



# Halloysite nanotubes as a nature's boon for biomedical applications

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## Abstract

The arena of biomedical science has long been in quest of innovative mediums for diagnostic and therapeutic applications. The latest being the use of nanomaterials for such applications, thereby giving rise to the branch of nanomedicine. Halloysite nanotubes (HNTs) are naturally occurring tubular clay nanomaterials, made of aluminosilicate kaolin sheets rolled several times. The aluminol and siloxane groups on the surface of HNT facilitate the formation of hydrogen bonding with the biomaterials onto its surface. These properties render HNT pivotal in diverse range of applications, such as in environmental sciences, waste-water treatment, dye removal, nanoelectronics and fabrication of nanocomposites, catalytic studies, as glass coatings or anticorrosive coatings, in cosmetics, as flame retardants, stimuli response, and forensic sciences. The specific properties of HNT also lead to numerous applications in biomedicine and nanomedicine, namely drug delivery, gene delivery, tissue engineering, cancer and stem cells isolation, and bioimaging. In this review, recent developments in the use of HNT for various nanomedicinal applications have been discussed.

## Keywords

Halloysite nanotube, drug delivery, tissue engineering, cancer therapy, nanobiomedicine

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## Introduction

Nanotechnology is a rising field which holds plenty of applications in diverse fields of science and technology, industries, environment, energy, and so on. This field holds promising future prospects and hence a lot of research is going on in expanding the horizon of its applications. Nanotechnology has played some specific and crucial roles in environmental science like sensing and remediation of pesticides and in removal of pollutants, such as nitrates.<sup>1,2</sup> Other important applications can be seen in development of nanomaterials using nanoelectronics for applications, such as thin-film transistors, wearable electronics, as artificial skin and muscle, and in solar panels.<sup>3</sup> Many types of nanomaterials are available naturally or synthesized artificially through top-down and bottom-up approaches for applications in multiple arenas of sciences.<sup>4</sup> One of the nanomaterials is halloysite nanotubes (HNTs) whose assorted roles have been discussed in detail here.

Halloysite is naturally available in abundance and economically viable clay nanomaterial mined from deposits. The particles of halloysite can be found in variety of morphologies, such as short tubular, spheroidal and platy clays—kaolin and montmorillonite—and the most common being elongated tubes.<sup>5</sup> HNTs are the tubular forms of halloysite and are chemically similar to kaolin. They are layered aluminosilicates ( $\text{Al}_2\text{Si}_2\text{O}_5(\text{OH})_4 \cdot n\text{H}_2\text{O}$ ) with hollow tubular structure and high aspect ratio (Figure 1). The outer diameter is about 40–70 nm, inner diameter 10–20 nm, and length 500–1500 nm.<sup>7</sup>

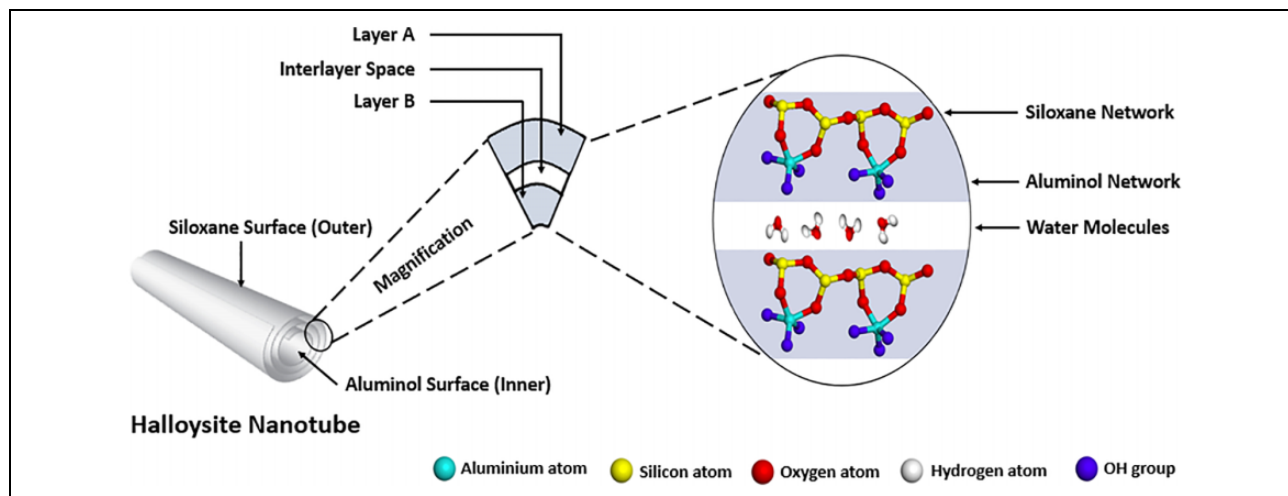
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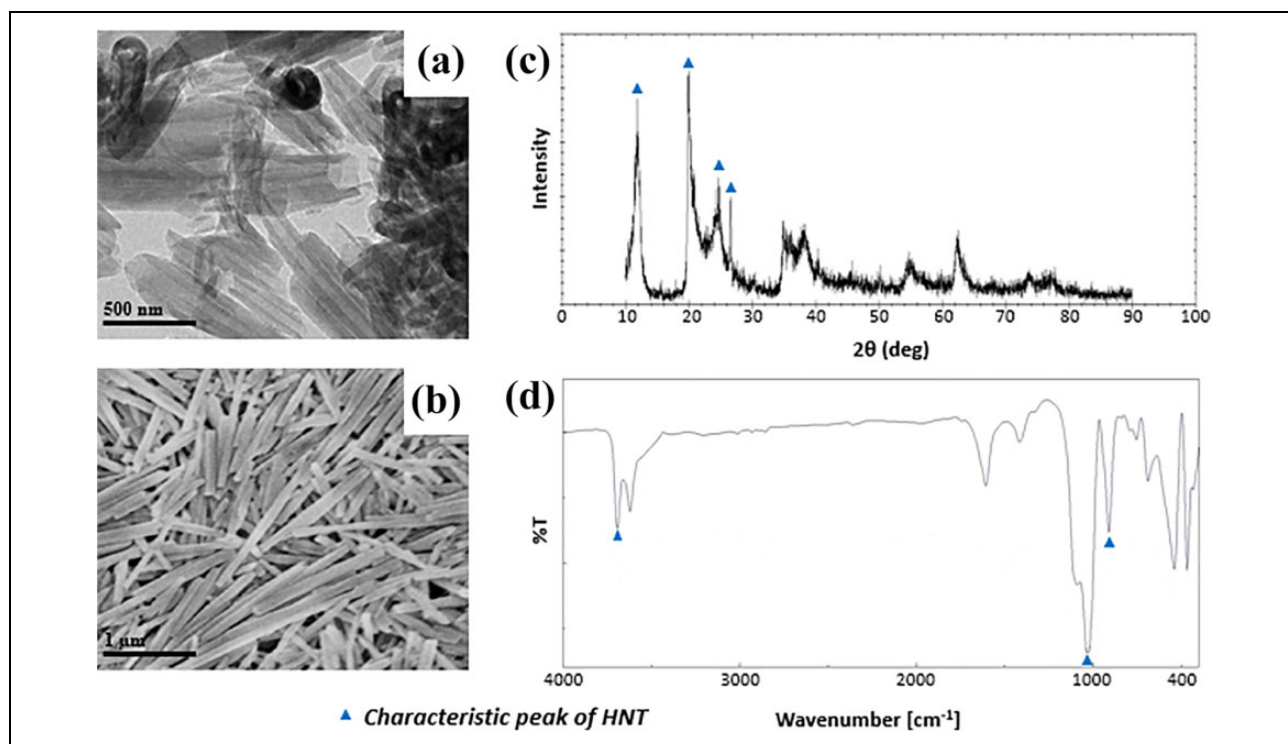
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**Figure 1.** Structure of halloysite nanotube.<sup>6</sup>



**Figure 2.** Characterization of HNT: (a) TEM, (b) SEM, (c) XRD, and (d) FTIR.<sup>8,9</sup> HNT: halloysite nanotubes; TEM: transmission electron microscopy; SEM: scanning electron microscopy; XRD: X-ray diffraction.

