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Cell damaging by irradiating non-thermal plasma to the water: Mathematical modeling of chemical processes

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ABSTRACT

Recently non-thermal plasma (NTP) is applied for many therapeutic applications. By NTP irradiating to the tissues or cell-lines, the water molecules (H_2O) would be also activated leading to generate hydrogen peroxide (H_2O_2). By irradiating plasma to bio-solution, its main output including vacuum UV to UV causes the photolysis of H_2O leading to generate hydroxyl (OH) molecules in couple forms with ability to convert to H_2O_2. Additionally, other plasma's output the oxygen atoms could also penetrate under the liquid's surface and react with H_2O to generate H_2O_2. In NTP applications for killing unwanted-cells of microorganisms (e.g. sterilization) or cancerous tissues, the H_2O_2 molecule is the main reactive species for cell death via inducing DNA damage in mammalian cells. In this paper we proposed a mathematical model for NTP application describing the formation of hydroxyls in the bio solution and other subsequent reactions leading to DNA damage in vitro. The instant concentrations of the OH and H_2O_2, the main species for DNA oxidation were obtained and investigated in this simulation. In order to validate the model, the cellular response to NTP stimulation was compared with some experimental findings from viewpoint of DNA damage to show the significant consistency.

Keywords: Modeling; DNA damage; hydroxyl; peroxide; non-thermal plasma

INTRODUCTION

Recently, the plasma which generated at room temperature and atmosphere pressure is applied in different medical fields (e.g., sterilization, wound cure, dentistry and cancer treatment) and known as plasma medicine that could be found in introductory reviews [1, 2]. Such kind of plasma could be generated by passing a noble gas (e.g. helium or argon) through a

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medium with an AC magnetic field (e.g. solenoid supplying by electricity in kV and KHz range). Such exhausting ionized gas looks like a flare but not hot, named as non-thermal plasma (NTP). The NTP includes electrons, ions, electromagnetic photons (e.g. visible and ultra violet light) and some reactive chemical particles e.g., reactive nitrogen or oxygen species (RNS or ROS) especially hydroxyl. The free radicals of NTP could destruct the membrane of the cells through affecting their proteins and lipids [3]. NTP could be provided through different techniques leading to variant size of affecting area (a few mm^2 to cm^2 range) for different applications [1-4].

The recent application of NTP is based on affecting plasma on the water known as plasma-activated-water (PAW) which could be executed 'directly' to the water of the tissues (mostly extracellular fluids) as shown in Figure 1. The 'indirect' PAW could be performed on some mediator water which would then be applied to the tissues.

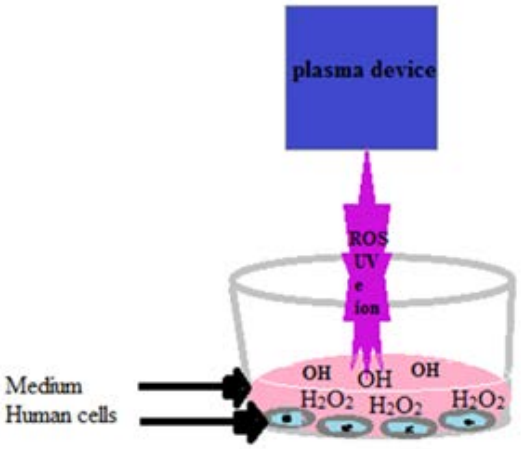

Figure 1: Schematic illustration for the interaction between NTP and cells in vitro. The reactive species formations in the culture medium (particularly hydroxyl) have been regarded as the main factors causing the death of cancer cells *in vitro*.

Here, hydroxyl effects of 'direct PAW' on damaging DNA (a less-noticed subject) were studied through mathematical modeling. This process makes the water to generate highly reactive species such as hydroxyl radical (OH). Since a significant percentage of the human body is water, including intracellular and extracellular fluids, the initial dominant reaction of any radiation with body is radiation-water interaction. In addition, cell culture mediums are water base, so attention to the radiation-water reactions for *in vivo* and *in vitro* NTP-studies is essential. Some NTP components including UV photons and oxygen atoms could interact with water to produce OH molecules [5].

The aim of this study was "mathematical modeling" of the dominant photochemical / chemical reactions for OH formation by NTP irradiating to a bio solution (e.g. cell culture medium) and subsequent reactions leading to DNA damage.

