

Water H₂O₂ Levels as Factor in Swimmers Melanoma

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Abstract:

Introduction: This study proposes a foundation which could demonstrate a published H₂O₂ hypothesis of cancerogenesis. A mechanism explaining the high incidence of melanoma in swimmers is described.

Methods: Human hairs immersed in drops of pure water with resistivity of 18.2 MΩ × cm (million ohms) were in indirect contact with adjacent drops of 35% H₂O₂. Digital microphotographs and video-recordings were obtained for further analysis.

Results: This approach allows visualization of details that could not be analyzed previously. The findings show that H₂O₂ penetrates hair follicles either at the sites of injury to external structures or at the intact shaft/skin junction.

Conclusions: In hairs immersed in pure water mixed with a low concentration of H₂O₂, sebum and contiguous dermal sheaths blocked exogenous H₂O₂. Conversely, in injured hair follicles and at the intact shaft/skin junction, H₂O₂ penetrated the tissue and was subsequently decomposed by catalase. This mechanism is proposed for the high incidence of Swimmers Melanoma.

Keywords: H₂O₂, Oxidative Stress, Swimmers Melanoma, Pig Melanoma Formation, Biophysics cancerogenesis

Introduction

Analytical measurement of the effects of hydrogen peroxide (H₂O₂) on tissues has been difficult^[1]. The objectives of the present study are to introduce an experimental method to reduce H₂O₂ substrate concentration in solutions in contact with human hair follicles; thus mimicking surface fresh and saltwater H₂O₂ levels. A second objective is to demonstrate the protective role of the external skin layers from penetration of reactive oxygen species (ROS), namely H₂O₂.

One factor impeding optical microscopy studies of the effect of H₂O₂ decomposition on tissue is the rapid decomposition caused by the enzyme catalase. Invariably, multiple layers of gas bubbles rapidly form, obstructing the viewing field (Figure 1). Reports of "swimmers melanomas" in adults and children, as well as reports of H₂O₂ formation resulting principally from the excitation of humic substances in fresh and sea water by the ultraviolet (UV) portion of sunlight, have been published^[2,3]. These studies raise the question of whether H₂O₂ present in surface waters is a risk factor for the development of melanoma in swimmers.

Successful attempts to suppress the rate of H₂O₂ decomposition reaction utilizing activated carbons have been described^[4]. The hair follicle has been described as a dynamic miniorgan^[5] with independent cell division and differentiation and adjacent sebaceous gland, as well as dermal sheaths^[6]. H₂O₂ vapors are air-bound and used for bio-decontamination through deposition on surfaces via micro-condensation^[7]. This manuscript introduces a simple method to use the human hair follicle as sentinel following immersion in pure water to slow down the explosive repetitive decomposition of H₂O₂.

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Abbreviations: Decomposition: Breaking down of a molecule; H₂O₂: Hydrogen Peroxide; RH: Relative Humidity- Measure of ambient water vapor; ROS: Reactive Oxygen Species; 18.2 MΩ × cm (million ohms): Pure water resistivity. This only accounts for the dissolved ionic impurities commonly found in water; Substrate: The substance acted upon by an enzyme. In this manuscript (H₂O₂). Enzyme activity is directly proportional to substrate concentration; DOM: Dissolved organic matter in fresh water. UV Excitation: UV portion of sunlight (limited to depth of UV in body of water).

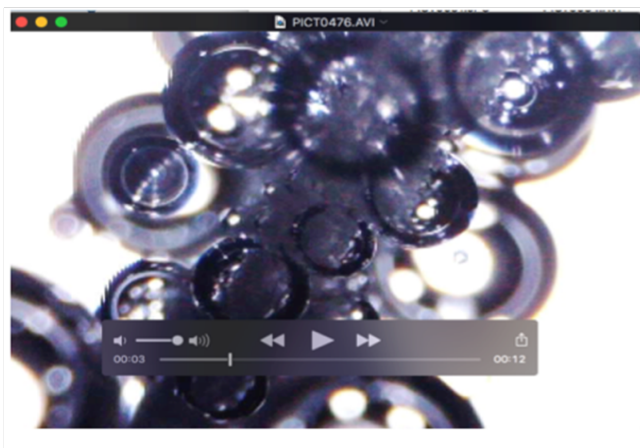


Figure 1: Microphotograph of 3% H₂O₂ spontaneous reaction with a hair follicle. Numerous oxygen bubbles obscuring the viewing field. See link for video-recording. <https://youtu.be/c-wrQdPK2pk>

Materials and Methods

Glass slides (25×75×1 mm, #1301) were purchased from Globe Scientific Inc. Certified food grade H₂O₂ (35%) and bovine liver liquid catalase (purified, thymol-free) was purchased from Sigma-Aldrich (StockC-40, one gram 11000 units). Very pure water with resistivity of 18.2 MΩ × cm (million 112 ohms) supplied by The University of Oklahoma, Health Sciences Center, was used. The equipment included a model OS425-LS non-contact infrared thermometer, Celestron model 44348 digital video microscope, 117 Acurite model 01538CDI remote weather station, Apple MacBook computer, and Apple Inc. iPhoto to 8.1.2 application.