HNTs are sufficiently able to bind with many synthetic and biological components because of the larger area of the surface, positively charged inner surface (Al–OH groups), and negatively charged outer surface (Si–OH and Si–O–Si groups). HNT is usually characterized through transmission electron microscopy (TEM), scanning electron microscopy (SEM), X-ray diffraction (XRD), and Fourier transform infrared spectroscopy (Figure 2). The functional groups present on the surface of HNT also aid in the loading of negatively charged biomacromolecules into the

positive lumen of the nanotube such as DNA encapsulation. Such an interaction of DNA and HNT has been used to study DNA damage using HNT-gold nanoparticles (AuNP) and HNT-silver nanoparticles (AgNP) composites.<sup>10,11</sup> Similarly, DNA damage and repair have also been studied by binding HNT with a DNA intercalator, acridine orange.<sup>12</sup> The outer surface of HNT is negatively charged, whereas the inner lumen is positively charged in most of the pH settings, hence allowing numerous modifications.<sup>13</sup> This makeup combined with increased biocompatibility

and lower cytotoxicity gives them an important part in the advancement of recent applications, such as in biomedical sciences, namely production of novel drug and gene delivery agents, as scaffolds for tissue engineering, in wound dressings, for tumor cells isolation, and for enhanced human cells adhesion.

HNTs are easily dispersed in water as single particles or in polar polymers without requiring exfoliation procedures. In aqueous biological liquids, the behavior and colloidal properties of HNT are similar to that of silica nanoparticles. The zeta potential of pristine HNT is lower than that for pure silica particles, but the process of loading negatively charged drugs often increases the magnitude of the HNT zeta-potential, resulting in colloids stable for months.<sup>14</sup>

The silica outer surface can also be functionalized by various methods allowing for additional drug binding.<sup>15</sup> HNT possesses a one-dimensional tubular porous structure on the mesoporous (2–50 nm) and even macroporous (>50 nm) scale and is considerably larger than many synthetic porous materials such as carbon nanotubes. This property enables versatile potential applications such as nanoscale support for the loading of functional entities.<sup>16</sup> The larger inner diameter of HNT allows for loading not only small drug molecules but also proteins and DNA.<sup>17</sup>

HNTs have two types of hydroxyl groups in its inside and outside, which can be used as active sites for functionalization and drug loading through modifications. Diverse types and classes of drugs have been loaded onto the inner lumen of HNT. Also, studies have shown that HNTs are a novel and potential material for gene and anticancer drug delivery in cancer therapy such as curcumin or adriamycin as reported in Liu et al.,<sup>18</sup> for enhanced anticancer efficacy.<sup>19</sup> There has also been a study in which HNTs were grafted with  $\gamma$ -aminopropyltriethoxysilane (APTES) and delivered as antisense oligonucleotides toward HeLa cells.<sup>20</sup>

The commercially available HNTs are inhomogeneous and long nanotubes (100 nm–2  $\mu$ m). It has been reported that long nanotubes might act as inducers of cell injury and inflammation.<sup>5</sup> Therefore, reports have recommended that nanoparticles that have diameter less than 200 nm are more suited as carrier for drug delivery; this is because of the high endocytosis in contrast to the larger diameter nanoparticles. However, this characteristic of HNT does not hinder its applicability as shorter diameter nanotubes can be achieved by treating the long HNT with ultrasonication.<sup>19</sup>

HNTs have evolved as a unique support system for immobilization of biomolecules since it allows higher loading of enzymes without compromising their activities<sup>21</sup> like it has been used as nanosupport for immobilization of  $\alpha$ -amylase.<sup>22</sup> Also, the curved surface of the HNT support can repress the interaction between the nearby enzymes and facilitates multiple bond formation. This helps with reducing the chances of enzyme aggregation on the surface of the support which aids in regulating the structural integrity and biocatalytic activity of enzyme to a great extent.<sup>23</sup> The

catalytic activity of HNT-based nanocomposite for biological and environmental applications was enhanced by the incorporation of metallic nanoparticles onto HNT. This holds significant potential for catalytic and biosensing applications.<sup>24</sup> HNT-based nanocomposites have been discussed in many other areas as well such as optical, electronic, and magnetic applications due to their exceptional properties.<sup>25</sup>

There have been researches which have reported drugs, DNA, proteins, antiseptics, anticorrosives, and flame retardants into the HNT with long shelf-lives and release profile of 5–12 h in water. This increased release rate was notably commanded by tube openings by means of diffusion. Also, capping the tube ends or embedding drug-loaded tubes into bulk polymers even allow for slower release up to days, weeks, or even months.<sup>26</sup> HNT can enwrap enzymes for increased storage time under high temperatures and with prolonged functionality, which aid the delivery of small substrate molecules into the inside of the tube for biocatalysis. Loading DNA into HNT is another prospective as functional nanoblocks. HNT can be used for enhancing the shells of microorganisms with extra functions (e.g. formation of spore-like microbial).<sup>21</sup>

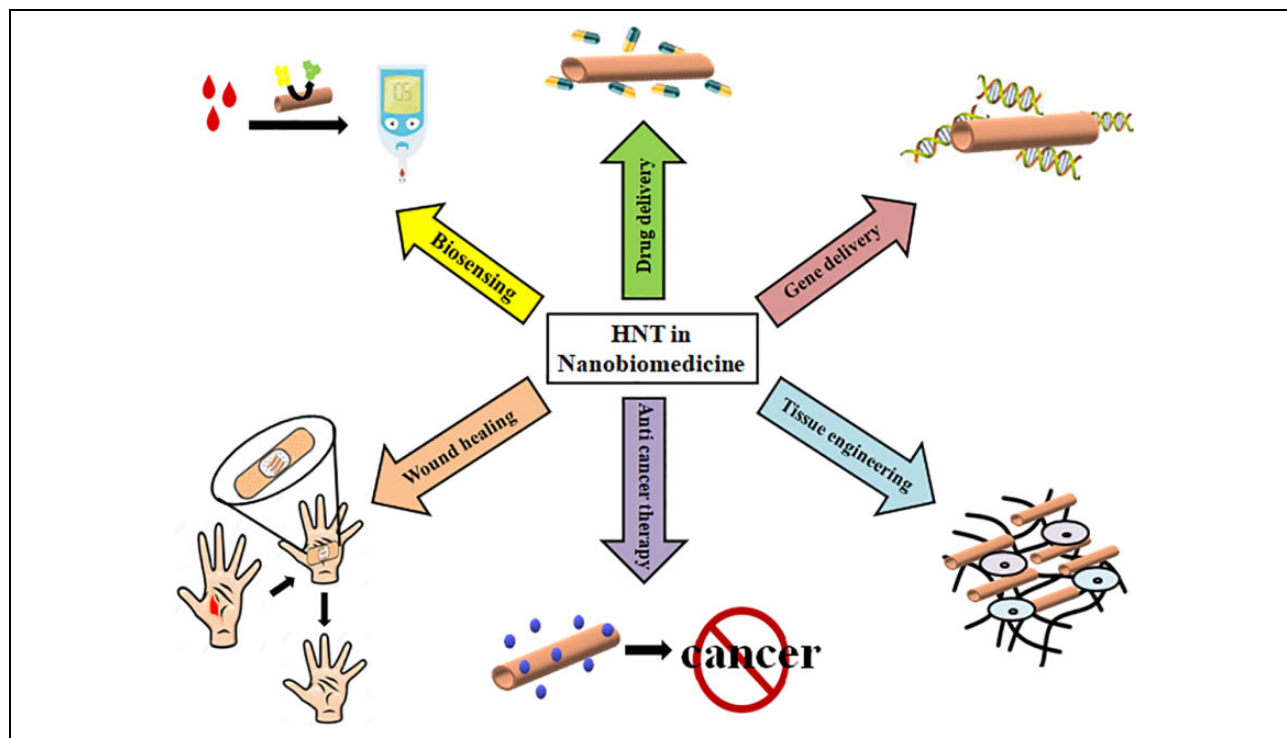
HNT can induce processes related to cell growth and proliferation, elusive cell responses to infection, injury and irritation, improved antioxidant ability, along with an overall accommodative reaction to exposure. This was analyzed through bioinformatics analysis of variably expressed protein profiles.<sup>27</sup>

