating a closed chamber environment, standardized images were taken at all study visits. A region of interest of 12 mm was selected and kept analogous throughout. Skin erythema is expressed as CIELab a* value in arbitrary units.

2.4.2.2 Wound healing parameters

In the observational study, dimensional wound healing parameters (e.g. wound surface, diameter, volume) were explored using the 3D stereo camera system (LifeViz QuantifiCare, Valbonne, France). In the interventional study, 3D image analysis could not be performed due to a technical malfunction. In both studies, clinical images were taken of the suction blister-induced wound.

2.4.2.3 Skin barrier function

Skin barrier function was measured using TEWL as described above in the method section 'characterization of wound healing model'.

2.4.2.4 Skin perfusion

Skin perfusion was measured using D-OCT as described above in the method section 'characterization of wound healing model'.

2.4.2.5 Skin surface biomarkers

In the interventional study, skin surface biomarkers interleukin-8 (IL-8), matrix metalloproteinase-9 (MMP-9), interleukin-1 receptor antagonist (IL-1RA), vascular endothelial growth factor (VEGF), tissue inhibitor of metalloproteinases (TIMP-1, and TIMP-2) were measured exploratively using FibroTx patches (FibroTx, Estonia). A patch comprises a multiplexed capture-antibody micro-array that is fastened to the skin via a dermal adhesive bandage. Upon application to the skin for 15 minutes, the antibodies imprinted on the micro-array selectively capture skin biomarkers via immunological recognition. The captured biomarkers were then subjected to a quantitative analysis through the utilization of a spot- ELISA (enzyme-linked immunosorbent assay).

2.4.2.6 Safety and tolerability

Safety and tolerability of INM-755 was frequently monitored in the interventional study by tracking adverse events, taking vital signs, and conducting standard blood analysis. In addition, local application site reactions were monitored by scoring erythema, oedema, scaling, and asking subjects about a stinging/burning sensation using well-defined criteria. The results are displayed as local tolerability assessments (LTA) and reported as a percentage from total. Physician-reported LTAs (erythema, oedema, scaling) were scored by the same blinded physician throughout the study and consisted of categorical

scores ranging from 0-3. Scoring was performed directly after blister induction and on each subsequent study day. Red-yellow-black (RYB) scores were given to assess the color and humidity of the blister wounds. RYB was scored after bandage removal and before drug application on each study day.

2.5 STATISTICS

No formal statistical significance analysis was performed for the observational study considering the lack of treatment groups and the focus on feasibility of the techniques. All data displayed for the observational study is summarized in descriptive statistics and reported as means over time. For the interventional study, a statistical model was applied to analyze repeated wound healing parameters. A mixed model ANCOVA with fixed factors of treatment, wound number, time, and treatment-by-time interaction, and random factors of subject, subject-by-treatment interaction, and subject-by-time interaction was used. It also included the baseline value, taken immediately after blister formation, as a covariate. The following contrasts were calculated within the model: LD INM-755 vs. vehicle, HD INM-755 vs. vehicle, HD INM-755 vs. LD INM-755, LD INM-755 vs. untreated, HD INM-755 vs. untreated, vehicle vs. untreated. Categorical LTA parameters were summarized by frequencies and treatment. For OCT skin perfusion and IL-1RA measurements the data was log-transformed because of log-normal distribution.

3. RESULTS

Twenty subjects (12 in the observational study, 8 in the interventional study) were enrolled in the studies and all completed the trial according to the protocol. All study subjects were Caucasian and had a mean age of 26.2 (19-37) years in the observational study and 23.4 (18-32) years in the interventional study. INM-755 in all dose levels was safe and well tolerated. The most frequent reported treatment-related adverse event was application site erythema, which was present in 8 (100%) of the epidermal wounds

that received vehicle and LD INM-755, and in 7 (87.5%) of the epidermal wounds that received HD INM-755. No dose relationship in adverse events was detected.

Table 1: Subject demographics

Study	***Observational***	***Interventional***
Age (years)		
N	12	8
Mean (SD)	26.2 (4.3)	23.4 (5.0)
Median	26.0	22
Min, Max	19, 37	18, 32
Height (cm)		
N	12	8
Mean (SD)	176.6 (10.1)	177.4 (5.8)
Median	177.6	177.1
Min, Max	162.3, 198.5	169.5, 185.4
Weight (kg)		
N	12	8
Mean (SD)	70.0 (11.7)	67.9 (5.8)
Median	68.2	68.2
Min, Max	52.9, 92.3	60.7, 74.6
BMI (kg/m^2)		
N	12	8
Mean (SD)	22.4 (2.39)	21.5 (1.6)
Median	22.4	21.3
Min, Max	18.2, 27.4	18.8, 24.5
Sex		
Female	6 (50.0%)	4 (50.0%)
Male	6 (50.0%)	4 (50.0%)
Race		
White	12 (100.0%)	8 (100.0%)
Fitzpatrick Skin Type		
1 (always burns and never tans)	1 (8.3%)	1 (12.5%)
2 (often burns and tans lightly)	7 (58.3%)	2 (25.0%)
3 (burns moderate and tans gradually)	4 (33.3%)	5 (62.5%)

3.1 CHARACTERISATION OF EPIDERMAL WOUND HEALING

In all 12 subjects included in the observational study, a complete suction blister developed within 154 minutes following the start of the suction procedure. In each case, the epidermal layer was effectively detached using a combination of tweezers and scissors.