Harvesting and mounting of hair samples

Sixteen hairs were plucked from the scalp of the author using tweezers. Care was taken to select hairs with large visible roots. Using a fresh double-edge razor blade, ten of the hair follicles were gently injured after placing on a dry slide. This maneuver produced four partially transected follicles with different follicular injury patterns. The individual hairs (12 intact and 4 injured) were individually placed on the center of clean glass slides then covered by two drops of very pure water with a resistivity of 18.2 MΩ × cm. Two to three drops of 35% H₂O₂ were delivered via a micropipette to the same slide 4 to 5 mm distant from the pure water drops. Care was taken not to mix the drops. Each preparation was mounted on a digital video-microscope platform and observed for any changes of the hair follicles. Gas bubbles emanating from the hair follicles appeared slowly on the hair tissue. Once the slow bubbling started after approximately 8 ± 3 minutes (Figure 1A), microphotographs and video-recordings were obtained and the data were stored for subsequent analysis. Ancillary testing demonstrated the gradual transfer of H₂O₂ into the pure water drops.

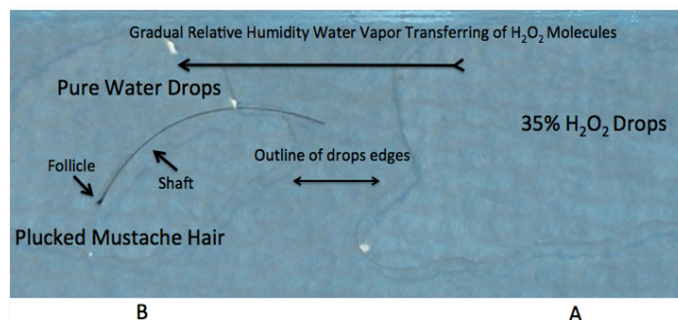


Figure 1(A): A= Drops of 35% H₂O₂ on right side of glass slide B= Plucked mustache hair in pure water drops Long black arrow showing theorized gradual transfer of H₂O₂ molecules penetrating pure water drops.

Results

All intact follicles showed bubbling activity; it occurred near the shaft/skin boundary in 12 follicles (Figure 2 & 3) and Supplementary Video #0043) and at the bulb and suprabulbar external areas in the four injured follicles. In all the injured follicles, O₂ gas was observed to be flowing slowly between the cortex and medulla, exiting at the injury site (Figure 4 & 5).

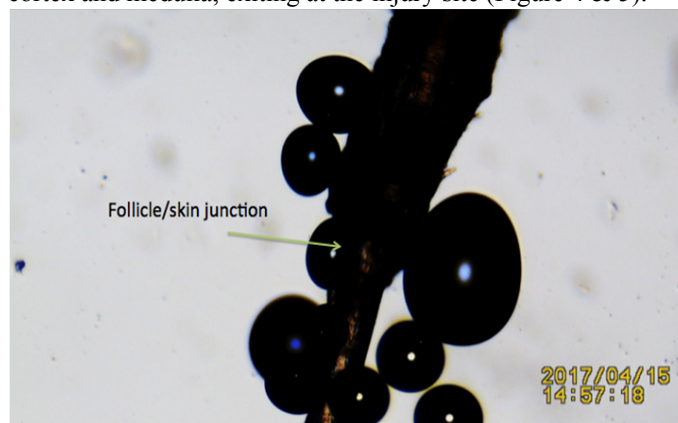


Figure 2. Showing O₂ bubbles emanating from the hair follicle/epidermis junction. Subsequent research (not shown) showed the bubbles originating from inside the distal shaft (not from the epidermal tissue). Please click on video link: <https://youtu.be/09tYp348jKM>

