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RECENT MODIFICATIONS OF CARBON NANOTUBES FOR BIOMEDICAL APPLICATIONS

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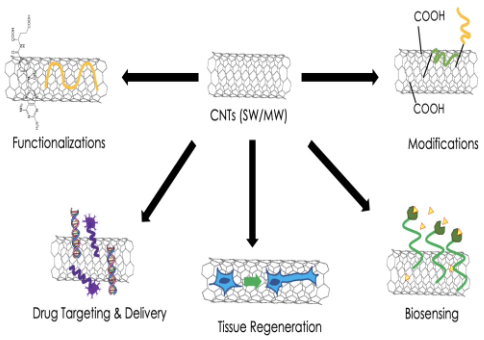
Graphical abstract



Abstract

Recent advances in the biomedical field have been remarkably achieved in the last few years, especially in the fabrication of nanomaterials that have various applications. Carbon nanotubes (CNTs) are carbon-based materials with cylindrical shapes that have an average diameter of less than 2 nanometre (nm) for single-walled CNTs (SWCNTs) and an average diameter up to 100 nm for multi-walled CNTs (MWCNTs). CNTs demonstrate unique chemical, physical and electronic properties, and these remarkable properties have led to the development of CNTs-based materials in the biomedical field. For the past decades, the functionalization of CNTs has been actively developed to increase their biocompatibility for application in antibacterial materials, dentistry, drug delivery, and biosensing. The surface functionalization enhances the capabilities, features, and properties by modifying the surface chemistry of CNTs to improve their biocompatibility. The functionalization of CNTs will enable the biomolecule loading on the surface of CNTs, and thus can be used for drug delivery for targeted cells or immobilization support. This review discusses the related literature on biomedical applications of CNTs such as antibacterial, dental materials, cancer therapy and biosensors from 2007 – 2022. We also review the antibacterial properties between SWCNTs and MWCNTs, functionalized CNTs-reinforced nanocomposite for dental applications, and the ability of CNTs to work as nanocarriers to deliver drugs directly to cancer cells. Moreover, the applications of CNTs-based biosensors in detecting biological and biomedical compounds are also discussed. This review is expected to provide guidelines for developing CNTs-based materials in the applications of biomedical field.

Keywords: Antibacterial, biosensors, CNTs, dentistry, drug delivery, functionalization

Abstrak

Kemajuan terkini dalam bidang bioperubatan telah dicapai dengan ketara dalam beberapa tahun kebelakangan ini, terutamanya dalam fabrikasi bahan nano yang mempunyai pelbagai aplikasi. Tiub nano karbon (CNTs) adalah bahan berasaskan karbon dengan bentuk silinder yang mempunyai purata diameter kurang dari 1 nanometer (nm) bagi dinding tunggal (SWCNTs) dan purata diameter sehinga 100 nm bagi berbilang dinding

(MWCNTs). CNTs menunjukkan sifat kimia, fizikal dan elektronik yang unik, dan sifat yang menakjubkan ini telah membawa kepada pembangunan bahan berasaskan CNTs dalam bidang bioperubatan. Sejak beberapa dekad lalu, pemfungsian CNTs telah dibangunkan secara aktif untuk meningkatkan biokeserasian mereka untuk aplikasi dalam bahan antibakteria, pergigian, penghantaran ubat, dan biosensor. Pemfungsian permukaan meningkatkan keupayaan, ciri, dan sifat dengan mengubah suai kimia permukaan CNTs untuk meningkatkan biokeserasian. Pemfungsian CNTs akan membolehkan pemuatan biomolekul pada permukaan CNTs, dan dengan itu boleh digunakan untuk penghantaran ubat untuk sel sasaran atau sokongan mobilisasi. Ulasan ini membincangkan literatur berkaitan tentang aplikasi CNT pada bioperubatan seperti antibakteria, bahan pergigian, terapi kanser dan biosensor dari 2007 – 2022. Kami juga mengulas sifat antibakteria antara SWCNTs dan MWCNTs, nanokomposit diperkukuh pemfungsian CNT bagi aplikasi pergigian, dan keupayaan CNT berfungsi sebagai pembawa nano untuk menghantar ubat secara terus ke sel kanser. Tambahan lagi, aplikasi biosensor berasaskan-CNT dalam mengesan sebatian biologi dan bioperubatan juga dibincangkan. Ulasan ini dijangka menyediakan garis panduan untuk membangunkan bahan berasaskan CNT dalam aplikasi bioperubatan.

Kata kunci: Antibakteria, biosensor, CNTs, pergigian, penghantaran ubat, pemfungsian

1.0 INTRODUCTION

For the past decades, the application of nanomaterials in various biomedical fields has drawn considerable attention. The ongoing research and development in the fabrication of biomaterials on the nanoscale have progressed to the production of medical components such as bioartificial organs, prosthetic devices, and dental implants. The relatively small sizes (<100 nm) of nanomaterials and large specific surface area have attracted scientists and researchers' interest in the application in the biomedical field [1 – 5]. Among the significant application of nanomaterials in the biomedical field is an antibacterial agent [6 – 8]. For example, it was reported that increased morbidity and mortality in the cause of drug-resistant is greatly linked to systemic infections related to multidrug-resistant (MDR) [9]. Based on the data and information from the National Surveillance of Antibiotic Resistance (NSAR) conducted in more than 40 hospitals in Malaysia, antibiotic resistance to bacteria such as *Acinetobacter baumanii, Klebsiella pneumoniae, Escherichia coli and Enterococcus faecium* have increased significantly in 2015 (Malaysian Action Plan in Antimicrobial Resistance MyAP-AMR) [10]. This study showed that the problem has increased the risk of multi-drug resistance for thousands of people in Malaysia. Therefore, these serious health problems have raised the concern to the health authority to govern the utilization of antibiotics wisely.

Another example of the emerging use of nanomaterials in the biomedical field is the use of nanomaterials as targeted drug delivery systems [11 – 13]. The small size and large surface area of nanomaterials have increased the solubility and improved the bioavailability, as well as the ability to move across the blood-brain barrier [2 – 4, 14]. One of the preferred nanomaterial in the application of biomedical is the carbon nanotubes (CNTs), which has attracted worldwide researchers due to their fascinating and unique physical and chemical properties [15, 16]. In general, CNTs are cylinder-shaped molecules that are made of rolled-up sheets of graphene. CNTs can be divided into single-wall carbon nanotubes (SWCNTs) and multiwall carbon nanotubes (MWCNTs). The diameter of SWCNTs is typically less than 2 nanometre (nm) while the diameter of MWCNTs can reach up to 100 nm (Figure 1(A)) [2, 5, 7, 8, 15, 16]. In other cases, the length or the diameter of the modified MWCNTs could reach up to micrometre or even millimetre, depending on the modification technique [2, 5, 13, 16, 17]. CNTs can be synthesized via three methods such as combustion chemical vapour deposition method (CCVD), laser ablation technique and arc discharge method [1, 2, 5, 15 – 17]. Among these techniques, CCVD is one of the most widely used owing to its several advantages such as economical, controllable synthesis and high-yield production. By controlling the parameters of synthesis, the desired properties of CNTs such as orientation and diameter can be prepared accordingly based on the specific applications [4]. Besides antibacterial and drug delivery systems, the CNTs have been also used in numerous biomedical fields including biosensors, cancer therapy, and tissue engineering (Figure 1(B)) [1, 2, 5, 12 – 15]. In most applications of CNTs material, functionalization is carried out in order to reduce absorption and desorption process [2, 5, 7, 15, 16]. In particular, absorption process can cause the attachment of highly branched molecules to the walls be it single or multiwall of CNTs [18].

