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Keywords: Bionic plants, Nanobionics, hybrid plants, Transformed plants

#### ABSTRACT

Plant bionics is a possible way to improve the natural plant system to handle the problem of urbanization, population explosion, food insecurity and for the betterment of human health safety and environment. Plant bionics is a specialized interdisciplinary science, which revolutionize the traditional plant research. This approach may also play a major role in replacement or modification of characters and functions of natural plant systems. Roots, stems, leaves and vascular networking system of plants are responsible for transmitting the chemical signals, metabolic activities, growth and functional activities. Now scientists are able to explore the unlimited possibilities of carbon nanotubes in biological and medical applications. Carbon nanotubes can append peptides, sugars, lipids DNA and RNA. These biologically transformed conjugates can be very handy to tackle the problem of reducing natural resources and medical treatments. The present article depicts proposed work flow for developing bionic plant and also discuss the infinite possibilities of plant bionics for the betterment of human being.



### Introduction

The survival, self-repair and self-energizing capabilities in harsh conditions are few of the unique features of plants which make them most advanced living systems (1-2). In the present era the fusion research area is very progressive and advance. Emerging plant bionics is one of them which have unlimited hidden possibilities. The bionics research is constantly in progress in animals but in the case of plants, the current research scenario is not so satisfactory although there are unlimited possibilities in nature where plants can create revolution (3). Plant bionics is one of the important topic of biological research, which facilitates to design the plantnanotech interfaces (4). It is generally believed that there is a need for substantial improvement and development of new technologies for the welfare of existing or upcoming human generations (5). Plants are capable for biodiversity maintenance to manage the air, soil, water, food, oxygen and maintain the balance in between them (6). Plant bionics can be very handy to monitor the environmental pollutants and mainly emphasizes the development of plants by manipulations in energy efficient and high yielding processes to fulfill the continuous rising requirement of human being (4). Researches has already proved that bionic plants can be used to tackle the issues like low photosynthesis rate, energy production, pollution, defense system and food insecurity etc (4, 7).

#### Rationale and mechanism behind the development of bionic plants

The main rationale behind the development of bionic plants is to detect harmful factors globally. Another emphasis behind the development of this technique is to develop improved natural resources by sustainable and eco-friendly methods. Therefore researchers have decided to make plants even more efficient and useful by boosting them with carbon nonomaterial. Carbon nanotubes (CNTs) can be categorized in to two types, single and multi-walled carbon nanotubes (MWCNTs)



respectively (8-9) (Fig 1). These CNTs could be incorporated with small chemicals or biological molecular material. The infusion of these transformed CNTs can incorporate changes in normal plants and convert them into living sensors (10). The scientists of Massachusetts Institute, Cambridge have already done some experiments on plant bionics and got success in embedding of coated CNTs into the leaves of plants and these experimented plants are able to sense chemical contaminants present in atmosphere/environment (4) (Fig 1). These Living-plant sensors are highly beneficial for the monitoring of chemical particularly in remote areas and can be good weapon to face chemical and biological disaster. Researches have already proved that these plants can sense nitro-compound and therefore they can detect underground hidden explosives (4). Sensors can be very useful for different targets therefore researchers wish to create the plants that could be used to check pollutants, microbial infection, pesticides and bacterial toxins. According to Giraldo "it's an opportunity for researchers of plant science and chemical nanotechnology to work mutually in this area that has a huge potential". Currently very less researchers are working in this emerging field. Therefore the main aim for writing this article is to motivate the researchers to work in this direction (4).





**Figure: 1.** Stepwise graphical representation for preparing bionic plant in Lab: (a) Types of CNTs that can be use to make bionic plant (b) Attachment of desired biological molecules or chemical sensors (c) Dilution of CNTS or preparing solution (d) Infusion of CNTs solution into plants (e) Vascular infusion (f) Developed bionic Plant (g) Scanning of working bionic plant activity through infrared sources.