The complete set of properties mentioned strengthen the role of HNT as innate nanocarriers for loading and controlled release of chemical and biological molecules like drugs, proteins, and DNA; agents for antibacterial, biocide, and antifouling activity; chemicals such as for anticorrosion, flame-retention, and polymers with self-healing property.<sup>21</sup>

## Toxicity studies

Biocompatibility of HNT is one of the main necessities for the usage of HNT as biomaterial in anticancer therapy and sustained delivery of biologically active substances in medical, pharmaceutical, and personal care products. The use of HNT has increased over the past few years, so it requires that the toxicity toward living organisms be assessed in detail. The toxicity assays of different human cell lines (e.g. epithelial adenocarcinoma cells, and dermal fibroblasts) to HNT are used to support the biocompatibility of the nanotube. The same was analyzed with animal cells like yeast, bacteria, algae, and microworms.<sup>14</sup>

Numerous reports have shown investigation of HNT for cytotoxicity in vitro when used on human cell cultures and microbial cells. When conjugated with iron oxide ( $\text{Fe}_3\text{O}_4$ ) nanoparticles, HNT reduced the cytotoxicity of iron oxide nanoparticles by changing their surface characteristics and suppressing the inherent toxicity on bacterial cells.<sup>28</sup>



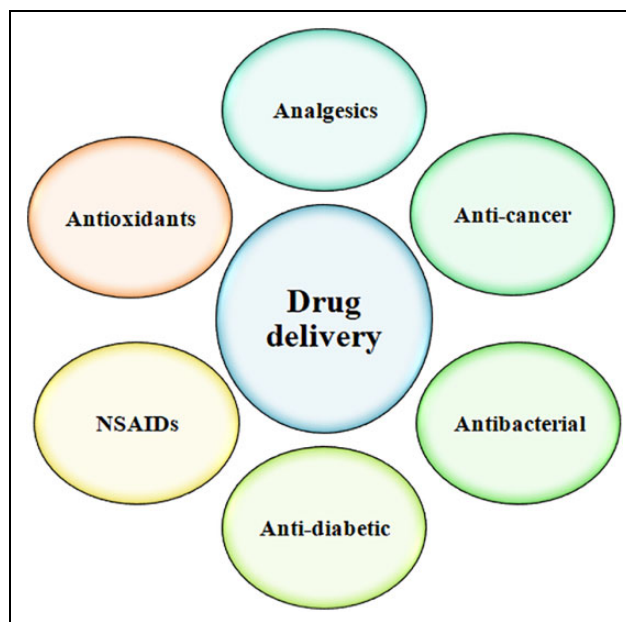
**Figure 3.** Applications of halloysite nanotubes in biomedicine.

There have only been limited studies which have examined HNT's cytotoxicity through in vitro studies against some cancer cell lines like MCF-7, HeLa, NIH-3T3, and A549 to name a few, but has shown promising results.<sup>29</sup> A very recent in vivo toxicity study in a soil nematode *Caenorhabditis elegans* has also demonstrated high safety prospects of this novel clay even at high concentration of  $100 \text{ mg mL}^{-1}$ .<sup>30</sup>

Furthermore, a newer study on the human peripheral lymphocytes by mitotic index assay had verified that HNT applied pertinent cytogenetic toxicity at  $100 \text{ mg mL}^{-1}$  only by hindering the passage of cell cycle. The same study also showed that the safety profile against the two model cell lines and human peripheral lymphocytes strongly back and validate for in vivo toxicity study of HNT and which makes an important case for commercial pharmaceutical and biomedical applications based on earlier evidence to sustain the release of loaded drugs.<sup>31</sup>

Moreover, toxicity effects of HNT and various other nanoclays and graphene oxide were evaluated on *Paramecium caudatum*. Toxicities of silica, graphene, montmorillonite, kaolin, and bentonite were compared with that of HNT; and the outcomes show that HNT is one of the safest clay for biological applications.<sup>32</sup> Hence, HNT has many practical and valuable applications in biological and related disciplines (Figure 3).

This review compiles the major purposes fulfilled by HNT in bio- and nanomedicine in the recent past and also about the future prospects of this novel nanomaterial in the biomedical and nanomedicine sciences.



**Figure 4.** Types of drugs loaded into halloysite nanotubes.

## Applications of HNT in biomedicine

### Drug delivery

There have been numerous studies and reports where HNTs have been used as nanocontainers or nanocarriers for drug delivery as well as targeted drug delivery (Figure 4). There are several ways of loading drugs into the lumen or onto the

surface of HNT, the major ones being adsorption, intercalation, and tubular entrapment (vacuum method).<sup>13,33,34</sup>

HNT is usually utilized as delivery agent by making them undergo some kind of modifications, either to the outer surface or the lumen. This is done because in many cases, natural HNT has shown weak bonding with the drugs, hence rendering sustained release impossible. But there have been reports otherwise, where natural HNTs were used as cores for layer-by-layer (LbL) encapsulation. This resulted in increased loading and continual release of drugs for up to 100 h and the formation of tube-end stoppers helped in prolonged drug release.<sup>35</sup> Natural HNTs were also used to design a biocompatible drug delivery agent for rabeprazole sodium (RAB) to tackle its acidic degradation in the stomach. The results were positive with sustained release and increased bioavailability of the drug.<sup>36</sup>

Prior to the loading of drugs on HNT, several strategies have been employed to modify its surface. One of the methods is modifying the surface of HNT with APTES. It is an organosilane and the technique is a renowned functionalization method due to their ease of usage and low toxicity. The organosilane acts as an intermediate medium for the binding of any desired molecule. APTES introduces silanol groups which bonds (hydrogen bonding) with the hydroxyl groups on the HNT surface.<sup>6</sup> HNTs modified with APTES were used as carrier for aspirin, and the results showed increased loading of 11.8 wt% of aspirin from 3.84 wt% (without modification). The restricted space of HNT resulted in change of material state of aspirin to nanocrystalline and amorphous, but this increased the dissolution rate produced by the first hour of burst release.<sup>37</sup> Release profiles of ibuprofen (IBU) loaded modified (APTES-HNT) and unmodified HNTs were studied. The APTES-HNT demonstrated enhanced drug loading and better release profile in accordance with the adapted model, Korsmeyer–Peppas.<sup>38</sup> APTES-HNT was then used as a nanocarrier for ciprofloxacin (CIP) for sustained release and to subdue the complexation of CIP with iron which reduces the drug's bioavailability. The functionalized HNT showed  $70\% \pm 1.7\%$  loading of CIP on it along with sustained release in Phosphate Buffered Saline (PBS) ( $92\% \pm 3\%$ ) up to 9 h. The functionalized nanotube also decreased the absorbance of iron by  $90\% \pm 1.3\%$  within 2 h.<sup>9</sup>

