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A CONCISE REVIEW ON CARBON NANOTUBES AS A NOVEL APPROACH FOR VACCINE DELIVERY

Anu Nair[1*], Viral Shah[1] and Dasharath Chaudhari[1]

[1]Dept. of Pharmaceutics, National Institute of Pharmaceutical Education and Research (NIPER), Ahmedabad, Gujarat, India.

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***Correspondence for Author**
Anu Nair
Dept. of Pharmaceutics, National Institute of Pharmaceutical Education and Research (NIPER), Ahmedabad, Gujarat, India.

ABSTRACT

Vaccines are the preparations given to conjure infectious agents or noninfectious conditions such as malignancies in patients, by stimulating body's immune responses through production of antibodies (humoral) or cell-mediated responses. Conventional vaccination regimen have raised concerns like low safety profile of live vaccines, weak subunit Vaccines immunogenicity and immunization failure due to poor patient compliance to booster doses. Currently profound research is focused towards the delivery carriers of vaccines with a goal to identify delivery system that can target antigens to the immune system and hold the potential to eliminate the administration of high booster and prime doses. A vaccine delivery system that can sustain and target the release of the antigen and could achieve temporal and spatial presentation of antigens to immune system thereby lowering the dose of weak immunogens is the present need of the hour. Carbon nanotubes (CNTs) are delivery carriers entirely made up of carbon and often referred as "zero dimensional" objects because of their geometry. CNTs have gained a tremendous focus as delivery system in the 21st century due to its unique properties of nano size that is small enough to be readily internalized by cells and amazing optical, magnetic and electric properties when used alone or with additions of metals. CNTs can be readily surface functionalized to achieve desirable properties as drug delivery carriers for vaccines and other proteins and peptides. The present paper reviews different conventional modes of delivery of vaccines along with different dosage forms for vaccine delivery along with their limitations. The review emphasizes on CNTs as potential drug delivery carrier for vaccines and provides a descriptive compilation

on different types of CNTs, their preparation and purification methods, surface functionalisation approaches and their characterization parameters.

KEYWORDS: Carbon nano tube, protein and peptides, vaccine delivery, dosage forms, drug delivery systems.

INTRODUCTION

VACCINE

Word vaccine was coined by Louis Pastuer and is derived from the latin word *VACCA* meaning cow. Vaccine induces active immunity by deliberate exposure of antigen to an individual. As per WHO vaccine is a biological preparation that improves immunity to a particular disease. A vaccine typically contains an agent that resembles a disease-causing microorganism, and is often made from weakened (attenuated) or killed forms of the microbe, its toxins or one of its surface proteins. The agent taken as antigen by body's immune system stimulates it to identify, kill, and remember it, so that the immune system can more easily recognize and destroy any of these antigenic microorganisms that it later encounters. Immune system basically produces T lymphocytes and antibodies on exposure to antigen. Once imitation infection goes away body is left with memory T lymphocytes as well as B lymphocytes which will remember how to fight the disease.[1]

Major diseases for which vaccines are marketed includes Anthrax, Diphtheria, Hepatitis A,B, Human papilloma virus, Influenza, Measles, Meningococcal, Mumps, Pertusis, Pneumococcal disease, Polio, Rabies, Rotavirus, Rubella, Smallpox, Tetanus and Yellow Fever. As depicted in fig.1 Every year 3 million deaths are prevented and 7 50000 children are saved from disability by vaccines. The global vaccines market is expected to reach $57,885.4 million by 2019 from $33,140.6 million in 2014, at a CAGR of 11.8% from 2014 to 2019.[2]

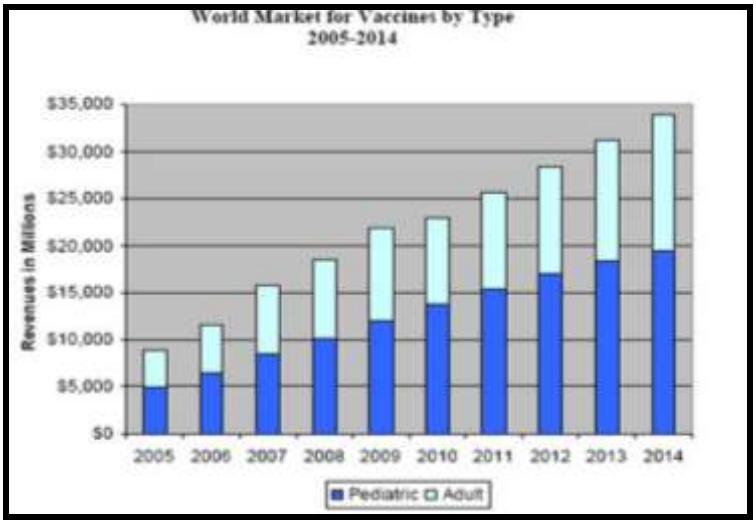

FIG. 1 World market of vaccines

DIFFERENT ROUTES OF DELIVERY FOR VACCINES

i) Intradermal delivery: This route is preferred as dermis and the epidermis layer contains Langerhans and dendritic cells which is crucial for immune response. Advantage of this route of administration is that small dose of the antigen which may induce response similar to the standard dose given by other routes is required. This would not only reduce the cost of therapy but also increase the availability of the vaccine when there is limited supply. BCG vaccine has been first developed by intradermal route and is being used since decades.

