Quercetin Delivery into Cancer Cells with Single Walled Carbon Nanotubes

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***Abstract*—Carbon nanotubes are made up of carbon atoms arranged in a series of condensed benzene rings and wrapped into a tubular form. They represent a new allotrope of carbon invented from fullerene family, which can be described as rolled up graphite sheets held together by van der Waals bonds. These materials due to some qualities such as high specific surface area, unique electrical and electronical properties are used in many applications as catalyst base, polymers mechanical strengthening, composites, electronical devices production and drug delivery. Especially, they are able to cross through cell wall and inter to cell nucleus. This work speculates on methods of design, synthesis, and quercetin delivery as an antioxidant agent that its anticancer function has been reported, by single-walled carbon nanotubes (SWCNTs) into cancer cells and their detection method. In fact, we introduce the concept of "functionalization partitioning" of SWCNTs, i.e., imparting various chemical species, such as poly (ethylene glycol) (PEG), quercetin with different functionalities into the surface of the SWCNTs. And we will discuss how binding of molecules to SWCNTs and their release can be controlled by varying the pH.**

***Index Terms*—Component; Carbon nanotubes; Quercetin, Drug delivery.**

I. Introduction

The discovery and subsequent widespread characterization of carbon nanotubes (CNTs) have opened a class of materials with unexpected electrical, mechanical, and thermal properties [1]. The structure of CNTs can be imagined as the cylindrical roll-up of one or more graphene sheets containing only sp^2 hybridized carbon atoms in a honeycomb arrangement [2].

Regardless of being single-walled carbon nanotubes (SWCNTs) or multi-walled carbon nanotubes (MWCNTs), CNTs present several remarkable properties such as high aspect-ratio, ultra-light weight, tremendous strength, high thermal conductivity and significant electronic properties ranging from metallic to semiconducting [3, 4]. CNTs have lengths that differ from several hundred nanometers to several millimeters, but their diameters depend on their class: SWCNTs are 0.4-3 nm in diameter and MWCNTs are 2-500 nm in diameter, depending on the method of synthesis [5]. The MWCNTs also consist several cylinders of graphitic shells with a layer spacing of 0.3–0.4 nm [5]. CNTs are currently one of the most popular nanomaterials which are used in number applications including electronics, energy storage, solar cells, molecular separation, sensing, biosensing, and drug delivery [6, 7]. CNTs can be used as a drug-delivery vehicles or 'nanocarriers' in cancer therapy and other areas of medicine without causing toxicity to healthy tissue and permits for a prolonged release period of the drug [2].

Flavonoids are a group of polyphenolic compounds widely distributed in the medicinal plants, vegetables, fruit juices and a variety of beverages (tea, coffee, wines and fruit drinks). Flavonoids, mainly quercetin derivatives, have received more attention as dietary constituents during the last few years. Experimental studies verified that they possess many beneficial effects on human health, including cardiovascular protection, anticancer activity, antiulcer effects, antiallergic, antiviral, and anti-inflammatory properties [8]. Mechanism of these anticancerous materials effect is different. Quercetin is one of the natural antioxidants (Fig. 1) and its anticancer properties have been proved by *in vivo* and *in vitro* experiments. These studies demonstrated that quercetin has a significant role in inhibition of breast, colon, prostate, ovary, endometrium, and lung tumor cancer cells. Nevertheless more researches are needed. Also quercetin is being used as ions chelating agent and prevent reactions between DNA and ions [8-10]. In addition to anticancer features of quercetin, we can mention that it can reduce blood LDL level, blood pressure in people with high blood pressure, prostate inflammation in men, atherosclerosis, allergy symptoms such as secretion of tears, urticaria, swelling of lips and face [8, 11].

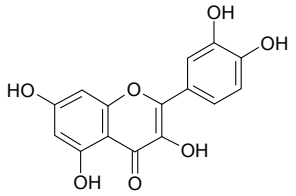

Figure. 1. Chemical structure of quercetin.