HNT modified with chitosan has also become an emerging porous microsphere for drug carriers. In this study, chitosan-modified HNT was loaded with aspirin for drug release studies. The loading of aspirin into the porous microspheres was 42.4 wt% which is around 20 times higher than the pristine halloysite (2.1 wt%).<sup>39</sup> Folic acid (FA)-conjugated chitosan oligosaccharide-magnetic HNTs were studied as a delivery system for camptothecin, which is an anticancer drug. HNT showed a high storage capacity of camptothecin and in vitro release results indicated that camptothecin outflow from the nanocarrier at pH 5 was much greater than that at both pH 6.8 and 7.4. This

pH-dependent release study is vital as the microenvironment in tumor tissues, endosomes, and lysosomes is acidic. For toxicity assessment, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays showed that the camptothecin-loaded HNT exhibited stronger cell growth inhibition against colon cancer cells.<sup>40</sup>

Doping of the HNT into a matrix is a well-exploited methodology as a support system and for controlled release of drugs. One such study made a ceramic skeleton or support system using HNT-doped polymeric matrix into which functional chemicals were loaded. Further variations, such as artificial nanocaps at tube endings and enlarged lumen by selective etching, were done to the nanotube. This improved the loading capacity (ca. 30 wt%) and release time from 10 to 200 h. It can be noted that no surface modifications were performed on HNT but it was still miscible with high and medium polarity.<sup>41</sup> In yet another study, sodium alginate/hydroxyapatite/HNT nanocomposite hydrogel beads were in situ generated as matrices for controlled drug release. Diclofenac sodium (DS) was loaded into the nanotubes and analyzed for release rate and entrapment efficiency, which was up to 75.11% with constant release rate.<sup>42</sup>

A different approach was reported, where HNT was analyzed for effects of alkali activation and then loaded with ofloxacin (OFL). This method showed prolonged release of OFL which was adsorbed onto the surface of HNT. This gave an efficient method to enhance adsorption and release of cationic drugs.<sup>43</sup> Also, HNT subjected to acid treatment and polymer-halloysite composite techniques were reported for release studies of sodium salicylate. The results in both the cases were improved loading and controlled in vitro release of the drug which was shown by four different drug release models: zero order, first order, Higuchi equation, and Korsmeyer–Peppas equation. The polymer/HNT composite showed sustained release over longer period than acid-treated and unmodified HNT.<sup>42,44</sup>

HNT was integrated into electrospun poly-ε-caprolactone (PCL) scaffolds to enhance the structural consistency. The HNT present in the scaffold acted as the nanocarrier for antibacterial agents like amoxicillin, chlorhexidine, brilliant green, doxycycline, gentamicin sulfate, iodine, and potassium calvulanate. These nanocomposites were observed to have inhibitory action against bacterial growth for up to 1 month. The results obtained show promising application of such nanocomposites for surgical dressings and sutures and with no damage to material properties.<sup>15</sup>

Biocompatible poly (*N*-isopropylacrylamide) was grafted onto HNT for thermo-responsive curcumin release studies. In vitro tests were performed which simulated the gastrointestinal transit of the HNT delivery system loaded with curcumin, a natural anticancer molecule. The results showed a targeted release of the active species into the intestine.<sup>45</sup> HNTs functionalized with cysteamine by

disulfide linkage were used to create a dual responsive nanocarrier which is loaded with curcumin and depended on intracellular glutathione and pH conditions. The direct chemical grafting with stimuli receptive linkage is an effective approach for nanocontainers with sustained drug release.<sup>46</sup>

Anticancer drug paclitaxel was encapsulated in HNT for intestinal and intracellular drug delivery studies. The polymer poly(methacrylic acid-co-methyl methacrylate) which is pH respondent was coated over the HNT for maximum drug release in the intestinal tract. At higher pH such as in digestive tract, triggered drug release was observed. HNT containing tablets loaded with paclitaxel were produced with controlled drug release properties. The anticancer effects of the drug were studied in vitro on human cancer cells with good results.<sup>47</sup> Another anticancer drug, doxorubicin (DOX) was loaded into multifunctional HNT and analyzed for targeted delivery and sustained drug release. In vitro studies were performed on targeted and nontargeted HNT which showed that targeted HNT sped up the apoptosis of cancer cells. In vivo studies reinforced that the targeted HNT did not have any side effects in the tumor-bearing nude mice as free DOX had damaged the tissues earlier.<sup>48</sup> (Poly-ethylene-glycol)-amine (PEG)-grafted HNTs decorated with carbon dots for additional fluorescent properties were used to enhance the loading and sustained release of quercetin, a low water-soluble drug. Quercetin has antioxidant properties, hence the aim was to target the tumor tissues. For the ease of targeting and increased cellular uptake, biotin was conjugated to the PEG's free amine groups.<sup>49</sup>

Dendrimer functionalization is a yet newer approach and has shown promising results in drug delivery studies. HNTs were functionalized with polyamidoamine dendrimer for drug delivery studies of chlorogenic acid, IBU, and salicylic acid. The dendrimer functionalized HNT showed higher absorption capacity for three drugs as compared to raw HNT and APTES-HNT. There was no damage to the living organisms seen in the in vivo toxicity studies of dendrimer functionalized HNT.<sup>50</sup>

Hence, HNTs are natural nanocontainers for drug loading and sustained release (in pristine or modified form) for both low water-soluble drugs (aspirin, IBU, CIP, curcumin, and DS) as well as water-soluble drugs (amoxicillin and sodium salicylate) with good results, therefore it shows the versatility of HNT as nanocarriers for loading and release of variety of drugs. Table 1 summarizes the modification strategies of HNT, types of drug loaded, site of drug loading, loading percentage, release rate, release models, and applications of the modified HNT. It can be used for oral formulations in tablet form or doped into polymeric matrices for other applications or modified in numerous ways for controlled release of drugs. Also, HNTs are safe and almost nontoxic for human and bacterial cell cultures in vivo.<sup>26</sup>

## Gene delivery

In the recent years, HNT has come forth as a nonviral gene delivery agent because of properties like biocompatibility, negligible toxicity toward human and bacterial cells, and high mechanical strength.

A study was conducted, where an HNT-based gene delivery carrier was developed for the delivery of intracellular antisense oligodeoxynucleotides (ASONDs) for targeting survivin (a protein belonging to inhibitor of apoptosis family). APTES-functionalized HNT was used to show the better intracellular delivery and improved anticancer activity of ASONDs (bound to the outer surface of functionalized HNT), hence rendering a potential vector for gene delivery.<sup>20</sup>

Polyethyleneimine (PEI) modification of HNT has been reported to be efficient for gene delivery, such as short-interfering RNA (siRNA) and plasmid DNA (pDNA). Such modified HNTs were used for the intracellular delivery of therapeutic antisurvivin siRNA. The Western blot analysis showed that PEI-HNT-mediated siRNA delivery decreased the target proteins PANC-1 cells levels by efficiently knocking down gene expression of survivin, thus reinforcing its potential as cancer therapeutic agent.<sup>51</sup> A recent study was conducted using PEI-grafted HNT (PEI-g-HNT) to bind green fluorescence protein labeled pDNA onto the surface of modified HNT. PEI-g-HNT showed less cytotoxicity than PEI and also had better transfection efficiency.<sup>19</sup>

Gene therapy holds great potential as a clinical treatment for cancer and genetic disorders. But to accomplish this, the gene carriers need to fulfill many delicate criteria, such as cytotoxicity and biocompatibility, which makes this route of treatment intricate. The use of HNT is still an ongoing exploration which has seen potentially viable results, hence opening up an imperative area of research in biomedicine.

