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SMART MATERIALS AND THEIR ADVANCED BIOMEDICAL APPLICATIONS: HNT AND HNT-POLYMER COMPOSITES

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Keywords

Halloysite nanotube
HNTs
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Abstract

The aluminosilicate clay minerals ($Al_2Si_2O_5(OH)_4 \cdot nH_2O$) known to exist in nature are called halloysite nanotubes (HNTs). HNTs, which are found in layered, spherical, flat and other forms, can be obtained naturally as well as synthetically. HNTs with an outer diameter of 50 nm and a length ranging from 500 to 1000 nm have a hollow and nanotube-shaped structure. It has natural deposits in Brazil, Turkey, New Zealand, China, the United States, Korea, Japan, and France, and it is a low-cost material that can be obtained through ore purification. Thanks to their high surface area, large pore volume, rheological properties, high interactions, and high binding capacities with biopolymers, HNTs are used in a wide range of areas. For example, HNTs have become a frequently used material in environmental applications such as wastewater treatment and removal of organic contaminants and dyes. It is also used in the production of nanoelectronics and nanocomposites, catalytic studies, flame retardants in make-up materials, forensic sciences and biomedical fields. The specific properties of HNT used in the biomedical field lead to numerous applications. In this review, it is aimed to present the advantages of HNTs for use in drug delivery systems, immune therapy, anti-infection applications, cancer therapy, bioimaging, biosensing applications, tissue engineering applications, implants and hygiene-cosmetics materials.

AKILLI MALZEMELER VE İLERİ BİYOMEDİKAL UYGULAMALARI: HNT VE HNT-POLİMER KOMPOZİTLERİ

Anahtar Kelimeler

Halloysit nanotüp
HNT'ler
Kil
HNT-polimer kompozit
Biyomedikal uygulamalar

Öz

Doğada var olduğu bilinen alüminosilikat kil minerallerine ($Al_2Si_2O_5(OH)_4 \cdot nH_2O$) halloysit nanotüpler (HNT'ler) olarak adlandırılır. Katmanlı, küresel, yassı ve diğer formlarda bulunan HNT'ler sentetik olarak elde edilebildiği gibi doğal olarak da elde edilebilmektedir. Dış çapı 50 nm ve uzunluğu 500 ile 1000 nm arasında değişen HNT'ler içi boş ve nanotüp şeklinde bir yapıya sahiptir. Brezilya, Türkiye, Yeni Zelanda, Çin, Amerika Birleşik Devletleri, Kore, Japonya ve Fransa'da doğal yatakları bulunan ve cevher saflaştırma yoluyla elde edilebilen düşük maliyetli bir malzemedir. HNT'ler, yüksek yüzey alanları, büyük gözenek hacimleri, reolojik özellikleri, yüksek etkileşimleri

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ve biyopolimerlerle yüksek bağlanma kapasiteleri sayesinde çok çeşitli alanlarda kullanılırlar. Örneğin, HNT'ler atık su arıtımı ve organik kirleticilerin ve boyaların uzaklaştırılması gibi çevresel uygulamalarda da sıklıkla kullanılan bir malzeme haline gelmiştir. Ayrıca nanoelektronik ve nanokompozitlerin üretiminde, katalitik çalışmalarda, makyaj malzemelerinde alev geciktiricilerde, adli bilimlerde ve biyomedikal alanlarda da kullanılmaktadır. Biyomedikal alanda kullanılan HNT'nin kendine özgü özellikleri çok sayıda uygulama için alternatif bir malzemedir. Bu derlemede, HNT'lerin ilaç taşıyıcı sistemler, immün terapi, anti-enfeksiyon uygulamaları, kanser tedavisi, biyogörüntüleme, biyoalgılama uygulamaları, doku mühendisliği uygulamaları, implantlar ve hijyen-kozmetik malzemelerinde kullanım avantajlarının ortaya konulması amaçlanmaktadır.

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1. Introduction

In the modern world, studies on protecting or improving human health have gained momentum with the development of technology. Depending on this rapid development, it is expected to produce new and superior materials in the biomedical field. In recent years, studies of many researchers around the world in the biomedical field have contributed to the development of new products. Scientists have benefited from materials that exist both in nature and subsequently produced for use in biomedical applications. One of these materials is halloysite nanotubes called HNT.

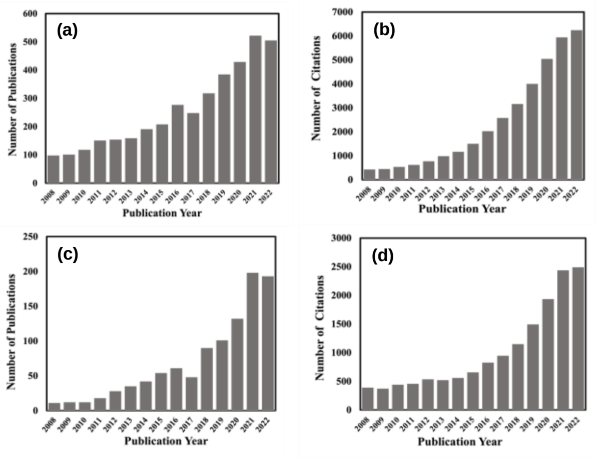

Figure 1. Schematic representation of the increase in the number of halloysite-related publications in the Scopus database from 2008-2022 **(a)**, and the number of citations in the studies on HNT in the last 15 years **(b)**, publications on the use of HNT, including the biomedical field, between 2008 -2022, according to the Scopus database **(c)**, number of citations in the publications about HNT in the biomedical field in the last 15 years **(d)**.

Various studies have been carried out on HNTs, which were named as Halloysite for the first time by M. Berthier, since the 1940s. In the Scopus database, it has been determined that the publications related to HNTs and their studies in the biomedical field have increased in the last 15 years, and the diagram of the increasing number of studies and the number of citations of the publications is presented in Figure 1. Based on this research, when studies in both academic and industrial fields are examined, it is predicted that the use of HNTs in different fields will increase in the future.

HNTs are clay materials obtained from aluminosilicate kaolin layers commonly found in nature. Nanomaterials attract the attention of researchers thanks to their prominent features such as being cheap and biocompatible, and having good thermal and mechanical properties. Halloysite clay minerals are used as corrosion inhibitor in implants, bioimaging, drug delivery, biosensor, tissue engineering, cancer diagnosis/treatment applications, etc. used in nanomedicine and biomedicine applications (Figure 2).

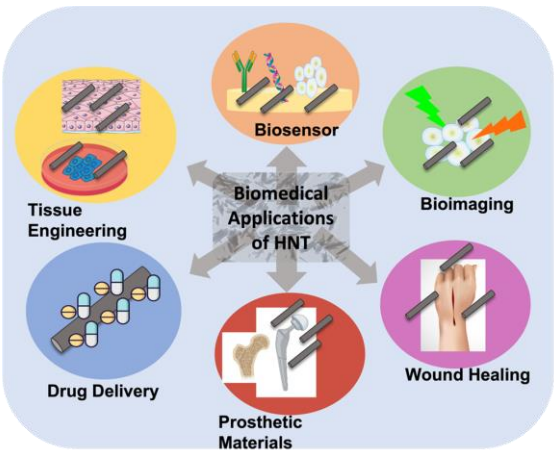

Figure 2. Biomedical applications of HNTs.

Halloysite is a clay mineral with the chemical formula $Al_2Si_2O_5(OH)_4 \cdot nH_2O$ and composed of layered silicates (Prishchenko et al., 2018; Guimaraes *et al.,* 2010; Yah *et al.,* 2012). Halloysite is a bilayer aluminosilicate chemically like kaolin and has a predominantly hollow luminal structure (Danyliuk et al., 2020). The approximate dimensions of the material consisting of these multi-walled tubes can be as follows; length: 200 nm-1.5 µm, inner diameter: 10-30 nm, outer diameter: 50 nm. (Guimaraes *et al.,* 2010). The hydrophilic voids of halloysite nanotubes are larger in HNTs compared to carbon nanotubes. These voids can be filled with chemicals and smaller nanoparticles. In addition, halloysites have the advantageous property that one of their surfaces is negative and one is positive, allowing the charged surfaces to be selectively modified. (Glotov *et al*., 2019; Wei *et al.,* 2014). The empty lumen that HNTs have in their structure allows different molecules to be loaded. The modification procedure can be determined for the selected application. Sahiner and Sengel (2017) benefited from the chemically modifiable properties of the HNT surface in their work. The TEM images of the studies using amine-based modification agents and the schematic representation of the modification step are given in Figure 3.

