Antimicrobial applications of clay nanotube-based composites

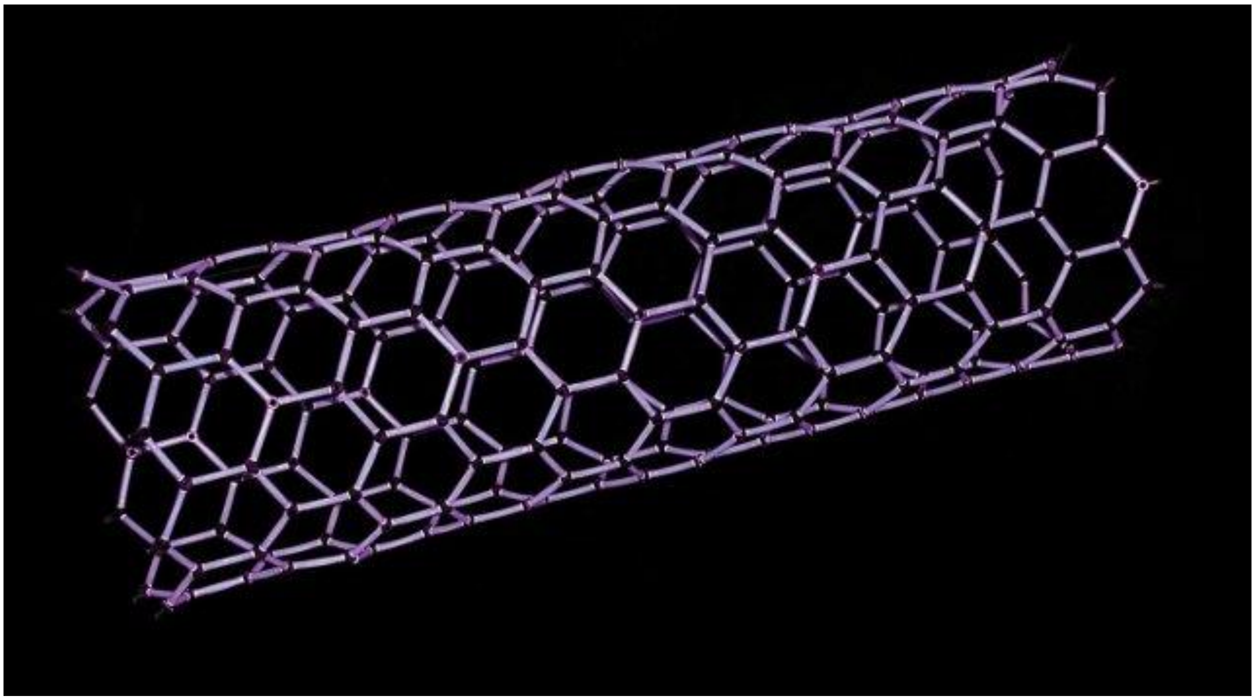

Table of Contents

1. Abstract

Due to their harmful effects, these nanomaterials are not designed safe for individuals and the surroundings as well. In recent years, there has been growing concern about the effect of carbon nanotubes on human health and the environment because of their toxic potential nature. These carbon nanotubes are synthetic in origin, nonbiodegradable, and non-biocompatible, hence harmful effects and environmental hazards. To overcome these drawbacks, green nanotechnology is one of the promising technologies that aims and advancing environmentally safe and less harmful nanoproducts.

Thus, clay nanotubes, nanocomposites, and nanopowders are emerging trendsetters in green nanotechnology. The clays are natural materials that possess great antimicrobial activity and proven biocompatibility and are easily available at very low prices. The important clays are Halloysite (HNT) which are naturally occurring clays of aluminosilicate and have been investigated as having excellent antimicrobial applications in medicine and clinical studies. Antimicrobial activity is a collective process of all such active principles which inhibit, prevent, and destroy bacteria, microorganisms, microbial colonies, etc. and industries need materials for doing such antimicrobial activity, preventing infections, and for the safety of their products. Keeping in view recent trends, in this chapter, various clay nanotube-based composites will be discussed with special emphasis on their antimicrobial applications.

2. Introduction

Clay nanotubes or HNTs (Halloysite nanotubes) could be mined from different mineral deposits because these exist naturally all over the world. That is why these are easily accessible and very cost-effective nanomaterials (Paul, 2008). These possess chemical similarities with kaolin clay with a two-layered aluminosilicate structure and tubular features. Their inner diameter is about 15 to 50 nm and their length is around 100 to 2000 nm. They possess huge surface areas which are why, they could be easily coated and loaded with various materials like biomacromolecules, drugs, and organic compounds. It is only due to their larger surface areas that a wide range of active agents like drugs, antibiotics, biological molecules, cancer drugs, marine biocides, and antimicrobial agents could be easily entrapped in not only their mineral inner lumen but also in void spaces of aluminosilicate and shells (Lvov Y. M., 2016). It is also their excellent property that these clay nanotubes are non-cytotoxic over various cell types which include dermal fibroblasts, chondrocytes, stem cells, osteoblasts, etc. (Santos, 2018).

It has been studied through a complete biocompatibility study in the model of rat dermal that clay nanotubes do not incite host immune response or cytotoxic response. Therefore, due to their high level of cytocompatibility, clay nanotubes represent themselves as the ideal candidate to be utilized for polymer additives, templates, and drug delivery systems in nanotechnology (Danyliuk, 2020). Due to their great biocompatibility, clay nanotubes have been widely used as antimicrobials by incorporating antimicrobial properties to them.

Antimicrobial properties have been imparted to clay nano-tube composites by various methods which include loading antimicrobial organic compounds like povidone, green iodine, amoxicillin, chlorhexidine, etc. inside clay lumen. Other methods include a coating of clay nanotubes with metal nanoparticles like silver, and copper to make them antimicrobial nanocomposites. These clay nanotube composites possess antimicrobial agents that provide a variety of antimicrobial applications in various fields like health and medicine, the food industry, clinics and hospitals, bone and tissue engineering, wound dressing, food packaging and food contact materials, dentistry, filtration membranes, and surface disinfectants, etc.

The objectives of this chapter include signifying how advancement has come in clay nanotube composites use and how various methods have been adopted with time to impart excellent antimicrobial properties to clay nanotube composites. It has been highlighted that those organic compounds loading methods that have been used in HNTs to impart antimicrobial properties to them were slow in drug release. But with advancement, various methods have been developed which include the coating of HNTs with metal nanoparticles which are more efficient than old methods and these also stand against the limitations of old methods. This chapter aims to find out and highlight not only the antimicrobial applications of clay nanotube composites but also to demonstrate the ways through which these antimicrobial properties have been imparted to clay nanotube composites.

3. Structure and Chemical composition of Clay nanotubes

Clay nanotube composed of alumino-silicate double layer which occurs in hollow spherical or tubular in structure a large amount clay silicate which contains thin plates. HNTs typically originate in soils formed from glass and volcanic ash such as volcanic deposits. It is present in the order of Andisol soil and is also called a clay mineral. The empirical formula of alumino-silicate mineral is $Al_2Si_2O_5\,(OH)_4\,nH_2O$. Halloysite clay nanotubes (HNTs) are occurring naturally in the tubular nanostructure, formed by rolling sheets of aluminosilicate kaolin several times. On the surface of the clay nanotube, the siloxane and alumina groups help in the hydrogen bonding formation on its surface with the materials of biomolecules. It consists of aluminium (20.90%), silicon (21.76%), hydrogen (1.56%) and oxygen (55.78%). Nano-clays are layered silicate nanomaterials.

