FUNCTIONALIZATION OF CARBON NANOTUBES FOR THE DRUG DELIVERY IN CANCER TREATMENT

by

Özde Zeynep Güner

BSc, Chemical Engineering, Istanbul Technical University, 2018

Submitted to the Institute of Biomedical Engineering
in partial fulfillment of the requirements
for the degree of
Master of Science
in
Biomedical Engineering

Boğaziçi University
2020

For M.S. theses

FUNCTIONALIZATION OF CARBON NANOTUBES FOR THE DRUG DELIVERY IN CANCER TREATMENT

APPROVED BY:

Prof. Dr. Hale Saybaşılı ..... (Thesis Advisor)

Prof. Dr. F. Seniha Güner ..... (Thesis Co-advisor)

Assoc. Prof. Bora Garipcan .....

Assist. Prof. Duygu Ege .....

Prof. Dr. Sevil Yücel .....

DATE OF APPROVAL: 24 June 2020

ACKNOWLEDGMENTS

This work would not have been possible without the support of my thesis advisor Prof. Dr. Hale Saybaşılı of the Biomedical Engineering Institute at Boğaziçi University. She provided me professional guidance and taught me much about both scientific research and life in general. I am especially indebted to Prof. Dr. F. Seniha Güner, who has been supportive of my career goals, and who worked actively to provide me with the protected academic time to pursue those goals. A crucial part of this work is accomplished with the help of generous Prof. Dr. Saime Batirel at Marmara University Medical School.

I am grateful to all of those with whom I have had the pleasure to work during my thesis. I would especially like to thank Dr. Elif Baysak, Serdar Bozoğlu, and Dr. Mehdi Meran for their sincere contribution. Besides, during this work, I had the support of some thoughtful colleagues that are Berkcan Arslan, Sıla Kılıç, Şevval Murat and Yeşim Yeniyurt.

Also, I would like to thank my lovely fellows at Boğazici University; Alamira Hajjar, Başak Dalbayrak and Sevde Bayrak who never left me alone trough this compelling two years.

Finally, I must express my very profound gratitude to my parents, Seniha and Haluk Güner whose love and guidance are with me in whatever I pursue. And also, my lovely grandmother, Aliye Erkal, who never left by my side. They are the ultimate role models. Lastly, I wish to thank my fiancée, Anıl Yılmaz, for providing me with unfailing support and continuous encouragement. This accomplishment would not have been possible without them.

Thank you.

Özde Zeynep Güner

ACADEMIC ETHICS AND INTEGRITY STATEMENT

I, Özde Zeynep Güner, hereby certify that I am aware of the Academic Ethics and Integrity Policy issued by the Council of Higher Education (YÖK) and I fully acknowledge all the consequences due to its violation by plagiarism or any other way.

Name :

Signature:

Date:

ABSTRACT

FUNCTIONALIZATION OF CARBON NANOTUBES FOR THE DRUG DELIVERY IN CANCER TREATMENT

Cancer is a significant health problem and the main cause of death worldwide. Targeted drug delivery is a possible replacement for classical cancer treatment, which includes chemotherapy, surgery, and radiation therapy. With this treatment, single-walled carbon nanotubes (SWNTs) are widely exploited. They have the advantage over other materials with their conductivity, large specific surface area, and chemical stability. However, the cytotoxicity of SWNTs is still a challenge in this field. The goal of the study is to obtain biocompatible SWNTs to use in targeted drug delivery. To reduce the toxicity of SWNTs and get an excellent drug carrier, we were modified SWNTs with a novel noncovalent functionalization method: adsorption of 9-fluorenylmethyloxycarbonyl (Fmoc)-terminated aromatic amino acid-functionalized with poly(ethylene) glycol (PEG) chains onto pristine SWNTs. For that purpose, among biocompatible agents, we used a Fmoc-Cys(Trt)-OH, Fmoc-terminated aromatic amino acid, due to operational simplicity, and PEG, an FDA-approved safe polymer, with two different molecular weights (PEG5000 and PEG12000). With this work, we can say that we successfully obtained functionalized SWNTs (f-SWNTs). In addition, we determined the most competent coating among synthesized f-SWNTs in terms the stability, binding efficiency, and suspending properties. Consequently, the in-vitro effectiveness of functionalized SWNTs was tested on fibroblast cells. Our results show that modifications improve the cytotoxicity of SWNTs, and f-SWNTs can be used in cancer drug delivery.

Keywords: Carbon nanotubes, Fmoc, Poly(ethylene) glycol, cancer.

ÖZET

KANSER TEDAVİSİNDE İLAÇ TAŞINIMI İÇİN KARBON NANOTÜPLERİN FONSİYONELLEŞTİRİLMESİ

Kanser dünya çapında önemli bir sağlık sorunu ve ölümlerin ana nedenidir. Hedefli ilaç taşınım sistemleri; kemoterapi, cerrahi ve radyasyon terapisini içeren klasik kanser tedavileri için olası bir alternatiftir. Bu yöntem için, tek duvarlı karbon nanotüplerden (TDNT'ler) yaygın olarak faydalanılmaktadır. TDNT'ler, iletkenliği, geniş spesifik yüzey alanı ve kimyasal stabilitesi ile diğer malzemelere göre avantajlıdırlar. Bununla birlikte, TDNT'lerin sitotoksisitesi, sağlık alanında kullanılması açısından aşılamamış bir engeldir. Çalışmanın amacı, hedefli ilaç taşınımında kullanılacak biyouyumlu TDNT'leri elde etmektir. Toksisitesini azaltmak ve mükemmel bir ilaç taşıyıcısı elde etmek için, TDNT'ler yeni bir kovalent olmayan fonksiyonelleştirme yöntemi ile modifiye edilmiştir: 9-fluorenilmetiloksikarbonil (Fmoc) ile sonlandırılmış aromatik amino asit ile fonksiyonelleştirilmiş poli(etilen glikol) (PEG) zincirlerinin TDNT'lerin üzerine adsorbsiyonu. Bu amaçla, biyo-uyumlu ajanlar arasından, operasyonel basitlik nedeniyle bir Fmoc-Cys(Trt)-OH, Fmoc sonlu aromatik amino asit ve FDA onaylı bir polimer olan PEG, iki farklı molekül ağı rlı ğında (PEG5000, PEG12000) seçilmiştir. Bu çalışma ile, işlevsel TDNT'leri (f-TDNT'ler) başarıyla elde ettiğimizi söyleyebiliriz. Ek olarak, sentezlenmiş f-TDNT'ler arasında en yetkin kaplama stabilite, bağlanma etkinliği ve süspansiyon özellikleri açısından belirlenmiştir. Çalışmanın son aşamasında, fonksiyonelleştirilmiş TDNT'lerin in-vitro etkinliği fibroblast hücreleri üzerinde test edilmiştir. Sonuçlar, geliştirilen f-TDNT'lerin toksik olmadığını ve ilaç taşıyıcı sistem olarak kullanılabileceğini göstermektedir.

Anahtar Sözcükler: Karbon nanotüp, Fmoc, Poli(etilen) glikol, kanser.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

CNT	Carbon Nanotube
CVD	Chemical vapor deposition
Cys	Cysteine
DCM	Dichloromethane
DNA	Deoxyribonucleic acid
DMEM	Dulbecco's modified eagle's medium
DMSO	Dimethyl sulfoxide
f-CNT:	Functionalized carbon nanotube
Fmoc	9-Florenylmethyl chloroformate
f-SWNT	Functionalized single-walled carbon nanotube
FT-IR	Fourier transform infrared
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MWNT	Multi-walled carbon nanotube
NMR	Nuclear magnetic resonance
PEG	Poly(ethylene) glycol
PTFE	Polytetrafluoroethylene
SiRNA	Small interfering ribonucleic acid
SWNT	Single-walled carbon nanotube
TGA	Thermogravimetric Analysis
TEM	Transmission emission microscopy
THF	Tetrahydrofuran

1. INTRODUCTION

Cancer is a disease described by uncontrolled cell growth and the spread of these cells leads to mutations in a tissue, organ, or cell DNA. If the spread is not controlled, it may result in death. Today, health problems are rapidly increasing due to industrial development and environmental problems on the one hand and rapid population growth on the other [1].

The classical cancer treatment includes chemotherapy, surgery, and radiation therapy. The main disadvantage of conventional therapies is the destruction of healthy cells as well as harmful cells during treatment [1]. Targeted drug delivery is a possible replacement for accepted methods. Therefore, efforts towards the development of targeted drug delivery systems capable of controlled drug release are continuing rapidly. There are many advantages of controlled drug delivery systems over conventional methods: site-specific drug delivery is possible, fewer side effects are observed, and lower drug concentration is needed [2, 3, 4, 5, 6].

