CARBON NANOTUBES – AN EMERGING THERAGNOSTIC DRUG DELIVERY NANO-CARRIER FOR TUBERCULOSIS

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Abstract:

Globally, tuberculosis (TB) is the main reason for fatality and it is a communicable illness,which significantly contributes to ill health conditions. In advance of the pandemic coronavirus (COVID-19), the prevalent infectious disease to cause mortality was TB, over HIV/AIDS. A recently developed category of materials known as carbon nanomaterials (CNMs) has been extensively employed with various applications on the therapeutic side, including cancer treatment, drug delivery, tissue engineering, and diagnostics.Carbon nanotubes (CNTs) is an ideal material for the detection and suppression of *M. tuberculosis*. These nanomaterials have a high photothermal conversion efficiency, the capability to generate reactive oxygen species (ROS), and are resistant to the majority of acids and bases.They have been found to be used in diagnostic and drug/gene delivery methods for fighting several infectious diseases, including tuberculosis. Properties, Drug loading, and delivery mechanisms of CNTs used in the theragnostic approach of TB are discussed in this review. The utilization of nanomaterials as anti-TB drug delivery systems seems to be an intriguing strategy to enhance TB therapy by lowering time and toxicity.

Keywords:

Carbon nanomaterials, Carbon Nanotubes, Tuberculosis, Drug delivery, Theragnostic Approach

Introduction:

Globally, tuberculosis (TB) is the main reason for fatality and it is a communicable illness,which significantly contributes to ill health conditions [1]. In advance of the pandemic coronavirus (COVID-19), the prevalent infectious disease to cause mortality was TB,over HIV/AIDS [2]. TB is caused by the gram-positive bacilli, *Mycobacterium tuberculosis*(*M. tuberculosis*)when the bacteria get liberated into the air(for example, through coughing) from the patients infected with TB.PulmonaryTB primarily infects thelungs, whereas extrapulmonary TB

affects othersites than the lungs. Almost 90% of individuals who contract the infections are elder people, as well as men, account for a greaterproportion of cases than women [3,4,5].

Recent years have seen a significant improvement in TB illness diagnostic testing.The WHO currently recommends a number of quick molecular assays as the primary TBdiagnostic test, meanwhile some of which identify the antimicrobial resistance pattern. At thelower levels of the healthcare system, these tests can be used [6]. Additionally, there aresequencing techniques that could offer thorough personalizedform of antibiotic resistance as wellas quick molecular tests designed particularly in order to identify the resistance for variousfirst- line and second-line anti-TB antibiotics [7]. Even though sputum smear microscopy(invented more than a century ago) is still frequently employed in low- income and middle-incomenations to diagnose TB, fast diagnostic techniques are gradually taking their place. The goldstandard for diagnosing tuberculosis is still culture methods [8]. After diagnosis, smear or culture(despite quick molecular testing) is required for tracking a patient'sresponse to therapy [9].Additionally, culture is necessary to identify the newer anti-TBantibiotic resistance as well as it could be employedfor confirmatory testsamong patients witha low pre-testchance of TB illness [10].

As per WHO, estimated TB cases in 2021 were 10.6 million, up from 10.1million in 2020 which shows a 4.5% increase. The number of newcases of tuberculosis (TB) per 100,000 people increased by 3.6% between 2020 and 2021,reversing annual decreases of roughly 2% for the most of the preceding two decades. Onlyhalf of the first End TB Strategy milestone was reached by the net reduction (10%) from2015 to 2021 [11].

A recently developed category of materials known as carbon nanomaterials (CNMs) has been extensively employed with various applications on the therapeutic side, including cancer treatment, drug delivery, tissue engineering, and diagnostics [12].It's interesting to note that CNMs have been shown to be effective against road-spectrum antibacterial efficacy, and as a result, they have recently utilized nanomaterials for developing novel tactics against drug-resistant species [13].CNMs have different sizes and shapes, and as a result, they interact with eukaryotic and prokaryotic cells in various manners. Additionally, CNMs frequently have functional groups added to their carbon skeletons to increase their solubility and/or characterize their interactions with large macromolecules [14].Recent studies on *M. tuberculosis* have elucidated a variety of characteristics of these infectious diseases, including their pathogenesis, diagnostics, and genomes as well as the infection and immunological techniques [15].

The association between the immunological activation brought on by infections of *M. tuberculosis* and the activation of immune responses brought on by carbon nanotubes (CNTs) that has not been detailed in any studies or research projects.The low density, flexibility, and exceptional mechanical strength of CNTs make them an ideal material for the detection and suppression of *M. tuberculosis*[16,17].These nanomaterials have a high photothermal conversion efficiency, the capability to generate reactive oxygen species (ROS), and are resistant to the majority of acids and bases.They have been found to be used in diagnostic and drug/gene

delivery methods for fighting several infectious diseases, including tuberculosis [18]. Properties, Drug loading, and delivery mechanisms of CNTs used in the theragnostic approach of TB are discussed in this review.