In the chemical composition and morphology of nanoparticles, HNTs polymer composites originate in various divisions including halloysite, hectorite, bentonite, and montmorillonite. HNTs clay nanotubes are usually formed by hydrothermal alteration of alumino-silicate natural resources. It has 1 1m in length, 50 to 80nm of its outer diameter and the lumen diameter is almost 10–15 nm. In clay nanotube (HNT) hydrogen bonding formed in the aluminium and siloxane groups on the HNT surface of biomaterials. Omaliusd Halloy is the first person who discovered clay nanotube and named it Halloysiteafter its discovery**,** the mineral in Belgium. In1826, Berthier describe the clay nanotube first.

Clay nanotube also called Halloysite, is abundant and economical. These are found in natural deposits in tonnes of almost tens of thousands. Chemically HNTs are comparably similar to another type of clay mineral which is kaoline. In HNT, in tubes, the alumosilicate sheets are rolled while a platy particle leads to kaolin. Halloysite reveals its dimensions on the nanoscale which shows nanotubular geometry. Clay nanotubes have nanotubular arrays having different regions in their structure. Clay nanotube has two important functional groups which have different pH ranges and surface charges, one is eternal (Al-OH) surface, and the other one is external (Si-O-Si) surfaces.

Halloysite clay nanotubes structure have tubule inner lumen has 8.5pH with positively charged and 1.5 pH of outer surface with a negative charge. Nifedipine, Furosemide, and Dexamethasone are negatively-charged molecules. These molecules have charge differences in their surfaces, due to this reason they can be loaded inside the clay lumen. The negatively charged outer surfaces of tubules are adhering to charge differences. Chay nano-tubes are also known as nanocontainers due to their functional groups which are used in their inner and outer surfaces (Abdullayev, 2011). Nano containers are used for controlled release by loading molecules like enzymes and drugs into polymers that are bioactive apart from their solubility. These bioactive molecules are loaded into clay nanotube-based composites through a variety of mechanisms like tubular entrapment, adsorption, and intercalation. The structure and chemical composition of HNT are shown in the figure.

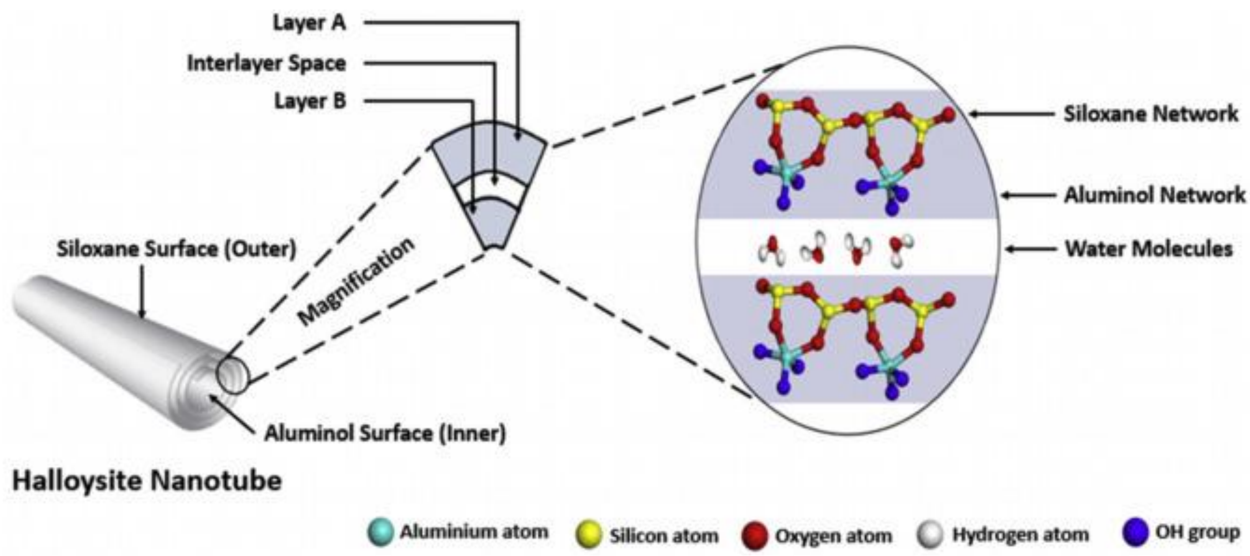

Figure 1.1: Structure and chemical composition of Clay nano-tube based composites

3.1. Composition of Antimicrobial Clay nanotube-based composites

In HNTs nanotubes there are two different accepted techniques are generally used to teach antimicrobial properties. First is HNTs lumen loading of organic materials occurs and adsorption takes place on the exterior surface and the second one is external grafting. There are various methods of loading molecules into halloysite nanotubes and these are given below.

3.1.1. Adsorption

In the thermodynamic equilibrium, the process of adsorption involves stirring of drugs and for one-day loading of halloysite nanotube. Oven drying and centrifugation processes are used to form loaded drug composites of polymer halloysite. The rate of equilibrium absorption is generally described and identified by an isotherm. Langmuir isotherm is obeyed according to the cationic drug (loading drugs) over halloysite nanotube through adsorption phenomena. Diclofenac sodium exhibited significant adsorption and is also a loading drug over the surface of poly-ionic minerals (Stavitskaya, 2019).

On the contrary, in fewer concentrations of drug solution, the Langmuir adsorption theory of isotherm is not constantly correct. In HNTs, the binding camber of diclofenac sodium is shaped abnormally showing that multilayers are occurring in its adsorption. In HNTs surface, adsorption phenomena have been exhibited by approving various types of experiments. In this mechanism, clay nano-tube composites act as a nano-adsorbant in which the absorptions of cationic molecules through pollutants treat water waste material.

3.1.2. Intercalation

HNT clay nanocomposites can be used to intercalate some bioactive materials of organic as well as inorganic molecules because they are the materials of the interlayer. In the process of d001 spacing 0.3–0.5 nm expands between the layers when the bioactive molecules are entering the interlayer space. The materials of intercalating clay nanotube form significant interactions of dipole and hydrogen bondings when interacting with tetrahedral silicone and silica sheets. Furthermore, HNTs are intercalated by the functional group of anionic interrelate with layers of alumina for example dimethyl sulfoxide, potassium acetate, amides, aniline, hydrazine, formamide, etc.

By increasing temperature, the halloysite nanotubes are dehydrated due to the absence of strong hydrogen bonding that causes low intercalation potential. The intercalation fact is associated with the interlayer water; the exchange of water molecules between wall layers of halloysite clay nanotube is the demand of intercalation. The drawback of this technique is the partial space between the wall layers of halloysite composite from loaded to prevent increasing molecular weight. Subsequently, in these applications, it might be accommodated by the use of smaller materials including drugs and corrosion inhibitors.

3.1.3. Tubular entrapment

Tubular entrapment is mainly the well-known loading of HNT composites also called as vacuum method. This technique opposite to intercalation allows for storing the different substances in the halloysite composite (HNT) lumen. Tubular entrapment has two different techniques; the general process of this technique is as follows:

The first one is stirring dried halloysite clay nanotube (HNT) with a highly concentrated solution of selected molecules. The molecules and walls of HNT have excellent solubility by the solvent which has less amount of viscosity. Particularly for the loading of substance which has bioactive molecules water has an appropriate solvent while organic substance contains desirable ethanol and acetone. It has been observed that due to the increase of the dielectric constant of the solvent the bioactive substances with negatively charged have a considerable quantity of drug loading. A homogenous mixture is formed from the suspension which is shifted through evacuating various times to a vacuum jar via a vacuum pump. In this process of vacuum, from the balanced nanotube composites, HNTs air is removed showing the sign of fizzing of minor solution of the lumen. Within 10–30 min of the process of vacuum, the suspended medium should remove air by atmospheric pressure by replacing air with a desirable substance.