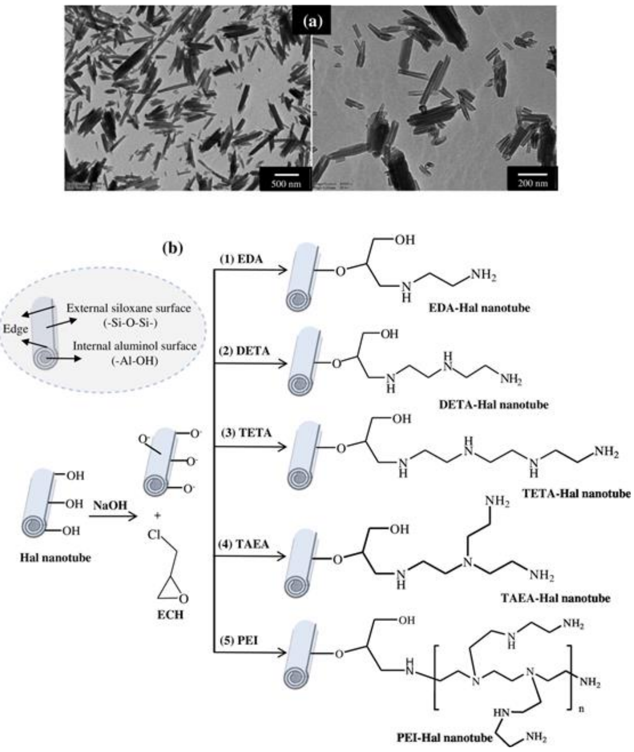

Figure 3. (a) TEM images of bare Hal nanotube at different magnifications, and **(b)** schematic representation of Hal nanotube modification with amine based modifying agents: (1) EDA-Hal nanotube, (2) DETA-Hal nanotube, (3) TETAHal nanotube, (4) TAEA-Hal nanotube, (5) PEI-Hal nanotube. (Adapted with permission from Sahiner and Sengel, 2017, Copyright (2017) Elsevier)

HNTs are very suitable for use in biomedical applications with their high biocompatibility (Biddeci *et al.,* 2022; Kushwaha *et al.,* 2021; Satish *et al.,* 2019; Cheng *et al.,* 2020).

2. Halloysite Nanotubes

Materials such as carbon, graphite, silica are among the nanofilling materials. Nano fillers improve the properties of materials. Halloysite nanotubes is one of these materials. Raw halloysite is obtained from altered rocks, saprolites and soil, in short from nature. Halloysite deposits are available in many different countries. In this way, HNTs are cost-effective materials. While some may be light red, most are white in color. In addition, halloysites are generally tubular in nature. Halloysite mineral is named after Omaliusd Halloy who discovered it for the first time. Halloysite nanotubes were defined by M. Berthier in 1826 as a hydrated polymorph of kaolinite type, dioctahedral 1:1 clay mineral. Although halloysite is chemically similar to kaolinite, the interlayers of HNTs are separated by a single water molecule layer. The molecular formula of halloysite is $Al_2Si_2O_5(OH)_4.nH_2O$. When n=2 in the formula, halloysite is in hydrated form and is called as "hallosite-(10 Å)". The structure of HNTs is presented schematically in Figure 4.

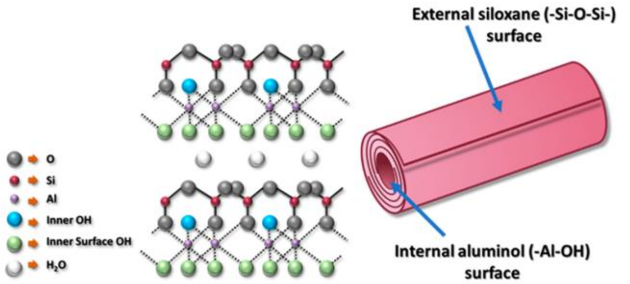

Figure 4. Schematic representation of the crystal structure of HNTs (Kamal *et al.,* 2019).

If n=0, that is its anhydrous structure, halloysite is called as “halloysite-(7 Å)” (Yuan, et al., 2015). The specific surface area of HNTs is between 22.1 and 81.6 $m^2.g^{-1}$, lumen area is between 10.7% and 39%, pore area is 14-46.8%, average pore size is between 79.7-100.2 A (Liu et al., 2014; Fizir et al., 2018). HNTs consist of a positively charged tubule lumen and a negatively charged outer shell. The fact that the tubule lumen and the outer shell have different functional groups facilitates modification. This provides an advantage for different applications such as drug release applications.

3. Biomedical application of HNT and polymer composites

3.1. Delivery System

In controlled drug release systems, the release occurs *via* a carrier. The carrier can be implanted in the patient's body or given by injection. Some factors can affect and slow the rate of drug release. Clay-polymer composites, hydrogel composites, etc. are some of the structures that can slow drug release. Halloysite nanotubes are natural minerals that can play a role in controlled drug release.

Shi *et al.* (2011) investigated a new gene delivery system. They functionalized HNTs with γ-aminopropyltriethoxysilane [APTES] for intracellular delivery and then used anionic antisense oligodeoxynucleotides [ASODNs] f-HNTs in the drug delivery system as a therapeutic gene. They formed f-HNT-ASODN complexes by binding to their surfaces. The cytotoxic effect of this complex on tumour cells was investigated. They observed that the obtained complex had low cytotoxicity and that its antitumor activity could be increased. Thus, they concluded that the HNTs studied in this study could serve as good carriers for gene therapy and biological delivery systems.

In another study reported by Long *et al.* (2017), HNTs were designed as a non-viral gene vector for gene therapy study and shortened to about 200 nm to increase the effect of endocytosis. Then, polyethyleneimine (PEI) grafting was performed to bind pDNA-tagged green fluorescent protein (GFP). PEI-g-HNTs showed low cytotoxic effect and high transfection efficiency and emerged as a promising study in applications such as gene therapy against some diseases.

De Kruif *et al.* (2016), designed the Nanoparticles-in-Microsphere Oral System (NiMOS) for protein delivery. Accordingly, BSA, selected as the model protein, was loaded into the magnified HNTs and the loaded HNTs were placed in microgels by the prilling method. This synthesized new structure revealed that even after *in vitro* digestion of the model drug, it did not undergo much enzymatic degradation and was preserved at a rate of 82%. Thus, this new approach has been a promising development for the delivery of proteins and macromolecules.

HNTs are the material used in drug delivery systems to penetrate the cell membrane of drugs and increase their solubility in the biological environment. This study as reported by Massaro *et al.* (2022) is the first example of the use of an HNT-based nanocarrier labelled with halochromic oxazine molecules. The reason for the use of HNT-based nanocarriers here is to ensure the delivery of a single-stranded peptide nucleic acid tetramer (PNATs) into the cell line. It was observed that despite the covalent binding of PNA to the outer surface of HNTs, its binding with different active species remained intact. In addition, covalent binding of PNA provided an oscillation in an acidic medium.

Long *et al.* (2018) synthesised polyamidomine-grafted halloysite nanotubes (PAMAM-*g*-HNTs) to be used to reduce the expression of the vascular endothelial growth factor (VEGF) gene in breast cancer cells through intracellular delivery of siRNA and gene therapy. The cytotoxic effect of PAMAM-*g*-HNTs was examined against HUVEC (84.7%) and MCF-7 cells (82.3%) and showed good cytocompatibility even at high concentrations. The efficacy of PAMAM-*g*-HNTs/siVEGFs in the treatment of cancer was tested in 4T1-carrying mice and found to have higher anti-cancer activity. Lisuzzo *et al.* (2021), provided the loading of drug molecules dissolved in ethanol to halloysite nanotubes by using ethanol as a solvent. In the study, drug loading performance and release kinetics were investigated depending on the pressure conditions (Figure 5). The work of Lisuzzo *et al.* provides a guide for the development of halloysite nanotube-based carriers with tunable drug delivery.