Clinical imaging of induced blisters showed gradual restoration of the skin barrier over time (Figure 1A). As part of the feasibility process, quantifying the variability among subjects before and after undergoing the blister procedure for the three parameters tested (surface, TEWL, perfusion) was crucial. Figures 1B, C, and D illustrate the coefficient of variation for blister surface, TEWL, and perfusion, respectively. Following the induction of epidermal wounds, variability remained within acceptable limits (CV < 30%) (Shechtman, 2013) for all parameters.

In terms of wound healing, re-epithelialization of the epidermis measured with 3D photo analysis started within 3 days and was complete within 9-11 days (Figure 1E). After induction of the epidermal wound, TEWL immediately increased and gradually decreased during the observation period over time (Figure 1F). TEWL did not return to the baseline status within the study duration. This pattern was also present for skin perfusion as measured with D-OCT. The timing of return to a normal perfusion of the skin was longer than 12 days (Figure 1G).

Skin morphology before and after the blister procedure was visualized using OCT. Figure 2A depicts normal skin, while Figure 2B shows skin post-epidermal sheet removal. Notably, Figure 2B shows visible rupture marks on the dermo-epidermal junction. No variation in intensity is observed, signifying complete epidermal removal. Additionally, the hyperreflective bands in Figure 2B denote blister fluid seeping from the newly formed wound.

3.2 **EFFECTS OF INM-755**

Clinical images of epidermal blisters are displayed in Figure 3A. Skin erythema quantified using multispectral imaging is displayed in Figure 3B. Immediately after the blister procedure, all treatment groups (pre-dose versus post-dose) demonstrated a noticeable increase in skin erythema. During the first five days after wounding a trend towards separation between treatment arms can be observed. Following

the initial increase in erythema, there was no longer a noticeable pattern, as the erythema in the untreated wound returned to baseline as rapidly the treated wounds.

A steep increase was noted in TEWL from pre-blister (baseline) flux measurements (approximately 10 g/m^2/h) to post-blister measurements (approximately 100 g/m^2/h) after the removal of the epidermis (Figure 3C). From Day 2 to Day 7, a steep decrease in the flux measurements was observed. After Day 7, the TEWL generally gradually decreased to a mean of about 15 g/m^2/h on Day 21, close to baseline TEWL flux of about 10 g/m^2/h. TEWL of epidermal wounds treated with INM-755 in both concentrations and the vehicle recovered slightly quicker than the TEWL of untreated wounds (contrast LD INM-755 – untreated p=0.0518, difference 4.2, (95%CI: -0.036, -8.409). Contrast HD INM-755 – untreated p=0.0512, difference 4.1, (95%CI: -0.023, -8.194). Contrast vehicle – untreated p=0.0335, difference 4.4, (95%CI: 0.0392, -8.405)).

Skin perfusion as measured with D-OCT is displayed in Figure 3D. An increase in blood flow (AU) was observed directly after blister induction from approximately 25 AU to 90-115 AU. Perfusion of the skin returned to the baseline status within 5 days of treatment for all treatment arms. It recovered significantly faster after treatment with HD INM-755 (contrast HD INM-755 versus vehicle (p=0.0139, difference: -15.3%, (95%CI: -25.5%, -3.8%)), contrast HD INM-755 versus untreated (p=0.0437, difference: -12.6%, (95%CI: -23.2%, -0.4%)).

Figure 4 depicts the quantity (ng/mL) of all six biomarkers collected over time. In the statistical model, time was a significant ($p<0.05$) predictor for all tested cytokines except IL-1RA. VEGF was not analyzed statistically because of too many samples below level of quantification (BLOQ). Data BLOQ has been set at ½ of LLOQ value for the graph. No significant treatment effects were observed for IL-1RA, IL-8, MMP-9, and TIMP-2. However, a significant difference was found for TIMP-1 between the untreated blister

wounds and the blisters treated with LD INM-755 (p=0.0085, difference -0.121, 95%CI: -0.207, -0.035). Throughout the healing process, the untreated wounds contained higher levels of TIMP-1.

LTA scores for all four parameters tested are presented in Figure 5A-D. Of the four potential local reactions (erythema, oedema, scaling, stinging/burning) scored in the LTA, erythema was the most reported in the study. Oedema and stinging/burning were only reported sporadically. Scaling of the skin occurred throughout the study without a relationship to drug doses. No clear difference between treatments was observed in the LTA scoring.

RYB scores (Figure 5E) show that only red wounds were present, and that wound disappearance took longer for untreated blister wounds. Humidity scores ranged from dry to wet with an emphasis on dry wounds 8-9 days after wounding for treated wounds (LD INM-755, HD INM-755, and vehicle) and 11 days for untreated wounds.