This technique is repeated several times to ensure that bioactive molecular solutions are present in HNTs lumen. HNTs composites lumens have almost 15 nm diameter and 10–15 wt% molecules of biomaterials and are very close to theoretical estimates. In the cycle of the vacuum process, attached bioactive molecules are removed by centrifugation of halloysite composite suspension weakly. By repeating the washing process, it is clear that nanotube composites are fully loaded with the most wanted substance which has active sites of biomolecules.

In the second procedure, targeted bioactive molecules of bioactive materials are mixed with HNT composites in equal amounts. As an alternative to diffusion, in this method, the final mixture is a thick paste that is cycled between atmospheric pressure and vacuum two to three times. The resultant product of the mixture has active biomolecules in a dried vacuum. In HNTs the maximum amount of targeted active biomolecules latter method is used to avoid solution wastage and bioactive molecules.

3.1.4. Halloysite nanotubes or polymer composites

Halloysite nanotube composites are more hydrophobic than layered silicates and other nano-silica because of their lesser density at the outer surface area of hydroxyl groups so the interaction of tube–tube halloysite is comparatively weak. There are many methods applied for the formation of polymer composites commonly including functional methods like melt-blending, solvent casting, electrospinning, etc. Additionally, clay nanotube (HNT) or polymer composites can b formed by compression molding, in-situ polymerization, coagulation, hydrogel preparation, injection moulding, and deposition of polycations and HNT.

Clay nanotube-based composites are synthesized by several economical methods. The way of method was used to enhance HNT distribution into a polymeric matrix with enhanced interfacial interaction. Due to polymer distribution, some basic properties of clay nanotubes are affected which include HNT in the polymer matrix, thermomechanical, and microstructure. The homogeneous dispersion of polymer composites into its matrix than aggregates promotes some microstructural features. The formation of improved compression molding and water-assisted injection moulding technology has some morphological properties of HNT clay nano-tube or polypropylene (HNT/PP) nanocomposites. With the improvement of the mechanical behaviour of clay nanotube (HNT) based composites, the number of polymer composites is increased in a polymeric matrix (Gorrasi, 2015). The increasing amount of polymer composites shows more mechanical characteristics due to intrinsic stiffness, its increasing ratio, and uniformed distribution of halloysite nanotube into the matrix of polymeric.

The enhanced tensile modulus and flexural are considered when a consistently HNTs distribution is included in polylactide (PLA) matrices. The enhancement may be ascribed to the rigidity improvement of the nanotube-based composites by HNTs, allowing at the interface of clay nanotube advanced stress transfer. Therefore, the polymeric nanocomposites have no remarkable improvement in their tensile strength observed due to weak interference between HNTs surface and PLA matrix. On the other hand, by applying the quaternary ammonium salt to treat with HNT nanotube (modified-HNT) the advantageous interface of PLA matrix and polymer composites is observed. Young's modulus and mechanical strength of clay nanotube-based composites improved much more advanced than an unmodified nanotube-based composite of HNT or PLA.

The mechanical behaviour of polyvinyl alcohol-HNT was observed with nanotube-based composites with increased tensile strength and its elongation up to 7.5 wt% loading HNT. Furthermore, by strengthening effects of clay nanotube with better mechanical behaviours thermal stability and flame retardancy have been reported.

For unique polymeric nanotube-based composites to expand some new horizons in biomedical applications the major characteristics of HNTs act as a carrier for conductive filler and controlled delivery release. Designing and engineering biomedical yields clay nanocomposites allow its flexibility. Polyacrylic acid (PAA) or clay hydrogel composites are used as an absorbent in water waste for the removal of ammonia. HNT clay hybrid beads are used as alginates and are very efficient to eliminate methylene blue. In these practical applications nano, polymer-based composites are used for the removal of dye.

4. Methods for preparing Clay nanotube-based composites

Clay nanotubes possess unique properties which make them disperse more eagerly in polymeric matric structures as compared to platy clay-like montmorillonite and Kaolin. HNTs possess hydrophilic properties which make them easily disperse in systems of polar polymetric which include polyvinyl alcohol, polyamides, biopolymers, and polymethylmethacrylate. However, clay nanotubes also possess compatibility with systems of non-polymers like polylactic acid and polypropylene utilizing surface-compatible agents with it. There are various methods available for preparing HNT or polymer composites (Fakhruddin, 2021).

4.1. Melt-Blending

It is generally utilized functional method for preparing clay-nanotube composites. In this technique, the polymer is first melted and allowed to combine with intercalated clay's required quantity via an extruder (Kim, 2020). This process is done utilizing inert gases like neon, argon, or nitrogen. The dried polymer is mixed with some intercalant and heated in a mixer where it is subjected to some shear sufficient to make the desired polymer of clay nanocomposite. It is an environmentally benign and friendly process which is why found various applications in industries.

4.2. Solvent Casting

Solvent casting is another standard technique for preparing HNT nanocomposites in a well-dispersed polymer mixture. In this, HNTs are cast and then allowed to be dried for creating the polymer nanocomposite films. It is a process in which HNT nanocomposite has been prepared by dissolving the polymer into volatile solvents for getting a homogenous solution possessing low viscosity. The solution could be either spread over the substrate or cast into the mold. Then polymer film is allowed to adhere to the mold by drawing off the solvent from it. This technique is advantageous because films of solvent cast dried by themselves without using any external factor like mechanical or thermal stress. Many additives could also be introduced into the polymer-solvent solution. The polymer films produced through this method possess great optical clarity and thickness which has homogenous distribution (Sharma, 2019).

4.3. Electrospinning

It is also another famous method for producing HNT nanocomposites. The electrospinning process includes an electrodynamic process in which a liquid droplet is allowed to be electrified for generating a jet which is followed by elongation and stretching to produce fibres (Xue, 2019). In this process, the polymer melt is charged and ejected into the spinneret utilizing the high voltage of an electric field for solidifying the coagulate to create a filament. This process is suited particularly for producing complex and large molecules and it ensures that no solvent is left over in the final product (Bhattarai, 2017).

5. Imparting of antimicrobial properties to clay nanotube-based composites

For imparting antimicrobial properties to clay nanotubes, various approaches have been used. These include organic molecules loading inside the lumen of clay-like halloysite and then adsorption of it over the external surface. The other approach which is generally accepted is outside grafting. Nowadays grafting of clay nanotubes with metal nanoparticles like Ag and Cu nanoparticles which act as antimicrobial agents is proved as a very effective method to give antimicrobial applications to HNTs. The clay nanotube composites which have been incorporated with antimicrobial properties by loading antimicrobial agents in it have been used for various purposes like in wound dressings, bone regeneration, nanofibers, and the electrospun membrane.

5.1. Organic Compounds loading inside Clay nanotubes

For the loading of antimicrobial agents like organic compounds inside the clay nanotubes composites and their adsorption on clay/halloysite, the two techniques assist it. These techniques are vacuuming and sonication which assists while stirring this drug solution of antimicrobial agents. The efficiency with which antimicrobial agents have been loaded inside clay nanotube depends on the size, charge, and procedure of the overall process. Some of the antimicrobial agents which have been widely used for loading inside clay nanotubes are green iodine, gentamicin sulfate, vancomycin, povidone-iodine, tetracycline, doxycycline, potassium clavulanate, amoxicillin, brilliant green, and chlorhexidine (Lvov Y. &., 2013). These all-antimicrobial agents have been loaded inside clay nanotube composites using vacuum and sonication. For having excellent antimicrobial properties in clay nanotube-based composites, the kinetics parameter of drug release is the most crucial one. The commonly used and practical antibacterial medicines are amoxicillin, anionic povidone-iodine, and cationic chlorhexidine (Stavitskaya, 2019).

