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Photocatalytic treatment to inhibit the growth of skin cancerous cells

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Abstract

A photocatalytic technique has been applied to study the inhibition of growth of cancerous cell . A suspension of semiconducting carbon (band gap 1.2 eV) in methanol (2 mg/ml in methanol) on administering to C6- Glioma (5 x 10^6 cells) tumor transplanted in Wistar pups and exposing it with visible light (15 Lux) for 30 min. inhibited the growth of cells while exposure of the cells alone either with light or semiconducting carbon did not show any appreciable inhibition. The mechanism responsible to inhibit of the growth of the cell is discussed. It is proposed that some medical practitioners would adopt this technique to cure skin cancer.

Key words:

Photocatalytictherapy, Carbon nanotube in phototherapy, skin cancer treatment.

1.0 Introduction

Number of patients affected by cancer specially skin cancer is increasing almost every day. There are various treatments developed to cure patients from cancer like chemotherapy, radiotherapy, photo-dynamic-therapy, immunotherapy, gene-therapy etc. Though these treatments are effective but patents undergo serous discomforts due to the side effect of these treatments. Hainfeld et al [1] have shown that injecting cancer cell into mice, followed by a salt solution containing gold nanoparticles and 2 minutes later, irradiating mice with X–ray (250 Kvolts) completely eradicated the tumour. Neither gold nanoparticle nor X-ray had any therapeutic effect on their own. Fujishima et al [2-3] have shown that by phtocatalytic technique using TiO_2 the growth of cancerous T24 cells and HelLa Cells can be inhibited. But TiO_2 being large band gap material (3.37 eV) it requires UV light to excite photogenerated electron/holes. To avoid using UV light one needs to use small band gap semiconductor for this purpose. Unfortunately, most of inorganic small band gap semiconductors are unstable in aqueous media [4].

However, Sharon et al [5] have reported that low band gap carbon semiconductor is stable in aqueous media. Recently, Sharon [6] has shown that when water containing pathogenic bacteria like *ecoli* and carbon nanotube (band gap 1.2 eV) is illuminated with visible light for 30 min, almost 98% of the bacteria are killed. They have suggested that membrane of bacteria being made of organic materials like protein gets damaged by the electron/hole pairs produced at the interface of the semiconductor carbon and aqueous media. Encouraged with these experiments, it was thought to study the similar photocatalytic effect with cancerous cell using carbon nanotubes of band gap 1.2 eV. For this purpose, C6- Glioma tumor was selected.

Though photocatalytic process is a well established concept, but it may be useful to explain the photocatalytic process utilizing semiconducting materials. In general, whenever a semiconductor (for example, n-type) comes in contact with a material (either solid or solution) with a work function (i.e., Fermi level) of different magnitude (i.e. more negative to n-semiconductor) majority carriers (i.e. electrons of n-semiconductor) rush to the interface to balance the two Fermi levels (i.e. of semiconductor and the solution) [7]. Migration of electrons from n-semiconductor creates equal number of holes (i.e. positive charge) within the semiconductor. These two charges are separated by a distance (depletion region) of around few thousands of Å from the interface. This in turn creates a very high electrical field (~ 0.1 MeV/cm). Under this condition, if photons of energy slightly greater than the band gap of the semiconductor enter the depletion region, electrons from the valence band of the semiconductor get excited to conduction band creating a hole in the valence band. These photo-generated carriers are forced to get separated due to the presence of the electrical field present in the depletion region. As a result, hole finds its place at the interface while electron accumulates at the backside of the semiconductor (i.e. the side which is not illuminated). If a particulate n-type semiconductor is used for this purpose, then dark side of the particle would be accumulated with photogenerated electrons and illuminated portion would contain photogenerated holes [8]. These charges are highly reactive and have two alternatives: they would either annihilate or react with materials present around the surface of the particle. Annihilation is difficult due to the presence of high electric field in the depletion region. Alternatively, these photogenerated charges react with either water to produce OH^* or react with any organic materials present in the vicinity of surface. These photoreactions can be explained as follows:

Semiconductor + hv $\rightarrow$ e_{photo} + h^+_{photo} (at the interface of semiconductor and electrolyte)

O_2 + e_{photo} $\rightarrow$ O_2^{-*} (Formation of superoxide radical from dissolved oxygen in water)

H_2O + h^+_{photo} $\rightarrow$ OH^{-*} + H^+ (formation of hydroxy radical)

OH^- + h^+_{photo} $\rightarrow$ OH^{-*} (formation of hydroxy radical)

Thus, due to illumination of semiconductor particles in water, it can produce free radical like $O2^{-*}$ and OH^{-*} These free radicals or $e_{photo} + h^{+}_{photo}$ can react with membranes of cancerous cell in following fashion.