Challenges for vaccine delivery through intradermal route

- Lack of efficient intradermal delivery devices for vaccine delivery
- Low clinical effectiveness of biologicals because of limited bioavailability due to low permeation.
- Vaccine presentation and immunization session size.
- Regulatory and commercial issues.[3-5]

ii) Intranasal delivery: Mucosal vaccination can be delivered via a number of routes including oral, intranasal, pulmonary, rectal, or vaginal. Nasal route is considered to be straightforward and most suitable for vaccine administration as it offers many advantages in terms of high vascularization, bypassing GI and hepatic first pass metabolism, quicker onset of action, fewer side effects. Advantages of this route also includeneedle free delivery, small antigenic dose, induction of both systemic and mucosal immunity.

Challenges for vaccine delivery through intranasal route

- Rapid clearance
- Inefficient uptake
- Lack of human compatible mucosal adjuvant.[6]

iii) Buccal and sublingual delivery: Sublingual and buccal mucosa are attractive vaccine delivery sites that may poise advantages over other routes like dermal route in particular, because of anatomy and physiology of buccal mucosa which lacks keratinized epithelium. Thus vaccine permeation across buccal mucosa to systemic circulation is more efficient compared to dermal permeation. High vascularization, and bypassing hepatic first pass metabolism are some other benefits of this route. Sublingual and buccal vaccinations are available as drops, sprays, films.

Challenges for vaccine delivery through buccal/sublingual route

- Salivary flow may dilute the antigen or cause swallowing of the dosage form before the antigen is absorbed through the mucosa this is called saliva wash out.
- Mucosal turnover rate.
- Stable vaccine formulation.[7]

iv) Intramuscular delivery: Several vaccinesare given by this route like measles mumps rubella vaccine, varicella vaccine etc.

Challenges for vaccine delivery through intramuscular route

- Subcutaneous fat administration may lead to vaccine failure due to the poor vascularity which may lead to poor mobility and processing of antigen.
- Layers of fat do not contain the appropriate cells that are necessary to initiate the immune response (phagocytic or antigen-presenting cells).
- The antigen may also take longer to reach the circulation after being deposited in fat, this lead to delay in processing by macrophages and eventually presentation to the T and B cells that are involved in the immune response. Also there are chances that antigens may be denatured by enzymes if they remain in fat for hours or days.
- Also this is invasive technique.[8]

v) **Oral delivery:** Oral vaccination has several advantages when compared with other routes. Being needle free delivery, no requirement of any expert professional to administer thus having better patient compliance.Most common example of vaccine given through oral route is polio vaccine.

Challenges for vaccine delivery through oral route

Oral route for vaccine delivery have to address several challenges like overcoming the degradation in harsh, low pH gastric environment with the digestive enzymes and bile acids.Small quantity of antigen survives degradation and crosses intestinal wall, this can be considered major drawback of this delivery route.[9]

vi) **Intravenous delivery:** Onlyfew vaccines are administered by this route.

Challenges for vaccine delivery through I.V. route

Administered I.V. antigens may be dispersed and distributed failing to achieve sufficient concentration to activate immune system.

DIFFERENT DOSAGE FORMS FOR THE VACCINE DELIVERY

i) Oral reconstituble suspension

Prefilled liquid diluent is available for the reconstitution of the lyophilised vaccine product. But reconstituted products are not meant to be stored for more than 24hrs. It has also to be discarded if frozen.

ii) Nanoparticles

Nanoparticles employed as delivery vehicles or immune potentiators have been shown to improve antigen stability, processing and immunogenicity. Challenges for this drug delivery system is to attain physical stability.[10]

iii) Dry powder inhalable

Needle free delivery makes this system advantageous. Immune responses are equal to or greater than that created by the injected vaccine.Challenges for this system is maintaining dry form of the powder and post powder production handling. Moisture content is key factor in viral stability and maintaining the desired properties of the inhalable dry powders.[11]

iv) Liposomes

Liposomes are emerging as drug delivery system for vaccines. This is because they are rapidly uptaken by the antigen presenting cells and this protect the proteins from enzymatic breakdown by host cells.[12]

v) Emulsion delivery systems

Antigens are dissolved in the water phase and emulsified in oil in presence of emulsifier. Challenges are instability can result due to large droplet size and high oil content can cause unnecessary injection site irritation.[13]

vi) Edible vaccines

Subunit vaccines are relatively safer than the conventional ones avoiding the use of live viruses.