MATERIALS AND METHODS

Photochemical and chemical reactions: By exposing NTP to H_2O molecules within the cell culture medium, because of its UV photons, the photolysis reaction (i.e., photo dissociation and photoionization) occurs and generates extracellular OH (OH_{ex}) radicals (with half-life in ns range) responsible for initiating many reactions in the bio-solution [5]. In photo dissociation reaction, a water molecule is dissociated to OH and H atoms while in photoionization, it is ionized to produce the H_2O^+ (as shown in following Eq.1 and Eq.2, respectively). The H_2O^+ penetrates only a few microns into the water then generates hydronium (H_3O^+) and OH_{ex} through a charge exchange reaction as seen in Eq.3. Another significant mechanism of OH

production in bio solution is the reaction of NTP's oxygen atom with H_2O molecule at the liquid surface [6-7] as shown in Eq.4. Subsequent dominant reaction is OH_{ex} recombination and forming long live (12-24 h) extracellular H_2O_2 radicals ($H_2O_{2\,ex}$) as shown in Eq.5.

$h\nu + H_2O \rightarrow OH + H$ (Photo dissociation) (1)

$h\nu + H_2O \rightarrow H_2O^+ + e$ (Photo ionization) (2)

$H_2O^+ + H_2O \xrightarrow{k_C} H_3O^+ + OH$ (Charge exchange) (3)

$O^{\cdot} + H_2O \xrightarrow{K_d} OH + OH$ (Oxygen reaction) (4)

$OH + OH \xrightarrow{k_R} H_2O_2$ (Recombination) (5)

The radical of $H_2O_{2\,ex}$ is the major active component of NTP which could freely pass through the cell membrane to induce cellular injury through DNA damaging [8]. Unfortunately, it could also be produced within the gastric system by using diet or dietary supplements [9]. The $H_2O_{2\,ex}$ radical diffuses across the cell membrane leading to increase the intracellular H_2O_2 (i.e. $H_2O_{2\,in}$). The inserted $H_2O_{2\,in}$ itself is not very reactive with cellular constituents, but in the presence of iron ions, could be converted to OH via Fenton reaction [10] (Eq.6). The iron is the most abundant transition metal in biological systems and indispensable element for living organisms. It is also potentially toxic because its excess levels lead to the generation of the OH in the presence of H_2O_2 via the Fenton reaction. Like some other metal ions, the iron in the vicinity of DNA could also generate the OH_{in} radicals from H_2O_2. The generated OH could induce several classes of DNA damage including single/double -strand break, a basic site, and base oxidation [11] as follow reactions (Eq.7 to 8):

$Fe^{+2} + H_2O_2 \xrightarrow{k_F} Fe^{3+} + HO^- + HO$ (Fenton reaction) (6)

$HO + H_2O_2 \xrightarrow{k_Q} O_2^- + H_2O + H^+$ (Quenching reaction by H_2O_2) (7)

$HO + DNA \xrightarrow{k_{DNA}}$ DNA damage (DNA damage) (8)

Mathematical modeling of reactions: In order to investigate the mentioned reactions (Eq.1 to Eq.8), as usual, each concentration rate (i.e. variation velocity of any 'concentration as ***C***' or derivative of ***C***: d***C***/d***t***; in left sides) was expressed by other related concentrations (in right sides) as could be seen in the following differential equations (Eq.9 –Eq.15). The applied 7-variables and 11-parameters were defined in Table 1 with some typical values for the parameters and the relevant references.

$$\frac{d[H_2O]}{dt} = -I\sigma\varphi[H_2O] - k_d\,[H_2O][O] \quad \text{(Photo dissociation and Ionization)} \quad (9)$$

$$\frac{d[OH]_{ex}}{dt} = I\sigma\varphi[H_2O] + k_C[H_2O][H_2O] - k_R[OH]_{ex}[OH]_{ex} + k_d\,[H_2O][O] \quad (10)$$

$$\frac{d[H_2O_2]_{ex}}{dt} = k_R[OH]_{ex}[OH]_{ex} - k_{diff}\,\frac{n.V_{internal}}{V_{external}}([H_2O_2]_{ex} - [H_2O_2]_{in}) \quad (11)$$

$$\frac{d[H_2O_2]_{in}}{dt} = k_{diff}([H_2O_2]_{ex}\text{-}H_2O_2]_{in}) - k_F[Fe^{2+}][H_2O_2]_{in} - k_Q[H_2O_2]_{in}\,[OH]_{in} \quad (12)$$

$$\frac{d[Fe^{2+}]}{dt} = -k_F[Fe^{2+}][H_2O_2]_{in} \quad \text{(Fenton reaction)} \quad (13)$$

$$\frac{d[OH]_{in}}{dt} = k_F[Fe^{2+}][H_2O_2]_{in} - k_Q[H_2O_2]_{in}[OH]_{in} - k_{DNA}[OH]_{in}[DNA] \quad (14)$$

$$\frac{d[DNA]}{dt} = -k_{DNA}[DNA][OH]_{in} \quad \text{(DNA damage)} \quad (15)$$

