breakdown. They have sophisticated mechanisms of balancing inflammation, for instance, neutrophils have pro-resolution functions by inducing macrophage activation to be later phagocytized or formation of cytokine degrading aggregated NETs as shown in gout. Aim is to in-depth characterize functions of neutrophils in normal and disturbed healing and based on this to develop new strategies that modulate neutrophil activity in disturbed healing. We used full thickness wound healing model in wildtype and diabetic mice, analyzed appearance and localization of neutrophils, protease activity and inflammatory condition at different time. In wildtype mice neutrophils appear at first hours after wounding in the tissue, abundantly scatter in the surroundings for three days and completely disappear few days later. Neutrophil elastase shows two time point peaks, suggesting several subsets of neutrophils arriving into tissue that may have different functions, i.e. additionally contributing to resolution and wound closure. Comparatively, the diabetic mice wound healing is delayed for several days with a slow kinetic. Level of neutrophils is less but persists longer even after the wound is closed, although pro-inflammatory factors TNF and CXCL-1 are low. Elastase and gelatinase activity is higher than in wild-type mice, peaks at time of wound closure and remains high after that. All in all, unfavorable condition of diabetes leads to low pro-inflammatory factors, later appearance of PMN with inadequate protease activity, which hinders functions of PMN including resolution of inflammation and further wound healing.

Summary: To modulate adverse neutrophil activity in favor of pro-resolution functions we will develop multifunctional multiphasic wound dressing on the base of our previously employed modular hydrogel system based on starPEG and heparin. As one function to resolute pro-inflammatory neutrophil activity the wound dressing will contain microgels harboring neutrophil protease cleavage sites that will damp the excess protease activity on the wound. Depending on our understanding of neutrophil activity in wounds further immunomodulating functions can be integrated in the hydrogels.

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EXPLORING THE EFFECT OF AGEING ON THE HOST-MICROBIOTA AXIS IN SKIN REPAIR

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Abstract: The prevalence of perturbed wound healing, which is characterized by a dysregulated inflammatory response and often accompanied by bacterial infections, increases with progressing age. Pattern recognition receptors (PRRs) such as Toll-like receptors (TLRs) detect the presence of bacteria and initiate the inflammatory response. To understand whether age-related changes in PRR expression or function contribute to the dysregulated inflammatory response and delay in wound healing with increased age, the expression and function of PRRs were examined in vitro and in vivo and the effect of PRR stimulation on wound healing was examined histologically in incisional wounds from wild-type mice of increasing age. in vitro PRR stimulations demonstrated age-related differences in PRR and downstream cytokine (TNF, IL-1β, IL-6 and IL-12) mRNA expression in neutrophils and macrophages from aged (22-24 month old) wounded mice compared to young (5-7 week old) wounded mice, while expression levels in middle-aged (18 month old), wounded mice were similar to PRR and downstream cytokine expression levels in the young mice. Further, qRT-PCR results suggested differences in the temporal responses (in terms of PRR and downstream cytokine expression) to 3 and 24 hour PRR stimulations of macrophages from young and aged wounded mice. In vivo, TLR1, TLR2 and TLR4 mRNA expression were significantly increased in untreated wounds of aged mice compared to young, while the wound healing response in aged displayed an impaired response to treatment with PRR ligands Pam-3 cys (TLR1/2), LPS (TLR4) as well as MDP (NOD2), suggesting altered PRR expression or signaling may contribute to dysregulated inflammation and delayed healing in aged wounds.

Summary: The prevalence of aberrant wound healing is increased with progressing age. The work presented is aimed at contributing to our understanding of the significance of age in initiating an immune response and subsequent wound healing. Age-related changes in pattern recognition receptor expression and function were assessed by qRT-PCR and their potential impact on wound healing was examined histologically. in vitro and in vivo results suggest altered PRR expression and function with increased age as well as an impaired response to PRR stimulation during wound healing in aged.