5.1.1. Loading of Chlorhexidine gluconate inside HNT composite

The organic compound, chlorhexidine gluconate although possesses a positive charge but still could be easily loaded within clay nanotube composites. The loading of this organic compound within HNT shows only 25% of its sustained release within the first hour. However, this drug shows 75% of its release when it is present in its original untreated micro powder as an unloaded drug. That is why Chlorhexidine gluconate has been introduced into the coating of cotton fabric for its loading within clay nanotubes because it helps to improve the antimicrobial activities of Chlorhexidine gluconate without the need of using the silver particles in it.

It has been observed that the loading of Chlorhexidine gluconate with the help of cotton fabric coating increases its antimicrobial property and shows a reduction of bacteria, *Staphylococcus aureus, Pseudomonas, and Escherichia Coli* by 98%. It has shown its antimicrobial activity by 90% even if it gets washed 20 times because of chlorhexidine gluconate's slow release from clay nanotubes.

5.1.2. Loading of Amoxicillin and Povidone-iodine inside HNT Composite

Antimicrobial compounds Amoxicillin and Povidone-iodine have also been commonly loaded within HNT composites and possess a slow rate of drug release. These drugs have been first prepared in the form of their mixture for loading within HNT. The saturated solution of amoxicillin (50 mg/ml) and povidone-iodine (80 mg/ml) has been added into HNT as drug solutions and then allowed to be stirred for obtaining their homogenous suspensions. These solution suspensions are then placed within a vacuum for one hour in three cycles for ensuring their maximum loading inside HNT. This loaded clay nanotube is allowed to be washed twice for removing external/excess drugs if present inside the vacuum desiccator. It is placed overnight to make it in powder form so that these powder formulations could be stockpiled for their long-time usage and in the future, easily dispersed by mechanical stirring in water before usage (Stavitskaya, 2019).

The efficiency of loading of these drugs is then determined by using TGA (Thermogravimetric Analysis) and their release efficiency has been analyzed with the help of a UV-VIS spectrophotometer on 227 nm for amoxicillin and 224 nm for povidone-iodine. The curves of TGA show 8.3 and 7.6 wt.% of amoxicillin and povidone-iodine loading inside HNT. It has been identified that the loading of these drugs could be enhanced by clay nanotube lumen enlarging using sulfuric acid for etching inner alumina. These formulations are found efficient for *Escherichia Coli* and *Staphylococcus aureus* inhibition for a whole one week and their extended-release was found as 6-20 h. These types of formulations are found very effective for sprays in which clay nanotubes have been loaded with 3-5 wt.% antiseptics and these form a thin coating after spraying with extended antimicrobial activity (Stavitskaya, 2019).

5.2. Utilization of metals as Antimicrobials

It has been identified that organic antiseptics loading inside HNTs has its limitations because of its slow drug release. The pathogen's exposure to low concentrations of drugs progressively could become a risk factor in selecting drug resilient strains and this factor could not be ignored in any case. That is why, new strategies need to get adapted and this problem's possible solution is hidden in the utilization of metal nanoparticles as antimicrobial agents for producing the antimicrobial composites (Barot, 2020).

It has been studied previously that clay nanotubes (HNTs) possess huge surface area, which is why could be loaded with various materials like polymers, biomacromolecules, and drugs for extended and sustained releases. For fabricating uniform nanomaterials with a high yield in nanotechnology, it is important to know the antibacterial response of that nanotube and whether its nanofabrication procedures are cost-effective or not. It has been observed throughout history that metals are utilized as antimicrobials, and these are proven effective today against bacteria. However, advancements have been continuing to come with time in the synthesis of metallic nanoparticles and give rise to new antimicrobial fabrication technologies with age (Boyer, 2016).

5.2.1. Advancement in metal nanoparticles depositions over HNTs

There are various methods developed with time to deposit the metal nanoparticles over the inner lumen and outer surfaces of clay nanotubes by the use of particular metallic salt or compound with it in a multistep chemical reaction and at high calcination temperature. The utilization of metal as a support system with HNTs includes many advantages like large surface area, low cost, scale nanometre tubular morphology, and availability. These have been utilized as antimicrobials from Hippocrates' time till today and are very effective. In past, Ag electric colloids and synthetic antibiotics were common methods in antimicrobial therapies.

Currently, the processes of electrochemical nano-assembly perform a new role in depositing the metal nanoparticles over clay nanotubes for giving them antimicrobial features. However, in past fabricating methods of clay nanotubes, metallization involve multistep processes which comprise organic compounds, high temperatures, metal salts, and reducing agents to gain surface modifications in HNTs.

There are Cu and Ag particle technologies in wound dressings, disinfecting sprays, and hospitals. It is because metal nanoparticles like Ag and Cu have displayed extreme toxic behavior toward bacteria even though at many low concentrations. It was studied that these transition metals possess the ability to disrupt bacterial electron transport and respiration systems upon their absorption within bacterial cells. These transition metals are being incorporated into the inert materials of polymers for preventing infections. Recently Ag use has been increased as an antimicrobial agent within textile industries for antimicrobial washes and polymers (Barot, 2020).

5.2.2. Copper (Cu) nanoparticles as antimicrobial agents

The Cu nanoparticles act as redox-active metals which oxidize themselves during their interaction with bacterial cell membranes. The toxicity which has been induced by Cu involves multiple mechanisms such as ROS formation through free ions of Cu where cuprous and cupric ions participate in redox reactions. It has been studied that Cu^{2+} ions possess the ability to reduce into Cu^{+} in reducing agents' presence to catalyze the hydroxyl radicals formation by hydrogen peroxide. These produced hydroxyl radicals are extremely reactive and detrimental to processes like protein oxidation and cellular molecules of bacterial cells (Boyer, 2016).

5.2.3. Silver (Ag) nanoparticles as antimicrobial agents

In the case of Ag nanoparticles, these anchor within bacterial cells, penetrate them, and does structural damage to bacterial cells which eventually causes their cell death. It happens due to Ag ions released from Ag nanoparticles and it has also been observed under electron spin resonance spectroscopy that these metals also produce free radicals in bacterial cells present. These produced free radicals possess the ability to make bacterial cell membranes porous and lead to cell death (Barot, 2020).

5.3. Clay nanotubes nanostructured coating with antimicrobial metals

Clay or halloysite nanotubes could be utilized as a substrate material in doing nanostructured coating over it by metals as antimicrobial agents. For this purpose, electrolysis techniques have been identified as novel procedures for depositing metal nanoparticles over clay nanotube surfaces. Moreover, metal clay nanotubes showing antimicrobial activity have also been monitored against gram-positive and gram-negative bacteria with the help of a spectrophotometer. Antimicrobial applications have been given by such clay nanotubes which possess modified surfaces with pure Cu and Ag nanoparticles using electrolysis. Generally, the process of coating clay nanotubes with metals has been done in distilled water containing mixed HNTs in it. The constant direct current has been applied to electrodes of metals for synthesizing the metal nanoparticles and facilitating these metal nanoparticles' deposition over clay nanotube surfaces. The detailed fabrication processes of these clay nanotube coatings with various transition metals for giving antimicrobial properties to them are given below.

5.3.1. Silver-Clay nanotube (Ag-HNT) Synthesis

For preparing Ag nanoparticle coated clay nanotube composites, these Ag metal electrodes have been placed within 500 milliliters of distilled water by keeping a distance of 0.5 inches between these metal electrodes. This mixture was then heated over 95 Celsius and this whole setup has been connected to an outside power supply of direct current. Further 300 milligrams HNTs were also added to the heated mixture by constantly stirring this mixture with the help of a magnetic stirrer. After this, a direct constant current has been applied to this mixture for 40 minutes by keeping the current at 30 volts. Then power supply was disconnected when 40 minutes were passed, and this mixture is allowed to be centrifuged. The supernatant obtained was decanted and an Ag-clay nanotube which has been achieved as the final product then dried for 24 hours at 60 degrees Celsius. The variations in the use of HNTs mixture, distilled water and applied direct current period could be used to check the rate of antimicrobial activity of the same metal nanoparticle coated clay nanotube.