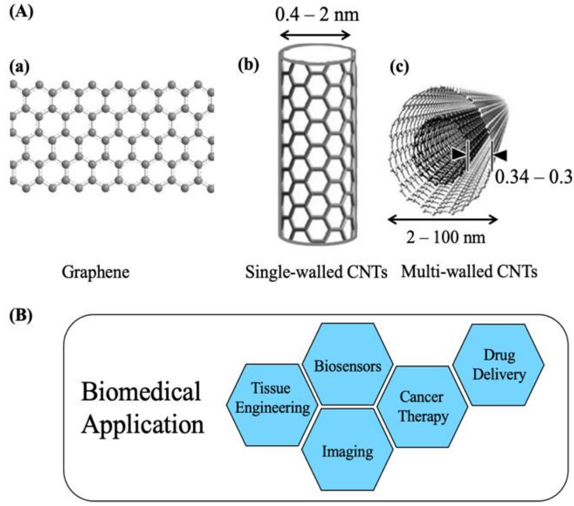


Figure 1 (A) (a) Structure of graphene, (b) structure of single wall carbon nanotubes (SWCNTs) and (c) multiwall carbon nanotubes (MWCNTs). (B) Examples of the applications of CNTs in Biomedical Fields. Figure 1(A) was reproduced (adapted) from an open access article [16] published by MDPI under a Creative Commons Attribution license (CC BY)

The functionalization of CNTs can be performed by using bioactive elements such as drugs, nucleic acid, peptides, or protein [19]. Figure 2 shows the several successful functionalization methods of CNTs and their associated functional groups that have been reported in the literature [20]. In addition, the functionalization of CNTs is divided into three categories as shown in Figure 3 [15]. Covalent functionalization of CNTs is performed by attaching the chemical moieties to the tubular structure via covalent bonding [15, 16, 21]. It has direct and indirect methods which direct method corresponds to a switch in the hybridization from sp^2 to sp^3. The indirect method is referred to chemical changes of carboxylic groups at the sidewalls of CNTs [22]. On the contrary, the non-covalent functionalization of CNTs offers various advantages for biomolecule immobilization on CNTs as it helps to maintain the intrinsic properties of CNTs upon functionalized. For example, this technique could retain the structure of the immobilized biomolecules after being added to CNTs. Compared to the covalent method, it could alter the CNTs' sp^2 structure and thus, affecting their mechanical properties [15]. Non-covalent functionalization typically uses surfactant of the polymer due to the interaction of the hydrophobic and adsorbed molecules with nanotubes sidewalls through Van der Waals force, π–π interaction and CH-π interaction [15, 16, 21]. While the endohedral filling is conducted to fill the inner part of the CNTs, best for application in the drug delivery system. For example, the CNTs can be filled with anticancer drugs [23]. In addition, the functionalization of CNTs is carried out to decrease the hydrophobic properties of CNTs via modification with protein or polymer materials. The enhanced hydrophilicity of CNTs is highly recommended, especially for the purpose of application in the biomedical field.

There are numerous reports in the literature reporting the successful applications of CNTs in biomedical fields such as in multidrug-resistant bacterial infections [25], drug delivery systems [12] anticancer materials [26], and biosensor [27]. In this article, an overview of different applications of CNTs such as antibacterial agents and bone-grafting/scaffolding in dentistry is discussed. It also briefly discusses the applications related to cancer and drug delivery. In addition, the application as biosensors and related to the electrochemical sensors are also being discussed.

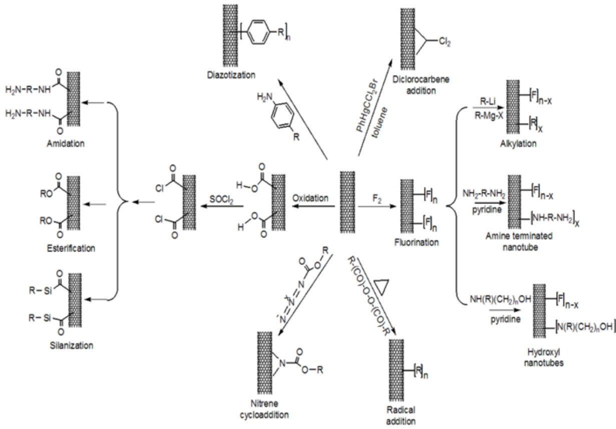


Figure 2 Functionalization of CNTs and associated functional group. This figure was reproduced from an open access article [20] published by MDPI under a Creative Commons Attribution license (CC BY)

Specifically, recent modifications with metals and metal oxides as well as biopolymers to increase toxicity and antibacterial effect on MWCNTs are detailed and the effect of different types of bacteria is also briefly discussed. Meanwhile, the addition of CNTs-based material to improve the flexural and mechanical strength and biofunctionalization of CNTs to improve their biocompatibility are also briefly detailed. Similarly, the biofunctionalization of CNTs is also briefly explained in the application of drug delivery and gene therapy for the transportation of drugs to targeted cancer cells. The use of biocompatible materials for immobilisation onto the surface of CNTs for a specific function in biosensing is also discussed.

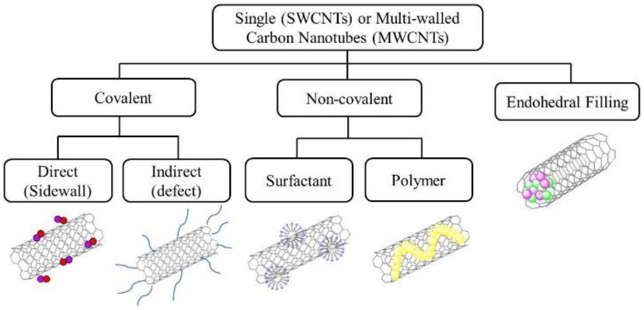


Figure 3 Methods of functionalization of CNTs

2.0 CARBON NANOTUBES FOR BIOMEDICAL APPLICATIONS

2.1 Application of CNTs as Antibacterial Agents

The first work on the antimicrobial activity of CNTs was demonstrated by Kang *et al.* in which they utilized the SWCNTs as an anti-bacterial agent in the treatment of *E. coli*. The cell membrane of respective bacteria was damaged due to the direct contact with SWCNTs, and the process was a mechanism leading to bacterial cell death [8].

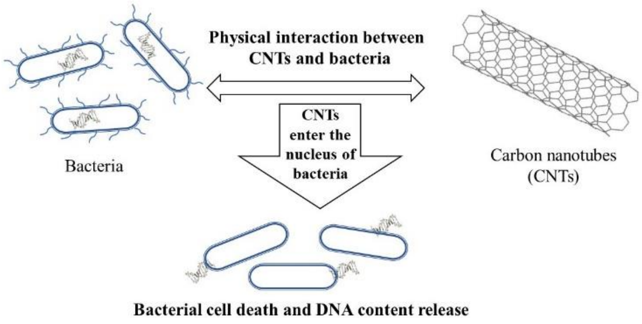


Figure 4 General representation of antibacterial activity by CNTs

As shown in Figure 4, the CNTs (SW/MW) enter the nucleus of bacteria and interact with the DNA, thus leading to the production of reactive oxygen species (ROS) which cause cell damage [2]. In addition, the induction of oxidative stress results in cell membrane damage, thus destroying the bacteria. Since then, the research work on the CNTs-based materials as antibacterial agents has been widely developed. There is a variety of methods reported in the literature on how CNT materials can be modified in order to enhance their antibacterial properties (Table 1).

For example, the modification of CNTs with metal and metal oxide nanoparticles has attracted particular attention for antibacterial treatment using photocatalytic disinfection methods. As zinc oxide nanoparticles (ZnO NPs) showed a remarkable effect on antibacterial properties [28], many research works have demonstrated the high antibacterial properties resulting from the combination of metal/metal oxides and CNTs materials. For example, in 2018, Al-Anbari worked on the modification of the MWCNTs with ZnO NPs to improve the antibacterial properties. They showed that the composite has a powerful bactericidal effect on Escherichia Coli (*E. Coli*) as the combination of ZnO NPs and MWCNTs altered their toxicity and thus, enhanced the antibacterial properties of nanocomposite [29]. However, studies by Ribut *et al.* revealed that ZnO NPs had their morphological changes when the pH of synthesis varied. They found that the ZnO NPs prepared at higher pH (11.0) provide effective antibacterial properties toward *E. Coli* [30].