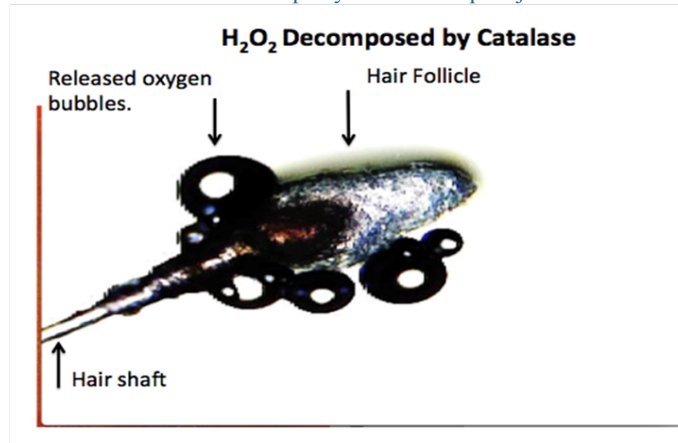


Figure 3: Microphotograph of video-frame from another experiment. On the top surface of the SDW, a human peri-umbilical hair was immersed in purified water. The H₂O₂ molecules transferred via water vapor (See Figure.1) were decomposed by the catalase present at the hair shaft/skin junction. Oxygen bubbles (arrow) are seen released by the hair follicle. Note: Notice hair shaft free of O₂ bubbles.

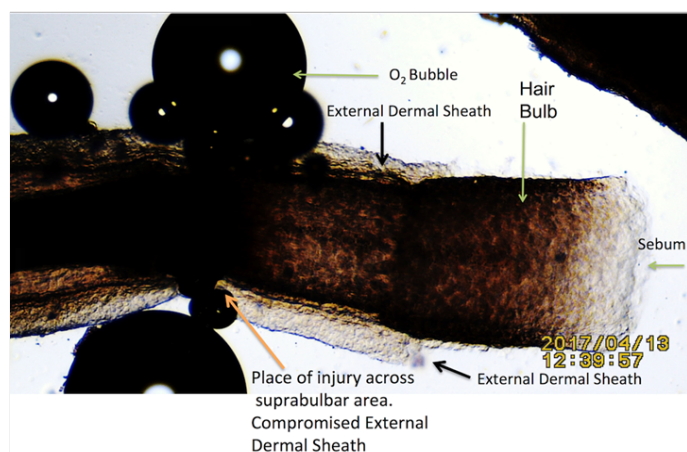


Figure 4: Injured hair follicle at mid bulb area showing place of O₂ bubbles origin. Hair immersed in pure water adjacent to drops of 35% H₂O₂. Black Arrows: External Dermal Sheath (EDS). Notice the absence of oxygen bubbles in areas protected by the EDS and sebum.

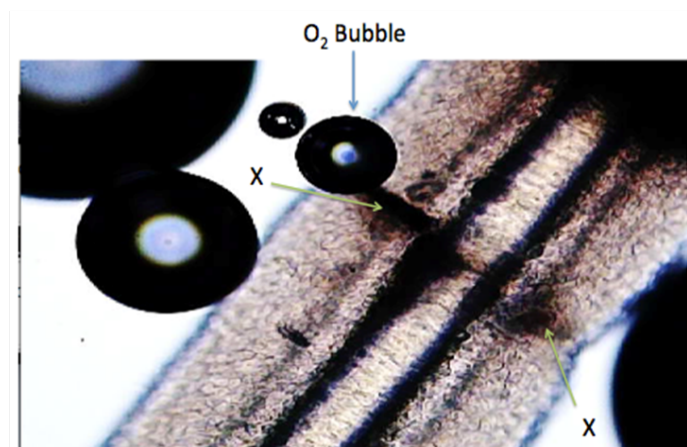


Figure 5: Transverse injury to another hair follicle via double edge razor blade X= Injured Tissue. Microphotograph of video-frame showing O₂ bubbles forming due to H₂O₂ molecules penetrating the pure water. The H₂O₂ decomposition caused by the protein enzyme catalase present in the hair follicle. The follicle's outer wall (sebum) integrity was disrupted by trauma, thus allowing penetration of the H₂O₂ molecules. Click on link for video-recording: <https://youtu.be/qRIV43Zphvc>

H₂O₂ water vapor transfer

Water vapor is known to transport molecules (including H₂O₂).

Concerning the cause(s) of the observed slow H₂O₂ decomposition by the hair follicles, it could be theorized that H₂O₂ molecules gradually penetrated the pure water adjacent to the 35% drops of H₂O₂ adjacently placed on the same glass slide as shown in Figure 1A.

