



INTRODUCING THE TRIGGERING OF ADHESIVE PROPERTY IN A HUMAN MINIORGAN: POSSIBLE IMPLICATIONS IN CANCER TREATMENT.

Clinical Science

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ABSTRACT

Introduced are findings of sequential addition of H_2O_2 and Tetracycline (TC) in contact with freshly plucked human hair follicles tissue. Antioxidants decompose H_2O_2 into O_2 molecules and H_2O . Observed was a slowing of the reactive oxygen species breakdown by TC that could be implied as a neutralization of the tissue intrinsic antioxidants. The observed inhibition phenomenon caused an immediate distortion detected via microscopy of the released oxygen bubbles from the breakdown. This distortion had been previously observed when exogenous H_2O_2 was added to sticky material from adhesive DNA precipitates. The mechanism explaining oxygen bubbles deformation is based on external pressures within an adhesive environment. Based on the similarities in O_2 deformation in experiments presented in this manuscript, as well as in previous published findings, is inferred that TC besides inhibiting exogenous H_2O_2 breakdown also triggers adhesive properties in a normal human miniorgan, thus reasoned to potentially having anticancer properties.

KEYWORDS

Human Tissue Metabolism, Human Tissue Adhesion, Fluorescent Studies Pitfalls, Oxygen Bubbles, Tetracycline, H_2O_2 Decomposition, DNA Precipitates, Cancer treatment, Oxygen Bubble Deformation.

INTRODUCTION

It has been said, "By studying the literature about Tetracyclines (TC), it becomes clearly evident that TC are very dynamic molecules. In some cases, their structure-activity-relationship (SAR) are well known, especially against bacteria, while against other targets, they are virtually unknown" (1). This manuscript introduces an unknown mechanism that could explain TC number of false negatives found from fluorescent tetracycline TC adhesion to cancer cells as a hallmark identifying carcinomas (2). Introduced is also an additional risk for misdiagnosis of TC fluorescence as false positives. In a previous manuscript *in vitro* experiments on freshly plucked human hair follicles documented the commonly used antibiotic TC deleterious effect on soft tissue, severe enough to allow for visualization of an underlying filamentous skeleton (3). This manuscript presents an *in vitro* glass slide optical microscopy methodology supporting a previously published sudden enzymatic inhibition of exogenous H_2O_2 breakdown by TC (4); and a property of TCs as an inductor of adhesive property in normal human tissue. This demonstration was achieved *in vitro* by direct application of TC to freshly plucked human hair follicles actively undergoing exogenous H_2O_2 irreversible dissociation by its inherent antioxidants. It is a known fact that during cell respiration, in order to achieve homeostasis toxic ROS i.e. H_2O_2 molecules are broken down by the ubiquitous protein enzyme catalase (5,6). Experiments presented will support TC as slowly inhibiting exogenous H_2O_2 decomposition and concomitantly observed as triggering adhesive properties. Only when TCs was applied to tissue undergoing ROS breakdown (cell respiration) is that the adhesive property ensued. The tissue adhesion was demonstrated by inference, in other words by comparing O_2 bubbles architectural changes between pure H_2O_2 breakdown by catalase and when post TC addition to tissue applied during the explosive reaction. Supporting the inference are observed O_2 bubbles similar deformations when moving through DNA precipitate mucous material (7). TC and its derivatives had been described to have anti-cancer properties (8). The newly described adhesion property induction is in support of TC potential as an additional tool as an anti-cancer drug. The findings herein presented may be of importance as additional factor in cancer research, since a cancer hallmark is loss of cell-to-cell adhesion (9). Still microphotographs and video recording are presented.

MATERIALS AND METHODS

MATERIALS

Hydrogen Peroxide 35%
Tetracycline capsules powder (500 mg)
25x75x1mm glass slide
Demineralized water bottle
Medicine dropper

Digital Video Microscope Celestron II model # 44341, California, USA. Still images and video recordings downloaded to an Apple Computer MacBook Pro Photo Application.

METHODS

The Single Side Preparation (SSP)

1- The SSP is an open-air technique where freshly plucked *in toto* scalp hairs (root and shaft) were placed on a clean 25x75x1mm glass slide; and covered by drops of 35% H_2O_2 .

