Elucidating the Mechanisms and Therapeutic Potential of Photodynamic Therapy in Wound Healing (光動力療法在傷口癒合中的效果與機制)

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Background of research

I. Background, motivation, and significance

Wound healing is a significant clinical challenge, particularly in the context of chronic wounds, which are a common complication of diseases like diabetes. Non-healing wounds such as diabetic foot ulcers (DFU) and venous stasis ulcers fail to progress through the normal stages of healing and can persist for months or even years, causing significant morbidity and reducing the quality of life for patients[1]. The management of chronic wounds places a substantial public health burden worldwide that is associated with high healthcare costs, long-term disability, and significantly reduced quality of life for patients.

Wound healing is a dynamic and complex process that involves a series of coordinated events, including hemostasis, inflammation, proliferation, and remodeling[2]. The inflammatory phase is crucial for protecting the wound from infection and also initiating the subsequent phases of healing. During the proliferative phase, new tissue, known as granulation tissue (GT), is formed to fill the wound gap, and new blood vessels are developed to supply nutrients to the healing wound. The final phase – remodeling - involves the maturation and reorganization of collagen fibers in the wound bed, leading to wound closure and the restoration of a functional barrier. Immune cells play a crucial role in these stages, not only defending against infection but also regulating the whole healing process[3]. However, **in chronic wounds, this process is impaired, leading to persistent inflammation and delayed healing**.

Photodynamic therapy (PDT) is a treatment that has gained significant attention in recent years due to its potential in treating various medical conditions, especially cancer[4]. It is a unique treatment modality that involves the use of light-sensitive compounds, known as photosensitizers (PS). When exposed to a specific wavelength of light, the PS becomes activated and undergoes a series of chemical reactions that result in the generation of reactive oxygen species (ROS). These ROS can cause direct cytotoxic effects.

The application of PDT in wound healing is an emerging field of research[5, 6]. It is minimally invasive, and can be targeted specifically to the wound site. Recent research has shown that PDT can promote wound healing. The ROS generated during PDT can kill bacteria, reducing the risk of wound infection. Additionally, ROS can also trigger a type of cell death known as immunogenic cell death, which can stimulate a robust immune response. This immune response clears dead cells and debris from the wound, reduces inflammation, and promotes tissue repair and regeneration. Therefore, the mechanism of PDT involves a combination of direct cytotoxic effects and the modulation of the immune response, making it a versatile therapy for a range of conditions.

Despite these promising findings, our understanding of the mechanisms underlying the effects of PDT on wound healing is still in its infancy. For instance, **it is unclear which types of PDT are most effective for promoting wound healing, and the immune mechanisms underlying the effects of PDT on wound healing are not fully understood.** Answering these questions is essential for designing of PDT for wound healing in different contexts, such as surgical wounds, burn wounds, abrasion wounds, surgical wounds in patients undergoing chemotherapy, or chronic non-healing wounds in patients suffering from conditions such as diabetes mellitus (DM). This proposal aims to address these gaps of knowledge for the design of novel therapy for wound healing.

Interestingly, tumors have often been described as 'wounds that do not heal' due to the chronic inflammation, tissue proliferation, and remodeling seen in both processes[7-10]. However, unlike in normal wound healing, these processes in the tumor microenvironment (TME) often lead to the promotion of tumor growth and progression. Given these parallels, insights gained from studying the immune mechanisms involved in PDT-mediated wound healing can potentially be applied to cancer therapy. Therefore, the proposed research could have far-reaching implications, not only improving our understanding of wound healing but also informing the development of novel cancer therapies.

II. Key questions addressed

1. **What type(s) of PDT is most effective in promoting wound healing?** There are two main types of PDT – Type I reactions generate radical and radical anion species (e.g. $O2^{\bullet-}$, $HO^{\bullet}$), while type II reactions produce singlet oxygen ($^1O_2$). Type II PDT is highly dependent on oxygen availability. Within each type, multiple PSs with varying properties can be utilized. It is unclear which type of PDT is best suited for wound healing applications. We will use acute and chronic wound mouse models in normal and DM mice to test the efficacy of a panel of PDTs.
2. **Does PDT influence the recruitment of innate immune cells to the wound site for wound healing promotion?** While several studies have shown the beneficial effects of PDT in promoting wound healing, little is known about the underlying mechanisms. Innate immune cells play pivotal role in all stages of the wound healing process. Using mouse models and cellular assays, we will track the recruitment of immune cells to the wound site following PDT, and assess the functions of these cells to delineate their role in PDT-mediated wound healing.