4. DISCUSSION

In these studies, we aimed to develop a novel epidermal wound healing model suitable for the comprehensive characterization of epidermal wound healing and testing of the efficacy of novel therapeutics in healthy volunteers. We successfully created an epidermal wound healing model based on suction blisters. The findings of the observational study indicate that the creation of blisters using under pressure devices was achievable in all subjects, and we demonstrated that the epidermal sheet could successfully be removed. With an acceptable coefficient of variation in all tested methodologies and detectable differences over time, the model was considered feasible to be used for the extensive characterization of wound healing and to test the effects of INM-755 in healthy volunteers.

In the first, i.e. observational, study we showed that a combination of several state-of-the art imaging techniques allows for better and quantitative assessment over time with high specificity to detect small physiological differences, compared to visual assessments of wound closure often used as pharmacodynamic endpoint in later phase clinical trials and in clinical practice(Wei et al., 2016). By using D-OCT, TEWL, and 3D photography, we were able to follow normal epidermal wound healing and quantify skin perfusion, barrier function, and wound closure. Interestingly, wound closure was complete within the follow-up period based on 3D imaging and visual inspection. TEWL and skin perfusion, however, did not return to baseline within the observational period indicating that skin restoration was not entirely complete at the end of the study (Day 12). This is in line with previous research showing that skin barrier function requires approximately 4 weeks to return to baseline (Kottner et al., 2013; Ten Voorde et al., 2023). This finding indicates that when relying on visual examination and clinical imaging, there is a tendency to suggest that the wound has completely healed, despite other wound healing parameters not having fully returned to their baseline levels.

By using the suction blister model, we were able to investigate the effects of INM-755 in healthy volunteers and found differences in erythema for treated (including vehicle treatment) versus untreated blister wounds. Treated wounds seemed to be more erythematous than untreated wounds, as quantified with multispectral imaging (high standard deviation). Notably, the untreated blister exhibited higher perfusion levels in the initial post-wounding days when compared to treated blisters. However, this pattern is reversed for skin erythema. Given the strong correlation between erythema and perfusion in biological processes, it is implausible that the difference is attributed to treatment effects and could potentially be caused by artefacts. One possible explanation is that differences can be explained by interference of the creams, given that both devices employ different methods of detection (color versus laser). Back reflectance or absorption of the light coming from the multispectral camera might have caused differences in detection.

Although 3D imaging proved to be a valuable tool in wound assessment, it also has its limitations. In the interventional study, a technical malfunction resulted in faulty analysis and thus data was not included in this article. In combination with the need for trained operators and analysts, the technique is not easily implemented in standard clinical care.

In the interventional study the skin barrier of all treated (including vehicle treatment) blisters recovered faster than those of untreated blisters, as determined by TEWL. The difference in moistness, i.e. a higher water content of the wounds, due to application of the cream, could have contributed to this finding. Furthermore, it is known from literature that moist wounds close and return to baseline status quicker, which is in line with the results of earlier studies (Dyson et al., 1988; Oudshoorn, Rissmann, van der Coelen, et al., 2009; Oudshoorn, Rissmann, Van Der Coelen, et al., 2009). Although some literature suggests that TEWL is not a useful tool in wound healing studies due to wound secretion, our previous study and the current study showed that TEWL can detect changes in water loss even when crust or skin

debris is present (Alborova et al., 2008; Ten Voorde et al., 2023). Especially in bullous diseases, this is useful considering the moist and disrupted environment.

Biomarkers were successfully captured through the exploratory FibroTx biomarker analysis in our wound healing model. Of all tested biomarkers, IL-8, MMP-9, TIMP-1, and TIMP-2 showed significant differences over time, demonstrating a link with wound healing. MMP-9 was directly elevated after wounding in all treatment arms without difference between treatments. The elevation of MMP-9 directly after wounding is in line with literature suggesting a role in cell migration early in the inflammation phase (Kandhwal et al., 2022). Interestingly, TIMPs were also increased directly after wounding but returned to baseline quicker than MMP-9, which even continued to increase up to Day 4. Considering the function of TIMPs, it is interesting to see MMP-9 continuing to rise over time, even with elevated TIMP levels. IL-8 was not elevated directly after wounding but showed a maximum response on Day 4. Elevated IL-8 after wounding was expected, considering the strong chemoattractant for leucocytes (Rennekampff et al., 2000). VEGF is known to be involved in stimulating vascular permeability, resulting in recruitment of inflammatory cells (Johnson & Wilgus, 2014), aligning with our observations: we noticed a tendency for VEGF levels to rise in all treatment groups during the initial inflammation phase and then return to normal within two weeks post-injury. However, it's crucial to note that the reliability of our VEGF data was limited due to numerous values being below the limit of quantification (BLOQ), preventing us from conducting statistical analyses. Although IL-1RA did not significantly change over time using the statistical model, visual inspection of the figure shows separation between treated blisters and untreated blisters on Day 4. From literature it is known that IL-1RA has a predominant role in the early phase of wounding and it could be that IL-1RA levels were even more increased in the initial 3 days, and that the increase observed on Day 4 is already a decline in elevation (Macleod et al., 2021).