On the other hand, another group (Rafique *et al.*) performed a modification of the CNTs with ZnO NPs to improve the antibacterial properties with variation in the pH of synthesis. They demonstrated that the synthesized MWCNT/ZnO composite has improved antibacterial properties against Staphylococcus aureus as the zone of inhibition test showed a maximum of 20 mm circular area. In addition, the studies also demonstrated that the antibacterial activity works best when synthesized at pH 10.0 compared to pH 6 and 7 [31]. In another approach, Mohammed *et al.* decorated the CNTs with metal-doped ZnO materials. They reported that the synergistic effect of the synthesized metal-doped ZnO NPs (Au and Ag) and CNTs have played an important role to inhibit the growth of the bacteria due to the formation of ROS that penetrates the cellular toxicity [32]. Also, the enhancement was owing to the correlative effect of the high surface area and strong adsorption capacity of the CNTs as well as the high antibacterial performance of the metal-doped ZnO NPs.

Besides ZnO semiconductor, another favoured semiconductor, titanium dioxide (TiO_2) is also widely applied in the modification with CNTs to apply as an antibacterial agent. Various research in the literature has reported the successful application of CNTs and TiO_2 as an antibacterial agent. TiO_2 has been a favoured choice owing to its unique properties and advantages such as non-toxic, low-cost, chemically and biologically inert, photostable and highly reactive. For example, Shimizu *et al.* reported the successful photocatalytic disinfection using CNTs-TiO_2 and they found that hydroxyl radicals play a major factor in the enhancement of the photocatalytic disinfection [33]. However, other experimental parameters (initial bacteria concentration and Scavengers) need to be explored in order to understand which parameters influence the photocatalytic disinfection properties.

Table 1 Recent works on antibacterial activity of CNTs-based material

No.	CNTs	Modifier	Bacterial	Principles and Findings	Ref
1	MWCNTs	ZnO NPs	*Escherichia Coli*	High antibacterial activity using MWCNTs-ZnO NPs via Photocatalytic Disinfection	[29]
2	MWCNTs	ZnO NPs	*Escherichia Coli*	Effect of pH on the morphology of ZnO NPs toward antibacterial activity	[30]
3	MWCNTs	ZnO NPs	*Staphyococcus aureus*	High antibacterial activity on ZnO NPs – MWCNTs prepared at the high pH (11.0)	[31]
4	SWCNTs/ MWCNTs	ZnO NPs – Au/Ag	*Escherichia Coli*	Synergistic effect of Au/Ag-ZnO-MWCNTs towards high antibacterial activity.	[32]
5	MWCNTs	TiO_2	*Escherichia Coli*	High antibacterial activity towards bacterial E.coli contributed by hydroxyl radicals	[33]
6	MWCNTs	TiO_2	*Escherichia Coli*	Effect of different scavengers towards disinfection of *E. Coli*.	[34]
7	MWCNTs	N^+ doped TiO_2	*Escherichia Coli*	Effect of lattice changes by doping N^+ to TiO_2-MWCNTs towards high antibacterial activity.	[35]
8	MWCNTs	Polymer-carboxylate-Ag	*Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Bacillus subtilis.*	Functionalization of MWCNTs with polymer to reduce agglomeration and increase stability of metal.	[38]
9	MWCNTs	cellulose acetate (CA) matrix - Ag	*Escherichia coli and Staphyococcus aureus*	Functionalization of MWCNTs with cellulose acetate (CA) and silver metal.	[39]
10	MWCNTs/ SWCNTs	Chitosan-Ag-Cu	*Escherichia coli and Staphyococcus aureus*	High antibacterial activity due to modification of CNTs with chitosan and metals	[41]
11	MWCNTs	Chitosan-Polymer	*Escherichia coli and Staphyococcus aureus*	High antibacterial activity due to modification of CNTs with chitosan and polymer	[42]
12	MWCNTs	Ag	*Staphylococcus aureus, Bacillus subtilis, Bordetella pertussis, Escherichia coli*	Antibacterial effect of Ag-MWCNTs towards gram positive and negative bacteria and fungi	[43]
13	SWCNTs/ MWCNTs	-	*Escherichia Coli*	Effect of single and multiwall of CNTs towards antibacterial properties	[44]
14	MWCNTs	-	*Vibrio parahaemolyticus*	Effect of functionalized and unmodified MWCNTs on antibacterial activities	[7]
15	MWCNTs/ SWCNTs	-	*Escherichia coli, Pichia pastoris, Saccharomyces*	Effect of aggregation on SWCNTs and MWCNTs on antibacterial activities	[49]

On the other hand, Mohamed *et al.* study the effect-of different scavengers on the disinfection efficiency of *E. Coli*. They found out that in photocatalytic disinfection, hydroxyl radicals and superoxide radicals played an important role in the high bacterial degradation. In addition, they revealed that bacterial degradation was high when a concentration of 100 mg/L was used while the degradation efficiency was reduced when a high concentration of bacteria was used [34]. In another approach, as visible light active materials have become particular attention in the past decade, modification of CNT-TiO_2 material towards visible light spectrum has become the main focus. In the research performed by Li *et al.* they doped the nitrogen ions (N^+) into TiO_2 lattice in the CNTs-TiO_2 composites to shift the TiO_2 absorption into the visible light region. Their visible light-induced materials showed that the photocatalytic degradation efficiency and antibacterial activity of CNT-TiO_2 composites were enhanced owing to better absorption in the visible light region by TiO_2 and the high absorption properties of CNTs materials [35].

However, there are several drawbacks presence that affects photocatalytic disinfection applications. For example, the agglomeration and instability of metal nanoparticle/metal oxides in CNTs composites have limited the applications [36]. In addition, the insufficient functional groups required for chemical interaction with silver cations (Ag^+) require CNTs to be modified with a chemical process in order to produce both hydroxyl (OH^-) and carboxyl groups (COOH) to confirm the sufficient adsorption of Ag^+ ions on the surface of CNTs [37]. For example, Lakourj *et al.* synthesized the nanocomposite of polypyrrole-co-polyindole doped with carboxylated CNT and showed that the composite was having good antibacterial properties with antioxidant activity up to 95% [38]. Similarly, Jatoi *et al.* prepared a composite of silver-CNTs (Ag-CNTs) and the Ag-CNTs were embedded inside the matrix of cellulose acetate (CA). The antibacterial assay ($0D_{590nm}$) of the synthesized nanocomposite demonstrated effective antibacterial properties up until 48 hours. The prepared CA/CNT/Ag3 sample demonstrated 100% bactericidal performance (0% viable cells) [39].

In the past decades, chitosan has been considered a promising polymeric and bio-based material and a prospective material for disinfection owing to being cost-effective and cheap. Moreover, chitosan alszo exists naturally as an antibacterial and its derivatives show good antibacterial properties against other microorganisms. Furthermore, chitosan is also shown to have low toxicological properties when exposed to the environment [40]. The modification of CNTs with chitosan and metal nanoparticles (Au and Cu) by Morsi *et al.* to treat two bacteria found in human faecal such as *E. Coli* and *Staphylococcus aureus* has demonstrated an excellent antibacterial activity. They found that 1% of the concentration of the nanocomposite was highly effective in destroying the microbes within 10 minutes of contact time, the log reduction value of *E. Coli* was found to be 1.59 and 1.87 for *Staphylococcus aureus*. The log reduction value represents the relative number of living microbes that are degraded by the disinfection process [41]. Another reported work on the modification of CNTs with chitosan and polymer material also demonstrates promising results as a large zone of inhibition was observed for both antibacterial evaluation towards *Staphylococcus aureus* and *E. Coli* [42].