Nanomedicine is a rapidly evolving field that revolutionizes the cancer diagnosis and treatment. Nanoparticles' exceptional biological properties are due to their small size and large ratio of surface area to volume. The use of nanomaterials to enhance the delivery of available therapeutic agents represents one among the oldest and most established branches of nanomedicine (Figure 1.1 [7]).Loading conventional drugs into nanoformulations by encapsulation, incorporation, or alternative techniques could have an effect on solubility, stability, pharmacokinetics, and biodistribution, and thus could alter the therapeutic reaction of the product [8]. Nanocarriers can remain in the blood circulation for a long time and provide the drug release according to the adjusted dose. These very small structures diffuse the tissue system, expedite drug uptake by the cells, allow effective drug delivery, and enable activity at the targeted site. The nanostructures intake by cells is much faster than other particles of size range from 1 to 10 μm .Thus, it interacts directly with reduced or negligible side effects to treat

diseased cells [9].

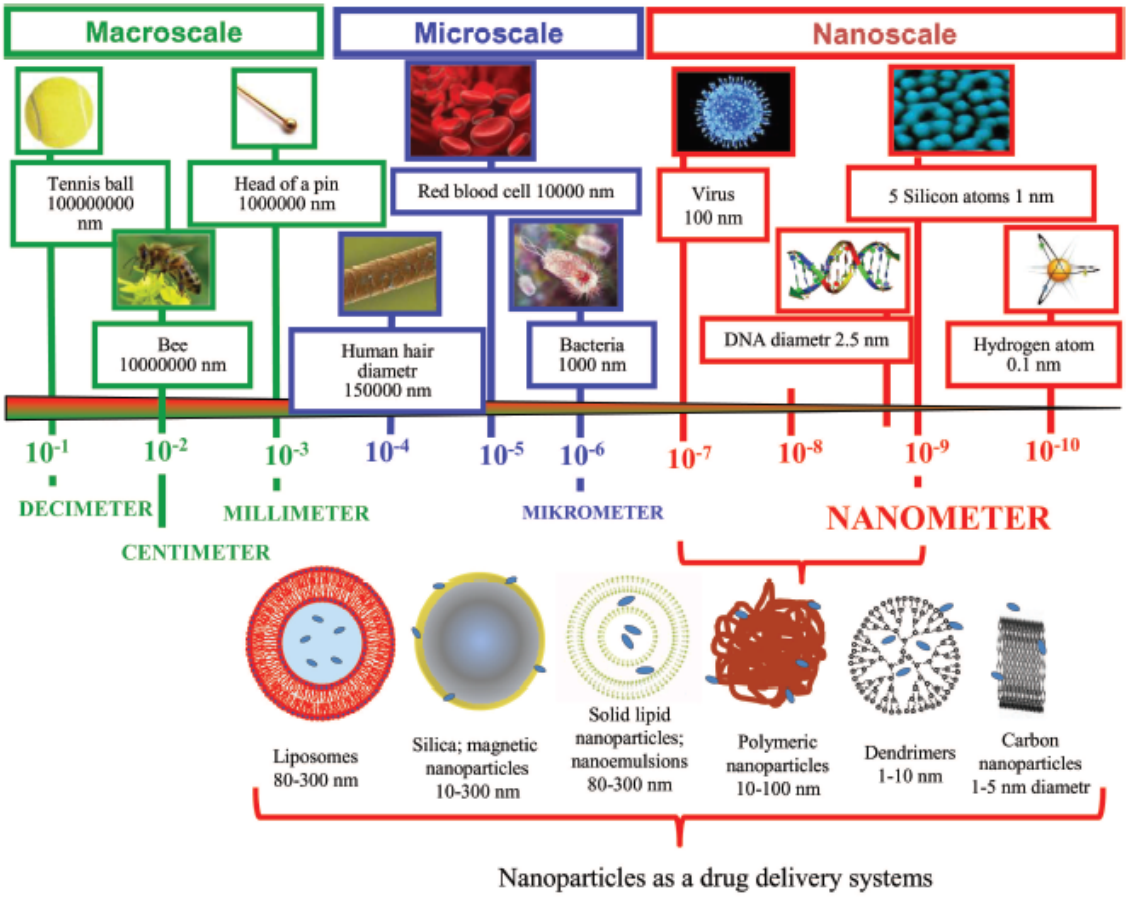

Figure 1.1 Particle classification according to their size. Scale shows size of nanoparticles used as drug delivery systems comparatively to other materials in macro, micro and nanoscale.

Besides the strong curiosity in nanoparticles as transporters of the active components, there are some struggles in unassisted active components usage. Certain active components are materials which are non-cellular permeable and susceptible to metabolic degradation. Another feature that limits their use is that they are extremely hydrophobic. Such components can be carried through the physiological environment by means of nanoparticles. By the use of nanotechnology for drug release, upgraded pharmacokinetic and pharmacodynamic assets of an active substance can be obtained. In the transport of this active component by nanoparticles, one of the most important parameters affecting the efficiency of the release system is particle size [10]. The drugs attached to the nanoparticles through adsorption, absorption, or covalent bonding.

Desorption occurs by polymer degradation, diffusion, physical or chemical polymer transformation (for example, protonation/deprotonation, low critical solution temperature), or a mix of those mechanisms. The advantages of using nanoparticles in drug delivery are more than its disadvantages, one of the benefits is their high dissolution rate, which leads to improved bioavailability and low drug dosing, reducing toxicity (Table 1.1). In addition, the success of nanoparticles in drug delivery can be credited to various aspects such as physical and biological constancy, simplicity of the production method, the possibility of effortless scaling, and suitability for freeze-drying and sterilization [11].

Table 1.1
Advantages and drawbacks of nanoparticles in drug delivery.

Advantages of nanoparticles	Disadvantages of nanoparticles
Controlled and sustained drug release	Production cost
Large amounts of drug loading	Not being able to characterize all features
Local targeting	Difficulty to adapt to large scale production
Variety of application methods	Biodistribution cannot be determined
The increased therapeutic effect of the drug	
A decrease in the side effect	
Incorporation of the drug without any chemical reaction	
Manageable particle size and surface characteristics	

Drugs transported by polymer-coated nanoparticles are released by diffusion or erosion procedures along the polymeric membrane. This membrane serves as a blockade to drug release. Hence, the drug solubility and diffusibility in the membrane is the determining factor for the release process. We need to concentrate on two essential elements developing a successful nanoparticle system: drug release and polymer biodegradation. The drug release rate depends on several factors: drug solubility, desorption of the absorbed drug, drug diffusion through the nanoparticle matrix, degradation of the nanoparticle, erosion/diffusion process. Therefore, to control the drug release process, we must focus on solubility, diffusion, and biodegradation of matrix materials.

The nano-carriers used in chemotherapy can be classified into two main categories: carriers using organic molecules as the primary building material for targeted or untargeted drug release, and carriers using inorganic elements (usually metals) as the core. Organic nano-carriers include lipids, dendrimers, liposomes, emulsions, and synthetic polymers.

Inorganic nanocarriers are extensively researched for imaging treatments recently because of their large surface area, better drug loading capacity, better bioavailability, low deadly side effects, and tolerances to most organic substances such as controlled drug release. Quantum dots, carbon nanotubes, porous silica, and magnetic nanoparticles have been widely used in cancer treatment in various ways [12]. Also, inorganic nanoparticles used as nanocarriers include silver, gold, iron oxide, and silica nanoparticles. Although the studies examining these have shown some potential applications, they are not as much as the other nanoparticle types mentioned in this section. Yet, just a few of the nanoparticles have been recognized for clinical use, with more than half still at the clinical trial stage [9].

Up until now, many nanocarriers were offered to use as drug delivery agents. Some of the widely used nanocarriers for chemotherapeutics delivery are liposomes, polymeric nanoparticles [13], dendrimers [14], carbon nanotubes [15], mesoporous silica nanoparticles [16], micelles [17], quantum dots [18], gold [19] and magnetic nanoparticle [20]. The schematic representation of the aforementioned commonly used nanocarriers is given in Figure 1.2 [21].

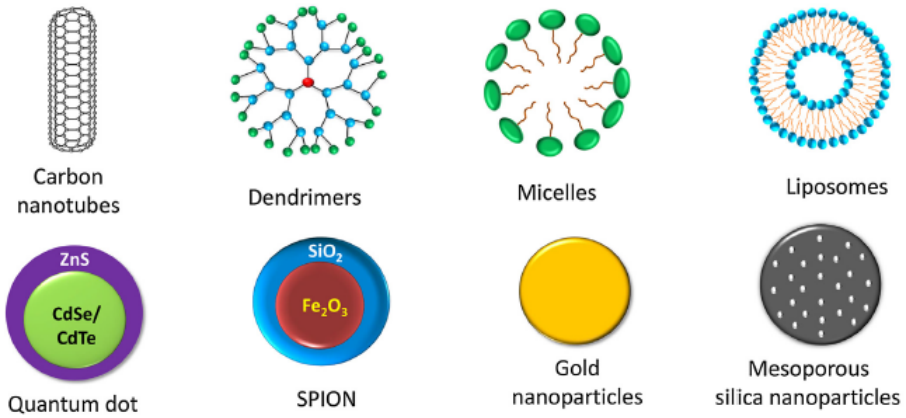

Figure 1.2 Schematic representation of mostly used nanocarriers.

