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# Antimicrobial photodynamic therapy: Single-walled carbon nanotube (SWCNT)-Porphyrin conjugate for visible light mediated inactivation of *Staphylococcus aureus*

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ABSTRACT

Due to the excessive use of antibiotics over the years, the microorganisms have developed resistance to numerous drugs. The growth of multi-resistant organisms (MROs) heads due to the insufficient treatment with the currently available medications which present a great threat to the biotic component of the environment as well as to the food technology sectors. The goal of this research was to develop a nano-composite made up of single-walled carbon nanotubes (SWCNTs) and amine-functionalized porphyrin, which could further be used for the anti-microbial studies in presence of visible light showing photodynamic effect to inactivate cells. Photodynamic antimicrobial chemotherapy is gaining significant interest due to its capabilities as an innovative form of antimicrobial treatment. The development of anti-microbial photodynamic therapy (a-PDT) is a non-antibiotic access to inactivate microorganisms. We examined the synthesis of amine-functionalized porphyrin and conjugated it to the oxidised single-walled carbon nanotubes (SWCNTs). By the use of appropriate amount of single-walled carbon nanotubes (SWCNTs), we have shown the interaction between the porphyrin conjugated nanotubes and the bacterial cells in presence of visible light led to the cell membrane damage, concluding that SWCNT-porphyrin conjugates can be used as an antibacterial agent. The characterization of the oxidised SWCNT and SWCNT-porphyrin conjugates was determined by field emission scanning electron microscopy (FE-SEM), which provides detailed information about the composition and the morphological analysis. The particle size measurements were carried out by transmission electron microscopy (TEM). On investigating under the florescence microscopy, red fluorescence was observed. Thus, these properties demand us to design this facile material comprised of SWCNT-aminoporphyrin conjugates that shows potent antibacterial activity.


## 1. Introduction

Antimicrobial photodynamic therapy (a-PDT) is a newly synthesized therapeutic substitute which incorporates the use of photosensitive molecules like porphyrins, chlorophyll and dyes combined with visible radiation to damage the microbial agents by oxidation. Due to the immense and exploitative use of antibiotics, some of the micro-organisms have began to develop resistance against the antimicrobial agents. This limitation has encouraged the researchers to develop new antimicrobial agents which can inhibit microbial activity. Among the bacterial species which normally dwell on human skin is the *Staphylococcus aureus* which are responsible for many deep skin infections like ecthyma, impetigo, etc [1]. *S. aureus* is present 30% in the nose of healthy adults and 20% in the skin. These bacteria can infect healthy person by spreading rapidly when they come in direct contact with an unhealthy person or by touching a contaminated object. *S. aureus* can spread through the bloodstream and infect distant organs. Infection caused by the bacteria is treated either by antibiotics or by the surgical removal of the skin of the infected area. Antibiotic are chosen based on whether they are effective against the strain causing infection. Thus, there is an urgent need for the development of effective

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antimicrobial agents. As newly synthesized carbonaceous materials, carbon nanotubes (CNTs) acquire some exclusive properties which distinguish it from the other conventional materials, such as the unique and distinct tubular arrangement of nanotube, the modifiable surface, tensile strength and stability [2]. The emergence of the nanomaterials has been incorporated in the optical devices, superconductors, fuel cells, catalysts and biosensors. According to some recent reports, the carbon-encapsulated materials which include carbon nanotubes, fullerenes, graphene oxide, etc. have been known to show some antimicrobial properties [3]. Thus, the antiseptic behaviour of the carbon materials can be employed for antimicrobial properties. Carbon nanotubes are made by folding a sheet of graphene into a folded structure. The length of the carbon nanotubes are directly correlated with their different properties. The shorter carbon nanotubes are more reactive and less flexible, whereas the longer carbon nanotubes are less reactive and more flexible. The incomparable physical, chemical and electronic properties of carbon nanotubes (CNTs) have aroused much interest in their potential applications. Carbon nanotubes have attractive magnetic, optical and thermal properties and have a plethora of other features which have applications in various fields. The single-walled carbon nanotubes (SWCNTs) are known for their properties as the building blocks in the manufacture of nanoscale electronic devices and for drug delivery in biomedical applications. For these applications, there must be a suitable enhancement of surface of the carbon nanotubes as they are insoluble in water. Owing to their neutral nature, they do not disperse well in organic solvents in their pristine state. Among the various surface modification techniques, the non-covalent surface modification is commonly known for maintaining the original properties. The carbon nanotubes exhibit their anti-bacterial activity due to their exclusive high surface/volume ratio, thereby decreasing the size of the nanotubes lead to improving their activity for interaction with bacteria [4]. Kang et al. determined that the SWCNTs were more toxic to the bacterial cells as compared to the MWCNTs, concluding that the diameter of the nanotubes played a major role in the antimicrobial activity. The same group remarked that the MWCNTs were more toxic when they are uncapped and dispersed in solutions. The interactions between the CNTs and the bacterial cells play an important role in the cell membrane perturbation, which eventually leads to the cell death.