The different types of bacteria are also reported to have influenced the antibacterial properties of CNTs. Hamouda *et al.* examined the effect of antimicrobial properties of Ag-MWCTs on both gram-negative and positive such as *Bordetella pertussis* and *Escherichia coli* (gram negative) *and Staphylococcus aureus* and *Bacillus subtilis* (gram-positive) as well as fungal (*Candida albicans* and *Aspergillus niger*). The antibacterial test showed that MWCNTs with moderate Ag loading (6%) led to a large inhabitation zone on *E. coli*. However, the high loading of Ag (24%) on MWCNTs shows no significant effect on the inhibition zone of microorganisms. In contrast, the large inhibition zone (17 mm) was obtained when gram-positive bacteria were evaluated using MWCNTs with 12% of Ag loading. But when Ag content was increased to 24%, no significant changes were observed with respect to the inhibition zone. On the other hand, MWCNTs showed different antibacterial properties toward fungal strains. Only a slight increase in antibacterial activity was observed when Ag was added to the MWCNTs. The size of the inhibition zone was observed to be increased from 11 to 15.9 mm, but no significant changes were observed when MWCNTs were applied to *A niger*. An inhibition zone at about 16.1 mm was observed when low loading of Ag (3%) was added to the respective MWCNTs. Moreover, increasing the Ag showed no significant changes as the inhibition zone remains at the average size of 16-18 mm [43].

On the other hand, it was reported that single and multiwall CNTs have a different effect on antibacterial properties. A study by Mohammed *et al.* revealed that single-walled CNTs of both before and after treatment with acid showed excellent antibacterial properties towards *E. Colis* compared to MWCNTs. They found out that the length of CNTs highly influences the interactions with the membrane cell of bacteria while tubes with short lengths tend to self-aggregate without interacting with bacteria. Meanwhile, the CNTs with long tubes agglomerate effectively as most of the cells were largely affected [44]. Similarly, Alavi *et al.* also reported that SWCNTs have more antibacterial properties compared to that of MWCNTs. They also suggest that the aggregation of MWCNTs in the solution has lowered its antibacterial properties [7]. Previous findings by Yang *et al.* revealed that the size of CNTs plays an important role in providing a critical effect on the microorganism. Moreover, as the size of CNTs decreased, their surface area increased. Thus, providing more reaction sites for cells to interact and uptake [45]. Since dispersed MWCNTs had moderate antibacterial activity, modification, and functionalization of MWCNTs have been conducted to improve their antibacterial activity. In particular, Ding *et al.* fabricated functionalized MWCNTs (f-MWCNTs) with carboxylate and hydroxyl. The antibacterial activity towards bacterial pathogen Vibrio parahaemolyticus demonstrated that individually dispersed f-MWCNTs showed higher antibacterial activity compared to raw MWCNTs aggregates. Their studies also suggest that the dispersity of MWCNTs plays an important role in the antibacterial effect of CNTs [46].

Previously, Liu *et al.* showed that modification of MWCNTs with a high concentration of CTAB could deactivate the cells and lead to high antibacterial properties compared to SWCNTs [47]. It was also reported that MWCNTs are much more toxic when unbundled and highly dispersed in solution [48]. The π–π interactions and strong Van der Waals forces made CNTs bundled together in any solutions [15]. On the other hand, the partially unbundled MWCNTs with a diameter of *ca* 4 nm showed high antibacterial activity compared to MWCNT bundles with an average diameter of about 77 nm [49]. Contrasted to SWCNTs, the sizes are smaller with a diameter of around 1 – 2 nm, and this is the reason why aggregation in SWCNTs influence the antibacterial properties [49]. Since SWCNTs are difficult to de-bundle, SWCNTs aggregates that are suspended or deposited are significantly preferred compared to individually dispersed [50].

2.2 Applications of CNTs in Dentistry

Due to the fascinating and unique properties of CNTs, extensive study has been done over the past two decades on their application in dentistry. The CNTs have interesting and unique properties thus making them one of the most highly researched materials in the world [51]. Among the unique properties are high mechanical strength, hydrophilicity, and absorption. Moreover, the high tensile strength possessed by CNTs makes them potential materials in the applications of dental implants and bioartificial organs [52]. To date, CNTs-based materials have been used in numerous applications in dentistry (Table 2), particularly in dental restoration [52, 53], tissue engineering [54 – 56] and drug delivery system [11 – 14, 23, 57]. In this aspect, they make great candidates for scaffolding, targeted medication delivery systems, and reinforcement of dental materials [52, 58, 59].

Table 2 Examples of Applications of CNTs-based materials in dentistry

Substrate Materials	Type of CNTs	Application	Findings	Ref
SiO_2 - allytriethoxysilane (ATES)	SWCNTs	Dental resin composite	Increased flexural strength	[65]
Nylon-6 Nanofibers	MWCNTs	Dental resin composite	Improved mechanical strength at low concentrations (2.5% and 5%)	[66]
GICs	SWCNTs	Dental restorative material	High compressive strength (31.8±8.0 GPa)	[67]
GICs	MWCNTs	Dental restorative material	Low compressive strength	[[69]
Graphene oxide (GO) - nanohydroxyapatite (nHAp) (nHAp/MWCNT-GO)	MWCNTs	Dental restorative material	Prevent the de- and remineralization process from damaging the dentin	[72]
Poly(3-hydroxybutyrate) (PHB)	MWCNTs	Tissue regeneration	Improved tensile strength	[78]
Bioactive glass/poly(etheretherketone)	MWCNTs	Bone tissue scaffold	-mproved the mechanical properties. Promote cell metabolic vitality and osteogenic differentiation of osteoblast cells.	[54]
hydroxyapatite	MWCNTs	Bone scaffold Materials	Increase the compressive strength (100.5 ± 5.9MPa)	[86]

Despite these favourable qualities, there is a limit to the amount of CNTs that can be loaded into polymers or other materials as reinforcement or filler [52]. This occurrence happens due to the agglomeration of CNTs, and this problem typically can be overcome by the functionalization method which would improve the dispersion of CNTs in the solution and thus, lower the agglomeration. This successfully increases the loading capacity of the matrix, which contribute to the improvement of CNTs' mechanical properties [51, 60]. The optimization of CNTs' loading in the matrix is performed with the goal to maximize the effectiveness of stress transfer to the CNTs' matrix interface. A variety of parameters such as alignment, dispersion, stress transfer and synthesis method would determine the effectiveness of the reinforcement [60].

In general, the application of CNTs will be greatly depending on the individual parameters such as size, type, arrangement, and morphological properties [16]. The MWCNTs have been considered a potential material in the application of drug delivery systems owing to their high loading capacity resulting from their large surface area. In addition, their ability to have a chemical interaction with the membrane cell has contributed to their versatility and increasing applicability [53]. Furthermore, their outstanding electrical and mechanical properties have indicated them as a promising bioactive material for the application as filler and scaffolds for bone tissue regeneration [55, 56, 59, 61]. The effectiveness in the oral cavity requires the bioactive material to have characteristics and the ability to control bacterial and viral infection, strengthen teeth, and bio-enhancing effect or bio-stimulant. Additionally, these materials must have the ability to reduce inflammation, tooth remineralisation, and tissue repair and regeneration [62]. Generally, CNTs can be applied in the field of dentistry [52] as shown in the Figure 5.