5.3.2. Copper-Clay nanotube (Cu-HNT) Synthesis

The same method has been used for preparing the Cu nanoparticle coated clay nanotube composites, Like Ag metal electrodes, Cu electrodes were also placed within 500 milliliters of distilled water by keeping the distance of 0.5 inches between these metal electrodes. This solution was then heated over 95 Celsius and this whole setup has been connected to the outside power supply of direct current. Further 300 milligrams HNTs were also added to the heated solution by constantly stirring this mixture with the help of a magnetic stirrer. After this, direct constant voltage has been applied to this solution for 40 minutes by keeping the current at 240 volts. Then power supply was disconnected when 40 minutes were passed, and this mixture is allowed to centrifuge. The supernatant obtained was centrifuged and then decanted. The Cu-clay nanotube which has been achieved as the final product is then dried for 24 hours at 60 degrees Celsius. The variations in the use of HNTs mixture, distilled water, and applied direct current time could also be used to check the rate of antimicrobial activity of Cu metal nanoparticle coated clay nanotube.

5.3.3. Surface Modification of HNT

The samples of Cu-HNT and Ag HNT have proved that the method of electrochemical deposition is a very effective way to make HNT-meta composites. It has been observed that the deposition of metal is based on the time taken for the electrolysis process and HNTs concentration utilized. For instance, Ag-HNT which has been fabricated by using 300mg HNT possesses a much higher weight% of Ag as compared to Ag-HNT which has been fabricated using 5 g HNT. Similarly, the fabricated Cu-HNT composite at 300 mg possesses a higher Cu % weight compared with fabricated Cu-HNT at 5 g. In the same way, the electrolysis which has been done under increased time amount would produce a meta-HNT composite with higher metal % weight deposition on their surfaces.

It has also been observed that Cu nanoparticles show clusters having irregular geometries and Ag nanoparticles are spherical mostly. It is because the voltage amount applied has affected the particle size of these metals. The results of TEM have shown that there are small particle metal sizes observed by applying a lower amount of voltage and with increasing voltage levels, the nanoparticle sizes, and their deposition on HNT also increase. The results of materials characterization have shown that process of electrolysis proved successful in creating the metal-HNT nanocomposites (Jee, 2020).

5.3.4. Advantages of using Electrolysis technique for HNT coating

It has many advantages of using the electrolysis technique for surface modification of HNT and these include scalability potential, simple setup, and fabricating ability using nominal starting materials. Theoretically, it has been concluded that utilization of larger norm electrodes which are designed with a greater surface area can be utilized to synthesize the industrial level metal-HNTs scale amounts. This method of electrolysis for metal nanoparticle coating over HNTs is not limited to only Cu and Ag nanoparticles. This technique could be used to deposit other metals like palladium, gold, and platinum and these metals show similar electrochemical behavior during their deposition over HNTs during electrolysis.

6. Clay nano-tube Composite Antimicrobial Applications

6.1. Biomedical applications of halloysite nanotubes

Biocompatibility is the most valuable attribute of Halloysite clay nanotube-based composites which have some huge potential applications including wound healing medical implants, biosensors, nanomedicine, tissue engineering, and some drug delivery, etc. In further research, HNTs has lacking toxicity levels from low quantity. The comparative analysis of the recent research showed that (MWCNs) multi-walled carbon nanotubes, cytotoxicity of halloysite against blood vessels of mice assays (in-vivo), and vein of human umbilical (in-vitro) endothelial cells were. As the result, halloysite is more biocompatible to blood vessels than carbon nanotubes or multi-walled. HNTs have applications in anticancer therapy in the field of biomedical studies. Coating of halloysite nanotube enhanced the microscale flow device. The flow device of micro-scale can capture the (CTCs) circulating tumor cells from the blood of the patient (Peña-Parás, 2018).

The consumption or efficiency of the halloysite nanotube enhanced its (micro-tube) performance in killing and capturing cancer cells. Therefore, in this study, HNT loaded doxorubicin with magnetic particles and folic acid into (HeLa) cervical adenocarcinoma cells. Doxorubicin which is used is very toxic. The halloysite clay nanotube treats cancer cells by release of targeted anticancer agents. Anticancer agents are potential bioactive material that was used. In HNT intercalation of anti-metabolite 5-fluorouracil developed the system of drug control release. For the treatment of cancer, it helps to remove drawbacks like quick metabolism, low absorption, and shortened half-life of 5-FU. Contrast ultrasound agents are used for exceptional delivery systems and echographic clinical imaging which includes wound care and patches of transdermal.

In biomedical applications, HNTs are used in the nonstop release of drugs, antiseptics, enzymes, and proteins. Nano-carriers in HNTs are called nano-bazookas. Continuously, nano-bazookas have therapeutic agents in which nano-bullets target cancer cells. HNTs are readily available and used for systemic administration (intravenously). They have enhanced thermal and mechanical properties, and good biocompatible as well as non-biodegradable. Potential material is formed by HNTs' non-biodegradability for the formation of nano-medicines, formulation of nanocomposites, implant developments, treatments of animals, etc. The HNTs can interrelate with polymers by the formation of polymeric composites through (H-bonding) hydrogen bonding, van der Waal, and electrostatic interactions. These relations of composite interactions are useful for their enhanced properties and the development of dispersed uniform composites (Danyliuk, 2020).