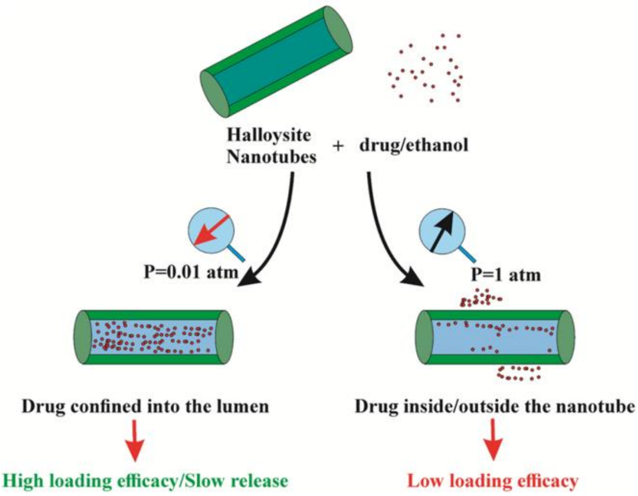

Figure 5. Schematic representation of the effect of different loading conditions on the properties of drug-loaded halloysite nanotube carriers (Lisuzzo *et al.,* 2021).

The synthesis of chitosan-coated nanocomposites for long-term drug delivery was recently published by Paul *et al.* (2022), According to this study, HNTs were loaded with diclofenac sodium and then a chitosan nanocomposite was prepared. The biocompatibility of the prepared HNT/diclofenac loaded chitosan composite was tested with human endothelial cells, and it was found that it showed more than 80% viability. According to this result, the usability of the synthesized HNT-based nanocomposites in wound healing and drug delivery systems has been demonstrated.

Halloysite is one of the clay minerals with the maximum efficiency in nano drug carriers. Therefore, Barmen *et al.* (2020) used norfloxacin as a model drug and used HNT to evaluate the antibacterial effect of this drug. In addition, *in-vitro* release studies were carried out by embedding chitosan into the norfloxacin-loaded HNTs to prepare bio-nanocomposite films. Accordingly, norfloxacin-loaded HNTs were found to be effective against gram-positive and gram-negative bacteria. In vitro drug release from FN (chitosan alone) and F4 (halloyside-loaded chitosan) samples were recorded at

6 hours as 41% and 31%, respectively, and it was shown that halloysite-loaded chitosan had a more sustained effect on drug release. The biocompatibility of halloysite/chitosan nanocomposites and their stability in the aqueous environment, as well as their usability in biomedical applications including drug delivery and wound healing, resulted to this study.

Saleh *et al.* (2021) used HNT as a drug carrier to penetrate the rat-brain microvascular endothelial cell membrane and deliver cargo. HNTs were labelled with rhodamine B isothiocyanate (RITC) followed by prolonged drug release using ionomycin. Accordingly, ionomycin effectively released its charges *in vitro* over a long period of time. The penetration of HNTs was tested with a blood-brain barrier model using endothelial and astrocyte cells, and it was observed that HNTs were able to cross this model barrier. Thus, it has been shown that HNTs can potentially be used in drug delivery systems.

In another study, Arshad *et al.* (2022) aimed to reduce the coagulation time by applying chitosan (CHT) and microcomposites (MC) together with HNTs. Accordingly, percent loading and percent release were investigated to confirm the sustained releases of ciprofloxacin-loaded CHT/HNTs MCs. A total of 75% penetration of ciprofloxacin into the rat intestinal membrane was observed within 3 hours. Bleeding disorders in LGIB have been controlled with another similar antibacterial agent, such as ciprofloxacin, to assist in the development of drug carriers.

Succesful preparation of cross-linked cellulose/HNT composite hydrogels were reported by Huang *et al.* (2017). These composite hydrogels were loaded with curcumin, which is used as a model drug, and its effect on cancer cells was examined. They reported a good inhibition effect on MCF-7 cells. In addition, its cytotoxicities in both MC3T3-E1 and MCF-7 cells were investigated, and it was observed that it exhibited good biocompatibility in both cells. As a result, the HNT-based hydrogels have emerged as a promising development in applications such as drug delivery systems.

Kurczewska *et al.* (2018) aimed to improve drug delivery capasity of HNTs by functionalization with polyamidoamine (Hal-PAMAM) dendrimers and 3-aminopropyltrimethoxysilane (Hal-APTS). In their study, in which chlorogenic acid, ibuprofen, and salicylic acid were used as model drugs, drug release and the effect of the synthesised material on living organisms were investigated. The release rate of chlorogenic and salicylic acid with Hal-PAMAM was observed to be quite slow but had no effect on ibuprofen release. They stated that Hal-PAMAM is a suitable material for use in biomedical applications such as the transport of drugs with small molecular sizes, since it does not have a negative effect on living organisms.

Cheng *et al.* (2020) synthesised a halloysite-based hydrogel by adding a fluorescent derivative carrying two arylboronic acid groups and used this material to create a H_2O_2-sensitive drug delivery system that produces fluorescein. Drug-loaded HNTs (DHNTs) have been characterized, and it has been proven that the drug is loaded into the nanotube cavity. In the study, it was seen that the H_2O_2 concentration affected the release, and the release was done completely and quickly with the increase in the concentration. In addition, it has also been shown that fluorescence intensity and oscillation were directly proportional each other.

Another study based on HNT as drug delivery system was reported by Hamedi and Koosha (2020). First, they loaded anthocyanins extract from black carrots to HNT. The release of anthocyanins from HNT in vitro was investigated by the pH-differential method and it was shown that the best mathematical model was the Korsmeyer-Peppas model. In addition, when the activities of anthocyanin-loaded HNTs against cancer cells such as MCF-7 and HT-29, the proliferation of cells was largely inhibited. Since the release of anthocyanin from HNTs is pH sensitive, it has been predicted that this system can act as a good drug carrier for inflamed tissues and cancer cells and can be used in blood sugar control.

A multitasking drug delivery system was designed by Dramou *et al.* (2018) Folic acid-chitosan oligosaccharide/magnetic HNTs (FA-COS/MHNTs) were succesfully prepared for intracellular uptake of anticancer drug carriers. Camptothecin (CPT) was loaded into the lumens of FA-COS/MHNTs to examine their release profiles at different pHs. While 83% of CPT release occurred at pH 5 in 24 hours, much less release was observed at pH 6.8 and 7.4 even after 48 hours. In addition, the high cytotoxic effect of CPT-loaded nanocarriers demonstrated an extraordinary usefulness in killing cancer cells. Thus, this synthesized nanocarrier has opened a new door for the delivery of tumour-targeted medicines and various biological applications.

Li *et al.* (2016) developed an innovative nanocomposite with HNTs for ibuprofen drug release. APTES modification was carried out to improve the performance of the material.

A novel HNT-based pH-sensitive drug carrier for the release of an anti-cancer drug, doxoribucin (DOX) was reported by Hemmatpour *et al.* (2022). HNTs were coated with polydopamine and then grafted with poly(N,N-dimethylaminoethyl methacrylate) brushes onto the HNT-PDA surface *via* electron transfer in atom transfer radical polymerization. It has been shown that synthesised modified HNTs cause a pH-sensitive release and the drug loading capacity is increased approximately twice (185 ± 15 mg.g^{-1}) compared to previous studies. It has also been observed that faster release occurs at pH 5.5 than at pH 7. It was thought that if the biocompatibility of the synthesized carrier could be proven, it would be a good candidate for the transport of toxic drug molecules to acidic sites.

3.2. Immune Therapy and Anti-infection

Shu *et al.* (2017a) reported antibacterial nanocomposites via preparation by incorporating Ag and ZnO nanoparticles into HNTs. The antimicrobial activities of gram-negative *E. coli* and Ag-ZnO/HNTs were tested and they obsnreved high activity and stability. These results showed that the synthesized nano-composite is a promising candidate for use in antibacterial domains.