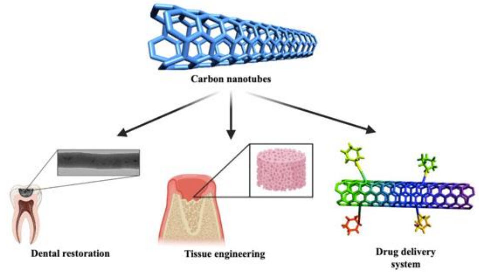


Figure 5 Application of CNTs in the field of dentistry. This figure was reproduced from an open access article [52] published by MDPI under a Creative Commons Attribution License 4.0 (CC BY 4.0)

In dental restoration, CNTs-based materials have been highly recommended owing to their high electrical conductivity and thermal stability, and outstanding mechanical properties. Hence, the modification of CNTs has been reported to significantly improve the strength of the composite [63]. As such, the CNTs have been proposed to be a promising candidate to be modified with the laboratory-processed composite resin [64] in order to enhance their flexural resistance. A study conducted by Pennisi *et al.* however, revealed that the incorporation of SWCNTs in the polymer composite did not improve the bending strength of indirect composite resin [65]. In contrast, the flexural strength was improved when SWCNTs were modified with SiO_2 and functionalized with allytriethoxysilane (ATES) before being incorporated into composite resins [65]. On the other hand, Borges *et al.* evaluated the mechanical properties of MWCNTs modified with composite resin filled with nylon-6 (N6) nanofibers at different concentrations. Their composites showed that at lower concentration (2.5 and 5%), the composite N6-MWCNT provide a composite resin with adequate strength associated with reduced film thickness [66]. Based on the findings from both Pennisi *et al.* and Borges *et al*., it can be concluded that each type of resin composite is determined by the amount of stress exerted on the restoration. Hence, understanding how CNTs enhance dental composites can aid in the development of new techniques in the preparation of durable and affordable material that could be employed in fixed partial dentures.

Glass ionomer cements (GICs) are an example of dental restorative materials that have interesting characteristics for the application as dental luting materials [58] due to their fascinating properties such as biocompatibility, good thermal insulation at oral temperature, good antibacterial activity and fluoride release [67]. However, the GICs materials suffer from poor mechanical properties for areas of a large force of mastication compared to that of composite resin [68]. Modification of GICs with different materials has been proposed in order to enhance the mechanical properties and CNTs materials have been highly recommended owing to their excellent mechanical, electrical and thermal properties. In addition, it has been reported that CNTs materials provide structural reinforcement to dental materials [52, 66].

In an effort to determine the effect of SWCNTs in GICs, AlMufareh *et al.* evaluated the different types of CNT with respect to GICs' compressive strength. They found out that incorporation of 0.03 wt% SWCNT into GICs lead to high compressive strength (31.8±8.0 GPa) compared to MWCNT (19 GPa) with the same wt%. They concluded that the nanofiller might be loosely attached to the GIC matrix and consequently affect the compressive strength [69]. Since MWCNTs have much better properties compared to SWCNTs, research has focused more on the study and development of MWCNTs-GICs. A study by another research group demonstrated that modification of MWCNTs has decreased the values of mean compressive strength and improved the diametral tensile strength regardless of the viscosity of cement [70]. Also, another work conducted by Goyal and Sharma, successfully prepare the novel MWCNTs reinforced GICs for the application as dental restorations. They found out that the mechanical properties of the composite were enhanced after incorporation of 2% w/w MWCNT as the composite hardness was increased from 2.19 MPa to 5.70 MPa. However, there is a limited amount of MWCNT that can be added as a reinforcement agent, as it leads to a formation of dark-coloured material, which can only be applied to posterior teeth [71].

Meanwhile, another research group, prepared the hybrid material composed of MWCNTs and graphene oxide (GO) combined with nanohydroxyapatite (nHAp) to prevent the de- and remineralization process from damaging the dentin. They found out that the nHAp/MWCNT-GO composite preserved the dentin from restoration owing to the production of an acid-resistant protective film [72]. All these findings support the ability and efficiency of CNTs as bioactive materials in restorative dentistry as well as preventive dentistry.

Another application of CNTs in the dental field is the utilization of CNTs in dental tissue regeneration, bone formation and tissue recovery [56]. In general, tissue engineering is the process of integration of scaffolds, cells and active biomolecules to create, maintain, replace, restore and improve the biological and functional tissues of whole organs [54 – 56, 73]. In dentistry, directed tissue engineering techniques for the treatment of periodontitis have shown favourable outcomes. Despite the promising outcomes, the reproduction of the distinctive structure of the tooth and periodontal system can be challenging work. In addition, the development and regeneration of oral tissue which is clinically safe are difficult to achieve. For example, the tissue that supports the teeth is heavily affected by gingivitis, resulting in periodontitis [55].

The tooth-supporting structure or periodontium is difficult to regenerate. The directed tissue and bone regeneration membrane use an obstructive membrane to sustain newly formed tissue, maintain a deficiency and restore tissue that has lost suitable cells [74]. Recently, CNTs and CNTs-based composites were found to be potential biomaterials for the regeneration of dental tissue. Additionally, with the outstanding strength and stiffness of CNTs, they have been considered promising materials to produce structural reinforcement for the production of scaffolds for bone tissue regeneration [75]. As CNTs are not soluble and high tendency to bundle up, their application in the biological system would be limited. Therefore, functionalization of CNTs and purification to remove residual metal catalysts are required prior to the integration of CNTs into biological systems [76]. In designing material for the application as a bone scaffold, one important criterion that needs to be considered is the mechanical properties of the materials. Provided the excellent mechanical strength of CNTs, they have been considered ideal materials to provide reinforcement in bone tissue regeneration [77].

Recently, Zarei *et al.* modified the MWCNTs with poly(3-hydroxybutyrate) (PHB) in order to increase their mechanical properties. Their studies showed that the addition of 1% w/v MWCNTs improved the tensile strength of PHB/1% MWCNTs composite compared to unmodified PHB scaffold and the tensile strength was near to periodontal ligament (PDL). Accordingly, they found that the PHB/1%MWCNTs scaffold mimicked the fibrous connective tissue of the PDL and holds a great potential to be used in periodontal tissue regeneration [78]. Meanwhile, another research group successfully incorporated the MWCNTs into bioactive glass/poly(etheretherketone) by using a compounding and injection-moulding process to prepare MWCNTs/BG/PEEK. They demonstrated that the addition of MWCNTs into BG/PEEK improved the mechanical properties. In addition, their prepared MWCNTs composite was revealed to promote cell metabolic vitality and osteogenic differentiation of osteoblast cells. Therefore, the composite has a high potential to be applied as a scaffold in dentistry [54].

Akasaka *et al.* in 2006 first demonstrated that CNTs have successfully induced the growth of the apatite in the solution containing calcium phosphate. Calcium phosphate which is the main component of bio-ceramics has widely recognized as biological safety material and scaffold [79]. Since Ibara *et al.* [80] and Murakami *et al.* [81] reported the use of b-tricalcium phosphate (TCP) nanoparticles coated on collagen scaffold have elevated the proliferation of bone grafting and attached cells of rat skull, Miyaji *et al.* coated the collagen scaffold with CNTs, and b-TCP nanoparticles and histological observation demonstrated that the penetration of blood cells into the collagen scaffold was improved. They concluded that the modification of scaffold with CNTs and b-TCP nanoparticles would contribute to the development of tissue engineering [82].

Hydroxyapatite, which is also a synthetic calcium phosphate used as bio-ceramic has been also widely used in dental applications [83]. It has been demonstrated that the addition of about 1% of CNTs into a matrix resulted in an increase between 36% and 42% of the stiffness of composite as well as tensile strength by 25% [84]. Khan *et al.* in 2017 showed that the addition of 3% CNTs into hydroxyapatite successfully increase the compressive strength (100.5 ± 5.9MPa). In addition, the composite also showed a better performance in terms of biocompatibility [85]. On the other hand, Fathy *et al.* synthesized the hydroxyapatite from natural cuttlefish bone and modified it with functionalized CNTs and chitosan. Their prepared composite showed that the addition of CNTs has significantly increased the compressive strength (0.82 ± 0.17MPa) and elastic modulus (11.1 ± 1.06 MPa). Their reported values were within the levels of human trabecular bone. However, the addition of CNTs in their studies has significantly lowered the value of cell viability percentage. Although the values for both cytotoxicity and proliferation ability on mesenchymal stem cells (MSCs) were low, they can be used as a bone graft or scaffold in low-stress concentration areas within the jaw area [86].