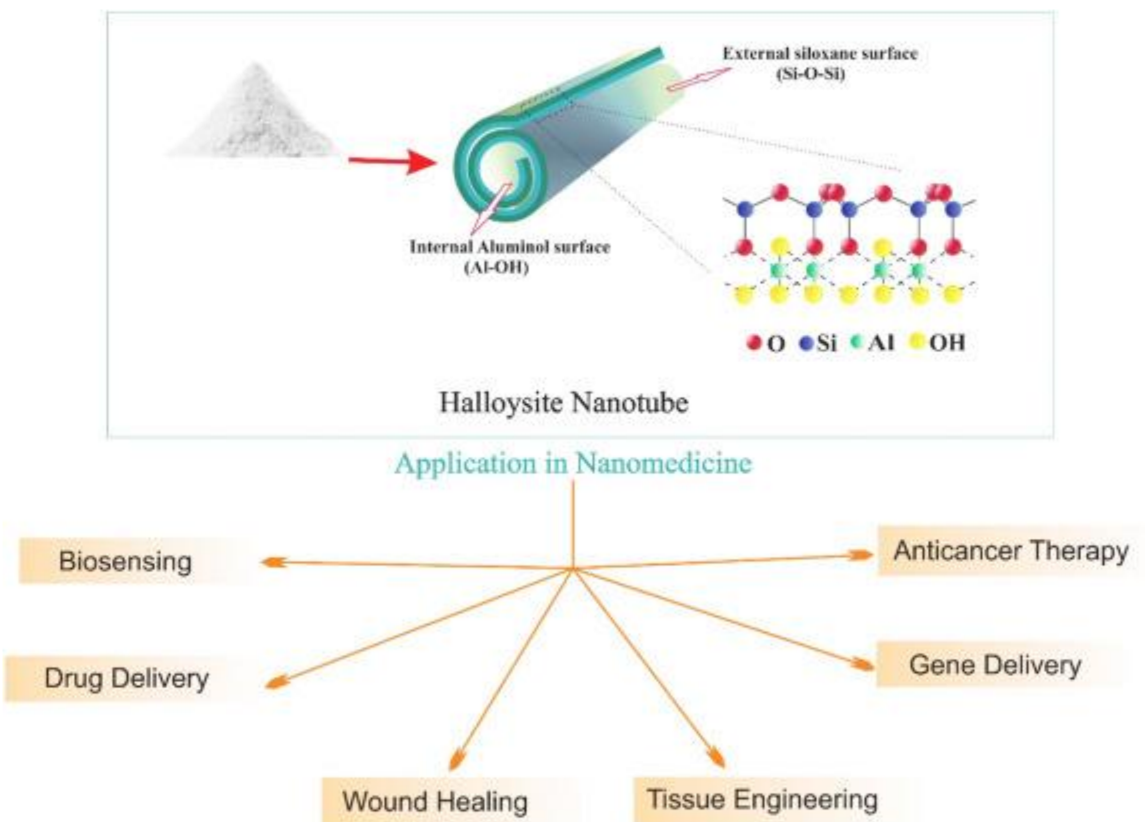

Figure 1.2: Biomedical applications of Clay nanotube-based composites

6.2. Liquids, and Surfaces Protection and Disinfection by Clay Nanotubes-based composites

There are an increasing number of infections that are acquired from the hospital. In health facilities, this type of infection is a very big issue. According to WHO (World Health Organization), the common way of transmission is by the indirect interaction in infectious diseases is transmitted with surface contaminants. These diseases are transmitted by sneezing, coughing, and talking of patients which produce infectious droplets. On surface contaminants, many different viruses and microbes can survive many days. The subsequent microbes are transferred to mucosal membranes of the nose, eyes, and mouth. Some reported infections and gastroenteritis outbreaks are spread by infected surfaces by hand contact. With the addition of microbial resistance, the effectiveness of antimicrobial agents is threatened seriously. World health organization WHO led a call for worldwide action in the 2016 General Assembly of the United Nations giving a political statement for antimicrobial resistance. Antibiotic resistance rate increases in highly developed nations among bacteria (Stavitskaya, 2019).

In Europe and the US, especially standard methods are used to stop long-time disinfection which includes disinfection solutions and wiping surfaces. Development with supportive antiseptic delivery could be economical and effective like aqueous chlorine dioxide spray. It has been reported that a definite quantity of durable antimicrobial schemes includes biocidal nano-materials like light-activated photocatalysts and silver nano-particles. These light-activated particles depend on phosphonium salts, TiO_2, and bactericide's surface-tethered quaternary ammonium compound. These nanomaterials are very effective against a broad array of microorganisms. HNTs Halloysite clay nanotube formed with nano-particles, loaded with medicine or drugs, and combined with paints to form the compound, or integrated into the hydrophobic surface. Zeta-potential halloysites do not accept long stable aqueous dispersions when it is ca.−30mV while the increases of HNTs zeta-potential to −50–60 mV is by loading forcefully with anionic drugs in internal nano-tube. Stable dispersions (water-based) are formed which are easily applied on surfaces relevant for favorable anti-bacterial sprays. For the purification of water, it has been seen that photo-catalysts are used for the degradation of bacteria in a state photo-induced. Halloysite clay nanotubes HNTs act as a stabilizer which was used to support photo-catalytic nanoparticles to prevent the accumulation of nano-particles. TiO_2 is a commonly used photo-catalysts and has some of its nanocomposites (Cavallaro, 2018).

For photo-catalytic disinfection, TiO_2 absorbs only wavelengths near to $\lambda < 400$nm UV region it is almost 3% solar spectrum while it cannot utilize visual light with efficiency and it has almost 43% of the solar spectrum. The modification in the bandgap is much expeditious visible light. A photo-catalytic method based on HNTs and TiO_2 were mainly tested for the degradation of waste product. It can also be used for anti-microbial applications. In UV-light anti-bacterial ZnO nanoparticles act as the photo-catalyst. Absorbed ZnO nanoparticles in HNTs are formulated as a UV barrier for the formation of bacteria on membranes.

In the bioactive properties of decorated halloysite nanotubes which is by the function of plasmonic inflammation of silver nano-particles. The measurement of optical absorption discovered wide plasmonic resonance for HNTs with silver nanoparticles in the part of 400–600 nm. The advanced samples were more noticeable under illumination which displays a bactericidal effect. Functionalized Ag clay seems for antibacterial treatments of surfaces and liquids. These are stimulated by exposure to visible light. Due to plasmonic effects, gold nanoparticles could be applied in the same way. Gold coating halloysite-based HNTs core-shell construction showed the interconnectivity, thickness, and morphology of the Au. It may specify the photo-thermal capacity and optical response of plasmonic materials (Danyliuk, 2020).

6.3. Tissue and Bone Engineering

In the recently developed research, halloysite clay which contains antibacterial particles has various functional groups used in many types of medical applications, polymeric films, and coatings. An electrospinning technique used to make nanofibers has been widely considered for different applications and tissue engineering. Due to the polymers used, drug-loaded fibers are biocompatible while due to polymer degradation they have antibacterial properties by the release of the drug slowly. Poly (lactic-co-glycolic acid), polylactic acid, poly (caprolactone) or gelatin, and polycaprolactone are used for making various fibers composites of halloysite polymer in the studied for antimicrobial protection. Lacticoglycolic acid (electrospun poly) nanofibers were changed with HNTs to form a system of drug delivery with a slow release of tetracycline hydrochloride. The drug loading efficiency was 42.65%. The inclusion of positively charged tetracycline hydrochloride into the electrospinning solution by halloysite loading shows a lower fiber diameter (Wong, 2022).

The halloysite nanofibrous mats exhibit useful antibacterial activity, revealed good cytocompatibility, and in both solid and liquid mediums, it can show bacterial growth. Drug-loaded halloysite with free percentages nano-fibrous mats in 42 days in low quantity than the first day which contains tetracycline hydrochloride and pure drug with nanofibers. In electrospinning drug-loaded halloysite clay nanotubes, due to constant drug delivery bone regeneration membranes were developed. Clay nanotubes doped into polycaprolactone or gelatin microfibers. Halloysite nanocontainers are used for advanced research in a clinical study by attaining new dental types of equipment with some properties of antimicrobial and bioactive in our daily life. As a delivery system of the drug, HNTs nanotubes play an important role in the establishment of dental coatings (Naumenko, 2019).

By using different types of alcoholic suspensions, the coatings of chitosan composite were deposited electrophoretically (EPD). Composites of chitosan coatings are also known as vancomycin-loaded Halloysite nanocomposites. Suspensions of Ethanol were selected by increasing the electrophoretically (EPD) rate as the best possible method and the coatings are formed with comparatively high violence and with high absorbent content or non-absorbed chitosan. Drug-loaded coating against S.aureus showed almost 27% (higher) anti-bacterial activity as compared to without loaded drugs with a similar coating (Stavitskaya, 2019).

6.4. Antimicrobial Applications of Zinc Oxide Nanoparticle

The excellent properties of (ZnO) Zinc oxide have attracted by its great interest worldwide. The understanding of the development of nano-particles has shown particular results. An appreciable survey of ZnO-NPs has been activated by using various types of synthesis methods for the production of nano-materials and its upcoming applications, high luminescent ratio allowed with the increasing number of excitation binding energy like 60 meV while the wide bandgap is almost 3.36 eV. In the packaging of food area (UV), ultra-violent protective agents are used while ZnO-NPs are commonly used as an anti-microbial. ZnO-NPs drive the synthesis methods of nanomaterials by increasing the focus on advanced improvement for its applications and functions.