### 1: Preparation of carbon nanotubes

The arc discharge, laser ablation, Plasma torch and chemical vapor deposition (CVD) are some of those widely used methods. Mostly these processes require vacuum or gas environment. Chemical vapor deposition technique prefers vacuum environment for the growth of CNTs at atmospheric pressure. These methods are very significant for the commercial production of CNTs (11). The strategy utilized in arc method is unchanged since the discovery of carbon nanotubes. The high release of temperature



resulted, in the deposition of carbon nanotubes at negative cathode. This strategy leads weight about 30% more in both single and multi walled nanotubes of up to 50 micrometers in length can be produced by this method (12). Arc release system utilizes higher temperatures (over 1,700 °C) for CNTs synthesis which commonly causes the extension of CNTs with slight structural imperfections in comparison with other methods (11-15).

#### 1.2: Laser ablation Method

Dr. Richard Smalley and colleagues at Rice University (Houston) introduced Laser ablation method. In this method, a beat laser vaporizes a graphite focus in a high temperature reactor while an idle gas is seeped into the chamber. CNTs created on the cooler surfaces side of the reactor as the vaporized carbon gathers. A water cooled surface might be incorporated into the framework to gather the CNTs. Later the same research group utilized a composite of graphite and metal impetus to combine single walled carbon CNTs. The laser ablation technique yields around 70% and produces principally SWCNTs with a controllable breadth dictated by the reaction temperature. The only drawback of this technique is high cost in comparison with arc discharge or chemical vapor deposition (16).

#### 1.3: Plasma torch method

SWCNTs can also be constructed by thermal plasma technique (17-18). SWCNTs made by this process are more effective and required minimal efforts compare to other methods. During the process, a gas mixture of argon, ethylene and ferroxine is passed into the plasma torch system, where it is atomized by atmospheric pressure and specific intense flame thereafter carbon nanotubes and amorphous carbon is formed in the form of exhaust. The collaborative effort of university of Sherbrooke and National Research Council of Canada also developed the similar technique in which ionized gas is used to obtain high temperatures required for vaporizing carbon containing substances. In this process, thermal plasma is started by a high frequency servicing in a coil and at the same time inert gas is maintained in the streaming.



Typically, a feedstock of carbon and metal catalyst particles is then nurtured in the plasma, which is cooled to produce single-walled carbon nanotubes (19). This method is comparatively better due to its superior production rate than arc discharge and laser ablation.

#### **1.4: Chemical vapor deposition (CVD)**

CVD have great importance for mass production of carbon nanotubes. In this process, two different gases are flown into a reactor, where one gas acts as a carbon source and the other behaves like a normal gas such as hydrogen and nitrogen. In this process the most commonly used gas sources for carbon are acetylene, ethylene, ethanol or methane. Carbon nanotubes develop at the sites of metal catalysts and carbon gas is separated on the surface of the catalyst-molecule at the end of the process. After this, the carbon transported to the edges of the particles and nanotubes finally get their shape. (20-21)



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**Figure: 2.** Development of CNTs by various methods (A) ARC discharge method (B) Plasma torch method (C) Laser ablation method (D) CVD: Chemical vapor deposition method.

#### 2: Transformation or functionalization of CNTs into sensors

Covalent, non-covalent (physical-absorption) or a hybrid approach are the main approaches often used for functionalization and biomolecular interaction of CNTs. The interaction takes place by a little "anchor" particle non-covalently connected to carbon nanotubes and then covalently connected to the bio-molecule of interest. In the next section the mechanism behind the chemical and biological transformation of CNTs in discussed (22). In the modification process of carbon nanotubes, several chemical reactions proceed in series. CNTs can transform (functionalized) according to the need which further can be utilized for wide range of applications. Covalent and noncovalent techniques are the most often used for funtionalization of CNTs (23).