Cancer's cell wall + $h+_{photo}$ _ _CO2 + cell wall residue

Cancer's cell wall + OH^{-*}. _ _CO2 + cell wall residue

Cancer's cell wall + O_2^{-*} _ _ _Oxidised part of the cell's membrane

These hydroxy radical or holes (i.e., positive charge) can oxidize organic materials present in their vicinity [8]. In both the cases the oxidized product [9-12] is CO_2 along with salts of halides, sulfides, phosphides, depending upon the nature of anions present in organic material.

Since, such types of photocatalytic processes have been applied to decompose various types of organic materials like, phenol [13], proteins[14], metals and organic chemicals[15] etc., it would not be wrong to visualize that constituent of the living cell membrane i.e., phospholipids may also get oxidized, resulting into the damage of the cell membrane. Because, all living cells have a 'skin', called the **plasma membrane,** which protects it from the outside environment, regulates the movement of water, nutrients and wastes in and out of the cell. If plasma membrane of the cell or the cell wall is damaged, it is possible to inhibit the growth of the cell. The major constituents of plasma membrane or the cell wall are proteins, phospholipids or in very general term carbonaceous material. Attacking these carbonaceous materials with highly oxidizing agents like hydroxy radicals or photogenerated holes can oxidize them, resulting into the damage of cell membrane [2,6]. Therefore, cell membrane of living organism (which is composed, of complex organic material) is also expected to decompose by the photocatalytic process, resulting into decay of living organism [6].

Fujishima [2] studied the decay of HeLa cell *in vitro* using high band gap TiO_2 for which they had to use UV light. It would be interesting to study this process *in vivo* with a semiconductor of low band gap using visible light. Sharon et al [16-18] has developed a process to synthesize CNT such that its diameter is within 50-100nm and its band gap 1.28 eV. TEM micrograph of CNT developed by them and which is used in the present work as well for killing pathogenic bacteria [6] is shown in figure 1. Since the band gap of this material was found to be in the vicinity of 1.28 eV, visible light can be used for the photoexcitation process. To confirm whether photochemical process can work satisfactorily in vivo, we studied the influence on the growth of C6- Glioma tumor transplanted in Wistar pups using a low band gap semiconducting carbon under visible light illumination.

2.0 Experimental techniques:

C6- Glioma tumor was transplanted in Wistar pups at the Tata Cancer Research Centre, who have the permission for carrying out such type of experiments. Since CNT is not soluble, slurry (2 mg/ml in methanol) was made in small volume of methanol. Two sets of slurry of carbon nanotubes were prepared.

A suspension of 5 x 10^6 cells of C6- Glioma tumor in 0.1 ml. of phosphate buffered-saline, pH 7.4 was injected in the back of newborn Wistar rat pups. The growth of tumors were monitored daily and those, which were non-necrotic, and of a size 1.0 cm. were excised and collected in sterile petri dishes. These were cut into fine pieces with sterile scissors, minced with a minimum volume of sterile phosphate buffer (pH 7.4) solution and then filtered through a sterile wire mesh. Aliquots (0.1 ml) containing 5 x 10^6 cells of C6- Glioma tumor were then subsequently injected into newborn pups. These pups were periodically monitored twice a week for palpable tumors. When the tumor size was 1.5 cm. these animals were subjected for the following studies.

The animals (each set comprising of 6 animals) were randomized into four groups to determine the tumor growth rate after the following treatments: (a) tumour was allowed to grow without applying the slurry of carbon nanotubes and without exposure to visible light) **taken as control**; (b) carbon nanotubes(2 mg/ml in methanol) was intra -tumor administered without exposure by light **taken as CNT control**; (c) another rat infected with the tumor was illuminated with visible light only (15 Lux) for 30 min.; **taken light control** and (d) to fourth set, carbon nanotubes(2 mg/ml in methanol) was administered and exposed to visible light (15 lux) for 30 min; **taken as photochemical therapy (PCT).** On 6^{th} days of these treatments, these animals were anaesthetized by ether and 0.2 ml of ^{99m}Tc-citrate (37.0 MBq) was administered through tail vein. These animals were subjected to scientigraphic imaging studies using gamma camera with low energy all purpose (LEAP) collimator with an energy setting centered at 140 keV and a 20% window. Almost identical behaviour was observed with each rat. A typical x-ray film is shown in Figure 2.

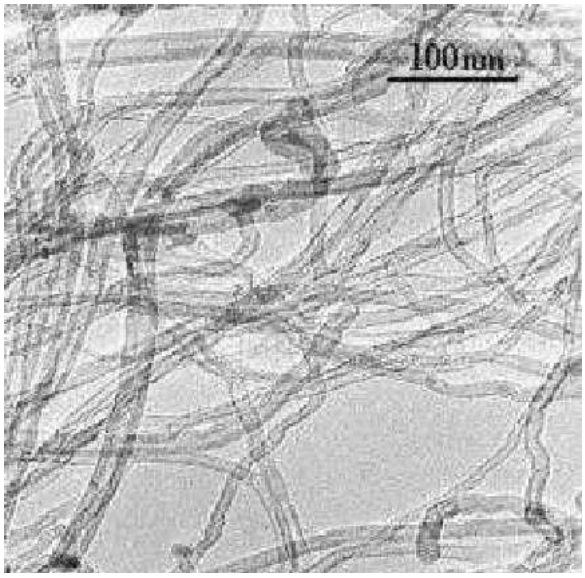

Figure .1 Carbon nanotube obtained from the pyrolysis of turpentine oil and purified [16] by refluxing with HNO_3