In another study by Shu *et al*. (2017b), A homogeneous co-precipitation method of CeO_2 and ZnO nanoparticles in the ethanol system was used. With this method, a modified and antibacterial CeO_2–ZnO/HNTs nanocomposite was obtained on the HNT surface. The antibacterial effects of ZnO, ZnO/HNT and CeO_2–ZnO/HNT nanocomposites were investigated with *E. coli* and CeO_2–ZnO/HNT nanocomposites showed the best activity with 8% cell viability. Based on this result, it has been predicted that the nanocomposites could be used as a disinfectant to prevent bacterial growth and spread. In another study, Zhang et al. (2013). AgNPs/HNTs were synthesized using AgNPs with antibacterial activity and HNTs with highly advantageous properties. They tested the antibacterial activities of AgNP/HNTs using gram-negative and gram-positive bacteria. When the results were evaluated, they observed that the growth of *E. coli* and *S. aureus* bacteria was largely inhibited. They reported that the synthesized AgNP/HNTs could be used in the field of biomedicine.

Majumder *et al.* (2022) used different methodology to locate AgNP in HNTs. They grafted silver nanoparticles onto halloysite nanotubes after modifying with tannic acid, and a new nanocomposite (GH-TA-Ag-NT) was synthesized. The bacterial activity of the nanocomposite was tested with *E. coli* ATCC 25922, *S. aureus* ATCC 25923 and *Salmonella enterica serovar Typhimurium* bacteria and showed very good antibacterial properties. In addition, when the effect of nanocomposite in curing gastrointestinal infection was examined, it was observed that *Salmonella* colonisation was significantly reduced within 24 hours. It has been observed that this biocompatible, economical, and safe nanocomposite synthesised is effective against antimicrobial resistant bacteria and could be a new alternative source for the overuse of antibiotics.

Usage of surfactants in the modification of HNTs was reported by Abhinayaa *et al.* (2019). They obtained modified-HNTs (SM-HNT) using surfactants such as cetyl trimethylammonium bromide (CTAB), sodium dodecyl sulphate (SDS), and Tween 80 and tested them against phytopathogenic bacteria. SM-HNTs were found to be quite effective in killing *A. tumifeciens, X. oryzae*, and *R. Solanacearum* bacteria. Among these modified-HNTs, CTAB-HNTs has been dtermined to be more effective in disrupting cell membrane integrity, suppressing growth and inhibiting biofilm formation due to its physico-chemical properties. Consequently, SM-HNTs have been shown to be a new nanomaterial that can be used to selectively control plant pathogenic bacteria. Figure 6 shows a schematic representation of the phytopathogen killing efficiency of intact Hal nanotubes and surfactant modified Hal nanotubes (SM-Hal nanotubes).

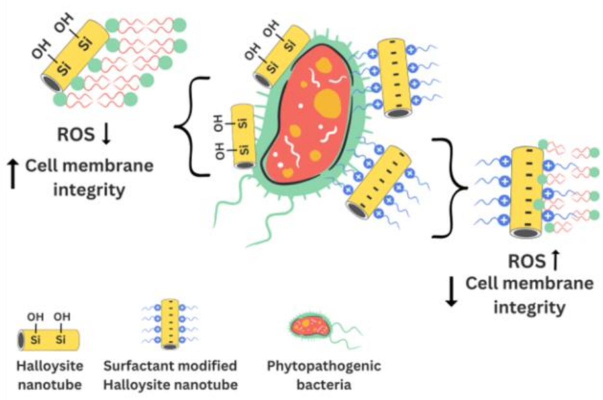

Figure 6. Schematic representation of the phytopathogen killing efficiency of intact Hal nanotubes and surfactant modified Hal nanotubes (SM-Hal nanotubes).

A new and viable method by designing carvacrol-loaded HNTs as an antimicrobial agent and water-based polyurethane nanocomposite coatings was demonstrated by Hendessi *et al.* (2016). The release and antimicrobial activities of carvacrol-loaded HNTs were investigated, and it was shown that carvacrol was effectively released for one week and inhibited the growth of pathogenic microorganisms. With the prepared carvacrol-HNT/PU nanocomposite films, the effect on *A. hydrophila* bacteria was investigated, and a decrease in the number of bacteria was observed. In addition, nanocomposite films have been shown to inhibit bacteria for two days. As a result, it has been predicted that prepared nanocomposites may weaken or prevent bacterial infections.

Fakhrullina *et al.* (2019) have studied halloysite-based nanocaps that are functionalized with antimicrobial effect. Accordingly, curcumin was loaded onto HNTs by the vacuum loading method and then coated with dextrin to obtain HNT's+Curc/DX nanocontainers. The antimicrobial effects of the synthesised nanocaps were investigated in vitro and it was observed that they effectively suppressed the growth of *S. marcescens* cells, while *E. coli* bacteria were not affected. In addition, the synthesised nanocaps successfully suppressed the infection in C. elegans nametodes infected with *S. marcescens* and extended the life expectancy of worms infected with pathogenic bacteria. According to these results, it is thought that new applications can be found in the treatment of dysbiosis by suppressing unwanted microflora.

3.3. Cancer Therapy

Guryanov *et al.* (2020) reported a promising nanoformulation that can be used in the anticancer treatment of Prodigiosin, which is effective against various cancer cells. The effect of prodigiocidone-loaded HNTs on malignant and non-malignant cells was investigated. They concluded that p-HNTs showed selective cytotoxic and genotoxic activity. By inhibiting the proliferative activity of Caco-2 and HCT116 cells, P-HNTs disrupted the F-actin structure and changed the cell morphology. As a result, the synthesised p-HNTs created an anticancer effect, and it was assumed that this effect would be effective in the treatment of living tissues in vivo.

Succesful synthesis of HNT/Fe_3O_4 nanocomposites was carried out by Abhinayaa *et al.* (2018). Bacterial interactions of nanocomposites and their effects on cancerous and non-cancerous cells have been studied by various tests. HNT/Fe_3O_4 was found to be less toxic to bacteria and biocompatible with non-cancerous cells. Additionally, it caused a cytotoxic effect on cancerous cells. As a result, the synthesised nanocomposite has been demonstrated to be a biocompatible nanomaterial with low toxicity to bacteria and high toxicity to cancer cells.

Liu *et al*. (2019) developed HNT nanoclusters (HNT's/siRNA complex) to efficiently deliver siRNA to target receptor-interacting protein kinase 4 (RIPK4) for the treatment of bladder cancer. HNT/siRNA complex has been shown to inhibit tumour growth and progression in cancer cells in vivo and in vitro and has no toxic effects on non-cancerous cells.

Tan *et al.* (2021) loaded HNTs with type II photosensitive indocyanine green (ICG) and labeled with Fluorescent isothiocyanate (FITC) for phototherapy. The HNTs-FITC-ICGs were then surrounded by a red blood cell membrane (RBCM). With the combination of these structures, the HNTs-FITC-ICG-RBCM nanocarrier was synthesized. We conjugated anti-EpCAM with streptavidin (SA) to HNTs-FITC-ICG-RBCM to improve specific recruitment of cancer cells. HNTs-FITC-ICG-RBCM-SA-EpCAM was designed for tumor targeting in breast cancer treatment. The photothermal and antitumor effects of the designed versatile nanoparticle were investigated. It has been reported that it exhibits anticancer activity in the presence of laser light and is highly effective in targeting cancer cells. Khodzhaeva *et al.* (2017) investigated the effect of binase with selective cytotoxicity against cancer cells immobilized on HNTs for antitumor therapy. Experimental work of immobilization of binase on HNTs is schematized in Figure 7.

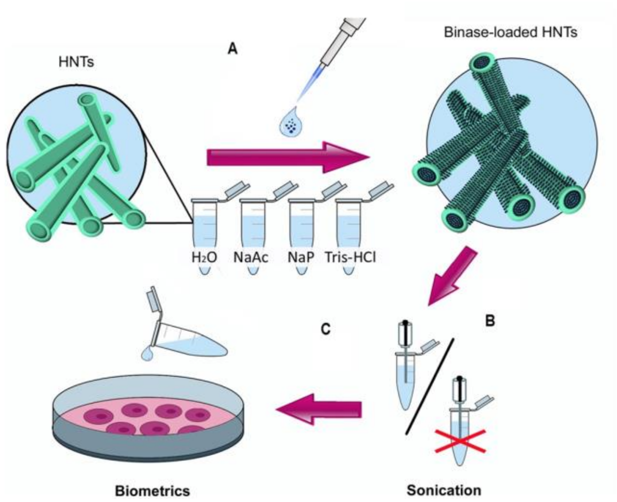

Figure 7. Immobilization of binase on HNTs and schematic representation of cell work (Khodzhaeva *et al.,*2017).