#### 2.1: Covalent Modification

Covalent modifications are exceptionally stable due to the presence of sp2 hybridization and sigma bonding between carbon atoms. Covalent modification appends a functional group connected on the side surface or end edges of the CNTs (23). The end caps of the CNTs have the most reactive segment because of its higher pyrimidization property compare to sidewall surfaces having lesser reactivity. Oxidation is another important and well studied technique for covalent functionalization for CNTs (24-27). In oxidation of CNTs, the carbon is present in the form of -COOH, C6H5OH and (C=O)–O–) groups (4-7). However, excessive oxidation may cause fragmentation of carbon nanotubes into small pieces termed as carbonaceous fragments. To overcome this problem Xing et al. (2007) introduced sonication assisted oxidation method for CNTs modification (28) to limit the damage of CNTs (29). It is also possible to functionalize the CNTs by esterification and amidation reaction (12). Esterification is a chemical process to derive ester from acid. In most esterification reactions the carboxylic acid acts as a major precursor and replaced least one hydroxyl group by alkoxy group to form ester. COOH (Carboxyl



group) is transformed into CH3COCl (Acyl-chloride) by SOCl2 (Thionyl- chloride) or C2O2Cl2 (Oxalyl chloride). Thereafter it reacted with the CO-NH (amide), -NH2 (amine) or -OH (alcohol) during the reaction. CH3COCl transformed CNTs can react readily with highly branched molecules and can be used to chelate silver nanoparticles (30-31). Halogenation of CNTs is an another interest in current research scenario for CNTs functionalization (32). It also allows us to functionalize CNTs according to our requirements. These reactions facilitate the subsequent covalent attachment between nanotubes and desired molecules. In halogenation reaction the CNTs can react with peroxytrifluroacetic acid to form essential carboxylic acid and trifluroacetic fuctional groups (33). The florinated CNTs can also be functionalized with urea, thiourea, amino saline and guanidine by different halogenated substitution reactions (34). The modification of CNTs is not limited to the chemistry of carboxylic acids. Several other methods such as cycloadditions, electrophilic and nucleophilic or radical additions have been also developed for the interaction of organic moieties directly onto the sidewalls of CNTs (35-41). Cycloaddition reactions provide an efficient and customized approach for preparing of CNT-based hybrid system that would be useful for a wide range of applications from animal to plant bionics (35). A wide range of chemical and biological molecules could be joined onto CNTs without any disturbance of its structure and functional groups. 1,3 cycloaddition reaction is often used reaction to study for carbon nanotubes functionalization azomethine yields react with carbon nanotubes. Cycloaddition also allows the coupling of pyrrolidine ring which further lead to a variety of functional groups such as PAMAM poly(amidoamine), phthalocyanine addends, perfluoroalkylsilane, and amino ethyleneglycol groups (30-31). Microwave-induced electrophilic addition for functionalization of SWCNTs was also reported with alkyl halides and by utilizing the Lewis acid as a catalyst followed by hydrolysis reaction which further revealed the attachment feature of alkyl and hydroxyl groups to the surface of the nanotubes (42-45). SWNTs can also be alkalized with alkyl halides with the help of lithium or



sodium metal and liquid ammonia. Radical addition reactions are used to make CNTS of small diameter. Small-diameter CNTs are formed due to the deposition of benzyl peroxide. These processes occur in the presence of alkyl halide. We can investigate the functional property and quality of CNTs made by this process with the help of gravimetric analysis. Modified CNTs developed by this process showed great solubility in organic solvents. The functional groups associated with these CNTs can be isolated by warming them in the presence of argon (25). Nucleophilic addition reaction is an addition reactant, termed as nucleophile. Researcher conducted several experiments on nucleophilic additions rections. Hirsch (2008) performed this process on organolithium and organomagnesium compounds to functionalize CNTs. After oxidation he found alkyl-modified CNTs. Hirsh also developed amino-modified carbon nanotubes by nucleophilic addition for amines to CNTs. (27).



**Figure: 3.** Chart summarizing options for the chemical modification/funtionalization of carbon nanotubes.



### **3:** Non-covalent modifications

Non-covalent attachment of CNTS with desired molecules can be achieved by wrapping the polymer chains around the nanotubes. Vander-waals interaction existed there in between nanotubes and molecule for maintaining the stability and the regular arrangement of attachment. The desired molecules can be physically bound to the surface of CNTs by this approach (3).