Challenges involved are expensive manufacturing methods and thermolabilenature necessitating cold storage from time of manufacturing till vaccination which increases overall cost of therapy.[13]

CARBON NANOTUBES (CNT) AS DELIVERY SYSTEMS FOR VACCINES

Carbon nanotubes also known as bucky tube were discovered by S Lijima.Carbon nanotubes belong to family of fullerenes in which carbon atoms are bound in the form of hexagonal mesh. One such carbon layer called grapheme is wound in the form of cylinder to form carbon nanotube as shown in fig. 2. These nanotubes have different physical, chemical and electrical properties. Similar to graphite, nanotubes have SP^2 Hybridization and they arrange themselves in ropes which are held together by Van der Waals forces.[14]

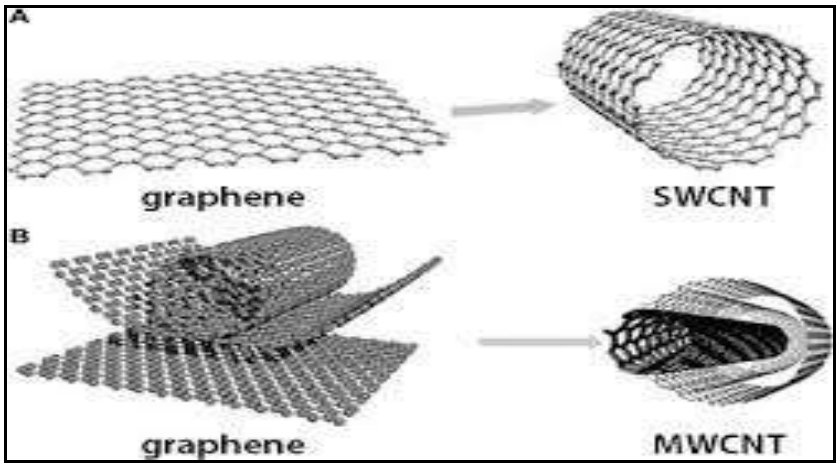

FIG. 2 Formation of MWNT and SWNT from graphene sheet

Types of carbon nanotubes

SWCNT(single walled carbon nanotubes): These are having diameter varying from 0.4nm to tube length which may be millions of times longer.

DWCNT(Double walled carbon nanotubes): It consists of concentric circles of graphene sheets as shown if Fig. no. 3. Properties are similar to SWNT but the chemical resistance is improved.

MWNT(multi walled carbon nanotube): This consist of many layers of graphite which are rolled into tube with an interlayer spacing of 3.4 Å as shown in Fig. no. 3. Outer diameter may range from 1 to 50nm.[14]

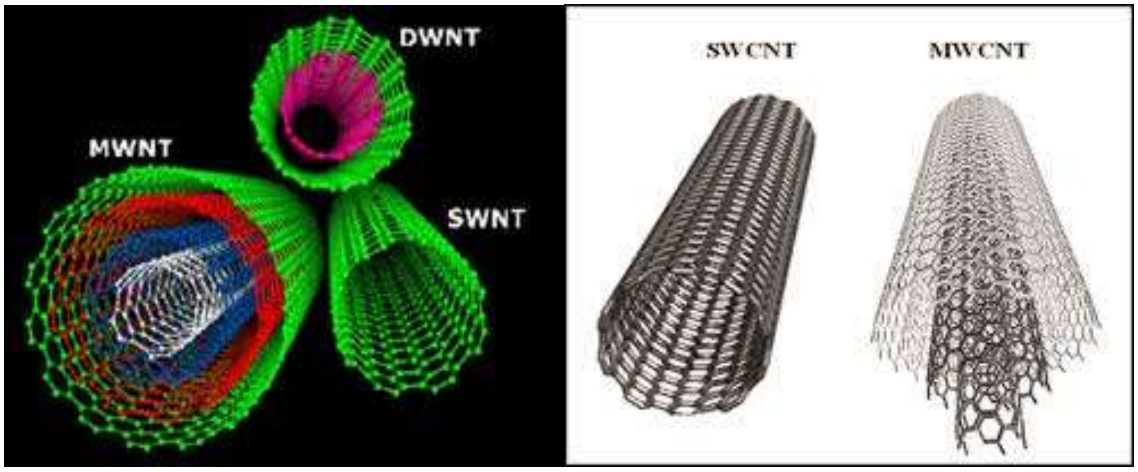

FIG. 3 Different types of carbon nanotubes

Properties of carbon nanotubes

- **STRENGTH:** Carbon nanotubes have high mechanical strength due to sp^2 hybridization.The bond in carbon nanotube is even stronger than the sp^3 bond found in diamond. Besides being strong the tubes are also elastic in nature.

- **ELECTRICAL PROPERTIES:** Carbon nanotube is highly conductive. The strong bonds between carbon atoms in nanotubes allow them to withstand higher electric currents even more than copper.

