

### L.11: Antimicrobial photodynamic therapy induces angiogenesis in wounds

Antimicrobial photodynamic therapy (APDT), is receiving a lot of attention for the management of antibiotic-resistant bacteria infection in wounds. It involves killing of target cells via formation of cytotoxic reactive oxygen species produced by the interaction of photosensitive drug and light of appropriate wavelength. APDT, in addition to the antibacterial effect, has also been shown to inactivate bacterial toxins, reduce proinflammatory cytokines, matrix metalloproteases and improve collagen remodeling in wounds. Therefore, APDT appears promising for the treatment of diabetic wounds where healing is impaired and the treatment is further complicated by bacterial infection. An important cause for impaired healing in diabetes is reduced angiogenesis. In this study, we have investigated the effect of APDT on angiogenesis process of wounds of diabetic mice.

APDT of wounds were carried using poly lysin conjugated chlorin p6 (pl-cp6). Cp6 is a chlorophyll-a derivative synthesized in-house. Because cp6 is anionic, its binding with anionic bacterial membrane is reduced. To enhance targeting to bacterial cells, cp6 was conjugated with a cationic peptide; poly L lysine (pl). The effect of pl-cp6 mediated APDT on angiogenesis of wounds of diabetic mice was evaluated by monitoring the levels of its markers nitric oxide (NO) and vascular endothelial growth factor-A (VEGF-A). In Fig. L.11.1, we show the comparison of NO and VEGF-A levels of uninfected wounds of diabetic mice subjected to PDT at different light fluences. Compared to untreated control (UI), PDT at a lower fluence (~60 J/cm<sup>2</sup>) leads to increase in angiogenic growth factors. However, at higher fluence (~120J/cm<sup>2</sup>), the levels of angiogenic factors decreases possibly due to the higher oxidative stress induced damage to inflammatory cells.

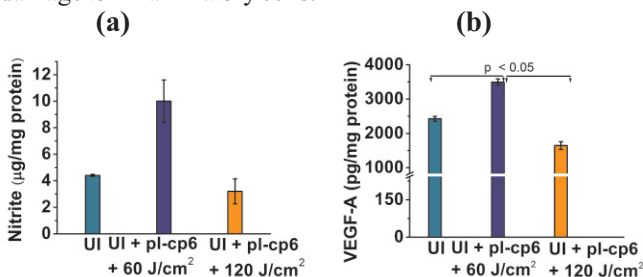


Fig. L.11.1: Effect of PDT fluence on (a) NO and (b) VEGF-A levels of wounds of diabetic mice on day 3 post wounding (p.w).

We also studied the effect of APDT on angiogenic markers in wounds infected with antibiotic resistant bacteria (Methicillin resistant *Staphylococcus aureus*) in diabetic mice and compared the efficacy of ADPT with other wound healing promoting agents such as aminoguanidine (AG) and

silver nitrate (Fig. L.11.2).

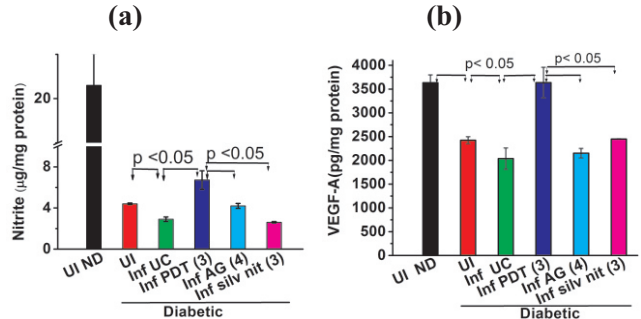


Fig. L.11.2: Effect of topical APDT and other treatments on (a) NO, (b) VEGF-A levels of wounds of diabetic mice on day 3 p.w. UI uninfected, Inf infected wounds, UC untreated control, ND wound, nondiabetic mice. PDT (3) refers to multiple PDT fluence of ~60 J/cm<sup>2</sup> AG= Aminoguanidine. Silv. Nit= Silver nitrate. ND = Non diabetic mice. Treatment frequency in parentheses.

Multiple APDT at lower fluence led to significant enhancement in levels of angiogenic markers than the untreated wounds and the wounds treated with AG and silver nitrate. Increased efficacy of APDT suggests that APDT not only inactivates bacteria but also leads to inactivation of bacterial toxins and increase macrophage recruitment at wound site which is required for proangiogenic response. Further, positive correlation was observed between nitrite level and wound closure time (Fig. L.11.3) suggesting that the increase in levels of angiogenesis marker leads to faster wound closure.

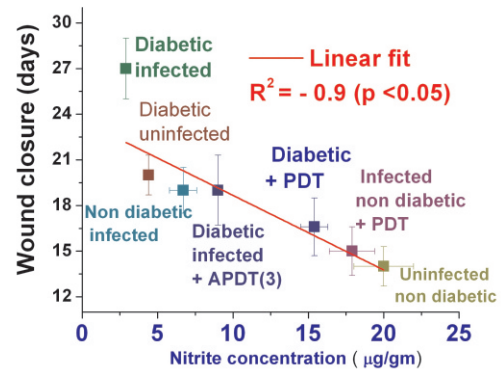


Fig L.11.3: Effect of APDT-induced increased nitrite level on wound closure of MRSA-infected wounds of diabetic mice.

These results suggest that APDT at optimal light dose enhances angiogenic response and improve healing of bacteria-infected wounds of diabetic mice. For more details please refer to K. Sahu et al., Lasers Med. Sci. (2015) 30:1923-1929.

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