2. CARBON NANOTUBES: DISCOVERY TO DRUG DELIVERY

The development of carbon nanotubes (CNTs) dates back to 1985. Harry Kroto of the University of Houston, USA, and Sussex University of the United States of America, Rice University, announced their investigation concludes on a very unconventional carbon allotrope. This C60, which contains 60 carbon atoms, is likened to a soccer ball with a total of 32 faces, 20 of which are hexagonal and 12 of which are pentagonal. While only two carbon allotropes of diamond and graphite are known prior to the present invention, a different path has been made in the carbon physical chemistry with the present invention. These newly developed molecules are known as 'fullerene '. Sumio Ijima, on the other hand, discovered carbon nanotubes in Japan in 1991 with the efforts to obtain cylindrical nanostructures from fullerenes [22, 23].

CNTs are cannulate carbon graphite nanomaterials with high surface area, high length-to-width ratio, and ultra-lightness. These tubes are between 1-100 nm in diameter and normally have both ends sealed with half of a fullerene molecule. Every atom is combined with three adjacent atoms, so the CNTs have sp2 bonds with an average of 1.4 ÅC-C distance, which gives molecules a unique power[24, 25, 26].

Carbon nanotubes are graphene layers generally known to have a high surface area, large aspect ratios, and unique mechanical strength. It is known that the tensile strength of CNTs is almost 100 times higher than that of steel and also exhibits electrical and thermal conductivity properties such as copper.

Carbon nanotubes consist of six-membered rings can be used as sensors, diagnostic devices, and carriers for drug delivery. It creates toxicity problems and is not entirely soluble in all solvents. Mounting functional groups into carbon nanotubes can make them water-soluble and functional so that active molecules such as proteins, and drugs can bind to peptides, nucleic acids [15]. Functionalization or conjugation of

CNTs makes them more soluble and biocompatible [15].

CNTs have strong potential as nanocarriers offering excellent electrical and thermal conductivity, durability, and also, they have applications in the manufacturing of biomedical devices and biosensors. Its physical properties, such as tensile strength, elasticity, and elasticity, make it possible to form a nanocarrier frame with outstanding mechanical properties.

Choosing the correct shape of carbon nanotube is essential to build up a drug delivery mechanism. There are two choices in this respect: single-walled carbon nanotubes (SWNTs), and multi-walled carbon nanotubes (MWNTs).

SWNTs can be regarded as a single long-wrapped graphene sheet. They commonly have a length/diameter ratio of about 1000 nm and are therefore conceived to be almost one-dimensional structures. SWNTs are excellent graphene sheets having a polyaromatic, mono-atomic layer in which a hexagonal ring wound into a cylinder is held in contact by a seamless assembly.

SWNTs have a diameter range of $0.4-3.0$ nm and length range of $20-1000$ nm. They are excellent conductors, but despite their great potential in various nanotechnological applications, the production of SWNTs is still costly and the advance of more cost-effective synthesis methods is very important to bring them into commercial-scale applications.

There are many advantages of SWNTs as drug delivery agents. Due to their large surface area they are qualified to cross amphipathic cell membranes, variable interactions with the drug, adjustable surface chemistry, and dimensions. Peptides, proteins, nucleic acids, as well as drug molecules, can be delivered by SWNTs for therapeutic purposes. Numerous advances have shown the functionalized SWNT-based drug delivery systems are effective for cancer tratments [27] and treatment of other diseases such as neurodegenerative diseases [28, 29].

MWNTs are formed by folding the graphene layer 2-10 or rolling only one graphene layer to produce a complex multi-wall structure [30].MWNTs have an average cylindrical tube diameter of 1-3 nm and an outer cylindrical tube diameter of 2 to 100 nm [4]. MWNTs consist of a plurality of layers of graphite that coaxially roll to form a tubular shape. They are also commonly used in drug delivery systems with their robust structure [31]; however, they can always be shaped with a high degree of structural defects.

2.1 Preparation and Purification of CNTs

In the design of drug delivery systems, the synthesis method of CNTs is also a parameter to consider. Synthesis of CNTs is performed using graphite and carbon black in a controlled flame environment [23].However, the nanotubes manufactured by this procedure do not have good quality and purity, appropriate size and shape, and mechanical strength due to its natural environment. There are three core techniques developed to manufacture CNT structures. High-temperature procedures, such as electric arc discharge and laser ablation, were initially considered; however, low-temperature chemical vapor deposition is nowadays commonly favored as it permits more precise control of the CNT structure [32].

Chemical vapor deposition (CVD) is a well-known synthesis method for high quality and purity solids in the reactive gas chamber [33]. It is one of the usual techniques for synthesizing CNTs and is intended for large-scale production of CNTs. In this method, initiator and CNT formation occurs on the surface of the catalyst particles. Much of CNT is produced by using a carbon source in the gas phase (methane, carbon monoxide, acetylene, etc.) and an energy source to energize a gaseous carbon. The synthesis of CNT by the CVD method is essentially a two-step process. The first step is the catalyst preparation, and then the actual synthesis of the CNTs is performed. The catalyst is usually used for physical vapor deposition, spraying, immersion coating, etc. The following stage is to heat the substrate to 500-1000° C in a carbon-rich gas medium. Fe, Ni, or Co nanoparticles are used as catalysts [34].

Compared to laser ablation, the CVD method is an economical and practical method for producing large-scale and highly pure CNT [24]. The volume of the sample produced in arc discharge and laser ablation operations depends on the carbon source size (anode in arc discharge and target for laser ablation). It also requires intensive purification steps, as previously described. These drawbacks have led to the development of gas-phase techniques. CVD has been recognized as the finest method for producing high-purity carbon nanotubes [35].

Following the step of synthesis is the purification of carbon nanotubes. Carbon nanotubes generally have an average purity of about $5 - 10\%$ since they contain large amounts of impurities such as metal particles, amorphous carbon, and small fullerenes. Therefore, there is a need for purification prior to the addition of drugs to CNTs [34].

Purification techniques of CNTs are divided into three groups: chemical, physical, and a combination of both. The chemical purification technique is based on selective oxidation, where carbon-related impurities oxidization rate is higher than CNTs, and metal-related impurities are dissolved by acids. Chemical purification effectively removes amorphous carbon and metal particles, excluding those impacted in polyhedral graphitic units. On the other hand, oxidation involved in the chemical method permanently affects the CNT structure. The physical method relieves CNTs from impurities based on physical variances such as aspect ratio, size, magnetic properties, etc. The physical method is employed to separate different diameter/length ratios of CNTs or take out graphene sheets, carbon nanospheres, and aggregates. Since this technique does not involve oxidation, it prohibits CNTs from severe damage. There are some handicaps of the physical method: it is complex, time-consuming, and less effective. Multi-step purification is the third technique, which involves the advantages of physical and chemical purification. This technique yields superior CNT products [36].

2.2 Functionalization of CNT surface

In order to use purified CNT as a drug carrier, we must solve a significant problem: the biodegradability. In order to do that and obtain a perfected drug carrier, SWNTs are functionalized with various biocompatible molecules by chemical methods such as covalent and noncovalent attachment. These chemical modifications improve the water solubility, decrease cytotoxicity, and advance drug targeting features of CNTs. For biomedical applications, surface chemistry or functionalization ensures the dissolution of CNTs, resulting in biocompatibility and low toxicity [15].

The functionalization process involves altering the structure of naturally pure CNTs by adding probable functional groups, ligands, nucleic acids, polymers, drug molecules, proteins and peptides, dyes, or radio-contrast agents for therapeutic applications. Despite the rewarding properties such as durable and water insoluble nature, CNTs are extremely lethal in nature, and accumulate in organs. The functionalization process gives the CNTs high resolution with improved biocompatibility. Accordingly, functionalized CNTs (f-CNT) are highly suitable for applications of encapsulation of therapeutic agents for targeted release studies [37].

f-CNTs have wide applications and take charge of increasing biocompatibility, solubility, encapsulation tendency, drug release in the body. According to various studies, drug molecule-loaded f-CNTs enter cells, and nuclei, indicating the peak concentration level of the drug molecule at the cell and molecule level [37].