- **THERMAL PROPERTIES:** The strength of the atomic bonds in carbon nanotubes allows them to withstand high Temperature. When compared to copper wires, which are commonly used as thermal conductors, the carbon nanotubes can transmit over 15 times the amount of watts per meter per Kelvin of heat.[15]

Advantages of carbon nanotubes as vaccine scaffold: Carbon nanotubes have some special features which make them suitable candidate for vaccine scaffold to carry specific antigenic target and facilitate presentation to immune system. Specifically when vaccines are delivered in the nano form they exhibit higher response. The vaccine when delivered in form of nano formulation can interact more with the cells leading to its higher efficiency. Carbon nanotubes have some specific properties as mentioned below which makes it a favourable device for delivering vaccines.

- Relatively inert and non immunogenic
- CNT can rapidly enter into dendritic cells which is required for the stimulation of the immune response.
- Non toxic
- High stable in harsh in vivo environment
- Unique structures allow conjugation of multiple antigens on one surface.
- Due to nanosize rapid entry into cells leading to stimulation of effective immune response.
- High thermal and electric stability leads to ease in manufacturing and scale up.
- Easy surface functionalisation may lead to targeted and more effective delivery of vaccines.
- CNTs can be made in particulate form which can act as depot for vaccines and promoteits in vivo engulfment by phagocytic cells which are involved in generating immune response.
- Functionalized CNT can be used to deliver the vaccines through routes with More patient compliance such as subcutaneous and oral without inducing severe, undesired toxic effects which strengthens the possibility of their usefulness as immunization strategies.
- Ability of carbon nanotube to attach different copies of antigens.[16-18]

Methods to prepare carbon nanotubes

Following technologies are used for the fabrication of CNTs

- Laser ablation
- Carbon arc discharge.
- Chemical vapour deposition.
- Silane solution
- Flame synthesis

- High pressure carbon monoxide reaction method

Laser ablation technique: Intense laser pulses are used to ablate or to vaporize carbon from graphite target.This laser ablation process is carried out in inert gas presence in a high temperature reactor. The resultant carbon nanotubes are collect from cooler surface of reactor were vaporized carbon condenses as shown in fig. 4.

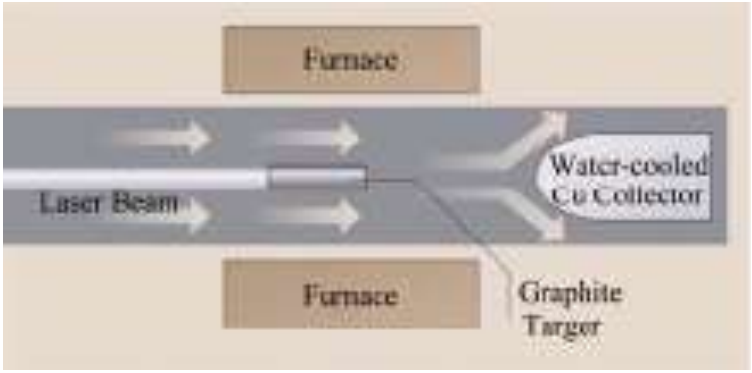

FIG. 4: Schematic diagram of laser ablation technique

Carbon arc discharge technique: This method produces best quality nanotubes. Current of 50 A is passed between two graphite electrodes in helium environment.This result in graphite evaporation which partly condenses on the cooler walls of reactor vessel and partly on cathode as shown in fig. 5. This technique is also used to produce SWCNTs by adding catalystslike Ni, Fe, Co in the anode.

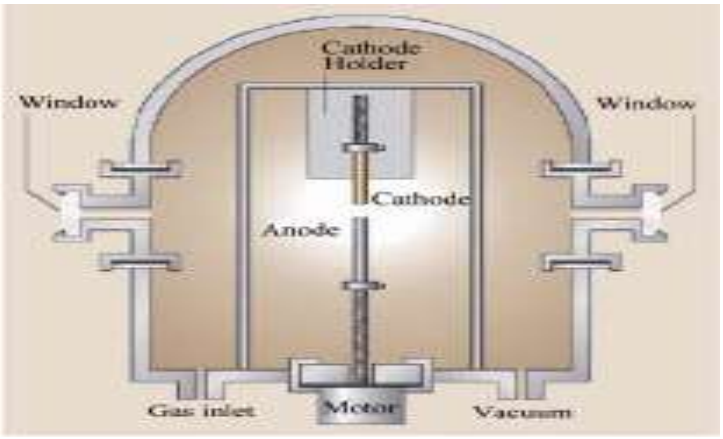

FIG. 5: Schematic diagram of carbon arc discharge technique

Chemical vapour deposition: In this Technique substrate with a layer of metal catalyst particles is prepared. Commonly used metals are nickel, cobalt, iron, or a combination. The size of the metal particles is taken related to diameters of the nanotubes that are to be grown.