2- The prepared slides were placed and focused on a video microscope where images and video recordings obtained for further analysis.

3- As the H_2O_2 molecule was being decomposed by the prevalent enzyme catalase in tissue, TC was delivered either by scattering a pinch of dry TCs powder, or diluting the powder in demineralized bottle water; and delivering two drops to the slide. Again, microphotographs and video recordings obtained.

RESULTS

The sudden chaotic presence of O_2 bubbles caused by the H_2O_2 decomposition by catalase (Fig 1) was readily inhibited by powder or diluted TC applied to human hair normal tissue (Fig 2). Images show deformed O_2 bubbles and tissue edges as if frozen in time (Figs 3, 4 and link to

URL: <https://youtu.be/H4xvi4AdJ3M>), similar to a picture of a still spring board diver in mid air

Control Image Showing Hair Follicle Irreversible H_2O_2 Decomposition.

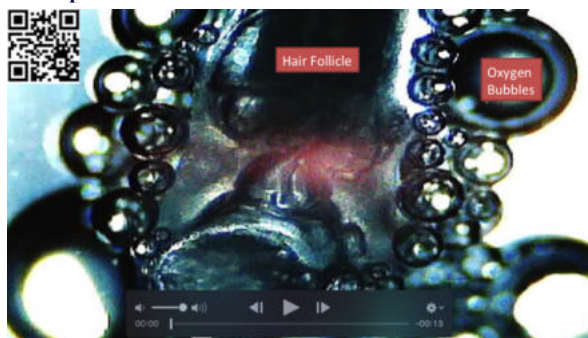


Figure 1. Selected frame showing intense chaotic H_2O_2 decomposition by enzymes present in a human hair follicle. Plucked beard hair placed in 35% H_2O_2 .

For details link to: URL <https://youtu.be/aqNbrBZPVok>
Or Scan QR Code in upper left corner of image.

Supporting Figure 1 above:

Link # 1: Dynamic presentation of merging oxygen bubbles. Please link to URL <https://youtu.be/cNkBW0934bw>
Or Scan QR Code below



Image Showing Powder Tetracycline Sudden Inhibition of H_2O_2 Decomposition

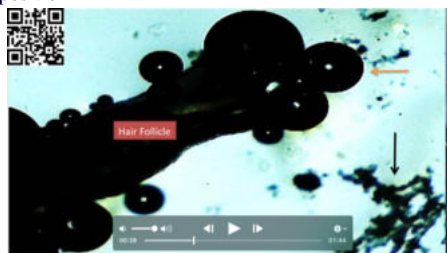


Figure 2. Hair follicle in 35% H_2O_2 showing breakdown. Sprinkled powder Tetracycline seen slowing reaction. Black Arrow: Tetracycline powder. For details link to: <https://youtu.be/bhQYgAjSG8E> Or Scan QR Code in upper left corner of image.

Supporting Figure 2 above:

Link # 2: Showing microphotograph of sudden inhibition of O_2 bubbles formation in a human hair follicle by Tetracycline powder. For further details, please link to URL <https://youtu.be/bhQYgAjSG8E> or Scan QR Code in below:



Image Showing Deformed Migrating Oxygen Bubble Post Tetracycline Notice Distorted Outer Root Tissue Edges

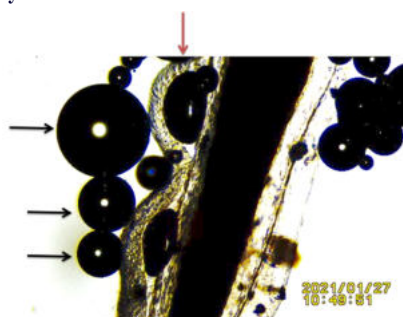


Figure 3. Showing image of mid hair follicle post Tetracycline powder sudden H_2O_2 breakdown inhibition. Black Arrows: Pointing at undeformed rounded oxygen bubbles outside of tissue. Red Arrow: Pointing at deformed oxygen bubble migrating through what appears to be inflamed tissue.