#### 3.1: Polynuclear fragrant compounds and dissolvability of CNTs

It was reported that polynuclear compounds have very significant role in the solublization of CNTs. Most oftenly polynuclear functionalized arenes (phenyl, naphthalene, phenanthrene, pyrene and porphyrin systems) with polar and nonpolar moieties are frequently used for the solublization of CNTs in liquid solvents (46). It was reported that sweet smelling amphiphiles like pyrene amphiles has greater dissolvability in comparison to phenyl amphiphiles in water (46). These fragrant structures can be modified with amino and carboxylic corrosive collection before functionalizing the CNTs (25).

### **3.2: Bimolecular Interaction of CNTs**

In bio-molecular nanotechnology, the CNTs has a crucial role because of it has a great potential required for the development of novel nano-biomolecular system of interest and this can only be accomplished by association of nanotechnology and bio-molecular system (47). The modification of CNTs is now possible and these nanotubes can interact with bio-molecules such as proteins, starches, and nucleic acids to form bimolecular sensors. Proteins have high binding affinity to carbon nanotubes because of their hydrophobic or hydrophilic nature. Polysaccharides are most widely used bio-molecules for the modification of carbon nanotubes (48). It was also reported that single and double tailed phospholipids has little conflict in their



binding behavior. The single tail of phospholipids easily tied the CNTs, but double tailed the phospholipids did not show the similar binding ability (49).

## 3.3: Stacking and electrostatic interactions of CNTs

The molecules having dual-functional activity are often used for the transformation of CNTs.  $\pi$ - $\pi$  stacking responsible for the one side interaction of CNTs and poly aromatics compound at the other side. One end of the particle is poly-aromatic exacerbates that interface with the carbon nanotube through  $\prod$ - $\prod$  stacking. The flip side of a similar particle has a practical gathering, i.e. amino, carboxyl, or thiol. For instance, pyrene derivative and aryl thiols were utilized as the linkers for different metal nanobeads, i.e., gold, silver and platinum (48).

## 4: Delivery of functionalized nanoparticles in to plants

There are two important methods for desired infiltration of CNTs into plants. The most often used method is vascular infusion in which solution of CNTs flowed by parts of plants and other is lipid exchange envelope penetration process which proceeded by lipid exchange mechanism (Fig 4).

### 4.1: Vascular infusion

Vascular infusion performed by infiltration the solution of functionalized nanotubes to the plant from underside of leaves. The solution is then penetrated into leaves by pressure force though stomata which are also known for O2/CO2 exchanging root. Researchers have already infused the various types of functionalized nonmaterial to various plants such Arabidopsis, spinach and *small flowering plants (50)*.





**Figure: 4.** Insertion of transformed nanaotube crossing cell membrane by LEEP method: Black arrow depicted the enveloped functionalized carbon nanotubes passing through cell membrane by lipid exchange mechanism and after the successful entry of CNTs inside the cell the membrane rearranged itself automatically.

# 4.2: LEEP (Lipid Exchange Envelope Penetration)

Leep is an important and very strong technique for transferring the functionalized nanotubes in to the plants. It requires chloroplast membrane root for infusion of CNTs solution. A special coated nano-material absorbed by membrane by lipid exchange mechanism. Researchers have already transferred the desired nano-particles by wrapping them in highly charged polyacrylic acid. Thereafter these desired particles can easily penetrate into the plants (figure 4) (51).



### Results

The suggested method is a multifaceted strategy combining nanotechnology, synthetic biology, and plant physiology was used to create a bionic plant for biosensing. Carbon nanotubes were first methodically incorporated into the plant's vascular system using a non-invasive procedure, harnessing capillary action for efficient dispersion. This aided nanomaterial movement throughout the plant structure. Following that, bioengineered nanoparticles were introduced, which were designed to detect certain target chemicals. These nanoparticles were expertly designed to respond to the presence of target molecules by generating an optical signal evident as changes in leaf colour or fluorescence. The creation of the bionic plant as a biosensing organism required an extensive research flow that integrated multiple approaches. Carbon nanotubes (CNTs) were created using a variety of procedures, including Laser Ablation, Plasma Torch, and Chemical Vapour Deposition (CVD). These approaches produced CNTs with a variety of characteristics and topologies, which were critical for later modification and sensor functionality. CNTs were converted into sensors using two basic strategies: covalent modification and non-covalent modification. Covalent alterations entailed chemically attaching certain molecules to the surface of carbon nanotubes, thereby altering their sensing capabilities to target analytes. Non-covalent modifications, on the other hand, used interactions such as - stacking or Van der Waals forces to attach sensor molecules to CNTs. This allowed for the creation of adaptable sensors capable of detecting a wide range of chemicals. These functionalized nanoparticles were then intricately administered into plants via a unique plant-based Vascular Infusion approach. The modified nanoparticles were distributed and incorporated throughout the plant structure via the plant's own vascular system, ensuring effective and extensive integration. The successful implementation of this research flow enabled the development of bionic plants with advanced biosensing capabilities, capable of detecting and responding to specific target compounds, representing a significant