There are two modification methods for CNTs: covalent and non-covalent modification (Figure 2.1 [38]). Covalent approaches include inoculation of polymer chains where durable chemical bonds are shaped. Covalent functionalization has the advantage of increasing the distribution of CNTs in polymeric structures and thereby greatly extending the efficacy of these polymer nanocomposites [39]. Due to the covalent attachment, strong chemical bonds are formed between polymer chains to CNTs [24]. The trajectories of the sp2 hybridized sidewalls of the CNTs are very reactive and tend to effortlessly covalently bind with the molecule. Nevertheless, the CNTs symmetry is

compromised. The carbon atoms of sp3 are changed from sp2, by this means electrical properties of CNTs in the polymer grafted nanocomposite is degrading. CNTs can be effortlessly functionalized through the carboxyl and hydroxyl functional groups through oxidation. Through strong acids such as air, oxygen or sulfuric acid, nitric acid, hydrogen peroxide. The benefit of this method is the usage of preformed polymer units of known molecular weight and multi-dispersion, thereby ensuring tight regulation of conformational symmetry. The foremost difficulty of this method is preformed polymer moieties primary bonding that sterically inhibits the diffusion of other polymers to be bonded on the CNTs, this guides to less binding density [40].

The most used covalent functionalization method is oxidation which is developed to functionalize carbon nanotubes. CNT oxidation is executed through oxidizing agents, for example, nitric acid. Throughout processing, carboxyl groups appear at the ends of the tubes and defects in the side walls. However, oxidized CNTs are collected in the presence of salts, although they are water-soluble, and therefore cannot be used straight for biological utilization as a result of the high salt amount of almost every biological solution.

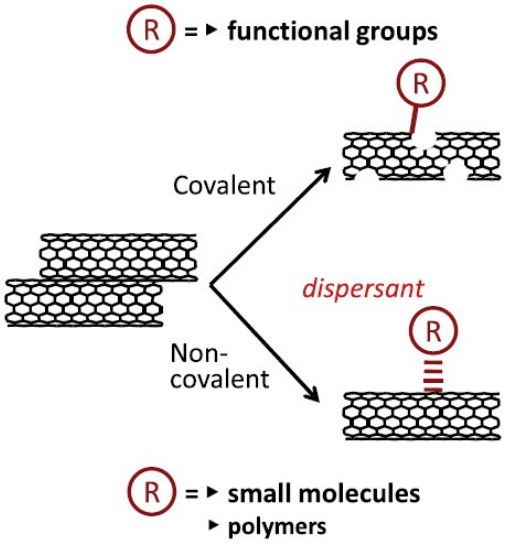

Figure 2.1 Schematic representation of bonding to CNT walls.There are two types of attachment: in covalent bonding, functional groups are covalently attached to the walls, on the other hand polymers and small molecules can bind to the surface by non-covalent interactions.

Additional modifications can be made in order to obtain stable CNT-polymer

conjugates in biological surroundings. Addition of hydrophilic polymers for instance poly(ethylene glycol) (PEG) to oxidized CNTs serves this purpose. Even with the strength of the covalent functionalization technique, the physical properties of CNTs are frequently demolished after chemical reactions as a result of the deteriorated nanotube structure and reduce the optical application potential of these materials [41]. Therefore, covalent binding cannot be utilized to functionalize CNTs used for photothermal ablation or imaging [42].

A relatively safer covalent functionalization method of CNTs is to combine drug molecules or functional groups. The covalent functionalization of CNTs obtained by the CNTs oxidation by strong acids causes reduction and produces carboxylic acid groups, which cause increased dispersibility in aqueous media. Alternatively, hydrophilic group addition to the outer walls and the ends of the CNTs results in water-solubility [37].

Such reactions frequently need extreme conditions for covalent bonding. In drug release perspectives, direct covalent modification of drug molecules to the CNT surface is less available in the literature. However, covalent functionalization has been usually applied for therapeutic biomolecules for example proteins, peptides, and DNA [37]. In most cases, covalent functionalization changes the internal properties of CNTs, such as conductivity and mechanical strength, because it destroys the normal graphene type structure [43].

In contrast to the covalent method, non-covalent functionalization can be accomplished via coating the CNTs with amphiphilic surfactants or polymers. Since the chemical structure of carbon nanotubes does not deteriorate, the physical properties of CNTs are mainly maintained via a non-covalent approach, with the exception of the shortening of the length due to the sonication used in the functionalization process. The non-covalent method includes the binding of therapeutic drug molecules to CNT surface through adsorption procedure. CNTs serve as nanoreversors to adsorb drug molecules through host-guest interaction. Methods of functionalizing CNTs relied on non-covalent interaction that can be completed without damaging the internal sp2 hybridized formation of the nanotube sidewall, thereby preserving the original elec-

tronic design and assets of the CNTs. Several types of non-covalent modifications were investigated. It is found that the non-covalent functionalization of CNTs can be accomplished by $\pi - \pi$ interactions between conjugated molecules and graphite sidewalls of CNTs [44, 45]. CNTs make considerable effort to preserve the final properties and increases their solubility considerably [46]. This behavior is clarified due to CNTs tendency to converge due to their strong internal van der Waals forces. To overcome this disadvantage, it is a fundamental approach to wrap the CNTs through non-covalent interactions with the polymer [43].

Non-covalent functionalization is crucial for improved biocompatibility and biomedical utilization of CNTs. With this modification, surfactants, polymers, and biomolecules such as DNA, siRNA, proteins, and peptides can be favorably loaded to the CNT surface. In addition, hydrophobic forces can inoculate lipophilic drug molecules into CNTs for targeted drug delivery utilizations. The existence of charge on the nanotube surface supports in the adsorption of drug particles.Non-covalent functionalization of CNTs is especially favored since it allows chemical modifications without influencing the electronic properties of the nanotubes.

In non-covalent functionalization, numerous polymers for instance poly(ethylene), nylon, and PEG are used. This method usually includes van der Waals forces and electrostatic interactions. PEGylation, one of the functionalization methods, has lately gained significant significance due to improved biocompatibility, solubility, and low toxicity. PEG-functionalized CNTs are broadly utilized in drug delivery because of their non-toxicity and enhanced solubility [47]. Nowadays, in the biomedical field applications of functionalized carbon nanotubes is mainly limited by chemical approaches that can make this material biologically compatible. The most straightforward procedures involve the physical adsorption of f-CNTs via several molecules, such as pyrene, naphthalene [48, 49].

One example of the limitations of non-covalent functionalization is showed applying it to a single DNA sequence by means of aromatic DNA base units. It has been revealed to be unstable because it is cleaved by nuclease, and as a result, the biological

application has been limited [42].

As mentioned above, the non-covalent binding of molecules to CNTs is the most commonly utilized drug delivery technique in literature. Regarding limitations, a non-covalently functionalized ideal CNT needs to have specific properties. This can be accomplished via forming micelle-type structures in which the amphiphilic molecules are coated to the CNT. Despite limitations, the non-covalent method has many advantages. Compared to chemical modifications, the non-covalent method has two core advantages: (i) being able to operate under comparatively mild reaction conditions, and (ii) maintaining the excellent graphitic character of CNTs [47].

With the non-covalent method, it is able to stabilize the dispersions of the nanotube in water without changing the conjugate system. Non-covalent modifications may make SWNT secondary properties such as molecular selectivity in addition to improved distribution [50]. One of the significant advantages of this approach is the protection of the conjugated electronic structures of the CNTs while improving their resolution in a remarkable manner [51].

In general, non-covalent functionalization enhances reactivity, catalytic activity, binding capacity, dispersibility, biocompatibility, and sensing behavior in nanomaterials [52, 53]. The above-mentioned modifications allow the CNT to cross the plasma membrane and encourage cellular uptake of tiny molecules and macromolecules such as nucleic acid [54], peptide [55].

PEG is an FDA approved polymer, and it is the most preferred material for functionalizing CNTs, enhancing dispersion in aqueous solution and biocompatibility of CNTs [56].

The challenge in using PEG is that the difference in hydrophilicity of the CNT surface and PEG. PEG is a highly hydrophilic polymer. In contrary, the CNT surface is hydrophobic. An intermediary agent to functionalize the CNT surface is needed.

In the last decade, studies on the active release of biomolecules and the non-covalent functionalization of CNTs for designing imaging agents have continued. In this study, non-covalent functionalization was performed to enhance the biocompatibility and water solubility of CNTs. For this purpose, 9-fluorenylmethyl chloroformate (Fmoc) -PEG complex was attached to the CNT surface. The reasons for using PEG as listed in the literature are as follows;

- Low toxicity and not perceived as foreign matter by the immune system,
- Soluble in the water environment,
- It is not a steric barrier to surface treatments or bioconjugation due to its high flexibility [47].

In the present study, by connecting the PEG chains to the CNT wall, the strengths of the PEG chain and CNTs are combined; a blood-compatible, and stable structure of nanomaterials can be obtained. After the SWNTs were functionalized, the cytotoxicity of f-SWNTs on healthy cells were tested. In order to do so, fibroblast cells were used. After all, the nanomaterials obtained in this way are promising great future as pharmaceutical vehicles with their superior properties.