Discussion

Oxidative stress due to tissue metabolism in hair follicles has been previously demonstrated^[11,12]. Hair follicles produce catalase in order to decompose ROS and achieve homeostasis. The present findings were possibly due to the very slow bubbling observed during the endogenous catalase-mediated decomposition of H₂O₂ that penetrated the hair follicles.

The first thought was that the presence of catalase in the scalp^[13] could be the cause of the intense bubbling observed at the shaft/skin junction site. High magnification views showed

that the bubbling originated away from the shaft/skin boundary surface. Since the shaft/skin junction area was uninjured, the observations prompted the consideration of whether the shaft/skin junction area in the skin was a point of spontaneous entry for H₂O₂.

Sebum/Dermal sheaths as barriers

Observed was that exogenous H₂O₂ penetrated deep into hair follicles. In seeking a mechanism for this observation, it was noted that when hair follicles were injured, the protective sebum coat and dermal sheaths were compromised (Figure. 6). This would allow the exogenous H₂O₂ to penetrate the internal tissue layers where it would be decomposed by the ubiquitous enzyme catalase.

The present study also presents an association of sebum combined with intact dermal sheaths acting as a barrier to ROS external toxicity.

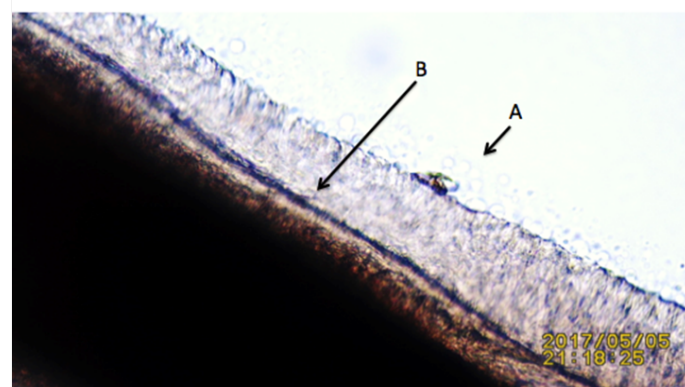


Figure 6: Plucked Beard hair mounted on glass slide- Black arrows showing A= Outer layer showing lipids globules B= Intact External Dermal layers.

Summary and Conclusions

Swimmers melanoma

Two decades ago, it was postulated that the recreational exposure to sunlight did not fully explain the current trends in melanoma Incidence^[14]. The authors suggested that the “positive association between a history of swimming and melanoma risk suggests that carcinogenic agents in water, possibly chlorination by products, play a role in melanoma aetiology”. Additionally, considering the skin dryness of swimmers, the same authors stated that this is “caused by a combination of the dilution of natural sebum and by the osmotic gradient produced when the body is immersed in water, drawing hydration from the outer skin layers.” Another study documented several dermatological problems related to prolonged or repetitive water immersion, including skin denuding of the protective sebum coat^[15]. The protective barrier function of the skin against ROS has been identified previously^[16].

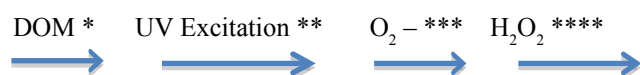
The experimental documentation presented in this manuscript supports the view that H₂O₂ fuels aging, inflammation, cancer metabolism, and metastasis^[17]. Furthermore, the present observations provide a basis for testing the hypothesis that ROS reactions are associated with cancerogenesis^[18]. We demonstrated that in hair follicles, compromised sebum/dermal sheath layers and uncompromised shaft/skin junction allow H₂O₂ penetra-

tion, resulting in repetitive ROS reactions and possible initiation of diseases. This could include melanoma.

Addendum

The data presented in this manuscript also supports that in fresh or seawater, the penetration of ROS into the submerged hairs occurs at the hair shaft/skin interface, as well as through the injured external structures. The risk for “swimmers melanoma” could be explained as follows: **“The formation of hydrogen peroxide results principally from the UV portion of sunlight exciting humic substances in the water and thereby leads to the formation of superoxide ion, which reacts with itself to form H₂O₂. Because this production is limited to the depth of UV light penetration, its vertical distribution provides a sensitive tracer for mixing processes”**^[19].

Factor affecting the formation of H₂O₂ in fresh waters



* DOM = Dissolved Organic Matter

** UV Excitation = UV portion of sunlight (limited to depth of UV in body of water)

*** O₂ - = UV portion leads to formation of superoxide ion, which reacts with itself to form H₂O₂.^[20,21]

**** H₂O₂ = Vertical distribution of Hydrogen Peroxide molecules now in body of water.

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