Fruits and vegetables especially citrus, apple, onion, parsley, green tea, olive oil, grape, cherry, black mulberry, dogberry, and raspberry are main food resources containing quercetin [12].

Enormous challenges have contributed towards development of new controlled drug delivery systems to

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different aims achievement, including: delivery of therapeutic agents to the desired site, enhancing bioavailability and drug protection. These challenges lead to a new emerging field called "nanomedicine' which involves the application of nanotechnology in medicine. It was recently recognized with potential to make the breakthrough in area of therapeutic delivery [13, 14]. Indeed, a range of new nanoscale materials have been investigated in recent years for drug delivery applications including: nanoparticles, nanotubes, nanofibers, dendrimers, liposomes, polymer micelles, nanogels, nanocrystals, viral vectors, and virus-like particles [13, 15-17]. Among them, CNTs that possess unique chemical and physical properties have received a great attention for drug delivery applications [18-21]. CNTs have been recommended as a promising substitute, offering advantages such as distinct inner and outer surfaces which are readily available by removal of the end caps, and an increased volume providing a high loading capacity for cargo molecules due to their innately high aspect ratios. Proposed filling techniques consist of immersing the nanotube in a solution containing the drug, attaching the drug to the inner tube wall surface or by insertion in particle form [22-24]. CNTs can be used as a drug-delivery vehicles or 'nanocarriers' in cancer therapy and other areas of medicine without causing toxicity to healthy tissue and permit for a prolonged release period of the drug [25-28]. Nowadays these nanotubes are being used in order to deliver cancerous and non-cancerous drugs to various cells *in vitro* and *in vivo* and researchers hope in the future they would be able to use them in gene therapy, vaccination, and different cancer treatments [29].

In the hypothesis presented here, we will discuss on methods of design, synthesis and quercetin delivery by SWCNT into cancer cells and its detection method.

II. Method

Functionalized SWCNTs with poly ethylene glycol (PEG) will be prepared by covalent method. Thus, in order to produce carboxyl end, SWCNTs should exposed to nitric acid (HNO_3) for 24 hours and remained acid must be removed by repeated filtration through 100 nm polycarbonate membrane and resuspension in water. PEGylation of carboxylic acid groups on the oxidized SWCNTs will be done by adding 1 mM poly (ethyleneoxide), four-arm, amine-terminated into the oxidized SWCNTs solution in the presence of 2 mM 1-ethyl-3-[3-dimethylaminopropyl]-carbodiimide hydrochloride under gentle sonication. After overnight reaction, unreacted reagents can be removed by repeated filtration and resuspension of the covalently PEGylated SWCNTs. These functionalizing groups increase carbon nanotubes solubility and biocompatibility [29, 30].

Arginine-Aspartate-Glycine peptide (RGD) capable of selectively binding to integrin $\alpha_v\beta_3$ receptors on a variety of cancer cells should be conjugated to PEGylated SWCNTs as described previously. Briefly, 1 mM sulfosuccinimidyl 4-N-maleimidomethylcyclohexane-1-carboxylate will be mixed with ~0.05 mg/mL (~300 nM) oxidized SWCNTs with covalently attached PEG-NH_2 solutions at pH 7.4 for 2 h. Upon removal of excess reagents by filtration, the SWCNTs will react overnight with 0.2 mM thiolated RGD [31].

Quercetin loading into RGD-PEG functionalized SWCNTs will be done by simply mixing 1 mM quercetin with the RGD-PEG functionalized SWCNTs at a SWCNTs concentration of ~0.05 mg/mL (~300 nM) at various pH values overnight. Unbound excess quercetin can be removed by filtration through a 100 kDa filter and washed thoroughly with water (over 10 times) [32].