3. EXPERIMENTAL SECTION

3.1 Chemicals and Properties

PEG monomethyl ether with a molecular weight of 5000 and 12000 g/mol, Fmoc-Cysteine (Fmoc-Cys(Trt)-OH), were obtained from Sigma Aldrich. Fmoc refers to the 9-fluorenylmethyl carbonyl. Dichloromethane (DCM) was obtained from Aldrich and distilled on P2O5 before use. Tetrahydrofuran (THF; 99.8%, J.T. Baker), diethyl ether, dimethylaminopyridine (DMAP) and N, N'-dicyclohexylcarbodiimide (DCC) were purchased from Sigma Aldrich. The chemical formula of PEG monomethyl ether and Fmoc-Cys(Trt)-OH is given in Figure 3.1.

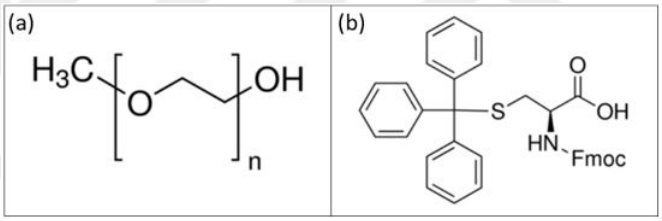

Figure 3.1 The chemical formula of (a) PEG monomethyl ether and (b) Fmoc-Cys(Trt)-OH.

3.2 Preparation of SWNTs

SWNT synthesis was carried out, under the direction of Prof. Dr. Nilgün Yavuz (ITU Energy Institute faculty member), according to the fluidized bed chemical vapor deposition method [57, 58]. The system where SWNT was produced is located in ITU Energy Institute Materials Production and Preparation Laboratory. The system consists of an oven and a quartz reactor that can operate at high temperatures. (Figure 3.2) The catalyst was placed in a quartz reactor with a disc, and fluidization occurs with the gas flow. During the production of the catalyst, iron nitrate [$Fe(NO_3)_3.9H_2O$] and magnesium oxide (MgO) was ultrasonically mixed in ethanol, and after the homogenous mixture was achieved, catalyst production was carried out by drying and grinding. The

amounts of iron nitrate and magnesium oxide were selected based on the mass ratios of iron and magnesium targeted in the catalyst. The iron nitrate in the catalyst was placed in the furnace turns into iron oxide (Fe_2O_3) at temperatures above 125°C during the heating process of the furnace, and thus the structure of $Fe_2O_3 + MgO$ takes its final form.

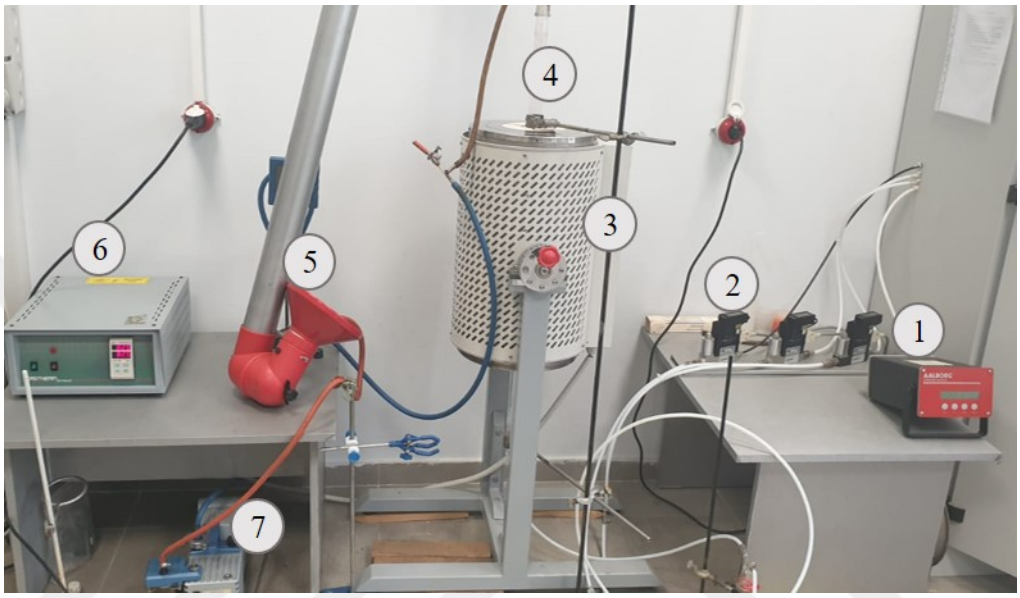

Figure 3.2 Synthesis system of carbon nanotubes. SWNTs are formed by CVD method. In the system there is a gas flow controller (1), panels showing gas flow rate (2), furnace (3), quartz tube (4), gas discharge unit (5), temperature controller (6), and a pump (7).

In the study, SWNT production takes place in the region of 5-10 cm above the disc in connection with the gas flow to the reactor at 800°C. The desired temperature was reached with argon gas, and acetylene with a specific flow rate was fed to the system. Argon was used as the carrier inert gas, and it was sent into the reactor with hydrocarbon. The most important reason for using inert gas was to provide the required fluid rate in addition to the hydrocarbon flow rate for the fluidization of the catalyst. In addition, at the beginning and at the end of the SWNT production, the inert gas flow was required to clean the inside of the reactor from the air and other gases, and thanks to this gas, structures formed outside the reaction zone of the furnace are flowed out of the furnace. The time required for the formation of carbon nanotubes has been determined by taking into consideration the previous studies in the literature and the group [59]. The flow of acetylene gas was then stopped, and the sample was

cooled to 200°C in an argon gas environment.

Following the synthesis, the SWNT was purified utilizing 6 M HNO_3. In order to gain short SWNTs, extremely concentrated HNO_3 handling was utilized during 7 h at a temperature of 120°C. Afterward, the nanotubes were filtered, carefully washed using distilled water just before the pH arrive ~ 7, and dehydrated in air.

3.3 Preparation of Fmoc-Cys(Trt)-OH Bearing Polymers (Cys-PEG)

A total of 2 Fmoc-PEG complexes were synthesized in the study. From the reaction of Fmoc-Cys(Trt)-OH with PEG5000 and PEG12000, Fmoc-PEG complexes were synthesized mainly by following the method specified in the literature [59]. For example, the synthesis using Fmoc-Cys(Trt)-OH and PEG5000 will be explained in detail. Other synthesis was carried out similarly with mole ratios of Fmoc-Cys:PEG:DCC:DMAP as 1.5:1:1.8:0.5, respectively. First, monometoxy PEG5000 (2.0 g) was dissolved in CH_2C_2 (30 ml) and mixed with Fmoc- Cys(Trt)-OH (0.45 g). DCC (0.37 g) and DMAP (0.122 g) dissolved in 10 ml of THF were added to the mixture, and the reaction was stirred at room temperature for 48 hours. At the end of the reaction time, the reaction solution was concentrated to precipitate the product in diethyl ether. The solid white precipitate was filtered, washed with ether, and dried under vacuum overnight. This procedure was repeated for PEG12000. Sample codes are given in Table 3.1.

Table 3.1
Codes of the samples.

Code	Fmoc-Cys(Trt)-OH	PEG5000	PEG12000	SWNT
Cys/PEG5000	+	+	-	-
Cys/PEG12000	+	-	+	-
Cys/PEG5000-SWNT	+	-	-	+
Cys/PEG12000-SWNT	+	-	-	+

3.4 Functionalization of SWNTs with Cys-PEG

The procedure to functionalize SWNTs, attach the prepared Fmoc-PEG complexes to the SWNT wall, is described below for the Fmoc-PEG5000 complex. SWNT (50 mg) and Fmoc-PEG5000 (0.25 g) were added to a flask containing 50 mL of dry THF. The mixture was sonicated in an ultrasonic bath for 30 minutes and then stirred at room temperature for 72 hours. The resulting mixture was filtered using a Sartorius PTFE filter with $0.2\mu m$ pore size to dispose unbound Fmoc-PEGs. A humid solid was remained on the filter. It was then washed with THF (500 ml) and acetone (500 ml) and filtered again. As a result, a black powder was obtained and it was dried for 24 hours under vacuum. The same process was used to get SWNTs functionalized with Cys-PEG12000. Schematic representation of Cys-PEG complexes preparation and functionalization of SWNTs is given in Figure 3.3.

3.5 Characterization of Materials

Transmission electron microscope. A JEOL 2100 device, operating at 200 kV, was used to obtain high-resolution transmission electron microscopy (HR-TEM) images. In order to prepare HR-TEM samples diluted solution (in THF) of the sample was dropped a on a holey carbon-coated copper grid.