Table 1: The definitions of variables and parameters applied in the Eq.9-Eq.15

Symbols	Definitions	Initial/Value	References
	Variables		
[H2O]	H2O conc.	500 µM	-
[OH] ex	extracel. OH conc.	0 µM	-
[OH] in	intracel. OH conc.	0 µM	-
[H2O2] ex	extracel. H2O2 conc.	0 µM	-
[H2O2] in	intracel. H2O2 conc.	0 µM	-
[Fe2+]	Intracel. Free iron conc.	80nM	-
[DNA]	DNA conc.	17µM	-
	Parameters		
σ	water molecule absorption cross-section	1×10^{-20} cm^2	[25]
Φ	photolysis quantum yield	0.33	[33]
K_R	Recombination reaction rate constant	$5.5\times10^{9}M^{-1}s^{-1}$	[34]
K_F	Fenton reaction rate constant	$4.4\times10^{4}M^{-1}s^{-1}$	[35]
K_Q	Quenching reaction rate constant	$2.7\times10^{7}M^{-1}s^{-1}$	[8]
K_{diff}	Diffusion rate	70 s^{-1}	[7]
K_{DNA}	damage reaction rate constant	$4.7\times10^{9}M^{-1}s^{-1}$	[8]
K_C	charge exchange reaction rate constant	$6\times10^{3}M^{-1}s^{-1}$	[8]
I	VUV-UV photons intensity	$1\times10^{8}s^{-1}cm^{-2}$	-
N	Initial cell density	$1\times10^{4}cm^{-3}$	-
Vin/Vex	intracell. To extracell. Volume rate	3.2×10^{-15}	[8]

Water molecules concentrations in bio solution decreases by two dominant reactions include; water photolysis reaction (first term in Eq.9 which depends on the light intensity, the water molecule absorption cross-section, photolysis quantum yield, and the photolysis time) and the oxygen atom reaction with water molecules on the bio solution surface (second term in Eq.9). Hence, $[OH]_{ex}$ is increased by these two mentioned reactions and decreased by recombination process of OH radicals to forming $H_2O_{2\ ex}$ (as shown with minus sign of third term of Eq.10 and in first term of Eq.11) [7]. On the other hand (second term of Eq.11), the $[H_2O_2]_{ex}$ is reduced by diffusing from outside to inside of the cells [8]. The resultant $[H_2O_2]_{in}$ is decreased by two processes of Fenton and quenching (second and third terms of Eq. 12 respectively). For *in vitro* systems, there is no $[H_2O_2]_{in}$ growth term due to inside production of the cells. In addition, the more cell density, the faster detoxification of the medium: as shown in the form of the relative cellular volume parameter in the second term of Eq. 11 [8]. Eq. 13 shows a decreasing manner for [Fe2+], due to Fenton phenomenon (assuming no production inside the cell in vitro). The $[OH]_{in}$ is decreased because of DNA consumption (the last term in Eq. 14). Hence, [DNA] is reduced (as shown in Eq. 15) leading to cell death.

Numerical simulation: For solving such differential equations (Eq.9 to Eq.15), a discretization technique known as finite difference method was applied. Since, all of them are first-order differential equations, the Euler method could be a proper selection in which the local error (error per step) is proportional to the square of the 'step size' (or 'sampling period: $\boldsymbol{T}$'), and the global error (error at a given time) is proportional to the step size [12]. Thus, in order to make the approximation error of this method to be a negligible value, $\boldsymbol{T}$ was assumed to a relatively small value of 0.1 ns (or 10^{-10} s). Thus continuous space with time-variant of $\boldsymbol{t}$ could be converted to a discrete-space with time-index of $\boldsymbol{n}$, since all variables are sampled at $\boldsymbol{t}$ equals to $\boldsymbol{nT}$.