CNTs can be also used as drug delivery carriers by delivering antibiotics in the case of gingival barrier is breached, or by delivering drugs to promote wound healing. One of the advantages of the drug delivery system is that the consumed drugs are aimed at specific bacteria in the peri-implantitis sites and prevent the development of multidrug resistance [56]. Compared to SWCNTs, the MWCNTs are more considered to be promising candidates for drug delivery particularly owing to the large surface area. The large surface area would allow the high loading of protein, a high level of stability in biological systems, and reaction with membrane cells [13]. It was reported in the literature that modification of CNTs with polymers such as polyethylene glycol (PEG) can enhance biocompatibility, suggesting that the respective composites are efficient drug delivery systems [53]. Another example as reported in the literature is the titanium alloy coated by CNTs was able to decolonize a *Staphylococcus epidermis* via the release of antibiotics kept in the CNTs [87]. However, since the amount of drugs is limited, it needs to be taken into consideration that the effect of localized drugs will decrease over time [56]. Although CNTs have been widely used in various applications in dentistry such as dental restoration and tissue engineering, little is known about the safety aspect of CNTs when used as drug delivery systems [11 – 14, 23, 57]. Furthermore, the toxicity effect of CNTs on dental tissues requires further investigations.

2.3 Applications of CNTs in Cancer and Gene Therapy

For the past several decades, cancer has become one of the deadliest non-communicable diseases affecting more than 7 million people globally [88]. Currently, various treatment methods such as chemotherapy [89], gene therapy [90], radiation therapy [91] and surgery [92] are being performed to treat the diseases. However, the aforementioned methods are failing due to several reasons such as the development of multi-drug resistance, toxic side effects [93], effect on the immune system [94] and targeting the wrong cells as well as the possibility of development of tumour [95]. To date, the growing interest in developing a treatment for cancer therapy is focusing on the implementation of nanomaterials due to their interesting features such as high surface area, solubility, good surface morphology and unique structural properties [3].

Since the discovery of CNTs by Iijima in 1991, [96] the applications of CNTs in the field of nanotechnology have expanded into the field of nanomedicine such as for the use as drug delivery to targeted tissues [97, 98]. Compared to other carbon-based nanocarriers like graphene, graphene oxides, fullerenes and etc [99], CNTs have gained wide attention due to their fascinating characteristics such as high surface-to-volume ratio, excellent conductivity and high mechanical strength, and intracellular bioavailability [16, 57] (Table 3). This section discusses recent findings and challenges of CNTs-based materials in cancer therapy and gene therapy (Table 4).

Table 3 Comparison of CNTs and others carbon-based nanocarriers

	CNTs	**Other Carbon-based nanocarriers**	**Ref**
Structural shape	Long cylindrical tubes or enrolled cylindrical graphitic sheet	i)Single and flat layer (Graphene) ii) Hollow sphere and ellipsoid (Fullerenes)	[17]
Size of Material	Length (1-2 mm) and diameter (10-20 nm)	0.1 nm – 1 mm	[16]
Loading Capacity	High	Low	[11]
Cellular uptake	Endocytosis; Passive diffusion; Phagocytosis	Endocytosis; Phagocytosis	[57]

Table 4 Examples of the Applications of CNTs-based materials in cancer and gene therapy

Functionalized CNTs	**Cancer Cells**	**CNTs Application**	**Findings**	**Ref**
Pyridine-MWCNTs	Breast cancer cell MCF-7	iC-9 Delivery	Eliminated a high number of MCF-7 cells	[100]
Pyridine-MWCNTs	Breast cancer cell MCF-7	DNA Delivery	Nucleic acids transferred to cells with minimal cytotoxicity	[101]
MWCNT-based nanostructured scaffolds	Breast cancer cell MDA-MB-231	Scaffold	Enhanced cell adhesion to the scaffold	[102]
Ultra-short - SWCNTs	MCF-7 cell line xenograft BCM-4272 patient-derived xenograft	Cisplatin Delivery	enhance the therapeutic efficacy and greater efficacy in suppressing tumor growth	[106]
PEG-CNTs	A549 lung cancer cells	AT737 Delivery	Induced Bcl-2-mediated apoptosis and generated intracellular ROS	[108]
CY7 dye conjugated SWCNTs	Pancreatic cancer cells	Optical imaging-guided photothermal therapy	Improved tumor retention rate	[111]
PEI-SWNT/pHSP-shT	Breast cancer cell MCF-7	Gene delivery system	Improved molecular-targeted gene therapy	[112]

One of the main difficulties in creating nanocarriers for application in drug delivery systems is to reduce the toxicity of therapeutically active chemicals while improving the therapeutic efficacy. CNTs have become more biocompatibility as the surface of the tubes was modified and it is capable to penetrate through cells which are difficult to transfect. Thus, it leads to a high interest in cancer and gene therapy research. Mohseni-Dargah *et al.* stimulated a certain death to a breast cancer cell MCF-7 lines via gene transfer based on suicide gene, inducible caspase 9 (iC9) which transfected using pyridine-functionalized multi-walled carbon nanotube, *pf*-MWCNT combined with chemotherapeutic drugs. According to their work, this approach efficiently eliminated a high number of MCF-7 cells. However, both subpopulations can be affected by this technique in addition to the malignant cells. Hence, they suggested a cell line with more aggressive behaviour, for example, MDA-MB-231 cells cultured in 3D were suggested to enhance the number of cancer stem cells number [100].

In another work, Kaboudin *et al.* conducted research on the preparation, characterization, and application of modified CNTs and they reported that *pf*-MWCNTs are capable of transferring nucleic acids to cells while having minimal cytotoxicity [101]. The iC9 gene was originally delivered through viral vectors [102]. However, as the approach can have negative effects such as carcinogenic, immunogenic, or even leads to inflammation, CNTs are currently being utilized as a safe alternative to deliver drugs to the human body [52, 57]. According to their finding [102], a 3D nanoscale multi-walled carbon nanotube, scaffolded MWCNT has been fabricated in order to observe the proliferation, migration, and invasion in cells of human breast carcinoma MDA-MB-231 lines. They fabricated the scaffolds of MWCNT with nanosized using the plasma-enhanced chemical vapour deposition, PECVD method and seeded them with MDA-MB-231 on both MWCNTs-scaffolds and pristine silicon surfaces. The samples were analysed using SEM analysis and immunocytochemical staining using P-I3-K, AKT, MMP 2, MMP 9, and NF-kB primary antibodies and their findings demonstrated that MDA-MB-231 breast cancer cells adhered to the MWCNT nanostructured scaffold more successfully than it did to a pristine silicon surface. However, the immuno-histochemistry activity of MDA-MB-23 for both materials stained similarly with the primary antibodies against MMP 2, MMP 9, AKT, and kB. The MWCNT-based scaffolds have a high possibility to be employed for *in-*

vitro research of breast cancer cell lines due to their physiological adaption and biomimetic features they displayed, according to their findings, which also revealed that they will aid in cell adhesion [103]. A study on the investigation to evaluate whether CNTs could be applied for tumour progression has been conducted by Akinoglu *et al.* in 2017 [103]. The application of CNTs as a diagnostic component in cancer therapy has been studied by Chan *et al.* in 2022 [104]. They used CNTs with a photo-assisted AC pulse sensor to distinguish between luminal breast cancer, MCF-7 cell line, and normal breast epithelial cells, MCF-10A cell line. Due to the excellent photocurrent properties of CNTs, where it enhances the current flow with the exposure of light, including the CNTs' low band gap value and its high electron transfer, it can also convert the absorbed light into current by photocurrent mechanism in separating and dividing the electron-hole pairs [105]. These CNTs' exceptional properties with the combination of distinctive MCF-7 cell bioelectrical signals will enhance cell-specific current signals, hence, be able to identify MCF-7 cell lines inside the population of the heterogeneous cell as shown in Chan *et al.*'s research. The study proposed that a clinically relevant CNTs optoelectronic-pulse approach can be developed to identify the cancer cells in a 3D cell structure that mimics malignant tissues developed in the human body [104].