advancement in plant-based biosensors for environmental monitoring and diverse analyte detection.

## Discussion

Plant biology now crossing its limits by modern advancement, available biological resources and approaches such as plant genomics, plant metabolomics, plant nanotechnology and plant bioinformatics etc. These advances are good for human welfare to tackle food security issues and biodiversity maintenance under climate change circumstances. Researchers have developed the more advanced plants which have capacity to capture light 30 percent more than normal plants (8). This achievement was accomplished by embedding CNTs in the chloroplasts of plants resulting 30 percent more photosynthesis because the chloroplast act as major component for photosynthesis (8). Plant bionics started new era of plant biology which enables the fusion of robotics and plant system (52). Plant bionics has lot possibilities and aims to implant non-native functions in plants by interfacing them with specifically designed nano-material. The spinach plant has been already developed to detect the explosives (53) by forming a conjugate of near-infrared fluorescent nano-sensors (NIFNs) and SWCNTs to the peptide Bombolitin II (8). This conjugate of complex used by plants to identify the nitroaromatics compound via infrared fluorescent emission and polyvinyl-alcohol functionalized SWCNTs that is responsible for generation of invariant reference signals from mesophyll region of engineered plants (8). When the nitroaromatic compounds or contaminants particles are transported up via plant vascular system from the roots to stem and into leaf tissues, they accumulate in the mesophyll region, resulting in relative variation in emission intensity. These experiments suggested that the bionic plants can be used in different areas such as ground water pollution sensors, environmental sensors and can send directly real time report to the remote monitoring and communication devices at standoff distances. In another experiment the scientist engineered a bionic plant to boost their plant's capacity to capture light



by as much as 30 percent. This was accomplished by embedding CNTs in the plant's chloroplasts resulting 30 percent more photosynthesis. The earliest indicator of drought analysis for agricultural were very complex such as satellites imaging and sensors embedded in soil (53). These applications cannot provide the comprehensive information about the water potential of particular plant in that area. Recently the researchers of MIT lab are working with an aim to develop these sensors that can be used on crops. For drought analysis the team developed new sensor technology leverages the plants' stomata, present on the surface of leaf and that enables water evaporation for plant. Evaporated water from the surface of leaf reduces the water pressure in the plant which further enables it to draw up the water from the soil through transpiration (54). In order to develop these sensors, the MIT team employed an ink made of carbon which are minute carbon hollow tubes that conduct electricity. They then dissolved this ink in sodium dodecyl sulfate, an organic compound that does not affect the plants' stomata. An electronic circuit can be produced by printing this ink across a pore. The circuit remains intact even when the pore is closed and it can be connected to a device known as multimeter to measure the current. However, when the pore is opened, the circuit breaks down, which stops the current flow, enabling the measurement accurately when a single pore is closed or opened. There are infinite possibilities which have come up after the growth of plant bionics which enables the plants to act as highly sensitive living devices that can sense a lot of specific substances such as environmental pollutants, toxins, pathogens, explosives, self repair mechanism, can be used as an alternative of street lights, cell phone towers and most important in the enhancement in productivity and quality of plant product that can take care of the increasing population of human beings (52).

It has opened new vistas in the of area plant nanotechnology. There are lot of possibilities to extend the research work in the direction of plant bionics.



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