Overall, this proposal aims to identify the most effective PDT for wound healing applications and dissect the mechanism of PDT-mediated wound healing promotion. This research has the potential to significantly improve our understanding of PDT and wound healing, and could lead to the development of more effective treatments for chronic wounds. The insights gleaned can also be applied to the development of cancer therapies.

III. Expertise and previous relevant work done by the research team

The proposed research represents a powerful collaboration between two research groups with complementary expertise. The Chow (PI) group brings a deep understanding of the immune mechanisms in tumors, providing critical insights into the parallels between tumor biology and wound healing, as well as the role of immune cells in these processes (Figure 1). The group has successfully established an *in vivo* drug screening platform for wound healing (Figure 2). The Lo (co-PI) group, with their extensive experience in the development of PDT, contributes a wealth of knowledge on PDT techniques and modifications, the generation of ROS, and the application of PDT in different disease contexts. The two groups have a proven track record of successful collaborations in designing novel PDTs for cancer treatment (Figure 3). Collectively, the team has been recognized by several awards, including the **Croucher Innovation Award** (PI) and **Stanford's Top 2% Most Highly Cited Scientists** (co-PI).

(d) A maximum of two non-text A4 pages of attached diagrams, photos, charts and tables etc.

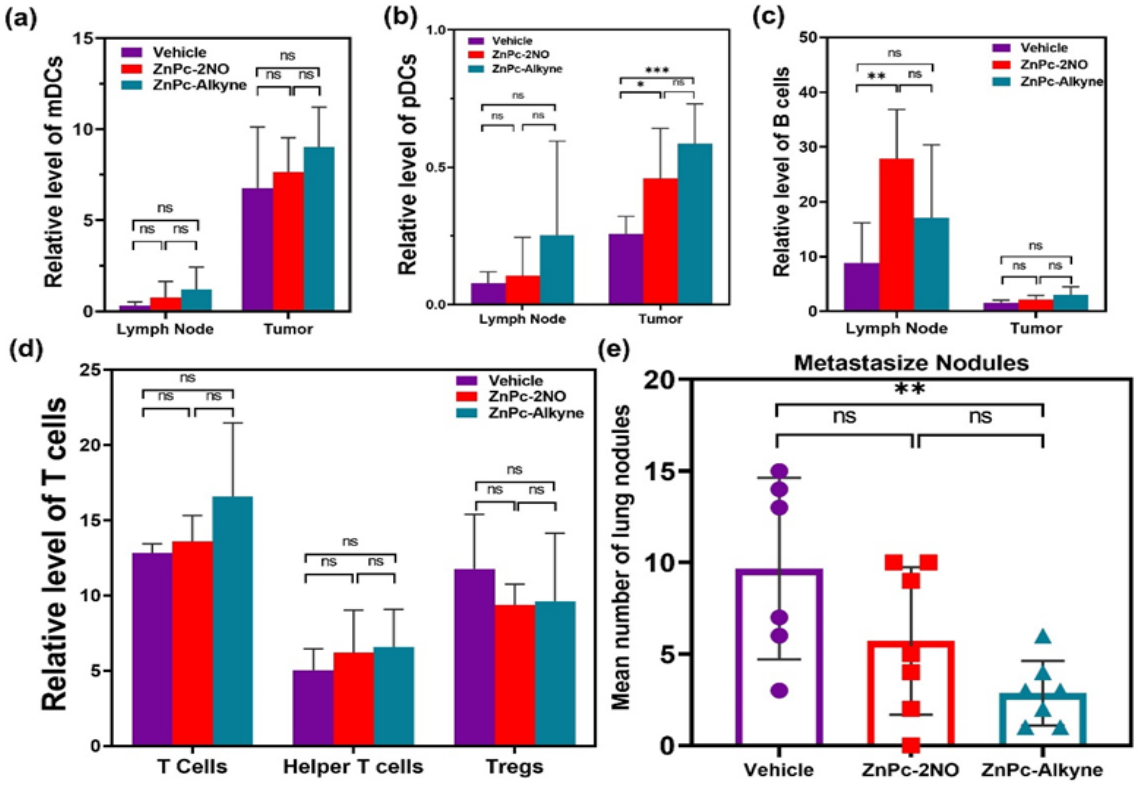

Figure 1: Immune profiling in the primary tumor, metastasized tumor, and draining lymph node in untreated and mice receiving PDT treatment. The relative level of (a) mDCs, (b) pDCs, and (c) B cells in lymph nodes and tumor sites in day 21. (d) The relative level of various T cells in tumor sites a in day 21. (e) Mean number of lung nodules in day 21. (*P<0.05, **P<0.01, ***P<0.001, ****P<0.0001).

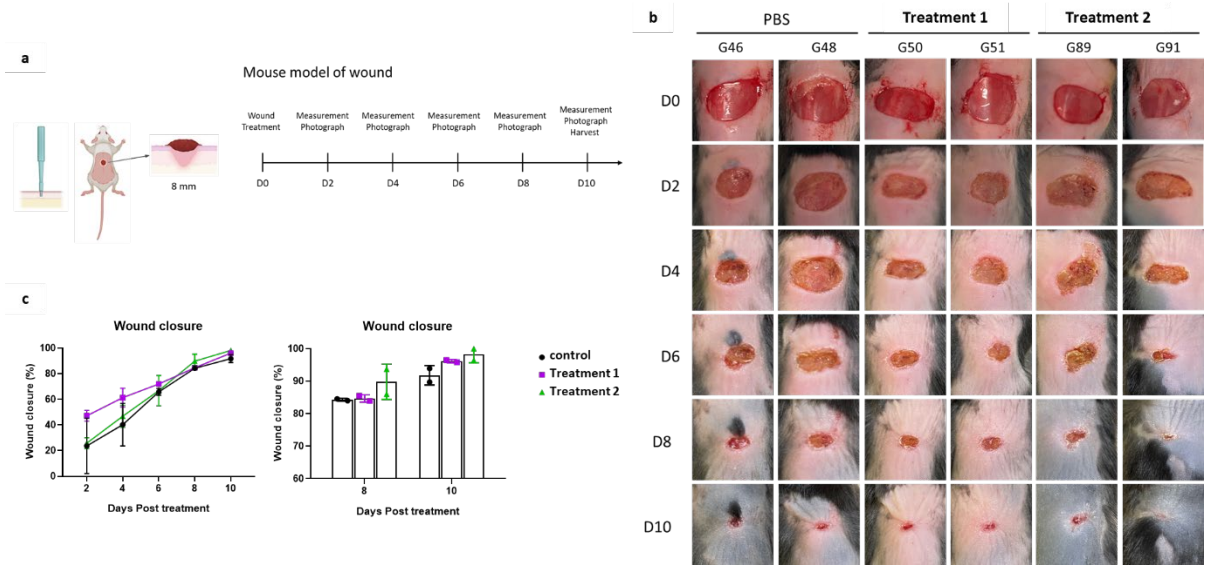

Figure 2: Screening drug treatments for wound healing *in vivo*. (a) Schematics showing the experimental setup for wound healing assays and drug treatment regimen. (b) Wound size was tracked throughout the course of healing progression. (c) Quantification of wound closure as a percentage of original wound size on Day 0.