The substrate is than heated to approximately 700 °C and two gases are bled into the reactor a process gas (such as ammonia, nitrogen or hydrogen) and a carbon-containing gas (such as acetylene, ethylene, ethanol or methane) which initiate the growth of nanotubes. The carbon-containing gas is broken apart at the catalyst particle surface, and the carbon is transported to the edges of the particle, where it forms the nanotubes. This mechanism is still studied and the residual catalyst particles depending on the adhesion between the catalyst particle and the substrate can stay at the tips of the growing nanotube during growth, or remain at the nanotube base, as shown in fig. 6

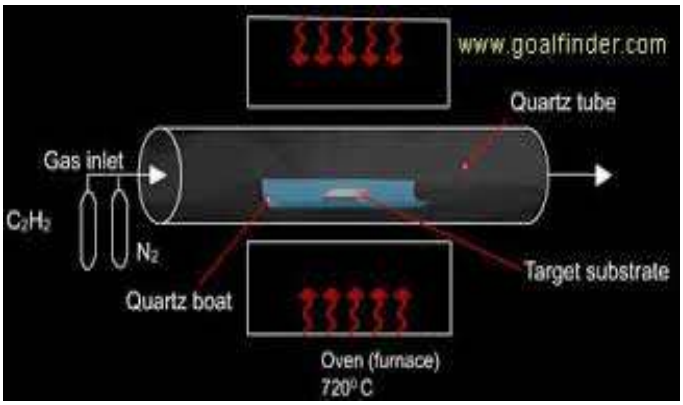

FIG. 6: Schematic diagram of Chemical vaporisation chamber

Silane Solution Method: In this technique a substrate such as carbon paper or stainless steel mesh is immersed in a silane solution of a metal catalyst, preferably Co: Ni in a ratio of 1:1 and a feedstock gas containing a carbon source such as ethylene is inserted through the substrate and the catalyst deposited thereon. During the entire process the substrate is heated continuously by applying an electrical current condensation of the gas leads to formation of SWCNTs.

Flame synthesis method: In this technique SWCNTs are formed in controlled flame environment from hydrocarbon fuels and small aerosol metal catalyst. Single-walled nanotubes have been observed in the post-flame region of a premixed acetylene/oxygen/argon flame operated at 50 Torr with iron pentacarbonyl vapour used as a source of metallic catalyst as shown if fig. 7.

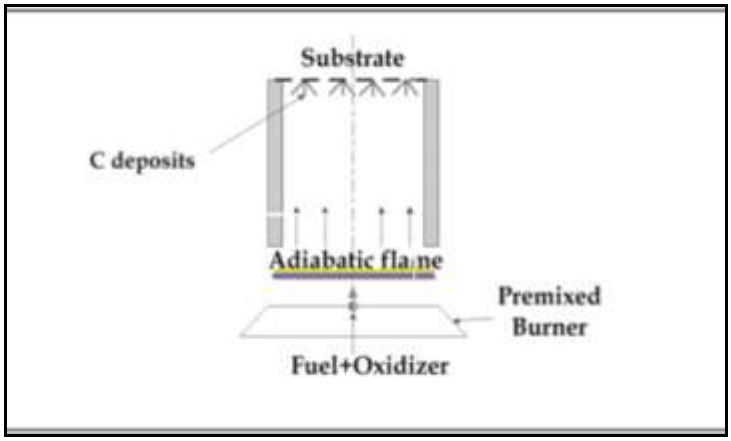

FIG. 7: Schematic diagram of flame synthesis method

High pressure carbon monoxide reaction method: This method is suitable for scale up synthesis of CNTs, because the nanotubes are free from catalytic supports and the reaction can be operated continuously. Unlike other methods in which the metal catalysts are deposited or embedded on the substrate before the deposition of carbon begins, in this method metal catalysts are introduced in the gas phase. The catalyst and hydrocarbon gas which is usually CO gas which reacts with iron pentacarbonyl [$Fe(CO)_5$] to form SWCNTs. This process is also called as HiPco.[19-22]

Purification of carbon nanotubes: All currently known synthesis methods for SWCNTs result in major impurities presence such as metal particles, amorphous carbon and multishell. There are many ways to purify nanotubes.

- **Air Oxidation:** is required for reducing the amount of amorphous carbon and metal catalyst particles. Optimal oxidation condition required is 673K for 40min.
- **Acid Refluxing:** Metal particles and amorphous carbon can be reduced by refluxing the sample in strong acid namely hydrochloric acid, sulphuric acid, nitric acid.
- **Surfactant aided sonication, filtration and annealing:** Efficient method to remove particulate impurities embeds inside CNTs.[14]

Functionalisation of carbon nanotubes to improve its efficiency in vaccine delivery

Functionalities can be used to attach antigenic peptides, proteins and biologics on the surface of the CNTs for more targeted delivery. Chemical functionalization can lead to better dispersion of vaccine and improve antigenicity in vivo which may lead to stable vaccine composition and unique PKPD properties.