CARBON NANOTUBES' PROPERTIES:

The structured generation of carbon atoms inside graphene cylinders leads to number of features. The enormous, cylindrical carbon nanotubes are constructed with sp2 hybridized carbon atoms that have a hexagonal arrangement [19].Single-walled carbon nanotubes (SWCNTs) (Figure 1), which are generated by rolling single sheets, because they have a single wall, while multi-walled carbon nanotubes (MWCNTs),because they have multiple walls, which are made up of layers of graphene sheets.MWCNTs have an average interlayer spacing of 0.34 nm between the graphene layers, resulting in individual tubes with wider outer diameters (ranging from 2.5 to 100 nm) over SWCNTs (0.6 to 2.4 nm).SWCNTs possessdistinct wall than MWCNTs, as they produce structural flaws and have less stable nanostructures as a result [20,21].

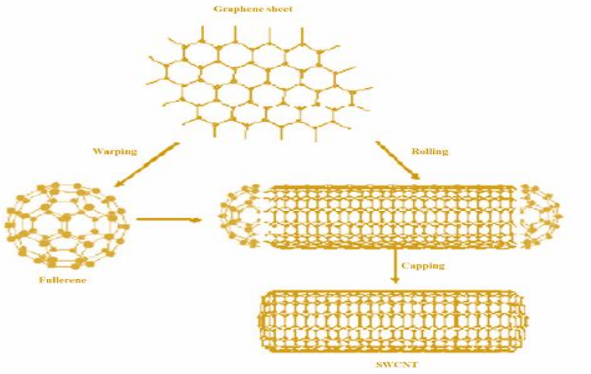

Figure 1: Single-walled carbon nanotubes closed at both ends

CARBON NANOTUBES: OPENING, FILLING, AND CAPPING:

As pointed out earlier, Carbon nanotubes are end-capped, as there exist basically two methods for drug loading: either filling CNTs at the time of synthesis or following synthesis.While post-synthesis could be more regulated and it produces 50–100% yields, by adding the contents of nanotubes in situ often results in a yield of just around 10%.Melting point, reactivity, surface tension, and material sensitivity are the criteria involved [22,23].

The ends of CNTs are supposed to be kept openduring post-synthesis production. This could be achieved by running electricity throughCNT, attacking with acidas it erodesthe tubes at the angled regions mostly, or oxidizing through carbon dioxide [24].There are two methods for introducing foreign materials into CNTs. The coating is one type of decorating, which involves the bonding of a functional group to CNTs and the functional groups are attached either to the

interior or outside of the walls of CNTs.Since carbon tends to be very inert, oxidizationhas beenutilized in creating an additional reactive surface for attachment as it is challenging [25,26].

Capillarity is the most typical method for filling CNTs. TheCNT's diameter and tension of the material's surface are the two factors that restrict capillarity.However, aqueous solutions also involve Van der Waals and hydrophobic forces [27]. If a CNT is filled, an appropriate composite that can be chemically reduced to the original material can be made to lower the surface tension of compounds with higher surface tensions [28].The impregnating fluid is given only a limited amount of solubility by the solution used to wash the CNTs, and as a result, it can only dissolve deposits that have been left outside the CNT.After filling, a current pass through the CNTs to fuse the ends together, capping them [29,30].

Drug loading and Targeting:

The CNT can deliver the drug to either an internal or exterior area. For insertion into the CNT, internalization or encapsulation depends on Van der Waals forces, and it functions best for therapeutics that are sensitive to external conditions and readily deteriorated [31,32].Drug delivery is inherently indiscriminate in conventional therapies utilizing therapeutic drugs. Therefore, toxic medication therapy is administered, which has the effect at best, causing the patient to experience a variety of unfavorable side effects and, at worst, resulting in the patient's death owing to the toxicity of the medicines themselves [33,34,35].It would be less harmful if therapeutic chemicals could be delivered, opening up the possibility to more potent, concentrated dosages and compounds that are toxic for conventional therapy[36].

Targeted nanoscaledelivery of drugs employs two fundamental methods. The first method is passive (or size-mediated) targeting, which depends on the specific dimensions of nanoparticles and the way diseases develop.Active targeting is the second method, that involves attaching an antibody or ligand to a specific target in order to deliver a substance only [37,38].