One constraint in this study involves using diverse imaging techniques at varying time points in both the observational and interventional study. This precludes a direct comparison of findings between the two

studies, even though such a comparison was not the main objective of our research. With the interventional study, we generated proof-of-concept for the developed wound healing model enabling us to explore the impact of INM-755 by assessing wound healing parameters.

Considering quantitative endpoints in early-phase clinical drug development, local tolerability assessments scored the progression of wound healing with little specificity. Although the assessment was included in the study as a safety measure for treatment with INM-755 and not a pharmacodynamic endpoint, it did give insight into erythema of blister wounds. The discrete nature of the scoring system does not allow for advanced statistics or nuances between wounds and no statistical differences were found. Based on LTA alone, no erythema differences in treatment arms could be identified, whereas with clinical imaging we were able to quantitatively describe differences in wound erythema over time as well as detect a small trend towards separation between treatment arms.

Although these two studies are a first step toward an epidermal wound model in healthy volunteers, the question remains whether the blisters in the model sufficiently mimic EB blisters. From literature, blisters are created at the dermal-epidermal junction, in line with the most prevalent types of EB (Vukmanovic-Stejic et al., 2008). However, these blisters are formed using force and trauma, whereas EB blisters are formed because of lack of cell adhesion. This difference in creation plus the different expression of keratin and MMP, might cause differences in the inflammation profile as well as the anatomical outlook. Furthermore, in this study, we removed the blister roof to administer INM-755 on open wounds, whereas in EB treatment, the epidermal sheet is not always removed because of the risk of infection. Lastly, anatomical location of wounds contributes to the time to healing and chance of infection(Bischoff et al., 1999; Degreef, 1998; Tahir Mahmood et al., 2023). Next to that, the disposition of immune cells differ across body locations and thereby influence the healing process. Lastly, the selected location of the wound in combination with practical challenges with the under-pressure blister device and application on the skin impacts the blister formation process dependent on location. The epidermal wound healing model

used in this research was conducted on the lower back, so caution should be taken when extrapolating these findings to other anatomical sites.

Over the years, several techniques have been developed to induce partial thickness wounds(Wilhelm et al., 2017b), and the development of imaging methodologies to measure skin responses has been progressing in parallel(Gaurav et al., 2024). Compared to other partial thickness models (e.g. tape stripping, abrasion, laser-induced wounds, split thickness) skin blister methodology as used in this study is time consuming and requires a complete seal and constant under-pressure. The procedural challenges make it difficult to draw blisters on certain body areas and movement of a subject can cancel under-pressure. In general, the selection of a partial thickness wound healing model should be based on the goal of the study and be as representative of the disease as possible.

Although there are limitations to the model regarding comparability to EB and differences among anatomical sites, the setup of these models can help in development of therapies targeting chronic and epidermal wound diseases. Bullous diseases are known to be rare and inclusion of patients in a clinical study is therefore difficult. By using a wound healing model in healthy volunteers, an early signal of efficacy can be found without the need for big multi-center clinical trials including a hand-full of patients.

Altogether, this study demonstrates the effective establishment of an epidermal wound healing model, providing robust groundwork for subsequent explorations into the wound healing process. With its capacity to monitor changes over time and discern variations between treatments, this model serves as a dynamic tool for assessing the effectiveness of novel treatments within the realm of wound healing.