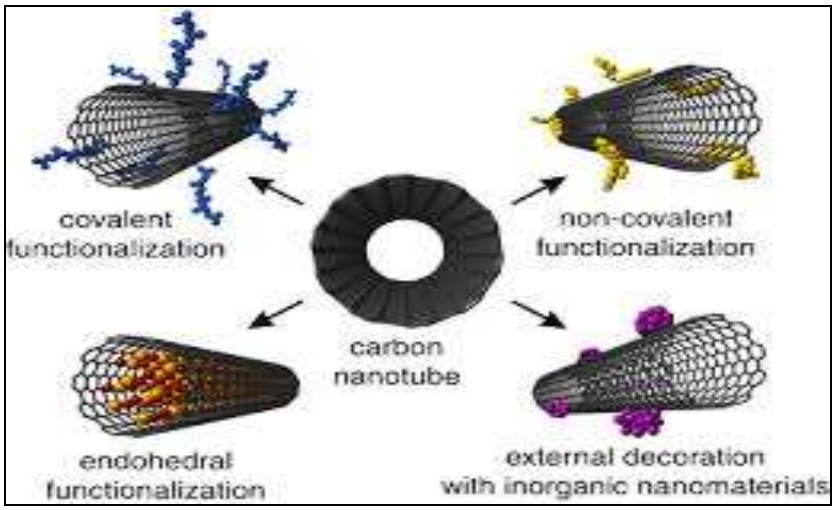

FIG. 8: Different functionalisation methods

Functionalisation can be covalent or noncovalent attachments. Covalent functionalisation includes covalent bonding of functional groups in the end or side walls of CNT which change the property of binding between the carbon atoms. While noncovalent process involves supramolecular Complexation due to various adsorption forces like Van der Waals and stacking interactions. CNTs functionalized with viral protein and molecular complexes were capable of generating specific immune responses in animal models and no cross reactivity were detected for CNTs. Tumor cell lysate conjugated to single-wall CNT was used as a therapeutic cancer vaccine in mouse model hepatoma. The conjugated vaccine improved cure rates as compared to lysates alone this was because of improved activation of cytolyses T cells.[25]

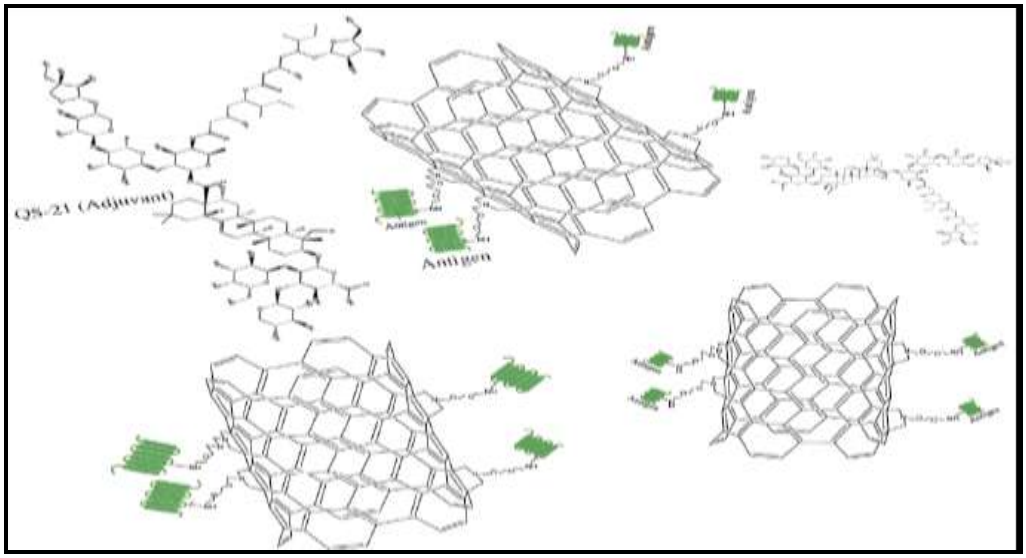

FIG. 9: Depiction of single-wall carbon nanotubes appended with multiple antigens and mixed with an immunological adjuvant, thereby constituting a vaccine.

Chemical functionalization includes the reaction with some molecules of high chemical reactivity. The end caps of nanotubes tend to be composed of highly curved and reactive hemispheres, compared with the side walls. Chemical functionalization like presence of carboxyl groups leads to a reduction of van der Waals interactions between the CNTs, which separates nanotube bundles into individual tubes which in turn enhances the solubility in aqueous or organic solvents. Flourine atoms in fluorinated CNT can be relatively replaced with functional groups like alcohols, amines, alkyl lithium compounds through nucleophilic substitution reaction. The CNTs can also be functionalised with diazonium salts, surfactants like SDBS(Sodium dodecyl benzene sulphonate)and triton X 100 or polymers of high molecular weight as shown in fig. 10 and fig. 11 to achieve steric stabilization yielding water soluble, stable and well dispersed functionalized CNT. [23-26]