Fourier transform infrared spectroscopy. FT-IR spectra were examined with an Agilent Technologies Cary 630 FT-IR device in the range of 4000-500 cm-1. FT- IR spectra play an essential role in determining the binding of Fmoc-Cys(Trt)-OH to PEG because it can directly reveal the formation of new bonds after binding.

Nuclear magnetic resonance spectroscopy. The 1H (500 MHz) spectra were recorded in CDCI3 using an Agilent VNMRS 500 device, and the structural characterization of the Fmoc-PEG complexes was performed.

Fluorescence spectroscopy. Fluorescence spectra were obtained using an F-4500 spectrophotometer. This study was carried out to verify the functionalization of SWNTs with PEG.

Thermal gravimetric analysis. Thermal gravimetric analyses (TGA) were performed on Perkin-Elmer Diamond TA/TGA with a heating rate of 10°C/min under an inert atmosphere in the temperature range of room temperature to800°C. The functionalization amount and thermal stability of SWNTs were determined.

3.6 *In-vitro* cytotoxicity studies

Cell viability assay. Samples are tested on fibroblast cell line, which was produced. Fetal bovine serum (%10) and 100 U/ml penicillin supplemented Dulbecco's Modified Eagle Medium F-12 (DMEM/F12, Gibco, UK) was used in cell culture at 37° C. For the cell viability assay, 96-well plate was used with $6x10^3$ cells per well. Before the assay, cells were treated with two different concentrations $(50, 100\mu g/ml)$ of coated SWNTs for 48 h. Cytotoxicity assay was done with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma, USA). After the treatment for 48 h, MTT (0.1 mg/ml) was added to each well. Then, the cells were exposed to MTT for 4 h at 37°C. Afterward, DMSO was added to wells, and the cells incubated at room temperature in the dark for 30 min. The cell viability assay was done in triplicates.

3.7 Statistical Analysis

All experimental studies were performed at least three times and each concentration was done in triplicates, to achieve independent experiments for the cell viability. Results are given as $mean \pm standard\ deviation\ (SD)$. In the evaluation of data, statistical analysis is done by two-way analysis of variance (ANOVA). Differences were considered significant from $p \leq 0.05$.

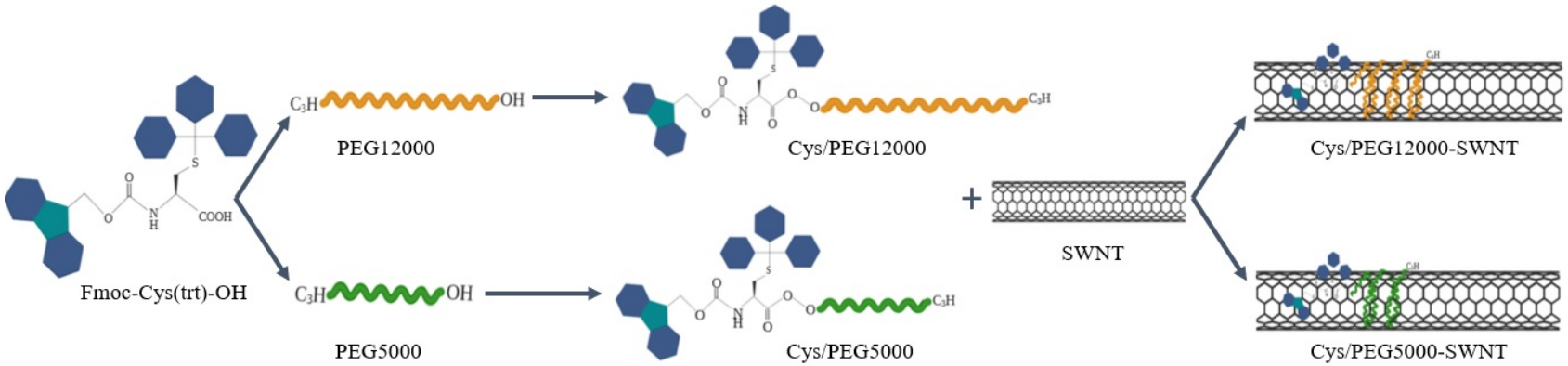

Figure 3.3 Schematic illustration of the functionalized SWNT preparation. Functionalized SWNTs (Cys/PEG-SWNT) were prepared in two steps. First, Fmoc-Cys(trt)-OH and PEG5000 or PEG12000 were reacted to form the coating for SWNTs. Cys/PEG complexes were prepared by Steglich esterification. For this reaction, DMAP and DCC were used as catalysts. Then, in order to obtain Cys/PEG-SWNT, SWNTs were coated by Cys/PEG complexes by mixing.

4. RESULTS AND DISCUSSION

4.1 Preparation of SWNTs

Single-walled carbon nanotubes were synthesized by the CVD method. In order to ensure the type of CNT and determine the characteristics; Raman Spectroscopy, FTIR, XRD, and TEM analysis were completed. The highest resolution technique is TEM which allows capturing the fine details of carbon nanotube samples. Single-walled carbon nanotubes with a diameter of 4 nm are visible in TEM images of synthesized CNTs which is given in Figure 4.1.

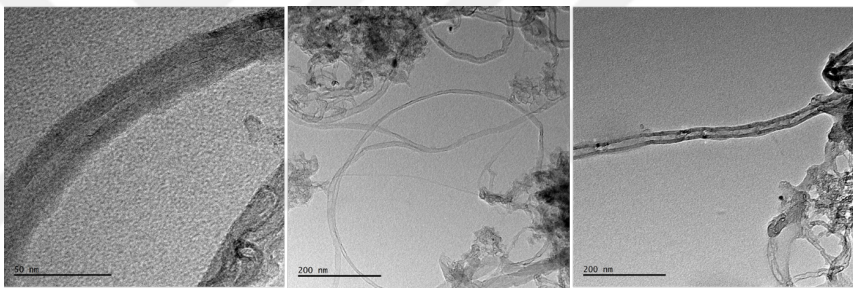

Figure 4.1 TEM images of SWNTs. The transmission electron microscope exposes the single walled constitution of the CNT.

4.2 Preparation of SWNTs

The molecule to be bound to PEG is 9-fluorenylmethyl carbonyl, which is also used as a protecting group in the organic synthesis of peptides [60]. Fmoc contains an aromatic fluorenyl group that can be easily attached to PEG. Structural characterization of Fmoc-Cys(Trt)-OH connected by different molecular weight PEGs was monitored by FT-IR spectrometry. The characteristic peaks observed in all spectra at approximately 2880 cm-1 in Figures 4.2 and 4.3 originate from the -CH_2CH_2 O-group contained by PEG [61]. Looking at the literature, around 1720 cm^{-1} bands seen in the Fmoc-PEG spectra correspond to the carbonyl carbamate of the Fmoc

group[62] . FTIR results support that we are successfully synthesized Cys/PEG structure. In addition, to support the FTIR data, the binding of Fmoc-Cys(Trt)-OH to

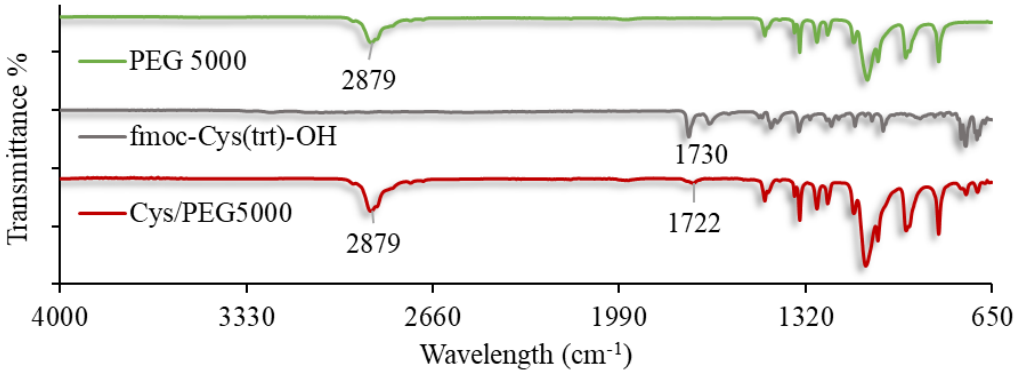

Figure 4.2 FTIR Spectra for PEG500, fmoc-Cys(Trt)-OH, and Cys/PEG5000. At the spectra, we see: characteristic peak of -CH2CH2O- repeat unit at approximately 2880 cm-1 for PEG, around 1720 cm-1 bands carbonyl carbamate peak for Fmoc group. Looking at Cys/PEG complex, both characteristic peaks of PEG and Fmoc are seen.