Carbon Nanomaterials against Tuberculosis

Using a transmission electron microscope, a team of Japanese scientists initially discovered CNTs in 1976 [39]. Due to their distinctive mechanical, thermal, and electrical characteristics, they have attracted an abundance of interest since Ijima first identified them in 1991 [40]. For instance, Korri-Youssoufi and colleagues utilized them to identify the DNA of the MtbrpoB gene.They created composite MWCNTs functioning as a detection platform that is coated in polypyrrole(PPy) and redox PAMAM dendrimers for this purpose.PPy-covered MWCNTs functioned as transducers while PAMAM dendrimers helped in enhancing the electrochemical signals [41]. The surface-attached ferrocenyl groups acted as redox markers.By observing the ferrocene redox signature, layers of MWCNTs-PPy-PAMAM-Fc was demonstrated to be one of the effective rostrums for the detection of DNA. Ability of the system to identify the DNA that has been targeted in a wide range of concentration (1 fM to 100 nM) with a reduced detection limit was demonstrated by cyclic and square wave voltammetry

[42].Later, ferrocene-modified oligo-methoxy-phenyl-acetonitrile is used as a transducing polymer and the same group created an additional MWCNTs-based TB biosensor.This nanocomposite with the detection of 0.08 fM has demonstrated excellent efficacy for DNA hybridization [43].

Chen et al. investigated the interaction with polyaniline, a flower-like CNT, the investigationinvolved the detection of TB by DNA using a relatively complex system with numerous signal amplification strategies.To produce the electrochemical signal, a tracer label was added to the CNTs-PANI nanohybrid [44]. A recycling method depending onnicking endonuclease-supported three-way DNA junction which is processed overan altered glassy carbon electrode with functionalized fullerene that resulted in C60 production by DNA probe hybridization.Following the hybridization among the tracer label and the cleaved capture probe, the electrochemical signal was produced. With a detection limit of 0.33 fM, biosensor that has been developed was usable throughout a broad linear range (1 fM to 10 nM)[45,46].

More complex devices have been made possible by recent improvements in electrochemical sensing methods paired with microfluidics and nanomaterials.In their study, Zribi et al. showed how to identify pathogenic Hepatitis C virus and Mtb DNA from the clinical isolates using a microfluidic technology on the basis of altered MWCNTs with the moieties of ferrocene [47].Inthe microfluidic device, the reported electrochemical biosensor's limit of detection was increased bulk solutionfrom picomolar to femtomolar, having broadrange from 0.1 fM to 1 pM.Additionally, it selectively enabled the direct identification of the rpoB gene of Mtb H37Rv from extracted DNA of the clinical isolate when operating under high flow [48]. For the purpose of detecting TB, metal oxide nanoparticles also have been connected with CNTs.For creating an impedimetric nucleic acid biosensor to identify Mtb, zirconia (ZrO2) grafted MWCNTs (crystallite size of ZrO2 28nm) has been collected through zirconium oxychloride’s isothermal hydrolysison behalf of CNTs, which actually have been depositedelectrophoretically onto indium-doped tin oxide which is coated with glass [49].With an increased detection limit of 0.01 nM, the developed nanocomposite used for the detection of IS6110 as it showedeffective performances ranging from 102 to 108 M [50].

The BCG vaccination is the most recent TB preventive vaccine that protects against TB in children between 60 and 80 percent of the time, but it is ineffective against pulmonary TB in elder people [51].In lieu of BCG vaccine, the only efficient vaccine among children, WHO advises drugs for TB in adults; the efficacy has also been observed to differ widely.Furthermore, BCG is a live vaccine, those with impaired immune systems may develop a disseminated infection as a result [52]. New vaccinations must be created immediately due to these restrictions. The history of anti-TB medicines that have been authorized and are utilized in therapy.In the1940s, the first efficient pharmacological therapies were created [53]. The most recent WHOrecommendations, which were published in 2022, strongly advised a isoniazid (H), rifampicin (R), ethambutol (E), and pyrazinamide (Z) for 6 months to drug-susceptible TB patients (pulmonary and extrapulmonary): for the first two months all the four drugs will be

given,subsequently by H and R for the rest four months. Additionally, it involves novel recommendations for the treatment of non-severe TB in children and adolescents between theages of 3 months and 16 years old. These recommendations call for a 4-month regimenconsisting of rifapentine (P), H, Z, and moxifloxacin (M), as well as a 4-month regimencontains rifapentine (P), H, Z, and occasionally also E, subsequently H and R for 2 months.However, as per WHO, the treatment success rate of patients from 194 states participating in the 6-month program was at least 85% [54,55].

Treatment for those who have been identified as having R-resistant TB (RR-TB) ormultidrug-resistant TB (MDR-TB) that are resistant to H and R, is more challenging as well asnecessitates the use of medications that have higher adverse effects. The global average hasbeen rising recently, ad it has reached 60% among majority cohort with new patients as they have statistics [56]. In the United States, treatment success rates for RR-TB are normally between 50%and 75 %. Treatment for pre-extensively drug-resistant TB (pre-XDR-TB)that is defined as TBresistant to R and any fluoroquinolone and extensively drug-resistant TB (XDR-TB) in which TB resistant to R, any fluoroquinolone, and at least one of bedaquiline or linezolid isconsiderably more challenging, and treatment success rates are often poor [57].