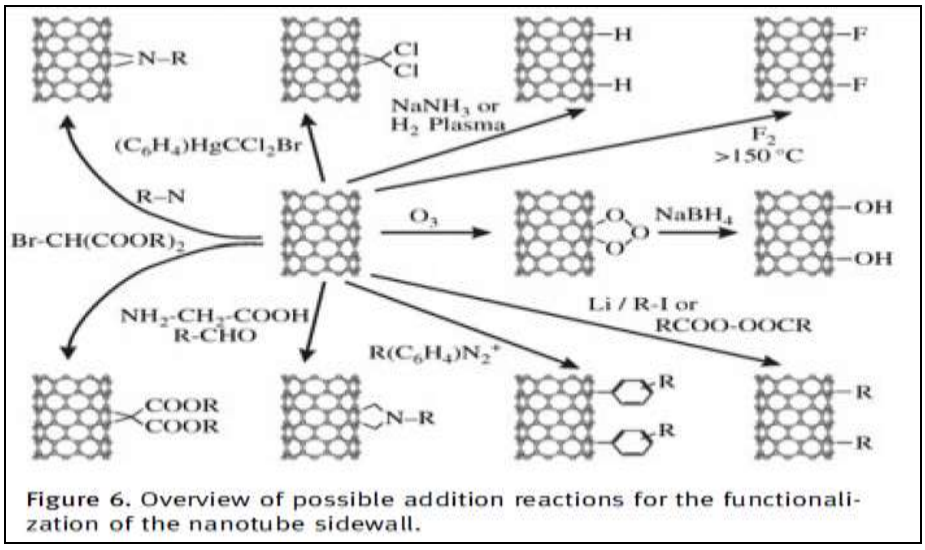

Figure 6. Overview of possible addition reactions for the functionalization of the nanotube sidewall.

FIG. 10: Overview of possible addition reactions for functionalisation of nanotube sidewall

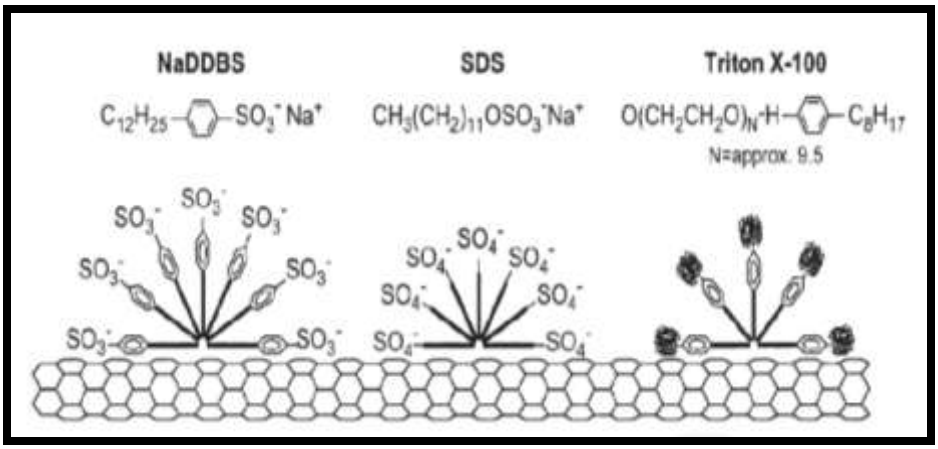

Fig. No 11: Schematic diagram of how surfactants adhere to CNT

Characterisation of CNTs

- **Surface Morphology** of CNTs can be characterised by TEM or SEM. XRD can be used to determine whether the structure of CNT even after modifying have the same cylinder wall structure as raw MWNT and the inter -plannar spacing remain same.
- **Functional group determination and stability** of the complex covalently attached to CNTs can be checked through spectroscopic techniques.
- Thermogravimetric analysis is done to determine the **growth of CNT surface**.
- **Toxicological studies** are performed for CNTs because they may persist in the body for long time. Various models are being used to study the toxicity profile. Wistar rats, Kunming mice, Swiss albino mice are being used for BAL analysis, gene expression analysis histopathological analysis. Various cell lines have been employed to detremine oxidative stress, cytokine assay, ROS analysis, NO analysis etc.[26]

CONCLUSION

Improvisation in delivery systems for vaccines is current focus area of scientific community due to three basic reasons. First, is the necessity in replacing whole-inactivated pathogens presenting a complex range of antigens, by newly developed vaccines based on selected target antigens. Secondly, to evoke cellular immune responses rather than surface antibody responses. Third, is a need to increase patient acceptability by developing a pain-free and safe needle-less delivery system in the future vaccine market. Physical delivery of antigen and formulation with microparticles to target antigen-presenting cells (APCs) are presently practiced delivery approaches that have demonstrated to be effective in animal models. CNTs since ‘90s have ignited a tremendous revolution in many fields including medicine, drug delivery, electronics and material science. CNTs have unmatched potential as carriers for delivery of active molecules, like therapeutic drugs or antigens due its unique properties and dimensions. With growing interest of nanotechnology research community in this field, it is expected that several applications of CNTs will be explored in future and the human community would be benefited by efficacious, safe and more acceptable delivery system for vaccines.