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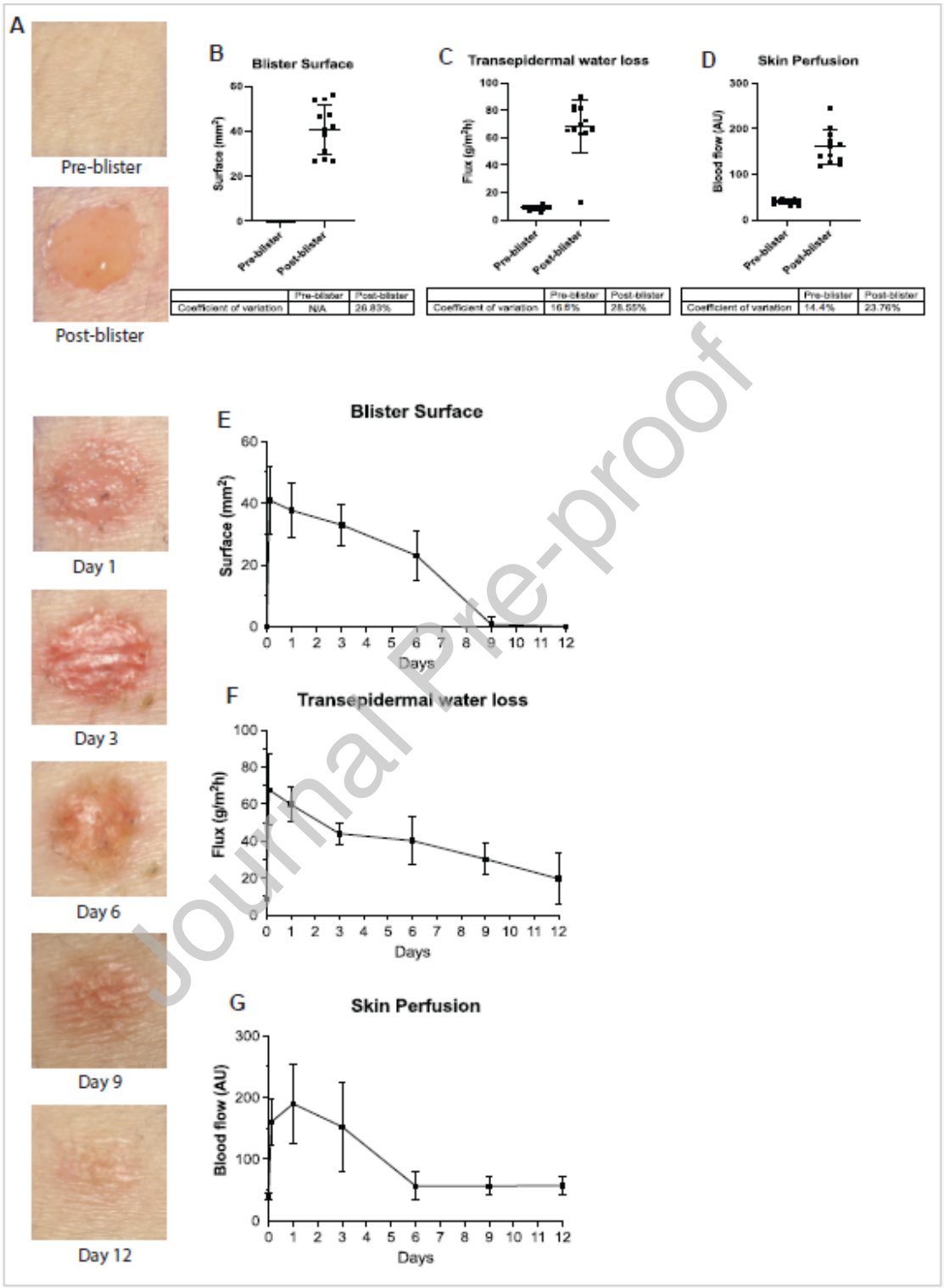


Figure 1: A) Representative images of epidermal wound healing over time. B-D) Variability within subjects measured before and after blister induction displayed as individual data points. Coefficient of variation is displayed for all parameters before and after induction, except for blister surface where no blisters were present. E-G) All data are displayed as mean +- SD, n=12.

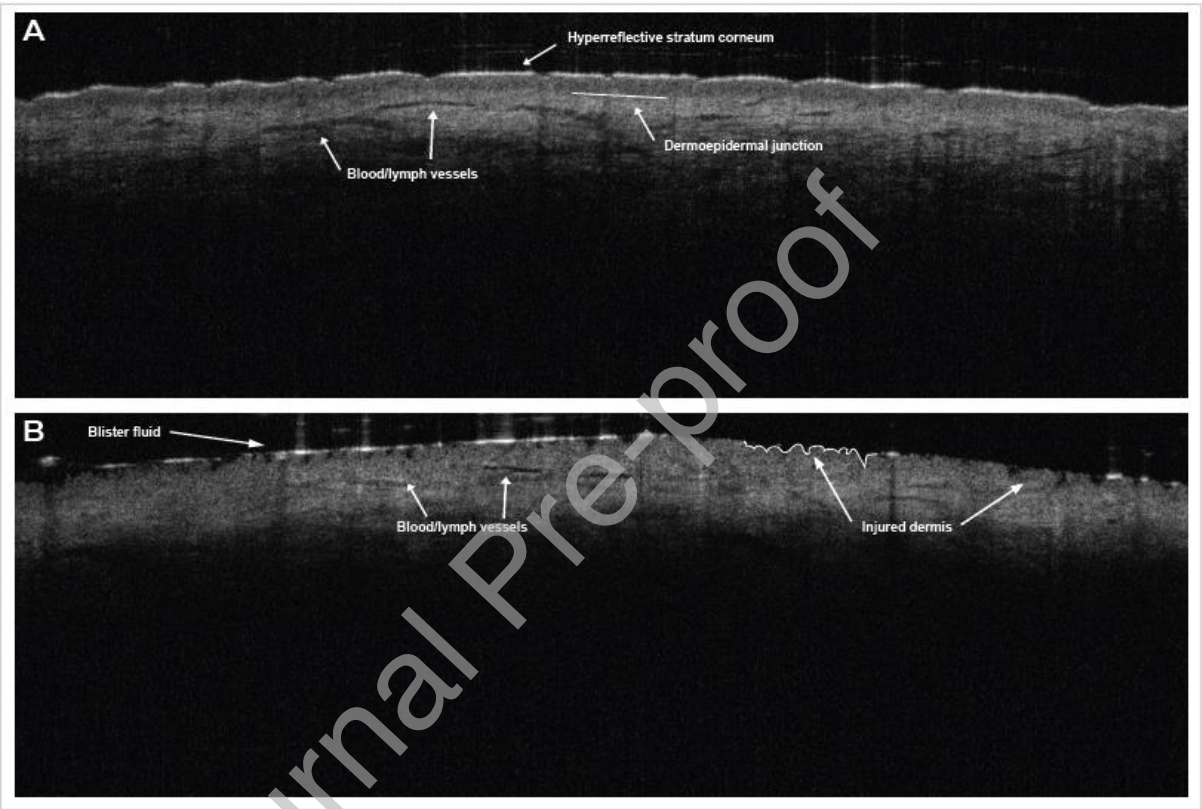


Figure 2: Cross sectional images as measured with D-OCT. A) Representative image of skin before blister induction. B) Representative image of skin after blister induction. Distinct anatomical structures are displayed in the image.