They reported that the Binaz-HNT composite has the potential to be used in clinical applications.

3.4. Bioimaging and Biosensing

The convenience of specific surface modifications of HNTs is utilized in various studies such as increasing drug release activities, anticancer activities and tumor targeting. In recent years, interest in HNTs in bioimaging studies has been increasing (Kurczewska *et al*., 2018; Mo *et al*., 2020).

Biosensors are the systems that perform analysis for the detection of a specific analyte. This analysis takes place by selective biochemical molecular recognition. In general, the system works as the recognition of the material to be analyzed, then the conversion of this analysis into a signal and the detection of the resulting signal (Kerman *et al.,* 2003). HNTs are promising materials as electrocatalysts due to their low cost, non-toxicity, compatibility with modification and thermal stability (Kokulnathan *et al*., 2022). In addition, siloxane groups, aluminol groups and other reactive groups in the structures of HNTs are effective in the immobilization of biological species. Therefore, the use of HNTs in electrochemical sensors is advantageous. (Tully *et al.,* 2016). HNTs are materials that have been widely used in biosensor studies in recent years.

Luo *et al.* (2020) have developed multifunctional nanoparticles for imaging and treatment in breast cancer by forming Fe_3O_4 nanoparticles fixed on the outer surfaces of HNTs, and composites made of doxorubicin loaded into nanotubes. A schematic illustration of the use of the HNT@Fe_3O_4@PPy@DOX complex in cancer therapy is given in Figure 8. As a result of *in vitro* experiments, it has been determined that this nanocomposite is a fast, effective, and biocompatible option for both bioimaging and clinical targeting therapy for breast cancer.

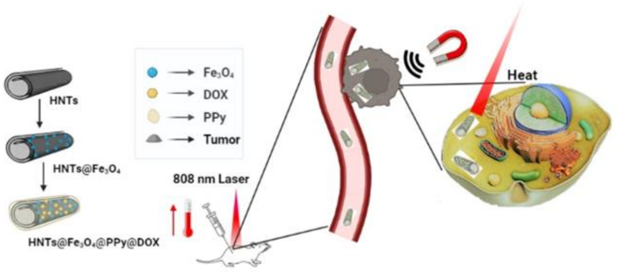

Figure 8. Schematic diagram showing HNT@Fe_3O_4@PPy@DOX expression and its use in cancer therapy.

Fluorescent gold nanoclusters (AuNCs) with an average diameter of 2.7±1.0 nm, stabilized with 11-mercaptodecanoic acid, on aminosilane-modified halloysite nanotubes has been successfully prepared by Gorbachevskii *et al.* (2021). As a result of *in vitro* studies, it was concluded that the synthesized composite material showed good uptake by cells. The material was found to cause no significant toxic effect and no visible membrane damage in the concentration range of 25–50 µg.mL^{-1}. This concentration-dependent mechanism of toxicity concludes that it is an effective material for using halloysite-stabilized AuNCs for halloysite visualization in biological objects, bioimaging, and cancer therapy.

Stavitskaya *et al.* (2018) performed a stabilized study on HNTs to investigate the fluorescence and cytotoxicity of cadmium sulfide (CdS) quantum dots (QD). In this study, they synthesized 6-8 nm CdS and $Cd_xZn_{1-x}S$ nanoparticles inside and outside the HNTs using a ligand-assisted method. They tested the synthesized composites on human skin fibroblasts and prostate cancer cells. At the end of the study, they reported that in human cell cultures, HNT-QD composites were internalized by living cells and showed intense and stable fluorescence with pronounced nanotube light scattering. As a result of fluorescence and cytotoxicity experiments, they reported that cadmium-zinc sulfide QD azine grafted on HNT is the most promising materials for bioimaging.

A laccase-halloysite nanotube and imidazolium zwitterionic surfactant-based biosensor for the determination of dopamine was developed by Decarli *et al.* (2022). Dopamine detection has been effectively studied using a modified electrode that offers the synergistic effect of laccase immobilized on HNT and a surfactant. It is concluded that this combination has remarkable sensitivity, wide linear range, selectivity and detection limit values. It was concluded that this biosensor, which was produced by showing a successful result in the determination of dopamine in the analysis made with real samples, is an efficient approach.

Sen *et al.* (2022) developed a magnetic halloysite nanotube-based SERS biosensor with Au@Ag core-shell nanotags for the specific detection of bisphenol A. A wide linear range of 0.001–100 ng.mL^{-1}, an R^2 value of 0.9944, and a detection limit of 0.75 pg.mL^{-1} were obtained. It was found that the prepared SERS aptasensor developed quite good Raman signal and exhibited SERS sensitivity. The developed magnetic halloysite nanotube-based aptasensor offers a wide variety of applications in the food safety and environmental health sectors.

An electrochemical immunosensor designed by Li *et al.* (2017) for the prostate-specific antigen (PSA) detection. In this work, cost-effective and biocompatible HNTs, conductive polypyrrole and electrocatalytic palladium were used. Modified glassy carbon electrodes (GCE) were prepared with nanocomposite of HNT, polypyrrole and palladium nanoparticles (HNTs@PPy-Pd). A schematic representation of the preparation of the immunosensor is presented in the Figure 9.

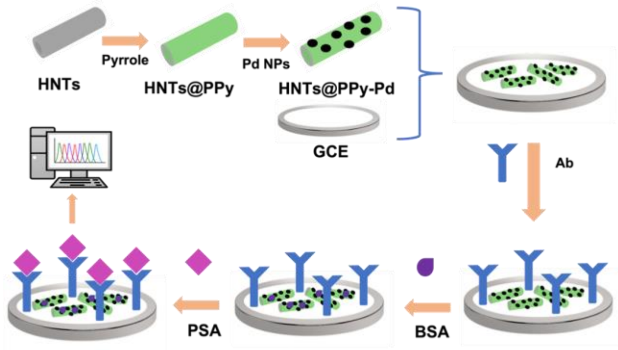

Figure 9. Schematic illustration of the preparation of HNTs@PPy-Pd nanocomposite modified GCE electrodes.

They reported that the immunosensor they developed showed a wide linear range (0.0001 to 25 ng/mL) and a low detection limit (0.03 pg/mL) for PSA detection.

3.5. Regenerative medicine

Tissue engineering is a multidisciplinary field in which sciences such as biology, pharmacy, medicine, engineering, and chemistry are used together to produce biomimetic composites. The main purpose in tissue engineering is to restore the tissue's function by repairing, protecting, or improving the tissue (Nekounam *et al.*, 2021). Developing an effective scaffold for tissue repair is an important part of the process, and therefore, developing scaffolds that can mimic the functional and structural nature of damaged tissue has been an area of much research in recent years (Mabrouk *et al.,* 2020). In the manufacturing phase of scaffolds developed for tissue engineering applications, natural polymers can be encountered, as well as synthetic polymers. While natural polymers have disadvantages such as weak mechanical properties, synthetic polymers have disadvantages such as cytotoxicity. It has been observed that successful results are obtained when the positive properties of both polymer groups are used in combination and the process is optimized (Gu *et al.,* 2016).

Many patients receive medical treatment for orthopedic problems and more than 2 million bone graft operations are performed annually. Halloysites are attractive materials for applications in bone tissues, implants and scaffolds (Campana et al., 2014). Contamination and infection are quite common in cases where bone defects caused by disease or trauma occur. This can seriously impair the normal function of bone tissues in the defects. Eventually, there are cases that lead to implant failure. Therefore, the control of infection is a very important and even vital factor in the success of bone regeneration (Wei *et al*., 2019).

Autograft and autotransplantation are traditional methods used in surgery for the treatment of damaged tissues. However, these methods are too limited for both bone and damaged tissue reconstructions due to high cost, organ failure, risks of immunological rejection, and postoperative complications Ou and co-workers (2020), designed a hybrid antibacterial hydrogel for bone regeneration. This study developed a strategy combining antibacterial and osteoimmunomodulatory activities with nanosilver particles, HNTs and GelMA (Ou *et al*., 2020).