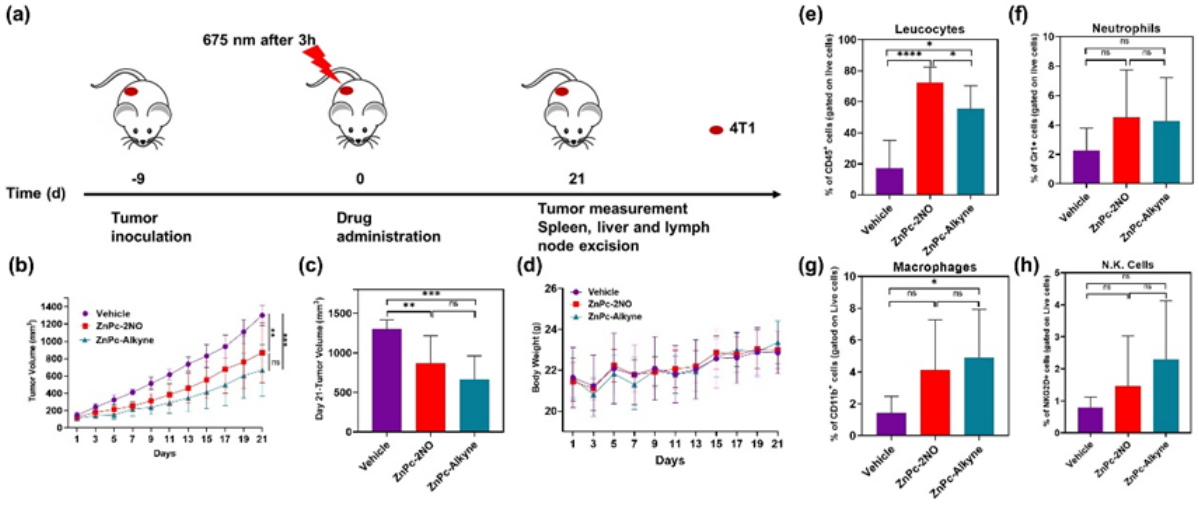

Figure 3: Newly developed PDTs were effective in suppressing tumor growth and enhancing innate immune cell infiltration into the tumor. (a) Schematics showing the experimental setup for administering PDT developed by the team to tumor bearing mice. (b) Effect of PDTs on tumor volume. (c) The tumor volume at day 21. (d) Changes in body weight with time after tumor cell inoculation. The proportions of innate immune cells in lymph nodes at day 21, including (e) leucocytes (f) neutrophils (g) macrophages and (h) natural killer cells (N.K. cells). (*P<0.05, **P<0.01, ***P<0.001, ****P<0.0001).

References

1. Graca Pereira, M., et al., *Wound healing and healing process in patients with diabetic foot ulcers: A survival analysis study.* Diabetes Res Clin Pract, 2023. **198**: p. 110623.
2. Almadani, Y.H., et al., *Wound Healing: A Comprehensive Review.* Semin Plast Surg, 2021. **35**(3): p. 141-144.
3. Chesko, D.M. and T.A. Wilgus, *Immune Cells in Cutaneous Wound Healing: A Review of Functional Data from Animal Models.* Int J Mol Sci, 2022. **23**(5).
4. Hu, T., et al., *Recent advances in innovative strategies for enhanced cancer photodynamic therapy.* Theranostics, 2021. **11**(7): p. 3278-3300.
5. Zhao, H., J. Sun, and Y. Yang, *Research Progress of Photodynamic Therapy in Wound Healing: A Literature Review.* J Burn Care Res, 2023. **44**(6): p. 1327-1333.
6. Ning, X., et al., *The photosensitizer-based therapies enhance the repairing of skin wounds.* Front Med (Lausanne), 2022. **9**: p. 915548.
7. Deyell, M., C.S. Garris, and A.M. Laughney, *Cancer metastasis as a non-healing wound.* British Journal of Cancer, 2021. **124**(9): p. 1491-1502.
8. Dvorak, H.F., *Tumors: Wounds That Do Not Heal-A Historical Perspective with a Focus on the Fundamental Roles of Increased Vascular Permeability and Clotting.* Seminars in Thrombosis and Hemostasis, 2019. **45**(6): p. 576-592.
9. Dvorak, H.F., J. Flier, and H. Frank, *Tumors - Wounds That Do Not Heal - Similarities between Tumor Stroma Generation and Wound-Healing.* New England Journal of Medicine, 1986. **315**(26): p. 1650-1659.
10. Ribatti, D. and R. Tamma, *A revisited concept. Tumors: Wounds that do not heal.* Critical Reviews in Oncology Hematology, 2018. **128**: p. 65-69.
11. Furman, B.L., *Streptozotocin-Induced Diabetic Models in Mice and Rats.* Curr Protoc, 2021. **1**(4): p. e78.
12. Michaels, J.t., et al., *db/db mice exhibit severe wound-healing impairments compared with other murine diabetic strains in a silicone-splinted excisional wound model.* Wound Repair Regen, 2007. **15**(5): p. 665-70.
13. Sanapalli, B.K.R., et al., *Preclinical models of diabetic wound healing: A critical review.* Biomed Pharmacother, 2021. **142**: p. 111946.