3.1 Discussion and Results

Photocatalytic effect of the carbon nanotube was studied in C6- Glioma tumor transplanted in Wistar pups. An avid concentration of ^{99m}Tc-citrate was observed (Figure 2B) in the tumor (i.e., blackened portion, Figure 2B) for treatments as described for groups (a), (b) and (c); while no uptake (negligible darkened portion, Figure 2A) was seen in C6-glioma tumors treated group (d). In other words, it was observed that growth of C6 tumor was inhibited when it was in contact with illuminated carbon nanotube (Figure .2A) and there was no effect of either alone carbon nanotube or alone the light on growth of cell (Figure.2B). From these experiments, it can be concluded that neither CNT nor light had any adverse influence on the growth of the cell. Moreover, for all these four sets of experiments, it can also be concluded that if there was any heating effect due to visible light illumination, the effect of temperature was not enough to inhibit the growth of the cell. Since amount of methanol used was less than 0.5 cc for all four sets of experiments, its effect on the growth was also negligible. However, when CNT was exposed to visible light, the growth of cell was inhibited. This suggested that when CNT was in contact with cell membrane a depletion region was formed at the interface of semi-conducting carbon and the electrolyte (or the media of the cell). When this interface was illuminated photo-generated electron/holes are formed, as one would expect with photocatalytic process. The holes caused the photo-oxidation of the cell membrane causing the inhibition in the growth of the tumour.

It also appears from our result that CNT on its own is not toxic to the cell's growth, because there was no inhibition to the growth when CNT was administered to the cell (set No b). This type of observation has also been reported elsewhere [19]. Macrophage-like cells were incubated in a growth medium containing single-walled carbon nanotubes in a surfactant. The nanotubes had an average diameter of 1 nm and an average length of 1 micron. The cells showed the same population growth as cell cultures containing surfactant but no nanotubes, and normal adhesion, morphology and confluence. That indicates that the nanotubes didn't adversely affect the cells

Though we have confirmed the inhibition of the growth of cancerous cell (in vivo) but there are still many questions to be addressed to, like which part of the organ of the cell really gets affected by the photo-generated holes and what are the products formed after the degradation. In order to get the insight of this process, experiments are under examination. Moreover, this process can be useful only for skin cancer, by illuminating only affected part by the help of optical fiber. This way normal cell will not be exposed to light.

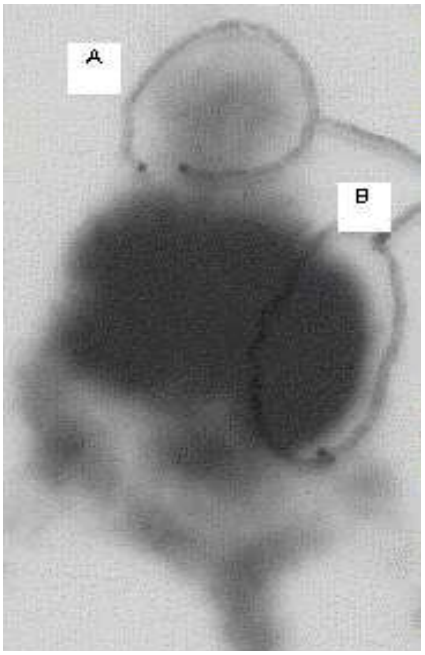

Figure .2 Scintigraphy imaging using ^{99m}Tc-citrate of C6- Glioma tumor (A and B) grown on Wistar pup. Portion (B) shows the normal growth of cancer while portion (A) shows inhibited growth of cancer cell.

Patient suffering from skin cancer like shown in figure 3, can be administered with slurry of CNT in methanol and then exposed to visible light by an optical fiber for some definite period, it is hoped that the cancerous cell would die leaving behind the regular growth of normal cell. It is hoped that some medical organization will adopt this technique to confirm its application to cure the patents from the skin cancer.

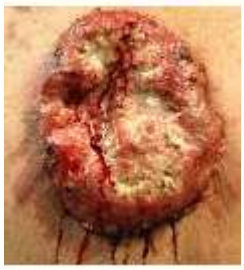

Figure 3 A typical growth of skin cancer which can be treated externally with CNT and light.

4. Conclusion

It is concluded that photocatalytic therapy can be used to stop the growth of malignant tumor. Since the growth of Glioma tumor was not affected when only CNT was administered, suggests that CNT alone is not toxic to the cell. CNT behaving like a semiconductor can be used for developing photocatalytic therapy for cancer treatment.

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