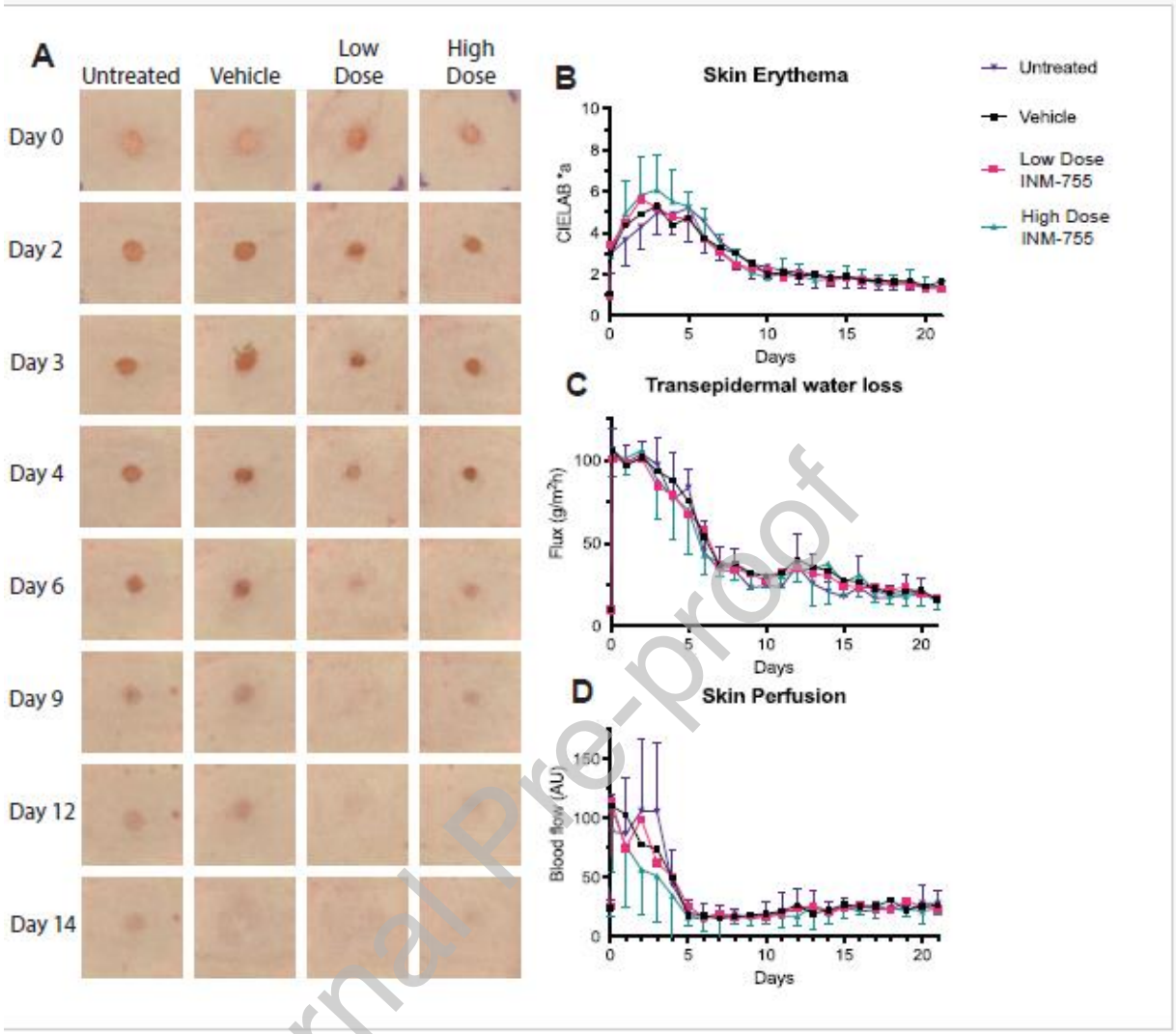


Figure 3: A) Wound healing progression displayed per treatment arm over time. Images shown are from a single subject B-D) Erythema, TEWL, and Skin perfusion displayed over time, respectively. All data displayed are mean +- SD, n=8.

