Often in science and medicine, there are views that are so deeply ingrained that few would challenge their validity. One is that antioxidants are always good, the more the better. Another is that reactive oxygen species (ROS) are always bad, one example being that they can cause oxidative damage which contribute to poor healing of wounds [1]. Hence, the removal of ROS should result in improved healing. However, if one was to critically evaluate all the scientific evidence available, one would find that this viewpoint has never been rigorously proven. In this article, I provide a brief review on the role of reactive oxygen species and antioxidants in wound healing and shed some light on the question of whether antioxidants or reactive oxygen species might help in wound healing.

ROS includes oxygen radicals and related non-radical species such as singlet oxygen and hydrogen peroxide \((H_2O_2)\). Closely related to ROS is the reactive nitrogen species (RNS) that arise from nitric oxide metabolism and its interactions with ROS. Collectively, they are known as reactive species. Certain reactive species can oxidize biomolecules such as nucleic acids, lipids and proteins and modify them. Depending on the target attacked, the oxidation process can decrease membrane fluidity, increase the leakiness of the membrane, inactivate enzymes or cause DNA base pair mismatch [2]. It should be noted that “reactive” is a relative term. For example, the hydroxyl radical reacts instantaneously with anything around it. On the other hand, \(H_2O_2\) is a stable liquid at room temperature. It reacts preferentially with the thiol functional group, especially if it is in the ionized thiolate form [3]. Because of its stability and reaction specificity, \(H_2O_2\) has been shown to function as a signaling...
molecule and the best studied mechanism is via its oxidative inhibition of protein tyrosine phosphatase [4]. In contrast, due to the lack of substrate specificity, the hydroxyl radical is unlikely to function as a signaling molecule. However, to make matters more confusing, H$_2$O$_2$ can react with the iron (II) ion to form the hydroxyl radical, transforming from a potential signaling molecule to one that is just destructive.

Why would reactive species be present in a wound? All wounds, even sterile wounds, undergo inflammation. Inflammatory cells such as macrophages and neutrophils produce reactive species as part of the defense mechanism against microorganisms. However, it should be noted that H$_2$O$_2$ can be produced in the wound even before leukocytes have infiltrated the wound. The H$_2$O$_2$ produced has further been shown to act as a chemoattractant for leukocytes in a zebrafish model of wound healing [5, 6]. During healing, cells within the wound transform from a quiescent state to one that is highly proliferative, migratory and angiogenic [7]. Growth factors and cytokines are important mediators of this transformation and H$_2$O$_2$ has been shown to be produced as a second messenger during growth factor signaling, particularly that which activates receptor tyrosine kinases [8]. Similarly, we have also found that low levels of H$_2$O$_2$ can replicate some of the effects of growth factor signaling, particularly cell proliferation and migration [9, 10]. Lastly, newly formed collagen fibers in the wound have to undergo cross-linking reactions to increase mechanical strength. The enzyme that catalyzes this process, lysyl oxidase, also produces H$_2$O$_2$ as a byproduct [11].

If ROS are produced in wounds, could excessive production be a cause of poor healing? One of the challenges of answering this question is that reactive species are very short lived and hence difficult to measure. Even if one did manage to measure production of reactive species in wounds [12, 13], it does not take into account whether the endogenous antioxidant defense and repair mechanisms are overwhelmed by this increased production. Hence the approach taken by our lab is to measure levels of oxidative damage using biomarkers such as the F$_2$-isoprostanes, protein carbonyls and 3-nitro-tyrosine. F$_2$-isoprostanes are formed when ROS attacks arachidonic acid, an essential component of cell membranes [14]. Protein carbonyls and 3-nitro-tyrosine are formed when ROS or RNS attack proteins [15, 16]. These compounds provide telltale signatures which indicate that reactive species have caused damage which was not mitigated by antioxidants nor repaired or removed.

The effects of oxidative damage in wounds have been inconclusive. Chronic wounds exudates have been shown to have higher levels of lipid peroxidation as measured by levels of F$_2$-isoprostanes [17]. Measurements of protein oxidation have been confusing. There was no difference in the absolute protein carbonyl content in acute and chronic wound exudate. However, chronic wound fluids was found to have lower protein content, thus the normalized protein carbonyl content in chronic wound was found to be 15% higher [18]. On the other hand, the Rac2 knockout mouse model showed delayed healing but also reduced oxidative damage [12].

Despite our incomplete knowledge of whether oxidative damage causes poor healing, there have been numerous studies on the benefits of antioxidants in wound healing. These studies claimed that plant extracts which showed potent antioxidant properties in vitro were beneficial in wound healing. However they either did not measure the effect of the antioxidants on oxidative damage [19] or used thioarbitruric acid reactive substances (TBARS) as an indicator of lipid peroxidation [20-24]. TBARS is an easy to measure but inconsistent indicator for lipid peroxidation. As an example, reported values for plasma TBARS in patients with diabetic foot ulcers range over several orders of magnitude from 1 nM [25] to 9 μM [26]. Hence, even though there are many reports on how antioxidants might improve healing, there is no proof to show that they do so by scavenging free radicals and preventing oxidative damage. In fact, our group has also shown that plant extracts can have both antioxidant and pro-oxidant activities [27] and it has been reported that grape seed extract improves wound healing by functioning as a pro-oxidant [28]. Ironically, many antioxidants can oxidize spontaneously and generate H$_2$O$_2$ as a byproduct [29, 30].

To fully understand the effect of oxidative damage in wounds, we applied H$_2$O$_2$ topically in a mouse model of wound healing. Consistent with the signaling role of H$_2$O$_2$ in wounds, we found that low concentrations of H$_2$O$_2$ (0.03%) promoted wound closure and angiogenesis. However, high concentrations of H$_2$O$_2$ (0.5%) delayed wound closure. These wounds were also more inflamed as measured by neutrophil infiltration and more proteolytic as indicated by higher levels of proteases and reduced connective tissue formation. Despite the delayed healing, we did not detect any increase in oxidative damage as measured by F$_2$-isoprostanes, 3-nitro-tyrosine and protein carbonyls.

What can we conclude from our knowledge of ROS in wound healing? I believe that excessive levels of ROS, particularly H$_2$O$_2$, might lead to prolonged inflammation and possibly neutrophil associated proteolytic damage. However, the amount of H$_2$O$_2$ needed to disrupt healing is insufficient to cause oxidative damage. In fact, oxidative damage (at least to the biomolecules examined) is unlikely to cause delayed healing, although it could be present as a consequence of poor healing. Very low concentrations of H$_2$O$_2$ might be beneficial in wound healing especially in poor healing wounds such as diabetic wounds. However more studies would be needed to validate this.

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References


Alvin Loo graduated with a Bachelor of Applied Science (First Class honors) from NUS in 2006. He joined Professor Barry Halliwell’s lab in 2007 as a graduate student. He was initially tasked by his supervisor to find out if antioxidants are beneficial in wound healing but instead he ended up investigating if reactive oxygen species, particularly hydrogen peroxide, are beneficial in wound healing.

Alvin was also an active student leader and has organized various student activities, including a students’ symposium. He also represented NUS at the 61st Meeting of Nobel Laureates in Lindau, Germany in 2011.

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