

Delivery of Quercetin as an Antioxidant Agent to Cancer Cells by Single-walled Carbon Nanotubes

Jafar Ezzati Nazhad Dolatabadi^{1*}, Ali Akbar Jamali²

¹Research Center for Pharmaceutical Nanotechnology, Student Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran

²Member of Young Researchers Club, Islamic Azad University of Tabriz, Tabriz, Iran

E-mail:ezzati_j@yahoo.com

Mohammad Hasanzadeh³, Nasser Razmarai⁴, Yadollah Omid⁵

³Drug Applied Research Center, Student Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran.

⁴Parasitology Laboratory, Razi Vaccine and Serum Research Institute, Northwest Branch, Marand, Iran

⁵Research Center for Pharmaceutical Nanotechnology, Tabriz University of Medical Sciences, Tabriz, Iran

Abstract— Carbon nanotubes are made up of carbon atoms arranged in a series of condensed benzene rings and wrapped into a tubular form. These materials due to some qualities such as high specific surface area, unique electrical and electronic properties are used in many applications as catalyst base, polymers mechanical strengthening, composites, electronic devices production and drug delivery. 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 nanotubes into cancer cells and their detection method. In fact, we introduces the concept of “functionalization partitioning” of single-walled carbon nanotubes (SWCNTs), i.e., imparting various chemical species, such as poly (ethylene glycol) (PEG), quercetin with different functionalities onto 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.

Keywords-component; Carbon nanotubes; Quercetin, Drug Delivery.

I. INTRODUCTION

One of the major inventions in nanotechnology is carbon nanotubes discovery. First time carbon nanotubes were discovered by Iijima in 1991 while he was studying carbon electrode surfaces during arc discharge [1].

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 [2, 3]. 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 [4, 5]. 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 [6]. The MWCNTs also consist several cylinders of graphitic shells with a layer spacing of 0.3–0.4 nm [6].

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 [7]. 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 [7, 8]. 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 [7, 9].

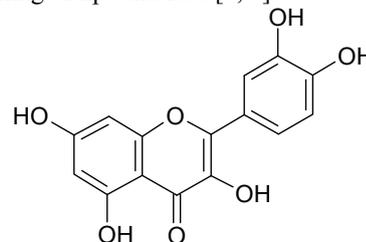


Figure 1. Chemical structure of quercetin.

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 [10].

Enormous challenges have contributed towards development of new controlled drug delivery systems to 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 [11, 12]. 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 [11, 13-15]. Among them, CNTs that possess unique chemical and physical properties have received a great attention for drug delivery applications [16-19]. 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 [20-22]. 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 [23-26]. 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 [27]future they would be able to use them in gene therapy, vaccination, and different cancer treatments.

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

II. METHODS

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 [27, 28].

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-NH₂ 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 [29].

Quercetin loading onto RGD-PEG functionalized SWCNTs will be done by simply mixing 1 mM quercetin with the RGD-PEG functionalized SWCNTs at a nanotubes 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) [30].

III. RESULTS AND DISCUSSION

A. Functionalization of SWCNT quercetin loading into it

Aqueous solutions of high-pressure CO decomposition (Hipco) SWCNTs can be functionalized covalently by PEGylation of $-\text{COOH}$ groups on oxidized SWCNTs generated by refluxing in 3 M nitric acid. After simple mixing of the SWCNT solution with quercetin at pH 8 overnight and then repeated filtering to remove free, unbound quercetin. Figure 2, shows the method of functionalization of SWCNT and quercetin loading into it.

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 [27].

B. Quercetin release from SWCNT

Controlled release of drugs from a drug carrier complex is required as a important aspects of drug delivery systems [31]. Liu et al found that the amount of doxorubicin, a widely used chemotherapy drug for treating various cancers, bound onto SWCNTs was pH-dependent [27]. 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 with in the cancer cells [29, 32].

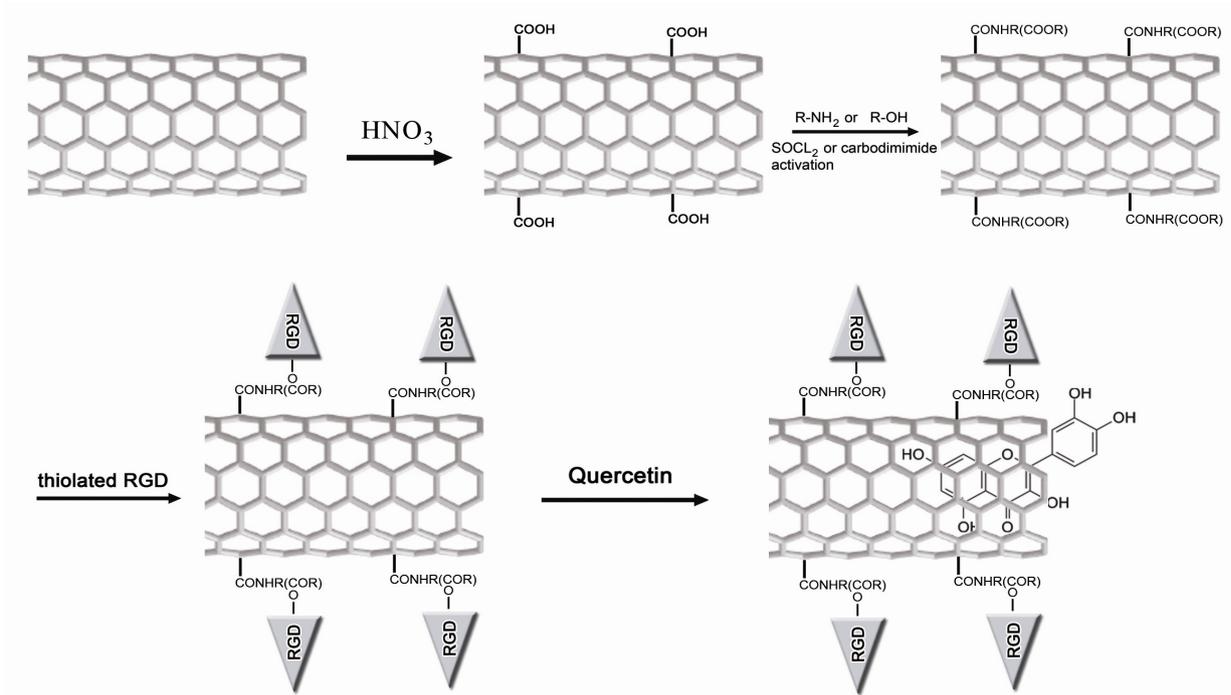


Figure 2. Method of functionalization of SWCNT and quercetin loading into it.

C. SWCNT–RGD conjugates

De la Zerda et al reported on the conjugation of cyclic RGD containing peptides to SWCNT (SWCNT–RGD) that is stable in serum [33]. In order to increase selectivity of SWCNTs to tumor cells cyclic RGD peptide should be conjugated on the terminal groups of PEG on SWCNTs (Figure2) [27]. These SWCNT–RGD conjugates bind with high affinity to $\alpha_v\beta_3$ integrin, which is over-expressed in tumor neovasculature, and to other integrins expressed by tumors but with lower affinity [33].

D. Detection method of RGD conjugated SWCNTs

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 [33]. 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 [34]. 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 mm³ sized tumor [34].

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. 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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