Another research group, Guven *et al.* works on the application of CNTs using ultra-short single-walled carbon nanotube capsules, CDDP@US-tubes in xenograft models to evaluate three individuals of human breast cancer established in mice. Their results showed that encapsulating cisplatin within CDDP@US-tubes, will enhance the therapeutic efficacy, prolonged the circulation time the permeability and the retention (EPR) effects dramatically increase the number of malignant cells [106]. Directly attacking the mitochondria of the cancer cells is one of the most efficient ways to treat cancer since it causes the cells to go into apoptosis [107]. However, the availability of the mechanism in clinical treatment is still limited. In 2017, Kim *et al.* prepared the CNTs nano drug ABT737 coated with polyethylene glycol (PEG) absorbed into the first endosomes by micropinocytosis and Clathrin-mediated endocytosis. Their result showed the released cytosol of nano drug will trigger the abruption of the mitochondrial membrane that caused the death of lung cancer cells. Hence, this leads to the formation of intracellular ROS. Their finding proposed a practical method for boosting the effectiveness of anti-lung cancer efficacy by elevating the rate of mitochondria accumulation of cytosol produced by anticancer drugs with the assistance of CNTs [108].

One of the most lethal malignancies worldwide is pancreatic cancer [109]. However, the existing treatment and therapy regimen in the clinic for advanced and inoperable cases are far from satisfactory [110], hence it is crucial to seek more effective strategies for anticancer therapy. To date, photothermal therapy (PTT) with assistance from CNTs has attracted considerable attention [111]. They used low-toxicity, bio-stable, and water-soluble SWCNTs to enhance photothermal conversion efficiency on orthotopic pancreatic cancer models, ASPC-1, BXPC-3, PANC-1, and SW1990. The modifications on SWNTs enabled visualization of the aggregate characteristics of CNTs at the level of macroscopic or microscopic in cancerous cells. They reported the targeting antibodies were bounded with dye-conjugated SWNTs that induced specifically targeting to pancreatic cancer cells performing dyes imaging-guided cytotoxic photothermal therapy. From the research, it can be concluded that this approach achieves excellent and precise therapeutic effects with minimal undesirable effects, thus providing a promising strategy for pancreas cancer and generally anticancer therapy [111].

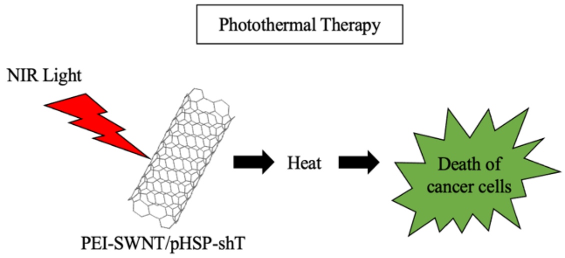


Figure 6 A schematic representation of biosensor. This figure was reproduced (adapted) from an open access article [27] published by Informa UK Limited, trading as Taylor & Francis Group under a Creative Commons Attribution license (CC BY)

A study by Ren *et al.* used a polyetherimide-modified single-wall carbon nanotube, PEI-SWCNT, engineered photoactivatable RNA interference, RNAi system may enable precise gene suppression under optogenetic control in cancer cells. PEI-SWNT consists of a stimulus-responsive nanocarrier, and a heat-shock protein 70B', Hsp70B'-promoter-driven RNAi vector, pHSP-shT. Their research finds out that, the heated PEI-SWNT in breast cancer cells due to irradiation of near-infrared (NIR) light will trigger the gene knock-down by targeting human telomerase reverse transcriptase via RNAi, in which the activity can be turned off by extinguishing the NIR light. Additionally, their research revealed that a combination of gene therapy and photothermal therapy (Figure 6) using a photoactivatable RNAi mechanism and PEI-SWNT resulted in greater antitumor efficacy, both in vitro and in vivo. A better understanding of molecularly targeted gene therapy in human cancer cells may be made possible by optogenetic control of RNA interference according to the CNTs carried which are activated by NIR light [112].

2.4 Applications of CNTs in Biosensors

The early first biosensor was introduced in 1906 when M. Cremer demonstrated that increasing levels of an acid in a liquid is proportional to the electronic potential that appears between parts of the fluid located on opposite sides of a glass membrane. Later in 1956, the biosensor was later developed by Clark *et al.* in 1956 and was regarded as the father of biosensors [113]. In summary, the biosensor can be classified as a device that determines the chemical and biological reactions by generating signals proportional to the concentration of an analyte in the reaction [114]. Sireesha *et al.* in 2018 provides an excellent review on the application of CNTs as biosensor which focuses on the recent development of CNTs in the biosensor field as well as various methods and techniques to improve the CNTs' performance with the latest designs [27]. Normally, a CNTs-based biosensor is made of two main parts which are biological sensitive elements and transducers. As shown in Figure 7, receptors can be described as the main instrument for biosensor technology. They bind with analytes of interest to form a signal which would be measured by the transducer. The transducer will convert the signal into one form of energy and this process is called signalization. The electronic system consists of an amplifier and processor which will process the received signal and convert it to display for reading [115].

In the past few years, CNTs materials have attracted particular attention in the application of biosensors in healthcare systems owing to their fascinated characteristic such as outstanding mechanical strength, good electrical conductivity, large surface area, high thermal and electrochemical stability in both aqueous and non-aqueous solutions [27]. Hence, these remarkable properties of CNTs make them a promising candidate for highly sensitive biosensor materials. However, the hydrophobic properties of CNTs and the strong intermolecular $\pi-\pi$ interactions have limited the development of CNTs-based biosensors [116]. Functionalization of CNTs has been performed in order to improve solubility and stability [27]. For the application of biosensors, immobilizing of biomolecules (bio-functionalization) onto the surfaces of CNTs in order to provide the surfaces with specific functions such as bio-specificity and/or catalytic activity are performed. Various kinds of literature have reported the successful bio-functionalization of CNTs with biomolecules such as antibodies, enzymes, proteins, and nucleic acids in numerous biosensor applications [15]. Furthermore, with CNTs' high surface-to-volume ratio, it will provide a large area for the loading of biomolecules that will lead to high signal amplification and exhibit biocompatibility [117]. In 2019, Zhou *et al.* provide an excellent review on the bio-functionalization of CNTs for the application of biosensors, focusing on the non-covalent functionalization of CNTs using various methods including physical adsorption, cross-linking, and polymer encapsulation.

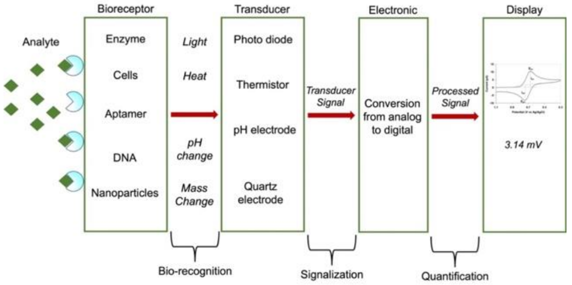


Figure 7 A schematic representation of biosensor. Adapted from Ref [27] under the terms of the Creative Commons CC BY license

The CNTs-based enzymatic electrochemical biosensors utilize the bio-specific of enzymatic reactions to control electric transmission between biomolecules and electrodes (27, 113 – 115). Table 3 shows the different types of CNTs-based biosensors, and the review is discussed in this part (biomolecules, particularly enzymes and CNTs bulk electrode materials.