Drug resistance must be tested byutilizing quick molecular assays, culturetechniques, or sequencing technologies, along with bacteriological confirmation of TB. Acourse of second-line medications is necessary for treatment. In comparison to prior regimensthat lasted 20 months or longer and the time period of the treatment has been shorten for all-oral drugs of MDR/RR -TB and pre-XDR TBto 6 months [58]. The WHO suggestsincreasing access toall-oral regimens and providing counseling and monitoring for negative side effects [54].In 2021, rifampicin resistance was tested in 71% (2.4/3.4 million) of those with abacteriologically confirmed diagnosis of pulmonary tuberculosis worldwide, matching thecoverage from the previous year (2.1/3 million) and increasing from 61% (2.2/3.6 million) in2019 [59]. A total of 166 991 instances of MDR/RR TB and pre-XDR or XDR TB showing an incidence of 25 038 caseswere found among individuals who underwent testing. The reports between 2020 and 2021 demonstrates that the total has 6.4% increaseshowing 156 982 in 2020, however, it was below 9.7% rise in total TBdiagnosis [60]. Additionally, it remained much less (by 17%)than the total (201,997) in 2019. In 2021, a total of 161 746 MDR/RR -TB patients wereengaged in therapy globally, an increase of 7.5% from 150 469 patients in 2020 but still asignificant decrease of 11% from the 181 533 patients who received treatment globally in 2019. Approximately, one in three persons acquiring MDR/RR TB each yearhad enrollment levels similar to those at this level [61,62].

In order to produce a partially closed or completely closed mesh, the carbon atoms in fullerene are joined using single and double bonds for creating hollow sphere. Fullerene is not easilysoluble in water [63].The basic fullerene was changed by the authors by adding a chain of tri-ethylene glycol monomethyl ether to improve solubility. Fullerene that has been altered has been shown anti-Mtb action after being methylated.According to molecular docking

experiments, SWCNTs may effectively transport the antitubercular drugs PZA and promote its therapeutic activity [64].

Considering its objective of producing dressing materials for burn wounds that are infected, multimodal bio-based hyperbranched polyester-amide (HBPEA) - functionalized multi-walled carbon nanotubes (f-MWCNTs) has beensynthesizedfor creating nanocomposite films having antibacterial capabilities as well as strong biocompatibility [65]. Theantibacterial tests results showed that f-MWCNTs was much efficient in preventing colonization of Gram-positive organisms overGram-negative bacteria. Additionally, the release of intracellular components was stimulated by f- MWCNTs [66]. As it has been shown for other carbon allotropes, the structural changes in these bacterial species' cell walls may be a major factor in determining how sensitive they are to CNTs [67].The effectiveness of f-MWCNT in decreasing the colonization of *Mycobacterium smegmatis* (Ms) has been demonstrated [68]. Peripheral blood mononuclear cells (PBMCs) intriguingly demonstrated improved proliferation and adhesion over the treated surfaces of f-MWCNTs, suggesting as CNTs might supports wound healing and by regulating or enhancing the host immune response [69].

Conclusion and Future Perspective:

The utilization of nanomaterials as anti-TB drug delivery systems seems to be an intriguing strategy to enhance TB therapy by lowering time and toxicity. In case of TB treatment, CNMs have lately been explored. By virtue of their high surface-to-volume ratio and inherent multimodal antibacterial activity, CNMs can greatly enhance the effectiveness of drugs against TB and vanquish drug resistance as they have an intrinsic antibacterial activity.However, most of researchers reported that CNMs' antibacterial properties failed to investigate their anti-TB properties. On the other hand, this is due to the need for specialized knowledge in order to analyze this pathogen, but on the other hand, similar to research on fungal cells. The large size and architecture of the distinctive cell wallof *M. tuberculosis* may make them more resistant to the physical damages caused by carbon nanoparticles.

Despite the administration of various drugs by employing carbon nanotubes, the difficulties surrounding CNT toxicity for infectious diseasesin CNT technology; remain unresolved due to the multiple contradicting research indicating both non-toxic and toxicbehaviors. Future toxicologists will need to pay close attention to this field because of the variety of factors previously mentioned that have been demonstrated to impact CNTs' toxicity.

ACKNOWLEDGMENT

None.

CONFLICT OF INTEREST:

The authors declare that there is no conflict of interest.

AUTHORS CONTRIBUTION

All authors hold significant and sincere participation in this researchwork and have accepted it for publishing.

FUNDING

Nil.

DATA AVAILABILITY

Not applicable.

ETHICAL STATEMENT

Not applicable

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