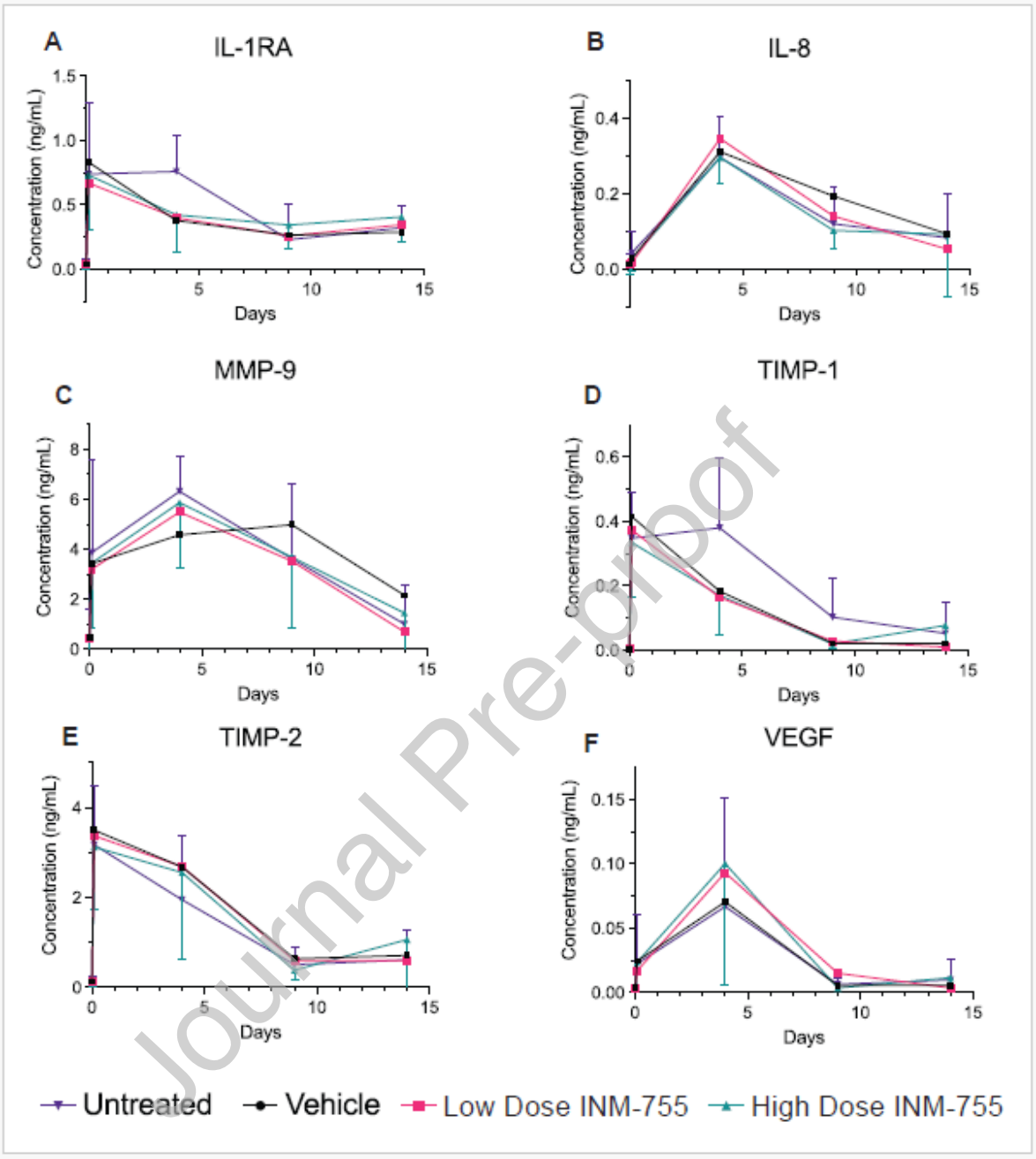


Figure 4: Concentration of exploratory biomarkers displayed over time in days. All data are displayed in mean +- SD. Data plotted in Figure 4F (VEGF) included data points set at ½ LLOQ.