HNT is also used in dental applications. HNT is often combined with resin dental composites (RDC) and used as a filler in dental treatments. HNT affects the biological and bioactivity properties, microhardness, bending strength and maximum polymerization rates of the materials it is included in (Gkouma *et al.,* 2021).

Skin wounds pose a significant problem with approximately 300 million chronic and 100 million traumatic wound patients worldwide. Although the treatments applied for acute and regional wounds are effective, there are some difficulties in the long-term care and treatment of large-area chronic wounds and burns (Das and Baker, 2016).

Various ways are sought to prevent deaths due to blood loss caused by injuries. For this purpose, Li *et al.* (2021) designed hydrogels for use in wound treatments. In the study, they synthesized the hydrogels using chitosan (CS) and oxidized dextran (ODEX). They aimed to improve their performance by adding HNT to hydrogels. They reported that while hydrogels can be injected, they can also form gel in situ by a Schiff base reaction between CS (amino groups) and ODEX (aldehyde groups). As a result of their experiments, they observed that the HNT-doped hydrogels effectively stopped the bleeding. Most importantly, they reported that the hemostatic capacity of hydrogels increased thanks to HNTs.

The effect and speed of kaolinite group clay minerals on wound healing are known. These materials, which have high hemostatic activity, are frequently used in wound treatments (Long *et al*., 2018). With this knowledge, HNT has become a very popular material in wound healing studies.

It can be said that by making the actively used and known biocompatibility materials more functional with additives such as nano-scale inorganic materials, filling materials or halloysite, it can be said that the existing tissue damage repair methods will be an alternative approach to all known problems and obstacles to be overcome. HNTs are among the most frequently used materials in regenerative medicine applications such as tissue engineering, bone regeneration, dental treatments, wound healing and the like. Some case studies using HNT in these areas are listed below.

Schmitt *et al*. (2015) prepared plasticized starch-based nanocomposite foams by melt extrusion using water as the blowing agent. They found that the addition of halloysite nanotubes to the plasticized starch matrix promoted cell nucleation and reduced cell size. They concluded that HNTs act not only as a nucleating agent but also as a barrier to limit cell growth leading to macroporosity in the material. As a result of the study, porous nanocomposites based on plasticized starch with a mixture of glycerol and sorbitol and containing 6% halloysite by weight, produced at a molding temperature of 117°C, have high porosity, macroporosity supporting the formation of cellular and extracellular components of bone and blood vessels, and adequate mechanical properties. They concluded that it would be very useful for bone substitutes or bone cement applications thanks to its important properties such as strength.

Khan *et al*. (2019) synthesized CTS/n-HAP/HNT (CHH I-III) nanocomposites. It was concluded that the mechanical properties of the membranes improved at 7.5% (w/w) HNT loading to the produced composite. In the second part of the study, low amounts of TiO2 nanoparticles (NPs) and TiO_2 nanotubes (NTs) were added to the CTS/n-HAP/HNT nanocomposite. The effect of TiO_2s on antibacterial activity and osteoconductivity was investigated. The CHH-TiT membrane was tested for growth studies of *S. aureus* and *E. coli.* The results showed that the CHH-TiT membrane was successful in inhibiting growth within 16 hours. They concluded that there is a potential to use CHH-TiT (7.5% HNT and 0.2% TiO_2 NT) membranes as a template for guided bone tissue regeneration.

To overcome the limitations of the CS/GP hydrogel, Vasheghani-Farahani *et al.* (2021) an injectable nanocomposite (NC) chitosan hydrogel containing modified HNT nanotubes (mHNTs) was synthesized. The synthesized hydrogels were aimed to be used for bone tissue engineering applications. mHNTs/CS/GP NC hydrogels were prepared as heat sensitive and injectable. Modification of HNTs with chitosan has been found to increase capture efficiency and loading capacity by reducing the initial burst release of Icariin from nanotubes. It was concluded that the NC chitosan hydrogel containing the produced IC@mHNTs is a suitable candidate for bone tissue engineering applications.

Kumar and Han (2021) synthesized polyacrylamide (PAM)/polyvinyl alcohol (PVA) (PMPV) based hydrogel by free in situ radical polymerization method. They added bioactive glass and haloysite nanotubes (HNT) to the hydrogels they synthesized to improve their mechanical properties and biomineralization. BGs provide enhanced cell affinity and bone-binding ability for the hydrogel system. HNTs improve their mechanical performance. It has been observed that the porosity decreases slightly with the addition of BG and HNTs to PMPV hydrogels, which have a porous structure. It has been determined that PMPV/BG@HNT hydrogels show higher mechanical strength when compared to other hydrogels. Cell growth studies of hydrogels showed cell growth between 3 and 14 days.

De Silva *et al.* (2018) reported fabrication of HNT-doped alginate-based nanofiber scaffolds by electrospinning technique. It is intended to be a material that mimics the natural extracellular matrix (ECM) structure useful for tissue regeneration. The HNT alginate-based matrix is loaded with an antiseptic drug, Cephalexin (CEF). Thanks to the structure of HNT that allows drug loading, it has been determined that CEF easily accumulates in the lumen cavity and outer walls. It is noted that the total loading capacity is 30% by weight. It was observed that the addition of HNT increased the tensile strength 3 times and the elastic modulus 2 times. It was also found that CEF loaded HNT-doped alginate-based scaffolds showed superior antimicrobial properties against *S. aureus, S. epidermidis, P. aeruginosa and E. coli*. It was concluded that the developed alginate-based electrospun scaffolds with superior mechanical properties and antimicrobial activity are a candidate material to be used as artificial ECM scaffolds for tissue engineering applications.

The preparation of mechanically strong and biocompatible cryogel composites based on hyaluronic acid (HA) and HNT in various compositions and their applications as scaffolds for different cell growth media has been stadied by Suner *et al.* (2019). Superporous HA: HNT cryogel composites were prepared under cryogenic condition in different HA: HNT weight ratios such as 1:0, 1:0.5, 1:1 and 1:2. It was determined that with the increase of HNT content in the composite structure, the mechanical and thermal stability increased in parallel. Bare HA and HA: HNTs cryogel composites were found to be non-hemolytic materials in the range of hemolysis rates of 0.63% to 1.39%. It was also found to be slightly hemostatic when in contact with human blood, with blood coagulation indices of 14.5–17.3, respectively. HA: HNTs cryogel composites have been determined to be as promising materials as tissue engineering scaffolds with their macroporous structures, high thermal and mechanical stability, good blood compatibility.

Barot *et al.* (2020a) have conducted a study for the physicochemical and biological evaluation of immobilized HNT-based resin composite silver nanoparticles for dental applications. In this context, they concluded that the mechanical properties of HNT/Ag added (1-5% by weight) dental resin composites were significantly improved thanks to HNT. It was observed that the addition of HNT/Ag more by mass (7.5-10% wt) did not cause any improvement in the mechanical properties of the dental resin. Finally, the corresponding HNT/Ag-based dental resin composites did not show significant cytotoxicity against NIH-3T3 cell lines. It has also been found that HNT/Ag added to the resin matrix provides additional microbial protection to the dental composite. Therefore, the use of HNT/Ag as a filler with antimicrobial properties is highly advantageous for the development of robust dental composites.

3.6. Prosthetic materials

Prostheses are biomaterials used to heal, fix or replace damaged joints and bones. The need for orthopedic biomaterials has been increasing over the years. Implants can be produced from ceramic, polymer, metal or composite materials. The most important issues in the production of prosthetic materials are; it is not allergic or carcinogenic, does not cause toxic effects and is biocompatible. Halloysite nanotubes are suitable for use in coatings of prosthetic materials. In addition, HNTs can be used as prosthetic materials together with polymeric materials. The combination of HNTs and polymeric materials provides some advantages: 1) improving the durability of the prosthetic material coating, 2) no need to carry out the sintering process, 3) accelerating the transmission of active ions.