Therefore, by substituting of $d\boldsymbol{C}(\boldsymbol{t})/d\boldsymbol{t}$ by a difference form of $[\boldsymbol{C}(\boldsymbol{n}+1)-\boldsymbol{C}(\boldsymbol{n})]/\boldsymbol{T}$, a differential equation with a typical form of $d\boldsymbol{C_1}/d\boldsymbol{t}=k_1.\boldsymbol{C_1}+k_2.\boldsymbol{C_2}$ would be converted to a recursive relation of $\boldsymbol{C_1(n+1)}=\boldsymbol{C_1(n)}+\boldsymbol{T}.K_1.\boldsymbol{C_1(n)}+\boldsymbol{T}.k_2.\boldsymbol{C_2(n)}$ which could be simply solved (by a 'for'-loop command of any computer language) based on the initial values of variables $\boldsymbol{C_1}$ and $\boldsymbol{C_2}$ and parameters k_1 and k_2.

All of the parameters and initial values could be seen in Table 1, thus the presented mathematical model could be programmed and simulated numerically by any calculation-software. Here, the MATLAB software (MathWorks: R2014a) was used to simulate the model and plotting the graphs.

In order to validate the presented model, the concentrations variations of main factors versus the time and also the manner of DNA damage in response to NTP stimulation were compared with some practical findings of other researchers to show the significant consistency.

RESULTS AND DISCUSSION

After applying NTP for a bio solution, as mentioned before, the OH is generated through two processes of photo dissociation (UV-H_2O reaction) and surface-plasma (oxygen reaction). A while after, the H_2O_2 is also produced in water due to two OH recombination reactions leading to reduce the ascending ramp of $[OH]_{ex}$ curve as could be noticed in Fig. 2. The H_2O_2 is an important molecule in biological systems because it can pass freely from outside toward inside the cells but not vice versa. Such unidirectional diffusion makes endogenous H_2O_2 to rise as could be seen in Fig. 3. On the other hand, within the cell, the inserted H_2O_2 molecules start to be decomposed to OH (by reaction with endogenous Fe2+) leading to generate endogenous OH. Hence, after a short time, the increasing slope of $[H_2O_2]_{in}$ curve starts to decrease while $[OH]_{in}$ is increasing as shown in Fig. 3.

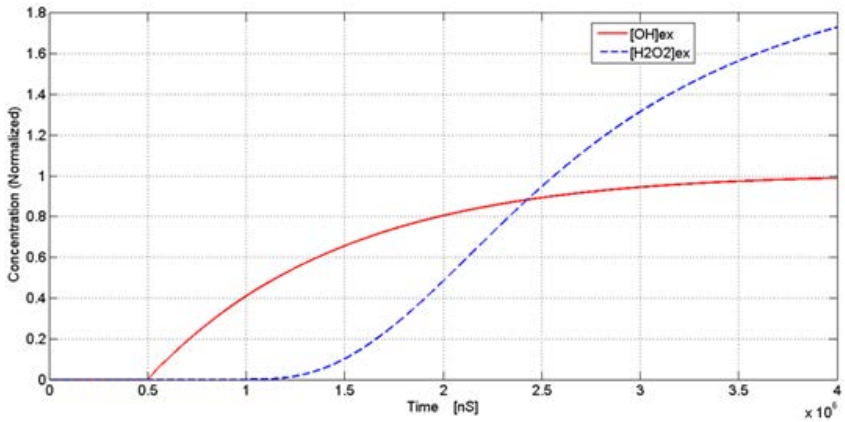

Figure 2: the time evolution of extracellular [OH] and $[H_2O_2]$ in the bio solution. It was assumed the uniform distribution for species in the bio solution. The OH molecule is produced and its concentration increased during the discharge due to the mentioned reactions leading to generate H_2O_2