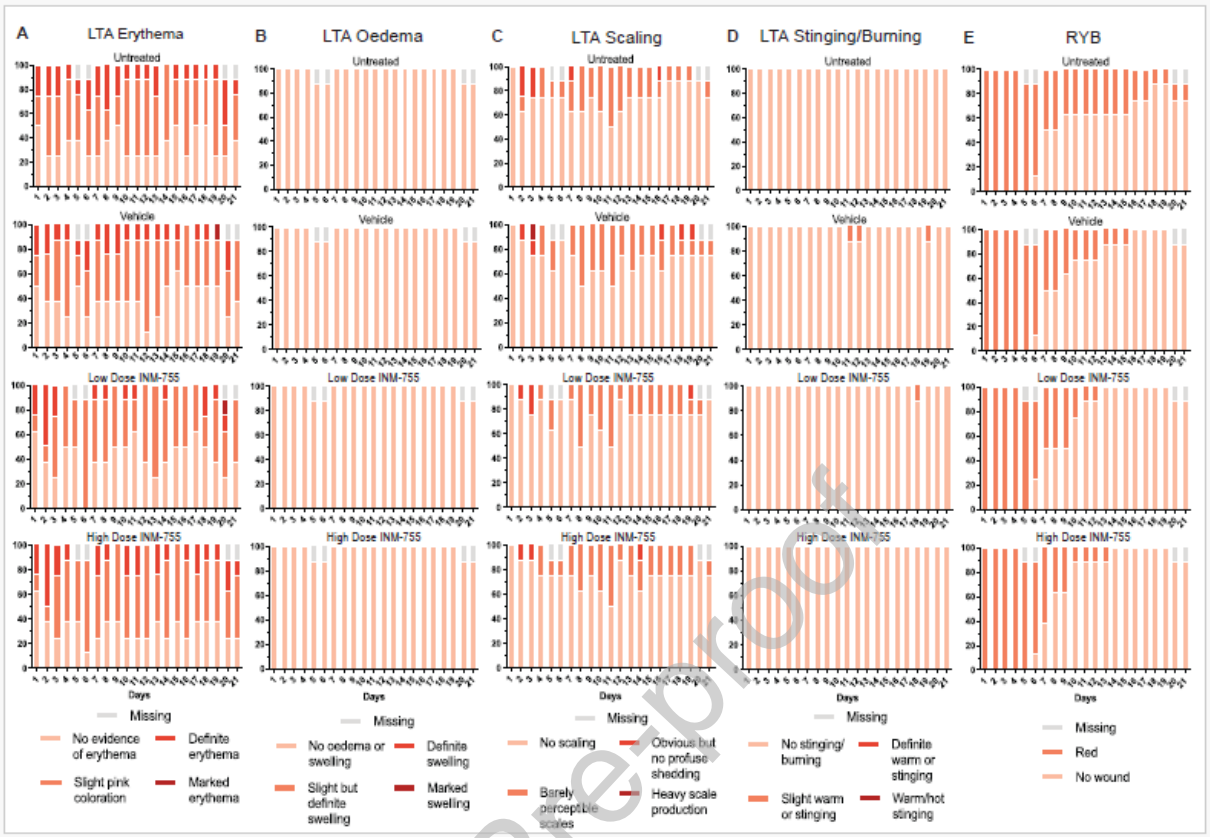
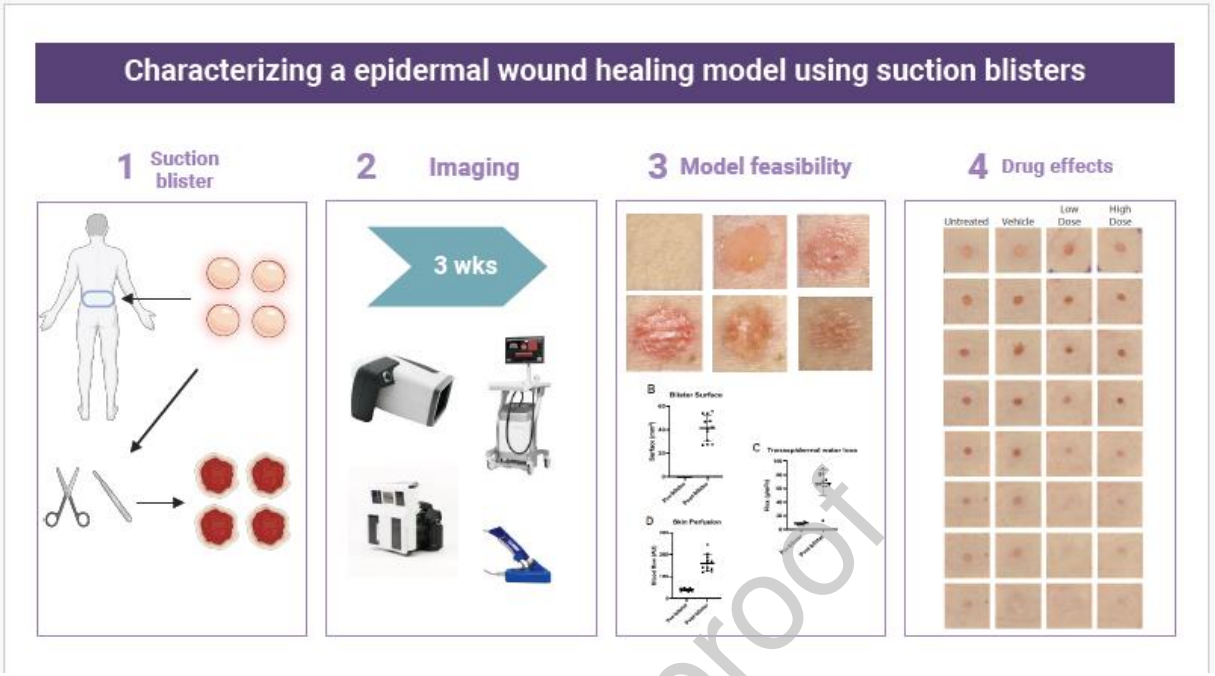


Figure 5: A-D) Local Tolerability Assessment plotted per parameter over time. E) Red-Yellow-Black score displayed per treatment arm over time. All data are displayed in percentages of total, n=8.



Graphical abstract