There has been great attention to design functional and advanced prosthetic materials. Acrylic materials, which are widely used in dentistry, are preferred in dental prostheses. Currently, there are carbon nanotube doped acrylic dental prostheses. However, these dental prostheses have very low mechanical strength. Also, carbon nanotubes are more expensive compared to HNTs. Gawdzinska *et al*. (2019), developed a composite nanofill material with silane-linked aluminum trihydrate (ATH-sil) and gelatin-modified HNT in 2019. In the study, it was observed that HNT was well dispersed in the gelatin polymer matrix. When composite prostheses containing HNT were evaluated, it was reported that their mechanical properties improved and they showed better mechanical strength (Gawdzinska *et al.,* 2019).

Some implants are used for short-term treatment, while others need to be used for much longer. In long-term implants, it is desirable that the material used should not cause any toxic effects. For this reason, one of the most important features that should be in implants for materials scientists is biocompatibility. By considering the biocompatibility requirement in implants, chitosan and HNT composite coating were applied on titanium substrate as studied by Molaei *et al.* (2016). With this study, they aimed to improve the corrosion resistance of the titanium substrate. It has been reported that chitosan/HNT film offers better apatite-inducing performance thanks to HNTs. One of the metals frequently used as implant material is titanium alloys (Ti-6Al-4V). Titanium implants release V and Al ions into the human body. This is undesirable for bone tissue. To overcome this situation, Chozhanathmisra *et al.* (2022) used a biocomposite coating consisting of HNT, alginate (ALG), hydroxyapatite (HAp), and polyvinylalcohol (PVA) on titanium implants. They noted that HNT-PVA-ALG-HAp coating improved osteoconductive and mechanical properties. In another study, HNT, modified hydroxyapatite (MHA), poly(3,4-ethylenedioxythiophene) (PEDOT) coating was applied on titanium to prevent bacterial infections caused by titanium implants. In the study, HNT-PEDOT-MHA coating provided good corrosion resistance and better biocompatibility for the titanium material (Chozhanathmisra *et al.,* 2018).

Thanks to the rapid development of technology, 3D printing technique has entered many different fields. By using 3D printing technique, Sa and co-workers (2019) worked on 3D antibacterial dental composite resins. Ag-containing HNTs were used to impart antibacterial properties to stereolithography resins. HNTs also improved the mechanical properties of the resins. It has been determined that Ag-HNT 3D composite material, which has antibacterial properties and does not cause toxic effects, has a potential for use in dentistry. In another study, a three-dimensional bioactive scaffold consisting of polydioxanone (PDS) and HNTs was prepared by electrospinning. An increase in fiber diameters was observed with the inclusion of HNTs in scaffolds designed for regenerative endodontics (Bottino *et al.,* 2015).

Azmi *et al.* (2017) synthesized PVA and HNT based hydrogels. PVA-HNT hydrogels formed by incorporating HNT into PVA hydrogels were kept in body fluid for one week. As a result of this experiment, it has been reported that PVA-HNT hydrogels are suitable for artificial cartilage material. If hydroxyapatite (HA) is desired to be used for bone replacement, it should be considered that it is not suitable for use alone because it is both fragile and does not show antibacterial properties. For this reason, Chozhanathmisra *et al.* (2016) benefited from the positive effects of strontium (Sr^{2+}) metal ion on bone formation and the antibacterial property of Sm^{2+} metal ion in their studies. Sr, Sm substituted HA (M-HA) /zinc (Zn)-HNT coating was applied on titanium alloy for use in orthopedic applications. It was stated that implant materials with good corrosion resistance were obtained with M-HA /Zn-HNT coating. Hasan and Ali (2018), in their study, they investigated the effects of adding HNT to the acrylic coating material used in prostheses. They tested the change with HNT added in the concentration range of 0.5% and 1.5% by weight. They reported that there was no difference in the thermal and surface properties of the coating material at both concentrations. However, they found that the mechanical strength was improved with the addition of HNTs. Vankatesh and co-workers (2019) have worked to develop 3D-printed implant materials. In this study, they strengthened poly lactic acid (PLA) which they used as the basic polymer, with biocompatible HNT. They reported that the material showed good mechanical properties thanks to HNTs. Molaei and Yousefpour (2019) investigated the potential for the use of chitosan-based nanocomposites in orthopedic applications. In their research, they worked with bioactive glass which helps bone repair, hydroxyapatite which has similar properties to bone structure and HNTs, which provide resistance to biomaterials. As a result, they noted that biodegradation is facilitated by HNTs.

Recent studies show that the need for orthopedic implants increases every year (Ji *et al.,* 2017). Ji and his team (2017) contributed to the field of orthopedic biomaterials by working on gelatin and HNT added composite biomaterials. In their studies, ibuprofen, an analgesic, antipyretic and anti-inflammatory drug for the treatment of bone diseases, was loaded onto HNTs. It has been observed that the mechanical performances of gelatin matrices are improved by adding HNT. They also reported that the HNT-doped gelatin system shows a better drug release profile compared to the gelatin scaffold alone. In another study, polycaprolactone-polyethylene glycol-polycaprolactone/gelatin (PCEC/gel) three-dimensional hydrogel scaffolds were prepared. Nanohydroxyapatite, HNT and iron oxide nanoparticles (Fe_3O_4) are included in the structure. The results showed that the PCEC/gel/HNT scaffold is biocompatible and has good mechanical properties. It has been reported to be suitable materials for use in bone treatment applications (Same *et al.,* 2022). In order to prevent infections caused by implants, a study was carried out using 3D polylactic acid (PLA), chitosan oligosaccharide lactate (COS) zinc halloysite-silver nanoparticles (ZnHNTs-Ag). In the study, the antibacterial property of the COS-ZnHNTs-Ag coating was tested (Humayun, *et al.*, 2022).

Tappa *et al.* (2014) worked on calcium phosphate cements (CPCs) to contribute to dental and orthopedic practice studies. CPCs were prepared in composite form with HNT and antibiotic additives. It was found that CPC-HNT composites showed both more effective drug release mechanism and good mechanical properties. In a different study, research was conducted on the improvement of dental resins after HNT supplementation. It was concluded that HNTs improve the mechanical properties of dental composites (Chen *et al.,* 2012). Torres *et al.* (2020) conducted a study aiming to show the behavior of hydroxyapatite and HNTs in different polymers. For this purpose, they worked with polyester poly (ε-caprolactone) (PCL), poly (lactic acid) (PLA) acrylate poly (2-hydroxyethyl methacrylate) (PHEMA), poly (ethyl methacrylate) (PEMA) polymers, which are widely used in the biomedical field. With this study, they aimed to prepare a new biomaterial for prostheses and at the end of the study they found faster degradation and improvement in mechanical properties. HNT-doped hydrogels were synthesized to improve the Young's modulus of gellan gum-based composite hydrogels for cartilage repair (Banifacio *et al.* 2018). Classical hydrogels show a compressive modulus of 10 to 60 kPa while HNT-doped hydrogels have been noted to show a modulus of compression from 116 to 143 kPa. In another study, a different method was presented to develop new dental restoration materials. In this study, it was aimed to prepare advanced dental biomaterials with chitosan and HNTs. When the results of the study were evaluated, it was found that HNTs strengthened the composites (Cho *et al.*, 2020).

Abdollahi Boraei *et al.* (2021) designed a nanocomposite scaffold with gelatin-hallosite nanotubes (HNTs). In their study, they combined the advantageous properties of both gelatin and HNTs. They determined that the bone regeneration ability increased thanks to the nanocomposite scaffolds loaded with strontium ranelate. They found that the addition of HNTs to the nanocomposite structure improved the porosity and mechanical properties. Wei *et al.* (2012) conducted a study on PMMA bone cement doped with gentamicin loaded halloysite nanotubes. They searched for the most appropriate HNT ratio. They observed that the mechanical properties of the composite structure were improved by the HNT addition of PMMA bone cement.