Table 1.1: Applications of Zn Nanoparticles

Applications of Zn or ZnO particles		
Sr. No.	**Application's Fields**	**Examples**
1	Electronics and energy	Nanogenerator power sensors based on ZnO nanowires, Low coast solar cells
2	Cosmetic's industry	Minerals cosmetics, UV filters in sunscreen, antimicrobial food packaging
3	Medicine and biology	Drug and gene delivery, Bioimaging
4	Materials and manufacturing	Chemical sensors based on ZnO, antimicrobial textiles, protections from exposure to UV rays

6.5. Wound Dressing

Halloysite nanotube is used for the manufacture of multi-layer flexible wound dressings which contains many different functions like tissue regeneration, fluid absorption, fungal protection, and antibacterial protection. The dressing might be used as a pad or topical gauze, or as packing of wound material for both therapeutic and prophylactic interventions. Clay nanotube HNTs are used for advanced properties of dressing, load numerous drug sets possibilities, or increased dominance over the release kinetics drugs all these are encouraging properties to deal with multiple infections of microorganisms, unhealing chronic wounds, and also used for behavior of multi-vector (Same, 2022).

The antiseptic elastic nanocomposite combined with dual drug co deliveries was formed by sulfate-loaded HNTs clay nano-tube composites polymyxin B and ciprofloxacin into the gelatin elastomer. polymyxin B sulfate was dispersed in the matrix by loading halloysite nanotube while the ciprofloxacin nanoparticles were distributed directly into the matrix. Ciprofloxacin nano-composites are those particles that proceed against both gram-negative and gram-positive bacteria. Both polymyxin loaded HNTs halloysite and ciprofloxacin were affected by the formulation of some basic physical characteristics like fibroblast adhesion, in vitro drug release, proliferation, and cytotoxicity (Gaskell, 2014). The properties of antibacterial were also investigated by the effect of these loaded halloysites. Drug-loaded halloysite showed the rate of dissoluble high polymyxin B sulfate which were liberated, and it also improved the strength of matrix tensile (Sandri, 2017).

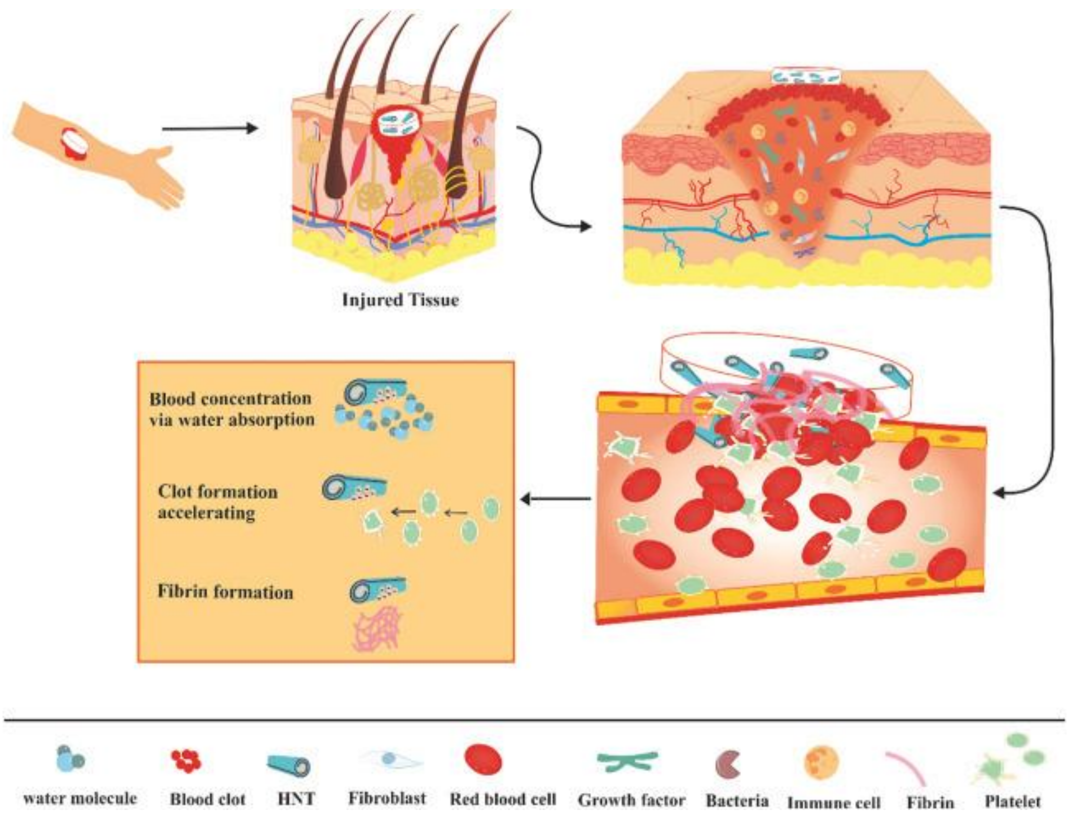

Figure 1.3: HNTs nanocomposites schemetic illustration of wound dressing

6.6. Filtration Membranes with Enhanced Antibacterial Activity

In this report, halloysite clay nanoparticles doped ultrafiltration membranes. These filtration membranes work with organic content in water. The high content of organic compounds increases consumption energy and decreases the lifetime of membranes by inhabiting biofouling. The composition of antibacterial agents with functionalized nanotube composites helps to control the evolution of biofilms. The membrane is formed by the use of a halloysite nanotube which allows for preventing bio-contaminants and for the antimicrobial properties (Stavitskaya, 2019).

a) The synthesized halloysite clay nano-composites (calamine) were added almost 1–3 wt% to the filter membrane (polyethersulfone).

b) The hybrid membranes have a lower rough surface than the membrane of pure polyethersulfone by thermos-mechanically stable membrane. It also had been increased the water flux. With the addition of HNTs halloysite, the membrane has enhanced hydrophilicity.

c) Clay nanotubes have preservative enzymes that act as antibacterial agents naturally. This creates another biofilms problem.

d) Covalently grafted lysozymes with carboxylic groups to functionalized halloysite HNTs. It was used to prepare ultra-filtration membranes of hybrid antibacterial by adding lysozyme into polyethersulfone.

e) The antibacterial test shows that hybrid HNTs membranes indicated the good performance of antibacterial. It was also used to treat wastewater.

f) Ultrafiltration polyethersulfone membranes produce qualified loaded halloysite which contains A nanoparticles. By phase invention, copper ions were processed.

g) In an early study, Ag-nanoparticles were grafted. Chemical modification of silane with halloysite is used for the formation of Cu^{2+} halloysite and the complexing of Cu- ions by mixing copper dichloride.

h) Tests of hybrid membranes showed that good anti-bacterial properties. Good antibacterial properties have more than 99% protective rates against S. aureus and E. coli nanomaterials.

6.7. Food Contact Materials

Materials of food packages that have polymeric films are susceptible to biofilm formation and bacteria colonization. Fruits, fish, bread, meat, cheese, and vegetables are particularly highly sensitive to fungi and bacteria. Polymer composites are developed with fillers of antibacterial which prevent food from decomposition and another important fact is the growth of micro-organisms. In the food packaging industry due to biodegradability and minimal environmental effect different variety of anti-microbial agents have been tested which contains plant extracts, natural polymers, and essential oils. Essential oils are very important and have well-known activity levels which are traced from plants. Due to essential oil volatility, it is difficult to integrate polymers (Sarfraz, 2020).