Supporting static Figure 3 above:

Link # 3. Figure 4 below. Showing deformed air bubbles and tissue moving towards the distal hair follicle (towards skin). Red Arrow: Pointing at distorted O_2 from TC sudden inhibition of H_2O_2 decomposition. For additional details please link to URL: https://youtu.be/uua_gX2vP1o

Or Scan QR Code below:

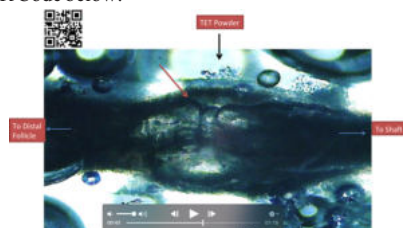


Figure 4. Showing deformed air bubbles and distended mid outer root tissue moving towards the distal hair follicle (towards skin). Red Arrow: Pointing at distorted O_2 from TET sudden inhibition of H_2O_2 decomposition. For additional details please link to: https://youtu.be/uua_gX2vP1o

Or Scan QR Code in left upper corner of image.

DISCUSSION

Bright yellow TC fluorescence in tumors under ultraviolet light was first introduced in animal experiments as early as 1957 (10,11); and subsequently tetracycline fluorescence was introduced as a diagnostic test for malignancy on harvested human gastric cells via gastric lavage. At the time it was observed via optical microscopy that TC was adhering to cancer cells and reports of false negatives criticizing contemporary clinical trials surfaced (12). The TC adhesion to cancer cells could be attributed to TC, since our present knowledge, is that loss of cellular adhesive properties is a hallmark of malignant cells. This manuscript now clarifies that conundrum as follows: When drops exogenous H_2O_2 are in contact with a freshly plucked hair follicle, the H_2O_2 molecules are decomposed into water and oxygen molecules (O_2). When Tetracycline powder is scattered over the active reaction, there is a slow inhibition of the decomposition as observed in the *in vitro* images above; this finding is supported by previous experiments (13). Video recordings show a dramatic stillness by displaying images of deformed merged O_2 molecules in the tissue, like frozen in time.

Distended Outer Root Tissue Rich in Stem Cells

Also appreciated are the hair follicle's outer root tissue distorted edges as if resembling inflammation (Figs 3,4). This cessation of activity is rationalized to imply a neutralization of antioxidants, such as catalase, that has been proposed to be essential in tissue life (14). The deformed O_2 bubbles are thought to be from environmental external pressure while migrating in dense mucous material similar to DNA precipitates as seen in (Fig 4 plus video below). As soon as the deformed bubble moved into a favorable environment (air), the deformation ceased and a rounded circumference observed.

O_2 Bubbles Deformation in Sticky DNA Precipitates

Prior experiments have shown that when H_2O_2 was added to a DNA precipitate on a glass slide, O_2 bubbles are detected moving within the dense mucous like tissue; due to external pressure within the sticky precipitate, deformed bubbles are seen. This deformation ceases as soon as the bubble exits towards a favorable air environment (Fig 5 + video) below.

DNA Precipitates External Pressures Deforming O_2 Bubbles in Transit

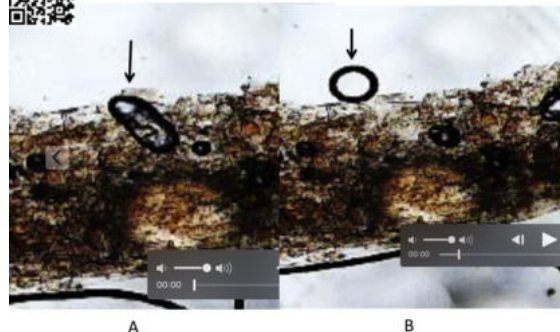


Figure 5. Selected photomicrographs of video recording showing: A: Black Arrow pointing at deformed O_2 bubble in mucous DNA precipitate. B: Black Arrow: Same bubble now free floating showing a symmetrical rounded shape.

Or Scan QR Code in left upper corner of image. Link to URL: Please link to URL <https://youtu.be/H4xvi4AdJ3M>

Above image from experiments by Abraham A Embi. "Introducing DNA Precipitates Adhesion to Normal Eukaryotic Cells Potential Application in Cancer Cells". *Acta Scientific Cancer Biology* 3.9 (2019): 37-41.