Mathi *et al.* (2020) presented an approach for orthopedic implant applications combining hydroxyapatite (HAP) with polyhydroxybutyrate (PHB) and halloysite nanotubes (HNT). With their work, they showed that the mechanical strength, easy accessibility, low cost and low toxicity of HNT are useful materials for bone implant. Xia *et al.* (2022) developed halloysite (HNT)/phytic acid (PA) coating material sensitive to pH stimuli. In their study, silk-HNT/PA coatings were used to improve the corrosion resistance and osteogenesis of Mg alloy osteoimplants. At the end of the study, they observed that HNT effectively increased the self-healing effect. The production of HNT added composite materials used in implant coatings is shown schematically in the Figure 10.

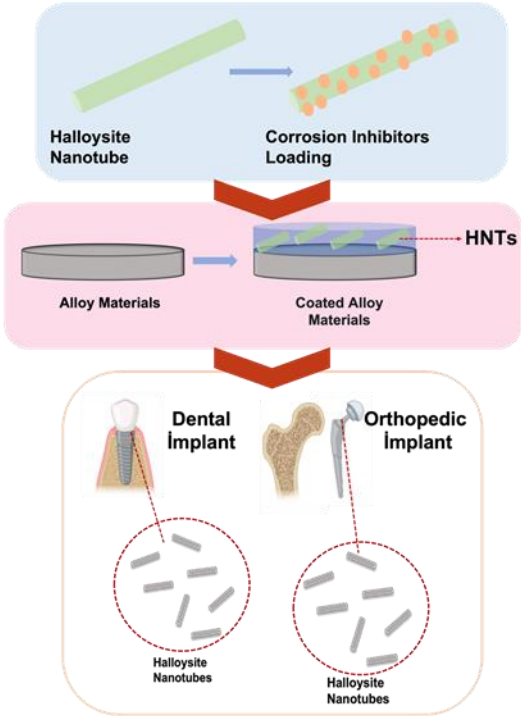

Figure 10. Schematic representation of the production process of HNT added surface coating materials used in implant materials.

Toxicity of prosthetic materials requires frequency of treatment and this reduces the quality of life of patients. In addition, biomaterials can undergo deformation during daily activities. Looking at the studies in the literature, it is seen that the use of HNTs gives biomaterials superior properties such as hardness and durability.

3.7. Hygiene and Cosmetic Materials

It has become popular for developing products. With the acceleration of studies in nanotechnology, various researches are carried out for new cosmetic formulations. Natural halloysite nanotubes have also taken their place in cosmetic formulations thanks to their prominent features. The inclusion of HNTs in cosmetic products improves the performance of the products. It can improve the textures of cosmetic formulations, extends shelf life and increases their effectiveness.

The expectation of new products for hair care, preventing hair loss or strengthening hair is increasing. HNT-added formulations are also beneficial for hair products. For example, Cavallaro *et al.* (2020) have designed a HNT and keratin based UV protective hair product for use in hair treatment. Through HNTs, HNT/keratin was immobilized on hair cuticles. The results showed that the keratin/HNT dispersion has a hair protection capacity of 50-60%. In another study, HNT was loaded into hair dyes and drugs to create a new hair care formulation by taking advantage of the long stay of HNTs on the hair surface. Lawson (2-hydroxy-

1,4-naphthoquinone), a dye type, was loaded onto HNTs. In this way, it has been observed that the hair dye maintains its effect up to six washes. This method has been tested on animals and effective results have been obtained (Panchal *et al.,* 2018). Bertolino *et al.* (2017) characterized HNTs in the presence of different biopolymers for medical and cosmetic applications. They investigated the surface and thermodynamic properties of HNTs in aqueous solutions of biopolymers such as chitosan, pectin and hydroxypropyl cellulose. Modified HNTs have shown potential for use in stimuli-sensitive release systems. Degrazia *et al.* (2018) conducted experiments on an orthodontic adhesive. For the experiment, they tested the antibacterial activity and bioactivity of orthodontic adhesives containing triclosan-loaded HNT (TCN-HNT). At the end of the study, it was noted that the bonding properties of TCN-HNT orthodontic adhesives increased and they showed effective antibacterial properties. Chlorhexidine (CHX) loaded HNTs were used in a study to investigate dental resin composites (Figure 11). It has been reported that CHX-HNTs, which were determined to be suitable as filling materials, showed high mechanical strength and very good antibacterial activity (Barot *et al.,* 2020b).

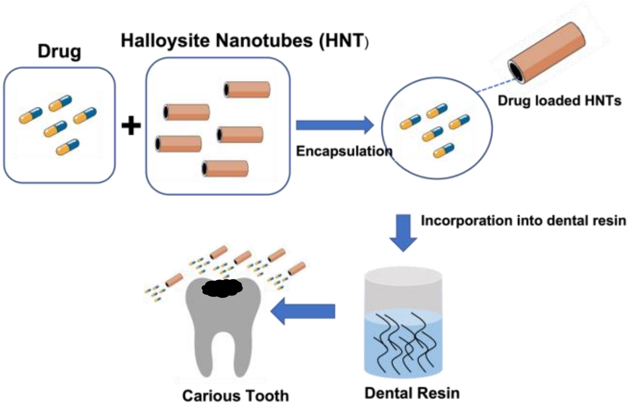

Figure 11. Schematic illustration of preparation of experimental dental resin composite containing HNT/CHX.

Wu *et al.* (2017) conducted a study on cotton textiles, which are also used as medical products. In this study, antibacterial composite was studied by loading HNTs with chlorhexidine gluconate (CG). The prepared HNT/CG composite was coated on a cotton fabric, and its antibacterial properties were investigated by the drug release zone and inhibition zone test. They reported that the drug-loaded HNT composite showed high bacterial inhibition activity, according to the study performed on *S. aureus, E. coli* and *P. Aeruginosa*. After one week, it was observed that the HNT/CG composite showed effective antibacterial properties. Koosha *et al.* (2019) developed HNT-enhanced chitosan/PVA nanofibers for skin tissue regeneration. The tensile strength of nanofibers increased by 2.4 and 3.5 times with HNT added to nanofibers at 3% and 5% ratios, respectively.

4. Future Perspectives

The possibilities that nature offers to people are so many that it can be said that they are innumerable. Halloysite nanotubes are one of these natural resources. It is used in scientific studies in different fields with its unique properties. HNT has also been the focus of attention in studies on human health, thanks to its features such as being biocompatible, abundant in nature, low cost and non-toxic effects. For example, it has been observed that the inner lumen in the structures of HNTs facilitates drug release by allowing a certain amount of drug and drug load. They can also be used as nanoplatforms to increase the immobilization efficiency of enzymes and nucleic acids with HNTs. In addition, when the studies are examined, it is concluded that they are suitable and very advantageous materials for biosensors, bioimaging and tissue engineering applications. Therefore, in the future, HNTs can be included more frequently in studies in the biomedical field, and more research can be done in this area. Most importantly, halloysite nanotubes can have different origins as they are natural materials. This means that they may have different toxicological profiles. When we look at the literature, it is seen that studies on the toxic effect of HNTs are at the initial stage. Therefore, in the future, the toxic effect of HNTs can be investigated in more detail by increasing the number of studies on the time-dependent use of HNTs on human health, and work can be started to purify the material.

4. Conclusion

Halloysite nanotubes, which are tubular in nanometer size, have been involved in many studies. In this review article, studies that prove halloysite nanotubes to be a versatile material for biomedical applications are presented. As a result of the studies, it has been understood that HNTs can be used safely in different biomedical applications. HNTs increased the mechanical strength of implant materials. It has created an affordable, easily accessible alternative for loading and releasing drugs in drug delivery applications. Halloysite nanotubes also improve the immobilization performance of biomolecules in biosensors. Thus, more sensitive, more selective and more reproducible results are obtained. HNTs have a structure that allows many different active ingredients to coexist. In this way, it is also advantageous to use in cosmetic products that can provide a healthy-looking skin. In conclusion, in this review study, we have seen that halloysite nanotubes are successfully used as versatile materials in biomedical studies. We think that the studies that have accelerated and increased over the years will increase even more in the coming years and that HNTs will appear in new techniques and materials.

Author Contributions

In this study; Sultan BUTUN SENGEL, editing, checking, writing, uploading the article, Nilay TUNCA, literature research, figure design Hatice DEVECI, literature research, figure design, Harun BAS, literature research, figure design, Vural BUTUN reviewing, editing, consulting the article contributed.

Conflict of Interest

There is no conflict of interest.

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