There have been various enzymes modified with CNTs for the application of biosensors.For example, to detect and analyse phenol in solution, in 2019, Wee *et al.* developed tyrosinase (TYR)-immobilized CNTs for sensing phenol compounds. The TYR was immobilized onto the surface of CNTs via enzyme adsorption, precipitation, and crosslinking (EAPC) approach. The EAPC together with CNTs on the SPE electrode showed excellent results for on-site detection of organic pollutants, especially phenol. Their new simple method in the preparation of TYR-based biosensors by highly dispersed CNTs in enzyme solutions will provide opportunities for the production of highly sensitive biosensors for biosensing applications [118].

Table 5 Recent development of CNTs-based Biosensor

No.	CNTs-based Biosensor	Type of Biosensor	Detection of Cells/Compounds	References
1	MWCNTs	Electrochemical	Phenol	[118]
2	MWCNTs	Electrochemical	Hydrogen peroxide (H_2O_2)	[119]
3	MWCNTs	Amperometric Biosensor	Phenolic Compounds	[120]
4	MWCNTs	Electrochemical	Cholesterol	[121]
5	SWCNTs/ MWCNTs	Electrochemical	DNA sequences	[122]
6	MWCNTs	Electrochemical immunosensor	Cancer marker	[123]
7	SWCNTs	Immunosensor	Biomarker	[124]

In another example, Huang *et al.* prepared an enzymatic hydrogen peroxide (H_2O_2) biosensor on a screen-printed carbon electrode (SPCE) using the composite of polymeric nanoparticles functionalized onto CNTs. These biosensors with a high surface area can immobilize enzymes and enhance electron charge transfer, thus resulting in excellent sensing activities,better selectivity, and stability. What is interesting is the biosensor can be prepared using a facile and simple method, thus, making it a potential low-cost biosensor for the application in biosensing of hazardous chemicals [119].

Another research group (Othman and Wollenberger, 2020) proposed an amperometric biosensor type for the detection of phenol compounds. The biosensor was prepared by chemically modified laccase from Coriolus hirsute (ChLa) immobilized on functionalized SPCE and the SCPE was modified with carboxyl functionalized MWCNTs. The mechanism of sensing was based on cyclic enzymatic oxidation of phenol under the utilization of oxygen and reduction of product and therefore, it is highly influenced by the suppressed direct electron transfer which is directed by the immobilization step. The advantage of this type of biosensor is its high sensitivity towards different phenolic compounds, which will be a great interest in screening polluted water [120].

Even though modified CNTs-based biosensors are favourable and encouraging, further and advanced development is required to be identified before they can be utilized as rapid, high-accuracy and economical biosensors. The fabrication of biosensors which is made from nanohybrid materials can be considered a systematic plan to develop future high-performing biosensors. For example, Ghanei Agh Kaariz *et al.* prepared an enzyme-based electrode based on an MWCNTs-based biosensor modified with gold metal and ZnO semiconductor and immobilized with cholesterol oxidase for the application of cholesterol biosensing. Their novel electrochemical cholesterol biosensor was shown to have fast electron transfer and excellent linear analysis performance. In addition, the biosensor is also highly selective in the determination of cholesterol over glucose and uric acid and the detection of cholesterol in human serum was also confirmed [121].

In addition, one of the challenging works in preparing CNTs-based biosensor devices is to integrate the CNTs into devices as it will affect the sensing capacity and sensitivity. Fu *et al.* overcame the problem by preparing the chemiresistive biosensors based on CNTs using semiconducting SWCNTs and nitrogen-doped MWCNTs for the detection of DNA sequences. The use of semiconducting SWCNTs in their studies is highly proposed as they play key roles in inducing the electronic structure of nanotubes. Furthermore, the semiconducting SWCNTs showed high sensitivity to gas and biomolecules which adsorbed efficiently onto the CNTs' surface. Meanwhile, the CNTs were also functionalized with DNA probe sequences attached to sidewalls. Their novel synthesized CNTs-based biosensor is able the detection in low concentration (2 pm to 2 nm) in 15 mins at room temperature. These properties suggest the CNTs-based biosensor can detect a wide range of low concentrations, is simple in preparation and is flexible. Hence, make them a promising biosensor for point-of-care testing, and clinical diagnostic [122].

The CNTs-based biosensor was also reported to work best as immunosensor for the early detection of cancers (cancer markers) such as breast cancer and prostate cancer. Rashid *et al.* prepared an electrochemical immunosensor based on gelatin modified electrode. The electro-active MWCNTs was functionalized with dopamine/mucin-1 and were used as signal to generate probes in the preparation of an electrochemical immunosensor to detect breast cancer. The function of the electrode was for the immobilization of antibodies (anti-MUC-1), while the electrochemical response of the probes was used for the measurement of MUC-1. The developed immunosensor demonstrated the detection of MUC-1 in the range of 0.05 – 940 U/mL, with a detection limit of 0.01 U/mL. The recovery values of immunosensor were found to be 96% for human samples, thus leading to its potential use in the biomedical field [123].

Another fascinating application of CNTs in biosensing is their use as biomarkers, which are typically used to identify the presence of the biological molecule in blood or tissue as a sign to indicate an abnormal process or disease [125]. For example, Kim *et al.* fabricated a densely aligned CNT film sensor using the Langmuir-Blodgett (LB) transfer technique and the well-aligned CNT monolayer was obtained by exerting the uniaxial force to the poly [(m-phenylenevinylene)-co-(2,5-dioctoxy-p-phenylene-vinylene)] (PmPV)-wrapped around nanotubes that suspended on water subphase. The CNTs-based biosensor demonstrated high precision, sensitivity and accuracy in the early diagnosis of Alzheimer's disease. With the low coefficient of variation and a high degree of recovery, the prepared CNTs sensor demonstrates the high potential uses for early diagnosis of AD diseases [124].

3.0 CONCLUSION AND FUTURE OUTLOOK

Carbon nanotubes own fascinating and unique properties that are different from other carbon-based materials. Their large surface area, excellent mechanical strength and outstanding electrical conductivity, high biocompatibility have made them an ideal material for application in the biomedical field. This article reviews the recent progress and achievement of CNTs-based materials in the application as antibacterial agents, dental materials (bone scaffolds, tissue engineering), anticancer and gene therapy as well as biosensors. The cytotoxic properties of CNTs have enabled them to be used as antibacterial agents. The modifications with metal (Ag) and metal oxide (ZnO/TiO_2) have enhanced their antibacterial properties especially when MWCNTs type was used. Due to their excellent mechanical strength and biocompatibility, CNTs have been actively used as nanomaterials in dentistry, especially in the fabrication of scaffolds and bone-grafting as well as tissue engineering. However, their practical application in tissue engineering has not been fully explored yet, as their relatively high toxicity has become a major concern for their application in the biomedical field. To overcome these challenges, they can be biofunctionalized to improve the CNT's biocompatibility in order to make it possible to serve as tissue scaffolding materials. On the other hand, the excellent transport properties of CNTs with the combination of applicable surface modifications to enhance its biosafety have led to the development of drug delivery systems for cancer therapies. However, one of the biggest challenges of the application of CNTs in the dental field and cancer therapy is the limited understanding of the biocompatibility of CNTs in human tissue. Therefore, current research is more focused on functionalization of CNTs to achieve the desired effect on the respective materials. Various reported results have demonstrated that modification with CNTs can improve the therapeutic effects and reduce toxicity, suggesting them as a promising material to be used as drug carriers in the future. The application of CNTs in the biosensing field has shown positive results as high sensitivity and reliability, simple and facile preparation as well as economically operated were reported. The versatility and functionality of CNTs-based materials could be potentially employed in the vast application of the biomedical field. Although it still needs a long time for research and development before it can be widely applied for practical use, it can be predicted that the CNTs-based materials will become one of the important materials and devices to serve the betterment of human health.

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