III. Results and Discussion

A. Functionalization of SWCNT

Covalently functionalized SWCNTs (SWCNTs-COOH) and MWCNTs (MWCNTs-COOH) have been reported by various researchers [29, 33]. Bhattarai et al. used MWCNTs-COOH for the synthesis of MWCNTs-hydroxyapatite nanocomposite for DNA complexation and Liu et al. functionalized SWCNTs-COOH with PEG [29, 33]. In order to formation of –COOH groups on SWCNTs, it can be refluxed in 3 M nitric acid. Then aqueous solutions of high-pressure CO decomposition (Hipco) SWCNTs can be functionalized covalently by PEGylation of –COOH groups on oxidized SWCNTs (Fig. 2).

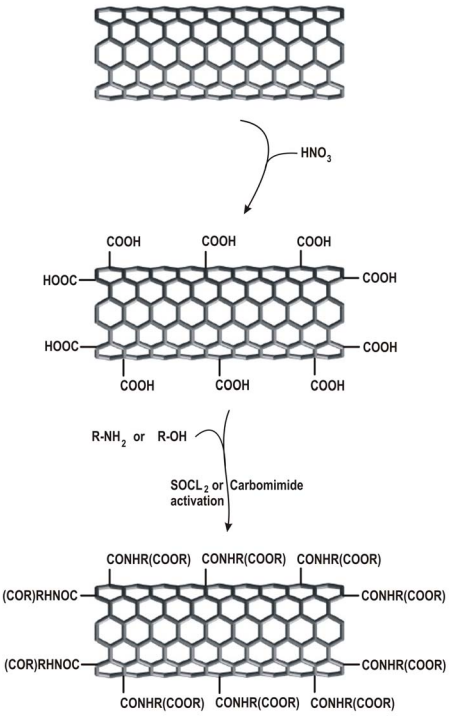

Figure 2. Method of SWCNT functionalization and PEGylation.

B. Quercetin loading into functionalized SWCNT

PEGylated SWCNT solution can be mixed with quercetin at pH 8 overnight and then should repeat filtering to remove free, unbound quercetin. Figure 3, shows the quercetin loading into functionalized SWCNT.

On the basis of optical absorbance data and molar extinction coefficients of quercetin and SWCNTs, it is easy to estimate the amount of quercetin that has been bound to SWCNTs [29]. Also after quercetin loading into SWCNTs, average diameter of it can be increased that can be shown with imaged by atomic force microscopy [29].

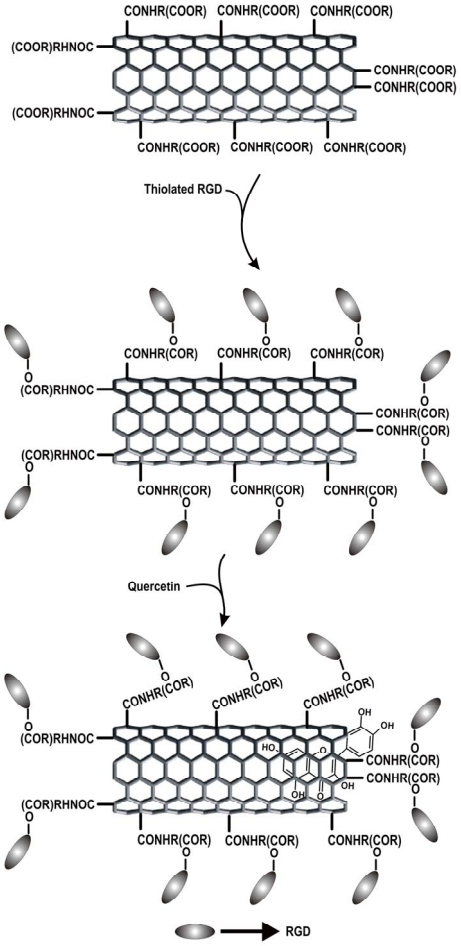

Figure 3. Functionalization of PEGylated SWCNT with RGD and quercetin loading into it.