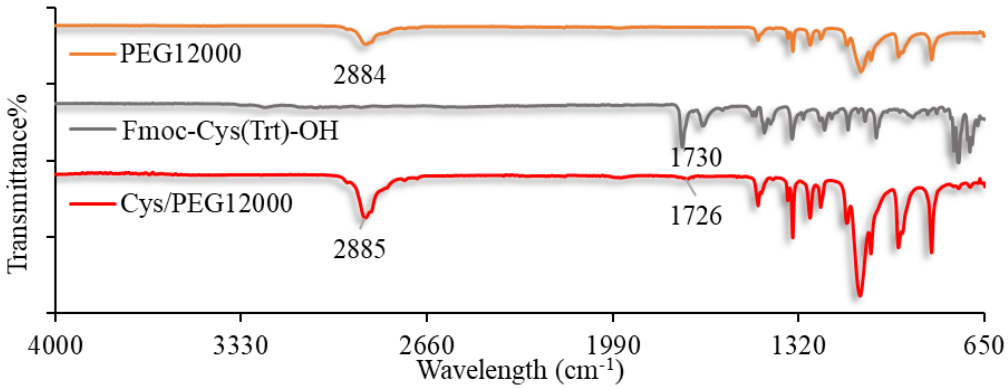

Figure 4.3 FTIR Spectra for PEG1200, fmoc-Cys(Trt)-OH, and Cys/PEG12000. At the spectra, we see: characteristic peak of -CH2CH2O- repeat unit at approximately 2880 cm-1 for PEG, around 1720 cm-1 bands carbonyl carbamate peak for Fmoc group. Looking at Cys/PEG complex, both characteristic peaks of PEG and Fmoc are seen

PEG monomethyl ether is examined by 1H NMR spectra. The 1H NMR spectra of Fmoc-Cys(trt)-PEG5000 and Fmoc-Cys(trt)-PEG12000 are given in Figures 4.4 and 4.5. Fmoc-Cys(trt)-PEG5000 spectrum gave signals representing the methylene protons of the PEG backbone at 3.6 ppm. According to the 1H NMR spectrum of the synthesized Fmoc-Cys(trt)-PEG12000, Fmoc proton signals and characteristic peaks are also described in Figures 4.4 and 4.5 [63, 64].

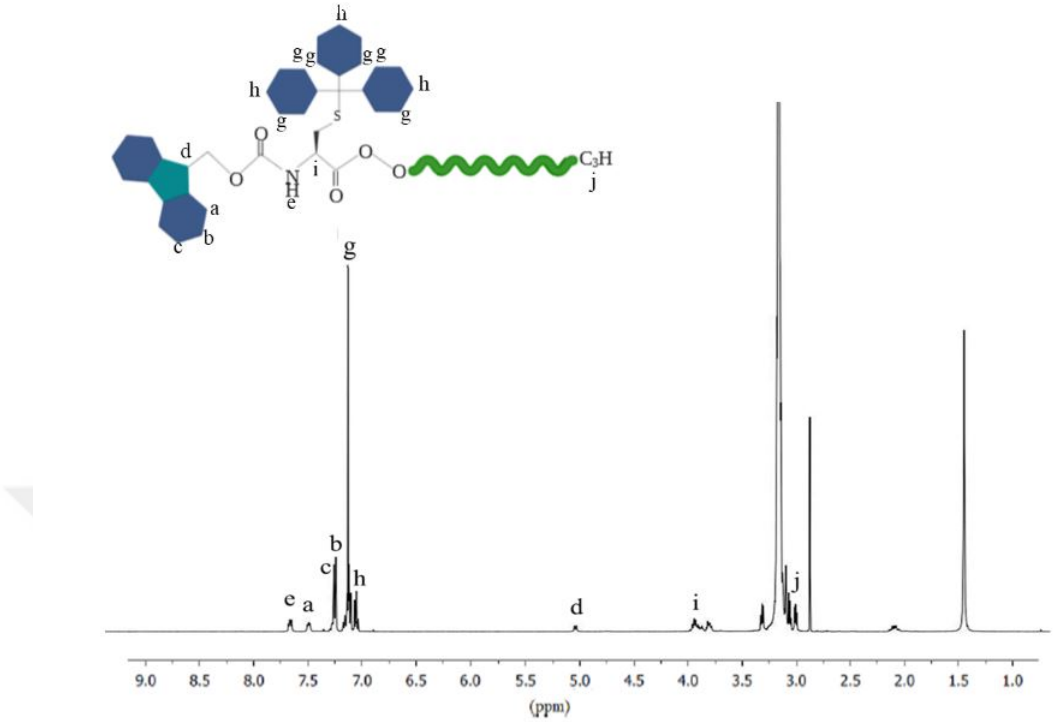

Figure 4.4 1H NMR Spectra for Cys/PEG5000. Peaks for zones are specified by letters.

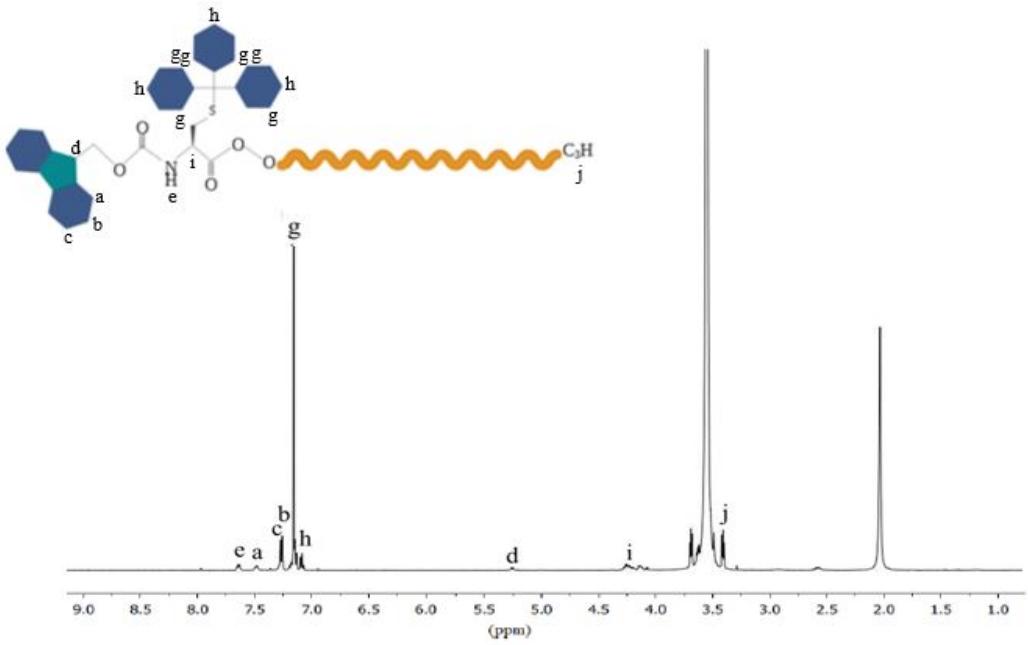

Figure 4.5 1H NMR Spectra for Cys/PEG12000. Peaks for zones are specified by letters.

4.3 Functionalization of SWNTs with Fmoc/PEG complexes

Fmoc-Cys(Trt)-OH was used for direct CNT immobilization. Also, it is known that Fmoc amino acids can interact via the $\pi - \pi$ bond because such molecules can self-associate with nanostructures [65]. In this study, Fmoc-PEG complexes are physically mounted to SWNTs for noncovalent functionalization of CNTs. With the computational studies carried out in the project numbered MGA-2019-41823 carried out within the scope of the ITU BAP Unit, it was found that the Fmoc-Cys(Trt)-OH have stacked on the surface of the CNT thanks to the aromatic fluorenyl groups. In this study, the yield of reaction preparing Fmoc-Cys(trt)-PEG5000 is calculated as 86%, and the yield of reaction preparing Fmoc-Cys(trt)-PEG5000 is calculated as 76%.

Figure 4.6 and 4.7 show the fluorescence spectra of Fmoc-Cys(trt)-PEG5000 and Fmoc-Cys(trt)-PEG12000. These fluorescence spectra are specific for Fmoc. In the Fmoc-PEG-SWNT complexes, the Fmoc groups fluorescence is quenched and provides evidence that the hydrophobic ends of this group in the ring structure are adsorbed onto the SWNT surface.