**Tissue engineering.** The formation of nanocomposite as support matrix for controlled drug release, polymer nanocomposite, nanotemplating, and catalytic support has also been reported.<sup>25,52</sup> HNT has been used as drug delivery agent for various kinds of targets as mentioned above, hence it also found its role as a delivery agent for tissue engineering. For instance, alkaline phosphatase (ALP) was incorporated into HNT for bone repair. HNT acted as a heat sink in the system which increased the thermal stability of the ALP. It also enhanced the activity of ALP highly, thus promoting the biomineralization process which was studied in vitro using a substrate, calcium glycerophosphate. This bioactive nanocomposite can also be incorporated into biomaterials used as scaffolds for tissue engineering.<sup>53</sup>

A crucial problem that needs to be attended to in tissue engineering is forming a scaffold, which is competent enough to support the three-dimensional tissue formation

**Table 1.** Applications of halloysite nanotubes in drug delivery.

S. no.	Drug	HNT and modifier	Advantage	Drug loading (wt%)	Site of drug loading	Release rate (wt%/mg g <sup>-1</sup> h <sup>-1</sup> )	Release kinetics model	Application	References
1.	DS	Sodium alginate/hydroxyapatite/HNT nanocomposite hydrogel beads	<ul style="list-style-type: none"> <li>Cumulative release behavior</li> </ul>	74.63 ± 1.65	Lumen	9.19 mg g <sup>-1</sup> h <sup>-1</sup>	—	Controlled drug delivery	42
2.	IBU	APTES-modified HNT	<ul style="list-style-type: none"> <li>Slow release due to strong affinity through electrostatic attraction</li> </ul>	14.8	Lumen and partially on outer surface	—	Korsmeyer–Peppas model	Controlled release of drug	38
3.	Aspirin	APTES modified or natural HNT	<ul style="list-style-type: none"> <li>Increased amount of aspirin loading</li> </ul>	11.98	Lumen pores	—	Higuchi kinetic model	Controlled release of drug	37
4.	Amoxicillin, brilliant green, chlorhexidine doxycycline, gentamicin sulfate, iodine, and potassium calvulanate	HNT-PCL scaffolds	<ul style="list-style-type: none"> <li>Enhancers of structural integrity</li> <li>Inhibition of bacterial growth</li> </ul>	—	Lumen	—	—	Sutures and surgical dressings	15
5.	Thermo-responsive curcumin	Poly(N-isopropylacrylamide)-HNT	<ul style="list-style-type: none"> <li>Brownian diffusion and electrophoretic mobility dependent on temperature</li> </ul>	3.6	Lumen	Approximately 10 pH 6.8	—	Biocompatible thermo sensitive materials for controlled release	45
6.	Curcumin	Dual responsive nanocarrier	<ul style="list-style-type: none"> <li>Covalent linking through GSH-responsive bonds.</li> <li>Good stability and retaining of antioxidant property of curcumin.</li> </ul>	2.9 wt%	Covalently linked to outer surface	Approximately 25% in acidic pH	Power fit equation	Controlled release of prodrug for anticancer therapy	46
7.	RAB	—	<ul style="list-style-type: none"> <li>Prevents comparative acidic degradation of RAB in stomach</li> </ul>	8.355 ± 1.542	Lumen	94% up to 24 h	—	Increased bioavailability and sustained drug release	36
8.	Paclitaxel	HNT coated with pH-responsive polymer poly(methacrylic acid-co-methyl methacrylate)	<ul style="list-style-type: none"> <li>Hindered release in acidic pH and increased release in basic pH</li> </ul>	7.5 ± 0.5	Lumen	40% at 120 h	Korsmeyer–Peppas model	Sustained release for anticancer therapy	47

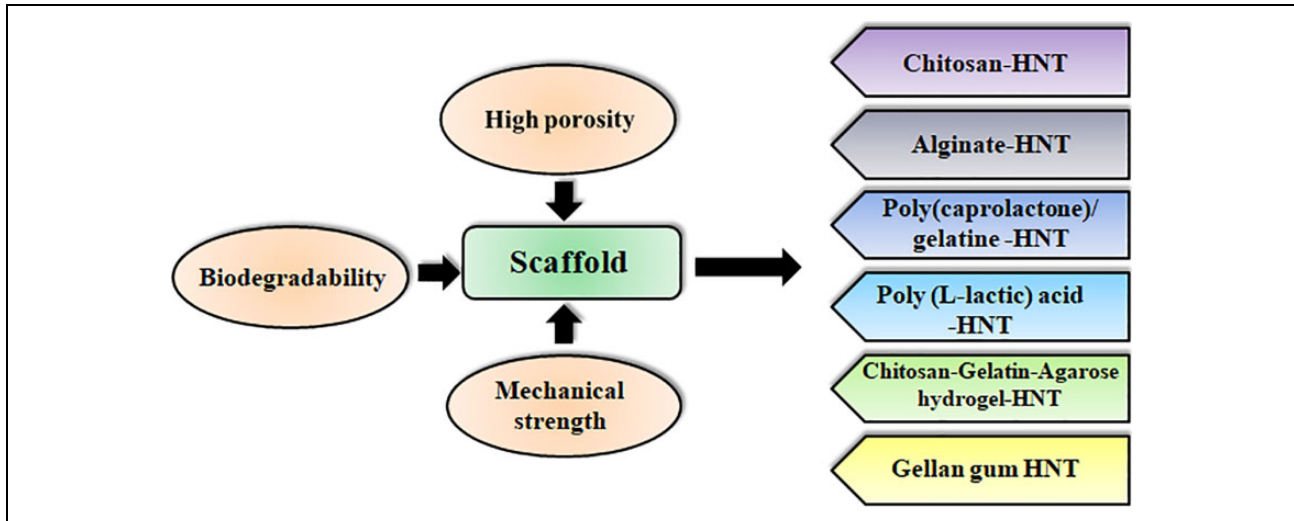
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Table 1. (continued)

S. no.	Drug	HNT and modifier	Advantage	Drug loading (wt%)	Site of drug loading	Release rate (wt%/mg g <sup>-1</sup> h <sup>-1</sup> )	Release kinetics model	Application	References
9.	Doxorubicin	MPTS-HNT	<ul style="list-style-type: none"> <li>Folate-mediated targeting and redox-responsive drug delivery.</li> <li>Promotes apoptosis of cancer cells in vitro</li> </ul>	14.8	Outer surface	40% at 79 h	—	Anticancer therapy	48
10.	CIP	APTES functionalized HNT	<ul style="list-style-type: none"> <li>Increased drug loading.</li> <li>Decrease in iron absorption after complexation with CIP</li> </ul>	70% ± 1.7%	Outer surface	92% ± 3%	—	Increased bioavailability of the drug upon administration	9
11.	CPT	Folic acid –conjugated chitosan oligosaccharide-magnetic HNTs	<ul style="list-style-type: none"> <li>Stronger cell growth inhibition against colon cancer cell.</li> </ul>	227.10 mg g <sup>-1</sup>	Lumen	Sustained release up to 60 h	—	Tumor-targeted drug delivery	40
12.	Chlorogenic acid, ibuprofen, and salicylic acid	Dendrimer functionalized HNT	<ul style="list-style-type: none"> <li>Carrier for low molecular size drugs</li> <li>No toxicity to living organisms</li> <li>Enlarged the lumen of HNT.</li> <li>Delayed the release of the drug.</li> </ul>	123.16 mg g <sup>-1</sup> (chlorogenic acid); 182.72 mg g <sup>-1</sup> (IBU); 39.52 mg g <sup>-1</sup> (salicylic acid)	Outer surface	—	—	Controlled release of drugs	50
13.	Sodium salicylate	Acid treatment and composite polymer halloysite modification	<ul style="list-style-type: none"> <li>Improved drug loading</li> </ul>	—	Lumen	—	Zero order	Controlled release of drugs.	44
14.	Quercetin	PEG amine-grafted HNT	<ul style="list-style-type: none"> <li>Improved drug loading</li> </ul>	278.36 mg g <sup>-1</sup>	Outer surface	—	First order Higuchi Korsmeyer–Peppas model	Controlled release with antitumor targeted drug delivered	49