C. *Quercetin release from SWCNT*

Controlled release of drugs from a drug carrier complex is required as a important aspects of drug delivery systems [34]. Liu et al found that the amount of doxorubicin, a widely used chemotherapy drug for treating various cancers, bound onto SWCNTs was pH-dependent [29]. Because of the acidic features of the micro-environments of extracellular tissues of tumors and intracellular lysosomes and endosomes, the pH-dependent drug release from SWCNTs could be used for drug delivery applications potentially facilitating active drug release from SWCNT delivery vehicles.

In an acidic solution of appreciable release of quercetin from Hipco SWCNTs attributed to the increased hydrophilicity and solubility of quercetin at this pH. Where in the quercetin can be released upon reduction at a low pH environment within the cancer cells [32, 35].

D. *Mechanisms of CNTs uptake by cells*

Functionalized CNTs that can cross cell membranes had been studied by various researchers [36, 37]. CNTs have the potential of crossing cell membranes which makes them efficient material for drug and gene delivery techniques. The first requirement for drugs delivery to cells is that the drugs should be attached to the drug delivery system by covalent or non-covalent bonding. After getting the targeted organs, tissues or cells, there are two options: (1) the drug is internalized (i.e., penetrate the cells) without internalization of the carrier or (2) both the drug and the carrier are internalized [2, 38-40]. The second internalization method has more efficient delivery system because after penetrating into cells, the intracellular environment will help either to the degradation of drug-carrier conjugate or releasing of drug molecules inside the cells but in the first internalization technique, the extracellular environment facilitates drug carrier conjugates degradation and the drug will then pass the lipid membrane to enter the cells [2, 38]. There are five methods to internalize macromolecules or nanoparticles in mammalian cells: phagocytosis, macro-pinocytosis, clathrin-mediated endocytosis, caveolin mediated path-ways, and clathrin/caveolin-independent endocytosis [2, 38, 41, 42]. The mechanism for cellular uptake of CNTs is not yet entirely understood. Nevertheless, two ways of internalization have been estimated: (1) through the endocytosis pathway and (2) via the endocytosis-independent pathway through passive diffusion across the lipid bilayer in a needle-like manner [23, 38, 43-45].

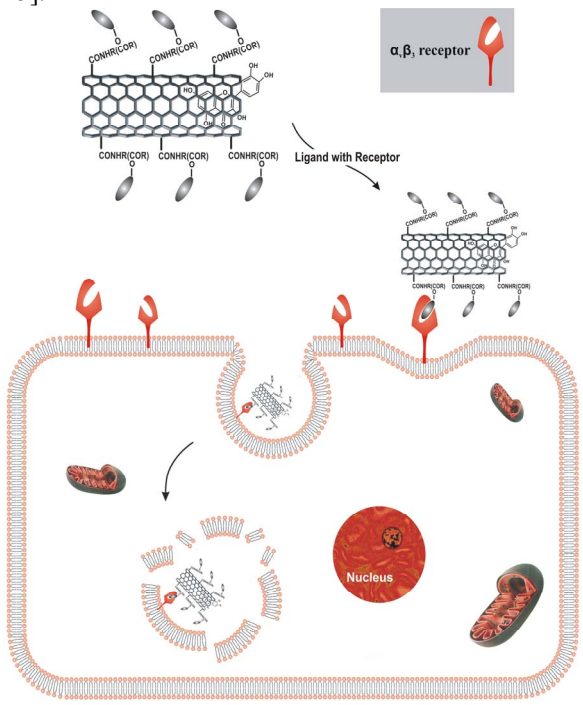

Figure. 4. The mechanism of SWCNT uptake by cell via endocytosis pathway.

Kam et al. reported the intracellular transportation of proteins and DNA by SWCNTs and showed the transporter capability of these materials. They also estimated that the clathrin-dependent endocytosis as the pathway for the uptake of a various SWCNTs conjugates with proteins and DNA [46].