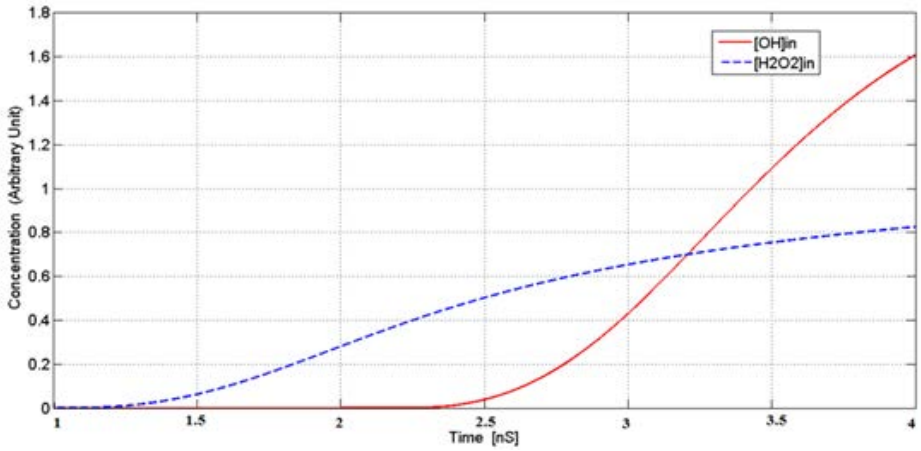

Figure 3: the time evolution of intracellular OH and H_2O_2 concentrations of species which are increased due to diffusion of H_2O_2 toward the inside of cell.

By stopping NTP irradiation, the exogenous OH generation is also stopped whilst the H_2O_2 generation is continued and diffused toward the inside of cells till diminishing $[OH]_{ex}$, as shown in Fig. 4. The last produced component, the OH is known as the killer agent of such treatment process which could be reacted by DNA leading to cell death based on some cancer treatment studies [13-16].

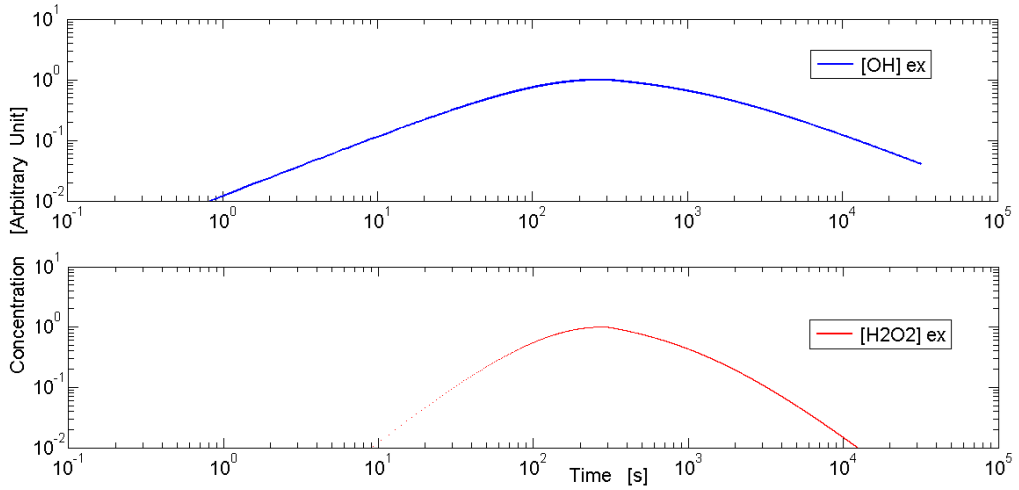

Figure 4: The steady state of OH and H_2O_2 concentrations within the cells after 300 s NTP irradiation

The magnitude of DNA damage versus $[H_2O_2]_{ex}$ was obtained and presented in Fig. 5. The obtained curve seems to be an ascending manner as well as appeared in the experimental findings of some researchers [8, 17]. The delay in rising makes the obtained curve to present a 'sigmoidal' appearance. Hence, there could be found a critical point on the horizontal axis with the maximum slope (i.e. zero second-derivative). It means that there is an optimum concentration for H_2O_2 with maximum sensitivity of DNA to be damaged. Such delay could be decreased by varying some parameters: increasing **n** the cell density, H_2O_2 diffusion toward the cell inside, free available iron concentration, and/or decreasing k_q.

The H_2O_2 molecule induces DNA damage which could decrease cell viability through activating of apoptosis pathways in a concentration- and time-dependent manner as mentioned in a review article for A549 cells [18].