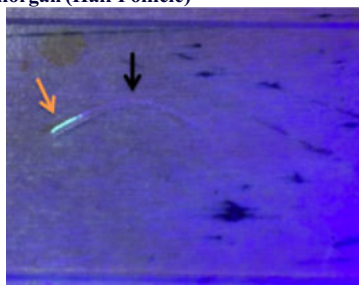
SUMMARY AND CONCLUSIONS

The experiments herein presented are in support of cellular adhesion property to TC when applied to living gastric cells as described in early clinical trials. Introduced is a newly discovered property of Tetracycline on a normal human miniorgan or hair follicle (15). In this

manuscript the data shows that only when Tetracycline is applied to living normal tissue undergoing irreversible H_2O_2 decomposition is that a sudden human tissue adhesive quality was found. Images also show deformed oxygen bubbles and tissue edges deformation resembling swelling (16). This tissue in turn is hypothesized to have similar adhesive qualities as DNA precipitates. Also reasoned is that no doubt there is a need for further research by well-equipped research facilities. Tetracycline has been shown to inhibit leukemic cells migration, this could be attributed to cellular adhesion (17). It is concluded that TC adhesion and fluorescence could also be observed in tissue from a normal human miniorgan (Fig 6).

The work herein presented is in support of a seminal and probably ignored paper when 61 years ago, TC fluorescence was documented in benign and malignant ulcers (18)

Demonstration of TC Adhesion to Metabolically Active Normal Human Miniorgan (Hair Follicle)



Hair follicle under UV light exposure post SSP liquid tetracycline showing: Orange Arrow: Evaporated tetracycline powder adhesion to normal tissue in human hair follicle. Black Arrow: Faint image of hair shaft (absence of TC adhesion).

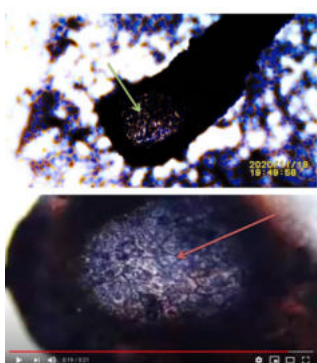
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MEDICAL IMPLICATIONS

The most salient implication is supporting TC as an anticancer agent in combination with H_2O_2 . While researching the literature, there is a resurgence of using TC fluorescence property in endoscopy fluorescence spectroscopic images in the gastrointestinal tract (19). Utilizing the data herein presented support advice for awareness that TC fluorescence could be triggered by other factors such as normal tissue when exposed to exogenous H_2O_2 as shown in this manuscript. Applying 33% H_2O_2 (HP₃₃) alone has been reported to reduce or eradicate skin cancers, including melanomas (20).

Tetracycline and Active Cellular Metabolism

The data shown in this manuscript and previous papers support an unexplained property of TC which is to concentrate in metabolically active tissue, such as in the distal dermal papilla area (see attached image below) and as herein recently reported in the stem cells niche in the middle part of the outer root sheath (21) demonstrated by a concentration of deformed air bubbles as shown above (Figs 3,4 + video).



Panel showing: A: Hair follicle in liquid tetracycline after evaporation- Red Arrow: Pointing at the biological active Dermal Papilla (DP) area showing tetracycline crystals adherence. B: Amplified image from attached video frame at 0:19". Test showing crystals adhesion to the DP. For additional details, please link to URL

<https://youtu.be/XC1XZdqJeqI> Or scan QR Code in upper right corner of image.

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Topical H_2O_2 and Skin Cancers

- Encouraging results were reported in a recent paper describing topically applied HP₃₃ as a neoadjuvant treatment aimed to totally eradicate or reduce skin cancer lesions. As reported "All lesions included in this study were reduced in terms of their dimensions after treatment with HP₃₃. Specifically, the length of lesions was reduced by a mean of 50%, while the width was reduced by a mean of 48%. Statistical analysis revealed a statistically significant reduction in both length ($p < 0.001$) and width ($p < 0.001$)".

Topical H_2O_2 and Tetracycline on Skin Cancers

Since a method where a combination of exogenous H_2O_2 and Tetracycline was shown in this manuscript to trigger cellular adhesion:

The Questions arise:

- If H_2O_2 and Tetracycline could do this to normal tissue what could it do in cancer tissue?
- What would the long-term effect of triggered cellular adhesion be when applying tetracycline post HP₃₃ decomposition to cancerous tumors?

Further research is warranted!

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