The combined potential of materials science and nanotechnology marching towards medicinal applications has aroused interest in overcoming various aspects of microbial infections [5–9]. Currently, photodynamic therapy is gaining much interest for its potential as anti-microbial agents. Photosensitizers such as porphyrins and pthalocynanines have been known to be effective against *in vitro* viruses, parasites, bacteria and yeasts [10]. Many studies carried out on the porphyrin macrocycles have demonstrated the great potentialities of this type of compound to be used as an antimicrobial agents. Porphyrins are platelike aromatic macrocycles and exists in life in the form of heme in haemoglobin, myoglobin, cytochromes etc [11]. The monometallic complexes of porphyrins are implicated in numerous key processes of life, such as metabolism and detoxification [12–14]. Presently, many new classes of porphyrins have been developed for biomedical applications and based upon their physicochemical properties, porphyrins are being used in a wide range of applications including artificial photosynthesis, oxidative catalysis, sensors, non-linear optics and development of nanomaterials for the treatment of cancer by photodynamic therapy and more recently it has been used for the photo inactivation of microorganisms [15]. The antimicrobial photodynamic therapy (a-PDT) can be used as an alternative potential to the conventional antibiotics used [16–20]. Antimicrobial photodynamic therapy functions by combining a non-toxic photosensitizer (PS), with visible light irradiation which in turn generates singlet oxygen and free radicals, which are known for their ability to kill the microbial cells [21,22]. Many different arrangement of photosensitizers exhibit an uneven degree of biocidal effectiveness depending upon the composition of their cell wall for instance the Gram negative bacteria is more opposed to photoinactivation in contrast to Gram positive bacteria [23]. In most of the microorganisms, for instance in bacteria, the major aims of a-PDT are the peripheral composition of the cells. The photosensitizer (PS) does not necessarily enter the interior of the microorganism; proper adhesion and specificity towards the site of attachment is sufficient for its light-mediated damage. The PS can be effective against bacteria only if it is co-administered while disrupting the outer membrane [24,25]. Therefore, the target microorganisms are unable to oppose the damage caused by fixing uptake, enhancing metabolic intervention or by altering the delivery of the drug [26].

## 2. Materials and Methods

### 2.1. Chemicals, bacterial strains and growth media

SWCNTs (Tuball) (purity ≥ 75%) were obtained as a gift from OCSiAl, USA. Sulphuric acid and N,N/-Dicyclohexylcarbodiimide (DCC) were acquired from HiMedia, India and incorporated as received. The different solvents exploited in this work were of analytical grade. The chemicals like Dimethylformamide (DMF) and Nitric acid were purchased from Rankem and S. D. Fine-Chem Ltd., India, respectively and employed in our work. All the other chemicals were bought from commercial sources and used. *S. aureus* (MTCC 737) was received from IMTECH, India. Deionized (DI) water was used in the preparation of all the samples. Bacterial growth media, such as nutrient broth (NB), Luria-Bertani broth (LB) and Agar-agar were obtained from Merck, Germany and HiMedia, India, respectively. The oxidative treatment of the single-walled carbon nanotubes (SWCNTs) was carried out on digital ultrasonic cleaner from Labman Scientific Industries.

### 2.2. Preparation of acid-Functionalized SWCNTs

In a typical procedure, 100 mg of the crude SWCNTs was supplemented to the solution of concentrated sulphuric acid and nitric acid in the ratio of 3:1 and bath sonicated for 6 h until a dispersed solution was obtained. The nanotubes suspension was diluted with DI water and was filtered through G4 crucible. This was followed by several washing with DI water. Finally the solution was rinsed with ethanol. The product was kept in a vacuum oven for overnight to get a form of dry powder which can be used for reactions.

### 2.3. Synthesis of 5,10,15-triphenyl-20-(4-aminophenyl)porphyrin ($H_2$TriMAPP)

$H_2$TriMAPP was synthesized from $H_2$TPMNPP (5,10,15-triphenyl-20-(4-nitrophenyl) porphyrin). $H_2$TPMNPP was synthesized by taking 180 mL of propionic acid in flask and warmed to 100° C. Then benzaldehyde (0.0324 mol) and *p*-nitrobenzaldehyde (0.0108 mol) and after 10 min, Pyrrole (3 mL, 0.0432 mol) was added and refluxed for 90 min. Towards the completion of the reaction, the solution was cooled to RT and kept in deep freezer overnight. Propionic acid was passed through the silica crucible and the product was washed several times with methanol to remove the traces of propionic acid. TLC was taken in which 3 bands were found, first band is *meso*-tetraphenylporphyrin and the second band is $H_2$TriPhMNPP. The obtained product was chromatographed using silica column in pure chloroform and dried under vacuum. $H_2$TriPMNPP (0.4gm) was dissolved in RB which contain 40 mL HCl and then purged under Argon for 1 h by keeping in ice. To this, $SnCl_2$ $2H_2O$ (16 eq.) in 20 mL HCl was added