DS: diclofenac sodium; IBU: ibuprofen; PCL: poly-ε-caprolactone; RAB: rabeprazol sodium; MPTS: (3-mercaptopropyl) trimethoxysilane; CIP: ciprofloxacin; CPT: camptothecin; PEG: polyethylene glycol; ATC: anaplastic thyroid cancer; APTES: aminopropyltriethoxysilane; HNT: halloysite nanotube.





**Figure 5.** Properties and types of scaffolds for tissue engineering.

(Figure 5). Such scaffolds should meet basic specific requirements such as:

- (i) High porosity with adequate pore size (to facilitate seeding and diffusion of nutrients).
- (ii) Biodegradability and degradation rate (scaffold should be absorbed by tissue surrounding it along with new tissue formation).
- (iii) Mechanical strength for strong support for growth of new tissue.

The microstructures of the scaffolds could be of various types like open pore structures, fibrous matrices, hydrogels, and other natural and synthetic materials.<sup>54</sup> The use of HNT for tissue engineering is a relatively new venture; hence, there have been only few promising researches during the past decade. The core idea while using HNT is to couple it with various compounds which will make potential scaffolds for tissue and bone growth.

The chitosan-HNT nanocomposite scaffolds showed considerably improved compressive strength, compressive modulus, and thermal stability as compared to pure chitosan scaffold. Although HNT did not affect the porous structure and porosity of scaffolds that much, it also did not exert any cytotoxicity to cells. Also, it was observed that the cells attached and developed well on the scaffold.<sup>54</sup> Chitosan-HNT was also produced by electrospinning with 0, 2, and 5 wt% HNT. The nanocomposite showed increasing Young's modulus and maximum tensile strength with increasing HNT concentration. This is an ideal membrane with improved mechanical properties and thermal stability for bone tissue engineering.<sup>55</sup> Another compound coupled with HNT was alginate to form a scaffold which showed high compressive strength, decreased water adsorption, and degradation rate. HNT enhanced the thermal stability of the alginate scaffold and also increased the cell attachments by

increasing the surface roughness and biocompatibility.<sup>56</sup> The physical properties of a sodium-alginate scaffold were improved by incorporating HNT and the composite was then cross-linked with calcium ions. At low HNT loading, the composite scaffold showed increased cell adhesion and proliferation in preosteoblast (MC3T3-E1) culture, which will be useful in bone tissue engineering.<sup>57</sup>

A study developed metronidazole-loaded HNT doped into poly(caprolactone)/gelatin microfibers by electrospinning as membranes with sustained drug delivery for guided tissue or bone regeneration. This inorganic-organic, anti-infective implant and drug delivery membrane can be used in various therapeutic applications requiring time extended functions, including pathologies demanding chronic drug treatments, wound healing, prevention of postsurgical adhesions, and tissue-engineering applications.<sup>58</sup> Electrospun poly(L-lactic) acid nanofiber scaffolds have been reinforced with unidirectionally aligned HNT increasing the tensile strength, Young's modulus, and fracture strain.<sup>59</sup> A multilayered polylactic acid (PLA)/HNT porous membrane encapsulated with aminoglycoside antibiotic (gentamicin) was prepared as an antibacterial membrane for bone regeneration. It was found to have good antibacterial efficacy against Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria, hence was proposed as a potential use in prevention of infection in bone regeneration applications.<sup>60</sup>

An in vitro and in vivo study was performed which strengthened the fact that HNT-doped scaffolds are biocompatible. Freeze drying method was used to dope porous biopolymer hydrogels at 3–6 wt% with 50-nm diameter/0.8- $\mu$ m HNT without any cross-linkers. A chitosan-gelatin-agarose hydrogel doped with HNT showed augmented mechanical strength, higher water uptake, and thermal properties. The scaffolds implanted in rats showed very

good resorption at 6 weeks also. The newly formed connective tissue placed near the scaffold showed complete restoration of blood flow due to neovascularisation.<sup>61</sup>

Yet another study integrated HNT in gellan gum matrices to develop composite hydrogels with controllable physical features. It showed a good human dermal fibroblasts biocompatibility when the cells were seeded on the top of the gels or encapsulated within the polymeric matrix. Fibroblasts onto the hydrogels with HNT exhibited high metabolic activity due to enhanced mechanical and topographical features because of HNT. These scaffolds prove to be suitable for different soft tissue engineering applications (pancreas, liver, skin, and chondral regeneration).<sup>62</sup>

Even though there are only few studies that have researched the role of HNT as bionanocomposites for tissue engineering, the current trend of studies is promising of a huge role of HNT in tissue engineering.<sup>63</sup>

### Wound healing

The tubular HNT has high mechanical strength, good biocompatibility, and hemostasis property which make them compatible for wound healing applications. HNTs were studied for their possible use as biocompatible nanocontainers for gradual and controlled release of antiseptics. Multiple studies have shown the use of the nanotubes for loading of antibacterial and antiseptic drugs for wound healing applications. Benzotriazole-copper coated HNT was loaded with brilliant green (antiseptic), which had shown sustained release (50–200 h) from the nanotube. The study also showcased controlled release of amoxicillin and iodine from the HNT.<sup>64</sup>

Another approach was to use HNT-based nanocomposite to make dressings to be used in wound healing. Three-dimensional, porous and flexible chitosan composite sponges were formed via the addition of HNT which had increased elastic modulus, compressive strength, and toughness. Also, it was noted that the HNT improved chitosan's blood clotting ability. In vivo tests confirmed the composite sponges to be cytocompatible with enhanced wound healing properties.<sup>65</sup> Better skin reepithelization and reorganization was demonstrated by chitosan oligosaccharides modified HNT, which were finer than that by HNT or chitosan separately. The wound healing by the composite is accelerated by enhancing the activity of inflammatory and repairing cells, which in turn is the resultant of the sustained release from HNT and chitosan oligosaccharides (homo- or heterooligomers of *N*-acetylglucosamine and *D*-glucosamine), making this nanocomposite a potential medical device for wound repairing.<sup>66</sup>

Likewise, HNTs were used as carriers of vancomycin in an alginate-based wound dressing. The demonstrated new dressing exhibited high stability and neutral character in relation to the living organisms applied in the bioassays. The results indicated that the designed potential wound dressing with optimized parameters can be an effective

strategy in long-term treatment of wounds.<sup>67</sup> CIP and polymyxin B sulfate-loaded HNT (HNT-B) were combined into a gelatin elastomer to form a double drug codelivery, elastic and antibacterial nanocomposite. The CIP with antibacterial activity was diffused into the nanocomposite matrix, whereas HNT-B was first loaded into the HNT and later dispersed in the matrix. The in vitro drug release behavior and the antibacterial properties improved significantly; this was attributed as the influence of CIP and HNT-B on the physical characteristics, cytotoxicity, fibroblast proliferation, and adhesion. This particular bionanomaterial has effective properties like high water absorbing quality, low cytotoxicity, adjustable biodegradability, and good elasticity, which are idyllic for wound healing application.<sup>68</sup> HNT also strengthened an elastic nanofibrous material made of PCL and gelatin which can be used as wound dressings with sustained drug release.<sup>69</sup>