Halloysite nanotubes HNTs in the recent research were used as active sites of carriers. During the high-temperature essential oil contains protective high anti-microbial properties and combinations of polymers in a specific time (Lagaron, 2005). Halloysite or carvacrol have a continuous anti-microbial activity and have less density than used polyethylene films. These antimicrobial properties showed against L. innocua and E. coli and they also showed fungicidal action. Halloysite nanotubes HNTs showed synergistic antibacterial properties when mixed with thymol and carvacrol. Rosemary oil with a hybrid of pectin-halloysite-based nano-composites has great potential for packaging. Thyme oil-loaded HNTs halloysite used in paper packaging process showing the powerful antibacterial property for 10 days processing against E. coli. By using many different processes halloysite nanotubes are loaded with organic antibacterial molecules. The natural process of kinetics from inorganic and organic hybrids of respective agents and clay nanotube-based composites are presented (Li, 2021).

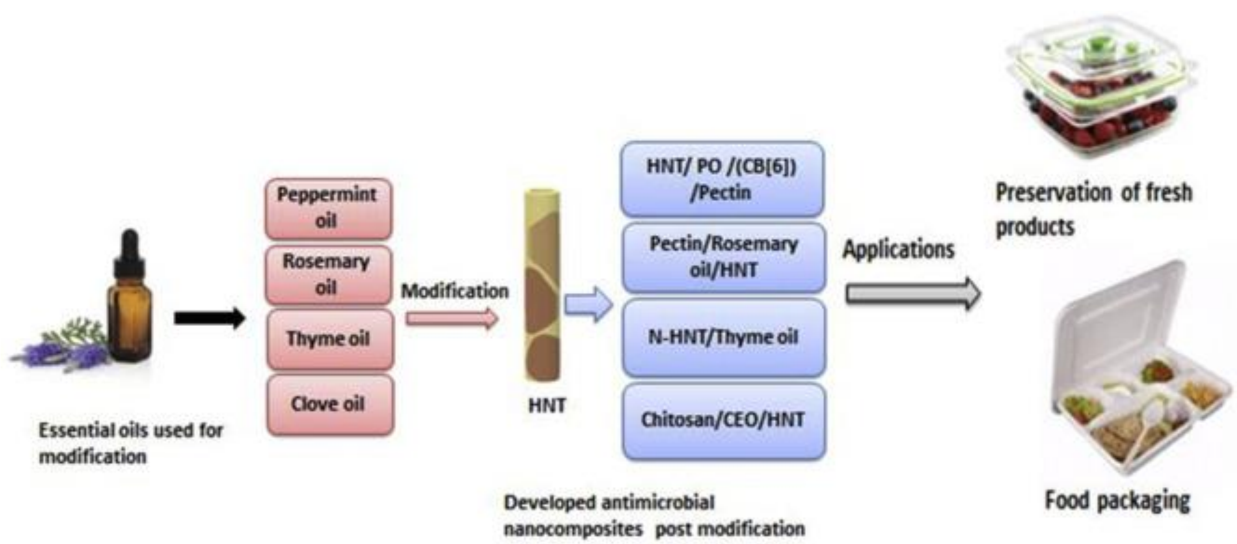

Figure 1.4: Antimicrobial HNTs nanocomposites for Food Contact materials

6.8. Dentistry

Dentistry is the science of studying which has two main terms oral medicine and dental medicine which are used to explain the conditions of the oral cavity, diagnosing, prevention of oral diseases, supporting structures and treatment, disorders, and particularly dentition. On the other hand, dentistry has (jaw and facial) maxilla facial region which contains oral mucosa, related and adjacent tissues, and structures. Dentistry study is not only related to teeth and gum diseases it also has some other diagnoses in the temporomandibular joint and supporting like nervous, anatomical structures, vascular, muscular, and lymphatic structures. HNTs nanocomposites are generally used in implants, resins, composites, and cement. Halloysite clay nanotubules HNTs make a potential target in dentistry material which includes antibacterial activities, non-toxicity, high mechanical strength, and biocompatibility (Barot, 2020).

In the root-canal treatment dead pulp and infected tissues were removed. This treatment is also known as endodontic therapy. Primary infections resist the process of antimicrobial which is associated with intercanal and its pathogens. Therefore, in the process of filling the root canal microbes targeted secondary infections. In various researches, the modification of polymeric resins has been studied which contains halloysite nanotube HNTs. By improving antibacterial activities, it is observed that the mechanical behavior is enhanced which contains breaking energy, modulus, and flexural strength. In another related study, the Farnesol-loaded halloysite HNTs are used as fillers to analyze their mechanical and physicochemical properties. The addition of Farnesol-HNTs mass fraction increases the conversion degree, compression strength, and flexural strength. According to this study, Streptococcus mutants have increased the zone of inhibitions and also have increased biocompatibility against the fibro-blast cells of mouse embryonic which are called NIH-3T3 cell lines (Peña-Parás, 2018).

Scientists also configured silver mobilized halloysite which has better mechanical behavior, and biological and physicochemical activities to examine dental problems. In which composite materials do not show fundamental cytotoxicity against the cell lines of NIH-3T3. In dentistry, dental resins are prepared with or without conventional glass fillers which are named TEGDMA or Bis-GMA. There is no significant increase in mechanical activity which was observed by increasing properties after having an increased (5%) amount of fraction. Dental resin TEGDMA or Bis-GMA have composites of substantial mechanical. HNTs clay nanotubes are used to deliver drug material to diseases affected in the field of dentistry. This technique reduces the side effects of related dosage by depositing a selected controlled quantity of drugs. It is beneficial to release the controlled decay of drugs at right time (Stavitskaya, 2019).

Minocycline microspheres (MM) are also known as Arestin. It delivers continuously releasing of minocycline MM to the periodontium. Ceramic bridges and crowns meet functional and aesthetic demands like alumina or zirconia. they have low elasticity with a brittle structure. Halloysite nanotubes gelatin-modified (HNTs-g) are used as an advanced nano-fillers system (ATH-sil) which has aluminum trihydrate with silane-coupling. Synergistically observed the methyl methacrylate monomer and methyl methacrylate (mMM/ MM) which affect acrylic materials. They analyze different studies like abrasion resistance, hardness, and solution of buffer absorption. In any case of examining dental applications, they performed fall tests (Same, 2022).

7. Conclusions

To conclude, clay nanotube is included in one of the emerging trends in nanotechnology because of its unique biocompatibility and excellent antimicrobial properties. These are double-layered spherical or tubular in a structure containing clay silicate with thin plates. Originally HNT formed from volcanic deposits containing glass and volcanic ash. The structural composition of HNTs is in such a way that on the surface of clay nanotube, hydrogen bonding formation in the aluminum and siloxane groups with biomolecules. Clay nanotubes are natural sources that provide biomedical applications because they are very cheap, biocompatible, cytocompatibility, and nontoxic materials.

The HNTs polymer composite contains halloysite, hectorite, bentonite, and montmorillonite. Functional groups of clay nanotubes also called nanocontainers to have different pH ranges such as 8.5pH with positively charged in tubule inner lumen and 1.5 pH with a negative charge. The surface charges of HNT include internal (Al-OH) and external (Si-O-Si) surfaces. Clay nanotubes used polymeric films and fibers for filling composite materials which allow bacterial protection in the study of SiO_2-surface chemistry. HNTs are used for the formulation of fabric coatings, wound dressings, dental materials, and fibers for tissue engineering.

Formulation of HNTs antibacterial is tested in research lab and they are medical utilize want more qualifier clinical studies. These halloysite clay nanotubes are also good for drug delivery systems when a topical effect is needed. In different biomedical applications, clay minerals have well-defined properties which include surface area, porosity, pore size, biomechanical properties, and physicochemical. The continuous release of therapeutic agents can be remarkably increased in an aqueous environment. With specific chemistry of halloysite, superior loading rates, and high surface area, the hybrid materials could provide for biomedical applications in tissue engineering, and dentistry to facilitate someone in enlarging the potential use of polymeric nanotube-based composites.

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