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REFERENCES

1. Elgert K D. Immunology: Understanding the immune system. 2nd ed. United States: Wiley-Blackwell., 2009; 629.
2. http://www.marketsandmarkets.com/PressReleases/vaccine-technologies.aspreferred on 3 April 2015.
3. Lambert P,Laurent P. Intradermal vaccine delivery: Will new delivery systems transform vaccine administration?. Vaccine, 2008; 26: 3197-3208.
4. ZehrungD,JarrahianC,Wales A. Intradermal delivery for vaccine dose sparing: Overview of current issues.Vaccine, 2013; 31: 3392-3395.
5. PuriN,SusanW,RahmanA,Sinko P. An investigation of the intradermal route as an effective means of immunization for microparticulate vaccine delivery systems. Vaccine, 2000; 18: 2600-2612
6. Fortuna A, AlvesA,SousaJ,Falcao A. Intranasal delivery of systemic-acting drugs: small-molecules and biomacromolecules. European Journal of Pharmaceutics and Biopharmaceutics, 2014; 88(1): 8-15.
7. Kraan H, Vrieling H, Czerkinsky C, Jiskoot W, Kersten G, Amorij J. Buccal and sublingual vaccine delivery. Journal of Controlled Release, 2014; 190: 580-592.
8. Zuckerman J. The importance of injecting vaccines into muscle. BMJ, 2000; 321(7271): 1237-1238.
9. Jones R. Oral vaccine delivery. Journal of Controlled Release, 2000; 65: 49-54.
10. Liang Z, Arjun S ,Nani W, Chun X, Neena M, Chenzhong Y, Anton P. Nanoparticle vaccines.Vaccine., 2014; 32: 327-337.
11. Burger J,Stephen P,Jessica L,Chad S, Braun, Mcadams D, Best J,Bhagwat P,Pathak P, Rebits L, Sievers R. Stabilizing formulation for inhalable powders of live attenuated measles virus vaccine. Journal of Aerosol medicine and pulmonary drug delivery, 2008; 21(1): 25-34.
12. Lacey M, Korsholm K, Andersen P. Liposomal vaccine delivery systems. Expert opinion in drug delivery, 2011; 8(4): 505-519.
13. Saroja C, Lakshmi P, Bhaskaran S. Recent trends in vaccine delivery systems: A review. International journal of pharmaceutical investigation, 2011; 1(2): 64-74.
14. Pavani R, Kodithyala V. Carbon nanotubes and potential applications. International research journal of pharmacy, 2011; 2(7): 15-21.
15. Dai, H. Carbon nanotubes: Opportunities and challenges. Surface Science, 2002; 500: 218-241.

16. Scheinberg D, Mcdevitt M, Dao T ,Mulvey J, Feiberg E, Alidori S. Carbon nanotubes as vaccine scaffolds. Advanced Drug Delivery Reviews., 2013; 65(15): 2016-2022.
17. Fahmy T, Pfefferle L, Haller G. US patent, US0216581, 2013.
18. Davide P, Charalambos D, Johan H, Fred B, Ed K, Jean P, Sylviane M, Maurizio P, Alberto B. Immunization with the peptide-functionalized carbon nanotubes enhances virus specific neutralising antibody responses. Chemistry & Biology., 2003; 10: 961-966.
19. Musaddique M, Javed I. Production of Carbon Nanotubes by Different Routes— A Review. Journal of Encapsulation and Adsorption Sciences, 2011; 1: 29-34.
20. Singh P,Tripathi R,Saxena A. Synthesis of carbon nanotubes and their biomedical application. Journal of Optoelectronics and Biomedical Materials, 2010; 2(2): 91-98.
21. Szabo, A., Perri, C., Csato, A., Giordano, G., Vuono, D., & Nagy, J.B. (2010). Synthesis methods of carbon nanotubes and related materials. Materials, 2010; 3: 3092-3140.
22. Digge M, Moon R,Gattani S. Application of Carbon Nanotubes in Drug Delivery: A Review. International Journal of PharmTech Research, 2012; 4(2): 839-847.
23. Balasubramanian K, Burghard M. Chemically Functionalized Carbon Nanotubes.Small, 2005; 1(2): 180-192.
24. JoenI,Cheng D, Kumar N, Baek J. Functionalization of Carbon Nanotubes. Carbon Nanotubes – Polymer Nanocomposites. Dr. Siva Yellampalli (Ed.).Intech., 2011; 92-113
25. Meng J, Duan J, Kong H, Li L, Wang C, Xie S, et al. Carbon nanotubes conjugated to tumor lysate protein enhance the efficacy of an antitumor immunotherapy. small., 2008; 4(9): 1364-70.
26. Lifei C, Huaqing X,Wei Y . Functionalization Methods of Carbon Nanotubes and its Applications. Prof. Jose Mauricio Marulanda (Ed.), Intech., 2011; 213-232.