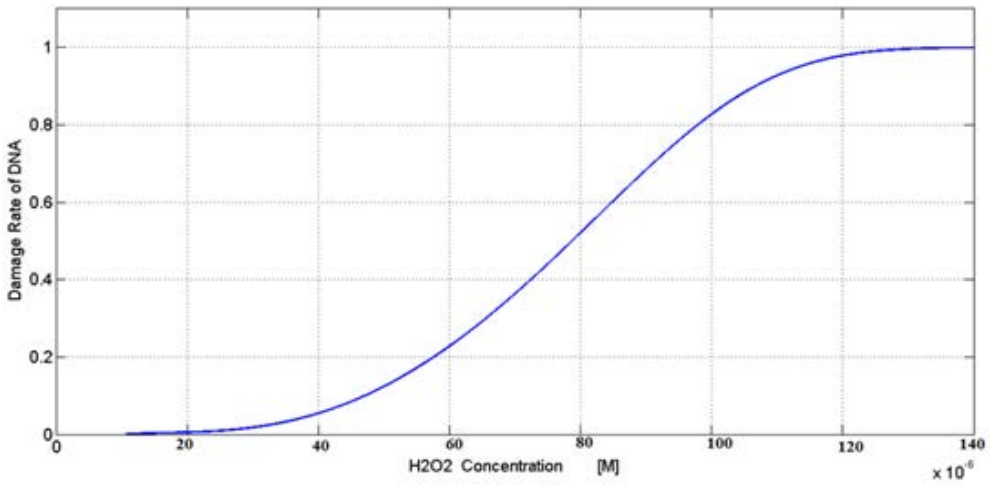

Figure 5: The relation between the DNA damage and the extracellular H_2O_2 concentration that shows an increasing manner which has a threshold with the maximum slope or zero second-differentiating (about $[H_2O_2] = 80$ µM)

The described 'DNA damage model' could be applied to variety of the NTP sources with any magnitude of VUV-UV intensity and relevant atom initial concentrations only by adjusting the model's parameters and initial values. Many studies on optical emission spectroscopy of several source kinds of NTP with different gas (e.g., argon, helium, or air) show VUV-UV and oxygen line in emission spectrum [19-24]. In treatment applications with irradiating plasma directly to the cells, the plasma output components could encounter with two types of targets: the cells, or the water around the cells. Other studies have noticed to the interaction of plasma components (mainly ROS and RNS) with the cells [19-24], whilst in this research we focused on the interaction with extracellular water through modeling. Some researchers activated a little water or mist by plasma irradiation, and then applied such activated product for killing microorganisms or cancerous cells [5-7, 25-26].

We considered in our model the main reaction of OH radical production in bio solution is water photolysis. Some researchers have shown that the VUV component of NTP irradiating to a bio solution has the main role in producing of OH radicals [2]. In this manner, some other researchers reported that by blocking UV/VUV photons, the density of OH radicals is reduced by more than 60% [12, 25]. The production of hydroxyl radicals has high efficient, since at the VUV range both absorption cross-section of water and quantum yield of OH radicals are high [12]. In addition, a little of OH radicals could also be generated by the reaction of the oxygen atom (found in NTP output) with the H_2O molecule accessible at the liquid surface [12, 26]. There is a bit of OH in gas phase solved in water which could be negligible relative to other sources.

Among components of NTP-based products, H_2O_2 has been shown to be main factor triggering the death of cancerous cells via induce DNA damage by endogenous OH through Fenton reaction in mammalian cells [27]. H_2O_2 alone with a relative high concentration or as the mediator of a series of anticancer drugs can selectively induce apoptosis in cancerous cells [28].

In different studies, it was shown that the effective H_2O_2 concentration in killing the cancerous cells depends on the type of cells. In one research, it was shown that H_2O_2 for 50–200 μM inhibits the proliferation of human breast cancer MCF-7 cells [29], but at 1–10 μM it increases the proliferation of hepatoma 7721 cells [30]. Interestingly, the proliferation of HT-29 colon cancer cells is enhanced at 10 μM whereas a higher concentration (about 1000 μM) leads to apoptosis [31]. The H_2O_2 for 50 μM also produced cell cycle arrest in A549 lung cancer cells [32].

Some studies worked on water photolysis and oxygen reactions [33-34] that we used their parameters in Table 1. A practical research showing the extent of DNA damage versus oxidative factors also confirmed our model's findings in fig.5 [35]. Since our model shows the chemical interactions *in vitro* studies, the presence copper atoms could decrease H_2O_2, whilst we did not imagine it because of following causes: The copper concentration is usually relatively low; the most of the copper-mediated hydroxyl radicals (obtained by H_2O_2 oxidation) are formed in the periplasm location, far away from DNA [8].

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Conflict of Interest: The authors have declared that no competing interests exist.

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