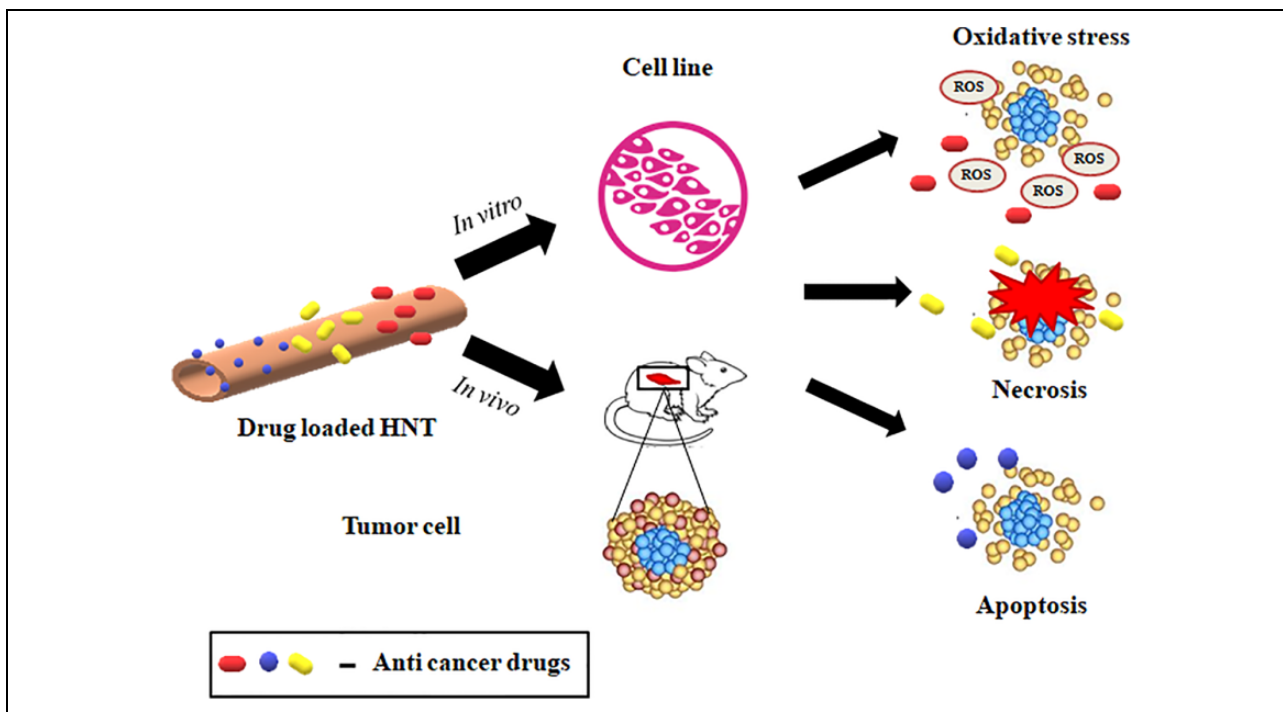
### Cancer therapy and isolation of stem cells

The capability to capture rare circulating tumor cells from the blood of cancer patients provides a significant advance in cancer study, diagnosis, and treatment on a patient-to-patient basis.<sup>70</sup> Chemotherapy has greatly made use of vehicles capable of targeted drug delivery; hence, naturally, HNT was also considered and studied upon for the same. Several approaches have been explored for such targeted drug delivery by HNT which have been discussed in Figure 6.

Firstly, direct loading of the drug onto HNT and its effects on a cell line was assessed. HNT was loaded with resveratrol and its delivery to the cancer cells was analyzed. MTT assays were performed with a neoplastic cell lines model system (MCF-7) which showed that the resveratrol-loaded nanotubes strongly increased the cytotoxicity, thereby leading to cell apoptosis. These findings were correlated with the concentrations and the incubation times of the drug loaded HNT.<sup>71</sup> Multicomponent HNT was examined as a vehicle to guide the delivery of anticancer drug DOX into cancer cells. The drug loaded FA-Fe<sub>3</sub>O<sub>4</sub> HNT was found to be toxic to HeLa cell lines leading to apoptosis of cells.<sup>72</sup>

Further, a multicavity HNT–amphiphilic cyclodextrin hybrid was formed for codelivery of natural drugs (silibinin and quercetin) into thyroid cancer cells. The interaction between cells and the carrier revealed that the materials were uptaken into the cells surrounding the nuclei. Therefore, it was surmised that the multicavity systems could transport drugs into living cells safely.<sup>73</sup>

Curcumin is an antioxidant property which has been utilized for anticancer efficacy; several techniques have been used to load curcumin into HNT for the same. In one such method, positively charged HNTs were functionalized with trizolium salts. These HNT-based carriers of curcumin were used for drug delivery to various cell lines. The



**Figure 6.** Halloysite nanotube in cancer therapy.

trizolium salts functionalized HNT loaded with curcumin were found to be active in many of the tumor cells.<sup>74</sup>

Also, biopolymer-grafted HNT was reported for the targeted delivery of anticancer drugs. In a study, chitosan-grafted HNT (HNT-g-CS) were studied for the delivery of curcumin to cancer cells. The HNT-g-CS loaded with curcumin demonstrated definite toxicity toward several cancer cell lines, such as HepG2, MCF-7, SV-HUC-1, EJ, Caski, and HeLa; out of which EJ cell line showed increased apoptosis. The content of reactive oxidative species (ROS) produced by curcumin loaded HNT-g-CS is more than by free curcumin, hence making a potential anticancer drug delivery vehicle.<sup>18</sup> HNT grafted with chitosan oligosaccharide was also used for delivery of DOX for breast cancer using MCF-7 cell lines. The DOX-loaded HNT-g-CS entered MCF-7 cells and triggered mitochondrial damage and attacked nuclei.<sup>75</sup> Another study was conducted on the cancer drug delivery efficacy of Chitosan modified HNT loaded with curcumin-gold hybrid nanoparticles. These HNT hybrid nanoparticles consisted of AuNP which have near-infrared (NIR) responsive property and pH-responsive curcumin release, hence making it a good candidate for targeted drug delivery of cancer cells with NIR imaging.<sup>76</sup>

In yet another study, nanotube-in-microsphere which is a fascinating new design that incorporated microfluidics, was implemented to make a drug delivery vehicle by encasing HNT in a pH-sensitive hydroxypropyl methylcellulose acetate succinate polymer. Atorvastatin and celecoxib were used as model drugs because they have dissimilar

physicochemical properties and interactive effect on prevention and inhibition of colon cancer. The HNT/pH-responsive polymer composite prevented the premature release of the drugs at pH 6.5 while it allowed faster release and increased drug permeability at pH 7.4. These observations were made simultaneously with the occurrence of inhibition of proliferation of the colon cancer cells.<sup>77</sup>

Another fast, low-cost, and effective fabrication method was reported for fabricating large rough HNT coatings by thermal spraying of HNT ethanol dispersions. The enhanced surface communications between HNT coating and tumor cells led to effective capturing of the cancer cells as compared to the normal cells captured by the HNT coatings (except with HeLa cells). The HNT coatings were also effective in capturing tumor cells in artificial blood and blood samples from patients with metastatic breast cancer. This superior capture capability holds good potential for early diagnosis and examination of cancer. The high killing ability of the DOX-loaded HNT was also confirmed and hence it can be used as an implantable therapeutic device for preventing tumor metastasis.<sup>78</sup>