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EPOXY-TIGLIANES INDUCE KERATINOCYTE WOUND HEALING RESPONSES VIA CLASSICAL PROTEIN KINASE C, PKCα AND PKCβ ACTIVATION

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Abstract: EBC-46 (tigilanol tiglate) and EBC-211 are novel epoxy-tiglianes occurring naturally in seeds of the Fontain's Blushwood tree, endemic to Queensland's tropical rainforest. EBC-46 is currently in clinical development by QBiotics Group, as an anti-cancer treatment in humans and animals. In addition to its direct effects on tumor cell viability, EBC-46 stimulates exceptional dermal wound healing following tumor destruction, manifested as enhanced wound re-epithelialization and closure. Our previous studies demonstrated that epoxy-tiglianes stimulate significant keratinocyte proliferative and migratory responses *in vitro*, mediated via protein kinase C (PKC) activation, thereby supporting enhanced re-epithelialization in treated skin. As classical PKC isoforms (PKC-α, -βI, -βII, -γ) are implicated in mediating the anti-cancer effects of epoxy-tiglianes, this study evaluated whether classical PKCs were also responsible for stimulating enhanced wound healing responses in epoxy-tigliane-treated keratinocytes.

Immortalized human epidermal keratinocytes (HaCaTs) were treated with EBC-46 or EBC-211 (0-10μg/mL), with/without Gö6976 (classical PKC inhibitor) or LY317615 (PKC-βI/PKCβII inhibitor). HaCaT proliferation was assessed by MTT assay over 72h. HaCaT wound repopulation was assessed using *in vitro* scratch wounds over 48h. Western blots assessed the impact of PKC inhibition on key keratin, cell cycle and migratory proteins over 48h; and the extent of phospho-PKC activation by epoxy-tiglianes over 24h. MMP activity assays quantified the impact of PKC inhibition on epoxy-tigliane induced MMP activities.

Gö6976 significantly attenuated epoxy-tigliane stimulation of HaCaT proliferation and wound repopulation. Although LY317615 also abrogated these responses, effects were less dramatic than Gö6976. Epoxy-tigliane-induced changes in key keratin, cell cycle and migratory proteins were prevented by both inhibitors, as were epoxy-tigliane-induced increases in MMP-1/-7/-10 activities. EBC-46 and EBC-211 induced the biphasic activation of PKC-α and PKC-βI/PKC-βII (at 0-30min and 6-24h post-treatment), while PKC-γ was undetectable in HaCaTs.

Summary: This study confirms that epoxy-tiglianes mediate enhanced proliferative and migratory responses in keratinocytes via classical PKC activation, especially PKC-α and PKC-βI/PKC-βII isoforms. Such findings provide further evidence for the mechanisms by which epoxy-tiglianes promote rapid re-epithelialization in treated skin.

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THE CENTRAL ROLE OF IRON AND MACROPHAGES IN CHRONIC WOUNDS: INVESTIGATING STRATEGY TO IMPROVE WOUND HEALING

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Abstract: In CVI patients accumulation of erythrocytes in the tissue and consequently iron overload, have been reported. Iron has been found in wound edge and wound bed of chronic venous ulcers (CVU). Moreover, macrophages (Ma) seem to be a critical responder to dysregulated iron homeostasis in the skin before and after ulcer formation. These and other findings suggest that iron is an important factor in the establishment and development of ulcers in the skin. In this project, we aim to investigate the molecular mechanisms of Ma activation via iron and modulate the pathological effects of iron in chronic wounds using biomaterial. To dissect the response of M1 and M2 Ma to erythrocyte overload we mimicked erythrocyte accumulation in the skin *in vitro* by co-culture of primary macrophages (M1 and M2) generated from human blood monocytes with autologous erythrocytes or Fe(II) up to 14 days. Cytokine analysis revealed that M1-like Ma released significant fewer amounts of pro-inflammatory TNF and IL12, after LPS, suggesting a down-regulation of the inflammatory response in M1-like Ma by erythrocytes and Fe(II). Interestingly, M2-like Ma showed a clear pro-inflammatory response with an increase of TNF and IL-6 and a decrease of IL-10 in presence of erythrocytes and Fe(II). Assessing intracellular iron accumulation by iron assay showed that M2-like Ma are more effective in iron uptake and has a better capability to effectively recycle iron. A similar amount of MitoROS was produced in the presence of Fe(II) by M1 and M2. To better understand the different functional response of M1 and M2 to iron-overload, regulation of iron was analyzed. Hence, in erythrocytes-overload condition, iron-transport proteins (CD163, TFR) are downregulated compared to control, however, iron-regulatory proteins (HO-1, IRP-1) are rather upregulated suggesting a complex iron regulation is needed when iron homeostasis is dysregulated. To modulate iron effect on Ma, DFX was added to Ma cultured with erythrocytes and we observed that cytokines response was modulated only partly in M2 Ma, and erythrocytes effect on