In general it is anticipated that the CNTs entrance into cell is influenced by the its dimensions (diameter, length) and surface chemistry [47]. In addition, uptake of CNTs can be considered as a dose- and time-dependent [48]. The mechanism of SWCNT uptake by cell has been shown in Figure 4.

E. *Detection method of RGD conjugated SWCNT*

RGD-conjugated SWCNTs can be used as the contrast agent for photoacoustic molecular imaging of cancer in a mouse tumor model. The basis of this method is sound

generation as a result of local heating by the absorption of laser light, which has higher spatial resolution than traditional ultrasound, and deeper tissue penetration than fluorescence imaging [49]. In this technique usually tissue is irradiated by a short-pulsed laser beam to generate thermal and acoustic impulse responses. Locally absorbed light is converted into heat; through thermoelastic expansion of the tissue produced heat converted to a pressure rise. The initial pressure rise transmits in the tissue as an ultrasonic wave, referred to as a photoacoustic wave, which is detected by ultrasonic transducers placed outside the tissue to generate electric signals. After that the electric signals are amplified, digitized, and transferred to a computer to form a photoacoustic image [50]. Photoacoustic imaging can provide higher spatial resolution, high sensitivity and slightly better tissue penetration in comparison with other optical imaging techniques. The major limitation of this prototype imaging system is relatively long time of data acquisition. More than 20 min was needed to create a single photoacoustic image of a 100 mm^3 sized tumor [50].

The intrinsic optical properties of SWCNTs make them useful as optical probes. Due to their quasi 1-D nature, SWCNTs exhibit strong high optical absorption and photoluminescence in the Near-infrared (NIR) range [31]. However, because of strong light quenching properties of SWCNTs, direct fluorescent labeling of SWCNTs has not been very successful for optical imaging applications. By wrapping SWCNTs with fluorescently labeled polymer like poly(vinylpyrrolidone) (PVP), individual SWCNTs can be observable by a fluorescent microscope [51]. Also conjugated SWCNTs with quantum dots (QDs) and the supramolecular assembly can be stably dispersed under physiological conditions and visualized by fluorescence microscopy [52]. NIR optical imaging in the range of 700-900 nm can provide prospects for rapid and cost-effective pre-clinical evaluation in small animal models, because the absorbance spectra for all bimolecules in the NIR region is minimum which gives a clear spectral window [50].

SWCNTs show strong resonance Raman scattering owing to their sharp electronic density of states at the van Hove singularities [31]. SWCNTs have numerous distinctive Raman scattering features containing the radial breathing mode and tangential mode (G-band), that are sharp and strong peaks and can be easily distinguished from fluorescence backgrounds, and therefore are appropriate for optical imaging [31, 53]. Raman spectroscopy potential for multiplexed imaging and lack of confounding background signal from autofluorescence can be considered as a major advantages of this method [54].

IV. Conclusion

Design of novel drug carriers with multi-functionalities is important in the drug delivery and controlled release field. CNTs, especially SWCNTs are highly promising in biomedicine and it is obvious that a bright and interesting future is predictable for all these carbon cylindrical nanomaterials. In addition, functionalized CNTs display greater biocompatibility with minimal cytotoxicity. We believed that SWCNTs quercetin delivery system will be reliable candidate for cancer treatment due to this fact that quercetin is natural antioxidant and anticancer agent and in comparison with synthetic anticancer agent such as doxorubicin maybe has low side effects. Besides, this drug delivery system has high selectivity to cancer cells and can be imaged through photoacoustic molecular imaging technique, NIR optical and Raman imaging. It is deemed that CNTs play a critical role as exemplary nanomaterials that can be clinically developed and constitute archetypal cases in the emerging field of nanomedicine. Hence, intensive research efforts, novel intriguing applications of CNTs in medicine and biology will be expected in near future.

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