HNTs have been creatively used in isolation of stem cells as it was seen advantageous in capture of tumor cells previously. A research showed three-dimensionally printed PLA pattern functionalized with a polydopamine interlayer, this helped in firmly binding the HNT on the surfaces of the PLA pattern in order for guided cell orientation. The roughness and hydrophilicity of PLA pattern were improved significantly by HNT and the in vitro human

**Table 2.** Halloysite nanotube in cancer therapy.

S. no	Drug loaded	Modification of HNT	Cell line	Target	Reference
1.	Doxorubicin	FA and magnetite nanoparticles grafted onto HNT	HeLa	Anticancer therapeutics	72
2.	Resveratrol	LbL polyelectrolyte multilayer functionalization of HNT	MCF-7	Breast cancer	71
3.	Curcumin	Trizolium salts functionalization	Several cell lines	ATC, hepatic cancer	74
4.	Silibinin and quercetin	HNT-amphiphilic cyclodextrin Hybrids	Human anaplastic thyroid cancer cell lines 8505C	Thyroid cancer	73
5.	Atorvastatin and celecoxib	HNT encapsulated in pH-responsive hydroxypropyl methylcellulose acetate succinate polymer	Caco-2 HT-29	Colon cancer	77
6.	Curcumin	Chitosan-grafted HNT	HepG2 MCF-7 SV-HUC-1 EJ Caski HeLa	Anticancer therapeutics	18
7.	Doxorubicin	Chitosan oligosaccharide modified HNT	MCF-7	Breast cancer	75
8.	Curcumin	AuNP in lumen and chitosan-coated HNT	MCF-7	Anticancer drug delivery	76

FA: folic acid; HNT: halloysite nanotube; AuNP: gold nanoparticle; LbL: layer-by-layer.

mesenchymal stem cells culture analyses showed that PLA pattern with HNT exhibited different ability to induce cell orientation according to different stripwidths.<sup>79</sup> Also, a multifunctional nanocomposite was made of supermagnetic HNT functionalized with chitosan (M-HNT) and was then adorned with the calcium phosphate 2-D nanoflakes (CaP). This was synthesized for increasing the osteogenic potency of human adipose tissue-derived mesenchymal stem cells.<sup>80</sup>

Anticancer therapy and stem cell study is an important and delicate field of research in biomedicine; therefore, the use of naturally occurring HNT with its myriad of properties for the same can be seen as an essential breakthrough with still a lot of potential for further exploration (Table 2).

### Biosensing

Nanoparticles are receiving increasing attention as future contrast agents (CAs) for ultrasound molecular imaging, especially when decorated on its surface with biological recognition agents for targeted delivery and deposition of therapeutics. Nanoparticles or nanomaterials have several advantages which make them beneficial as CAs like their magnetic and optical properties can be adapted by manipulating their composition, structure, size, and shape. Additionally, their surfaces can be easily modified with desired materials (e.g. ligands) to make them targeted enhancement of desired site.<sup>81</sup>

A study showed the parametric assessment of the effectiveness of HNT as scatterers for safe ultrasound-based molecular imaging. The results demonstrated the

possibility of employing a clinically available echograph to detect the ultrasonic backscatter produced by different concentration of HNT using both 5.7 and 7 MHzinsonification frequencies.<sup>82</sup>

A hybrid nanocomposite was obtained by selectively modifying the outer surface of HNT to support AgNPs. The key to design highly sensitive enzymatic electrochemical biosensor is to achieve direct electron transfer between the enzyme and the electrode surface which in turn can be achieved by improved enzyme immobilization with high loading capacity. The modified HNT with AgNPs were observed to be a good support for the immobilization and electrical wiring of redox enzyme glucose oxidase (GO<sub>x</sub>). The glassy carbon electrode was the platform on which the GO<sub>x</sub> immobilized HNT/AgNPs was deposited and this setup was used for the bioelectrocatalyzed electrochemical detection of glucose.<sup>23</sup>

Another study used HNT-based composite for sensing vitamins by functionalization. A polyaniline (PANI) functionalized HNT composites were reported as highly sensitive ascorbic acid (AA) sensor. The sensitivity of the composite toward AA was about 826.53  $\mu\text{A mM}^{-1} \text{cm}^{-2}$  within a linear range of 0.005–5.5 mM, and a low detection limit of 0.21  $\mu\text{M}$ . This is a good sensing ability and could be credited to the porous structure which formed efficient sensing channels. This in turn enhanced the electron transport and interactions between the PANI and AA.<sup>83</sup>

Bioimaging is a less ventured application of HNT but is being probed on due to ease of selective and unambiguous modification of the nanotube. A study was reported in

which HNTs were grafted with PEG amine and carbon dots were conjugated with the amino groups in PEG. The conjugation of biocompatible carbon dots into the PEG-NH<sub>2</sub> branches of HNT-g-PEG nanoparticles enabled photoluminescent activity for cancer cells imaging.<sup>49</sup>

Despite the limited number of studies reported on the use of HNT in biosensing and bioimaging, the biocompatibility, bioavailability, selective modification and targeting, and nanosize among other characteristics allow the nanotube to have significant potential to be used in this field.

## Conclusion

HNTs are naturally occurring, economically feasible nanomaterial, which are finding applications in many major areas as discussed here. The advantageous tubular structure of HNT has given it numerous roles as drug delivery and gene delivery agents or nanocarriers. In the assortment of studies reviewed in this work, diverse types of modifications/functionalization of HNT have been discussed, hence it can be reasoned that ease of changing the properties of HNT is possible due to their nature of origin. This is so reasoned because there are certain types of HNT that show some limitations due to their length, lumen volume, and uniformity which hinders their applicability in certain applications.<sup>84</sup>

They have been firmly established as a superior nanocarrier with higher loading capacity and sustained release kinetics for various drugs as well as biological agents. They have been extensively studied and used in drug delivery and targeted drug delivery using variety of drugs. The positive results of such studies have given them therapeutic applications. HNTs have also been established as a gene delivery agent for siRNAs, oligonucleotides, DNA, hence making them significantly important for biomedicine. The studies pertaining to cancer cell-targeted drug delivery are of utmost importance for their significantly positive results compared to other existing methods of targeted delivery. The biocompatibility of the modified HNT is one of the major reasons for the same.

Since the past decade, more research is being focused on widening the range of applications of HNT, and the results are very promising. The present and known properties of HNT are being manipulated for them in different fields such as biosensing and bioimaging, anticancer therapy, tissue engineering, and wound healing. HNTs have also found pertinent roles in various other fields of life sciences and medicine in the way they had been used in bone implants, dental filings, and tissue scaffolds; these applications are possible especially because the modified HNT has low levels of cytotoxicity as verified on various cell lines and bioassays. Apart from the biological aspect, HNTs have relevant roles in environmental area, waste-water treatment (e.g. removal of auramine dye),<sup>85</sup> catalytical applications, as glass coatings, in water purification, and in forensic sciences.<sup>86</sup>

The major hindrance in use of HNT is the absence of studies on humans for pharmaceutical applications. While HNT can serve as excipients for oral delivery of drugs and are not toxic in at least up to concentrations of 1000 µg mL<sup>-1</sup>,<sup>31</sup> it, however, is still nonbiodegradable in blood and therefore is not preferred for direct injections into the living systems as it may lead to thrombosis.<sup>27</sup> This opens up yet another potential area of research giving HNT wider range of applications which have been successfully tested on animal models, such as in topical cosmetic formulations, skin ointments, fighting lice, antimicrobial sprays, and traditional oral formulations, allowing for prolongation of the formulation of medical effectiveness.<sup>14</sup>

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