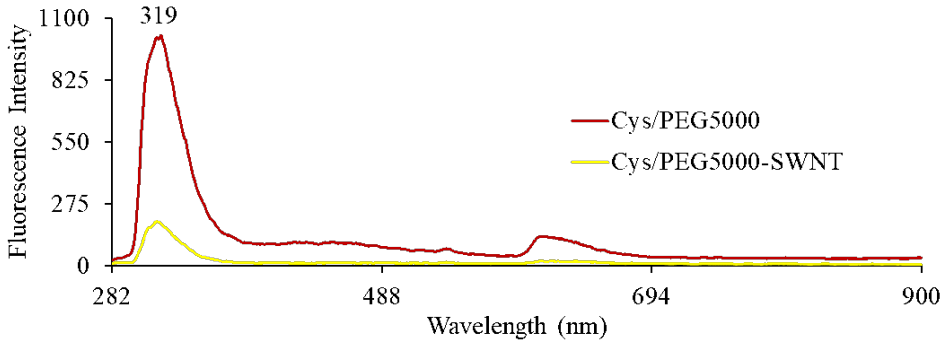

Figure 4.6 Fluorescence Spectra for Cys/PEG5000 and Cys/PEG5000-SWNT. Damping in the graph is seen after the Cys / PEG complex is bound to the SWNT.

As seen in Figure 4.6 and 4.7, the peaks at 315 and 319 nm in the spectrum of the Fmoc-PEG complex are damped after the step of binding to SWNT. The hydrophilic PEG chain is expected to extend into the solution. The $\pi - \pi$ bond between the aromatic rings has also been reported in the literature that may cause the fluorescence

spectrum to dampen in energy transfer[59].

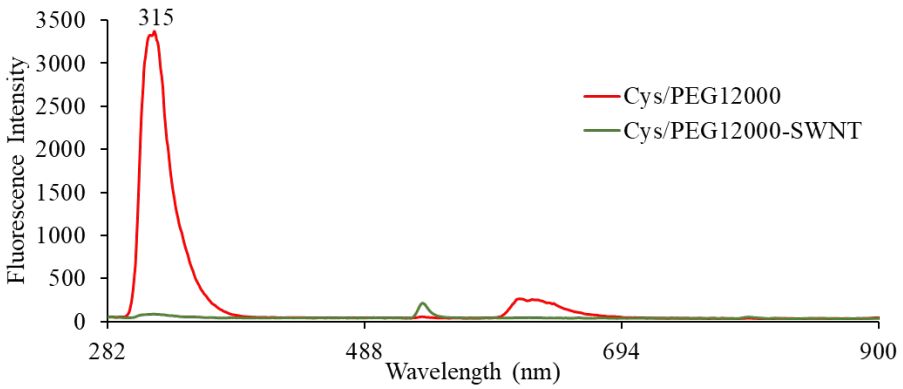

Figure 4.7 Fluorescence Spectra for Cys/PEG12000 and Cys/PEG12000-SWNT. Damping in the graph is seen after the Cys / PEG complex is bound to the SWNT.

The amount of Fmoc-Cys(Trt)-OH attached to the CNT surface was determined by TGA analysis (Figure 4.8). In order to confirm the attachment, and to eliminate the chain length effect of PEG, the amount of Fmoc-aa related to SWNTs was determined and TGA analysis was performed as specified in the characterization of the materials section. Also, we need to prove that the Cys/PEG complex bound to the SWNT surface. As shown in Figure 4.8, there is only a negligible difference in percent weight loss between Cys/PEG5000 and Cys/PEG12000, and it is clearly seen that weight loss is 95%. On the other hand, for Cys/PEG5000-SWNT and Cys/PEG12000-SWNT, a significant decrease in weight loss indicates that the Cys/PEG complexes bind to the surface of SWNTs. Investigating the difference in PEG chain length, Cys/PEG5000-SWNT has more percent weight loss than Cys/PEG12000-SWNT. Since we put the equal amounts of Cys/PEG5000 and Cys/PEG12000 when coating the SWNTs, we can say that SWNTs are more effectively coated with Cys/PEG5000 complex.

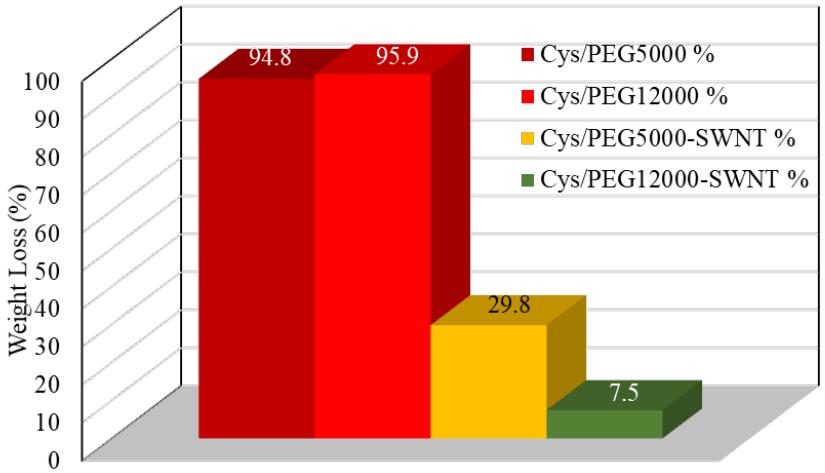

Figure 4.8 Percent weight loss for Cys/PEG complexes and functionalized SWNTs. Here, weight loss represents the organic material.

4.4 Dispersion in water

The dispersion of SWNT in water was examined in 0, 1, 3 and 5-hours. The results of the experiments performed to determine the dispersion behavior of SWNT modified with Cys/PEG complexes are shown in Figure 4.9. CNT dispersions were obtained by non-covalent modifications using Cys/PEG complexes with aromatic functionality mounted on nanotube surfaces with $\pi - \pi$ interactions. The CNT surface is hydrophobic, and when Fmoc amino acids are bound to the surface, polar groups of PEG (-OH and -COOH) lead to surfactant-like properties. As stated in the literature, supplementary interactions arise among the surface-bound and unbound Fmoc amino acids by$\pi - \pi$ stacking and hydrogen bonding; this can support the complete development of a stable nanotube distribution in the aqueous medium [65]. As shown in Figure 4.9, it was determined that Cys/PEG5000-SWNT provides the best dispersing feature.

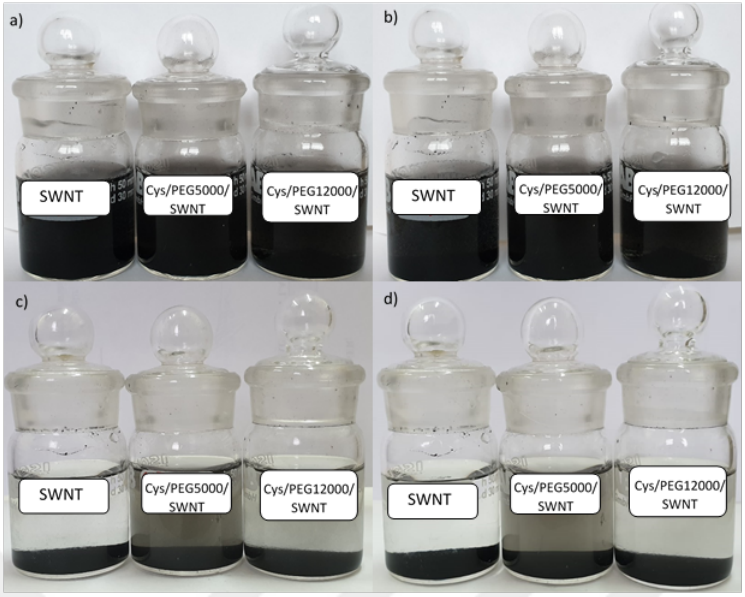

Figure 4.9 Dispersion of pristine CNT, Cys/PEG5000-SWNT, and Cys/PEG12000-SWNT at (a) 0 h, (b) 1h, (c) 3h, (d) 5h. Since the pH of blood and deionized water are almost equal, dispersion behavior of a material in a time period gives insight about the blood stay of that material.

4.5 *In-vitro* cytotoxicity studies

Carbon nanotubes are good drug delivery systems, they only have biocompatibility issues. Because they are not biocompatible, they are quickly expelled from the body before they reach the cancer cell shortly after they are given to the body [66, 67]. By functionalizing the carbon nanotubes with Fmoc/PEG complexes, we aimed to increase the duration of the drug delivery system in the body and thus the possibility of reaching the cancer cell. For this reason, cytotoxicity studies were performed on healthy fibroblast cells.

MTT assay was performed to detect the effect of biocompatible coating on SWNTs on fibroblast cells (PCS-201-012). Fibroblast cells were treated with Cys/PEG5000-SWNT and Cys/PEG12000-SWNT in two concentrations (50, 100 μg/ml). Results of this assay was evaluated regarding that pristine SWNTs are highly cytotoxic, approximately 5% cell viability[49]. MTT assay results are given in Figure 4.10. Results show that Cys/PEG5000-SWNT has higher cell viability. Comparing doses applied to the samples, 50 μg/ml has higher cell viability for both Cys/PEG5000-SWNT and

Cys/PEG12000-SWNT. We can conclude that Csy/PEG5000-SWNT has better cell viability performance. This result supports the better dispersion in water and more effective coating at TGA analysis.

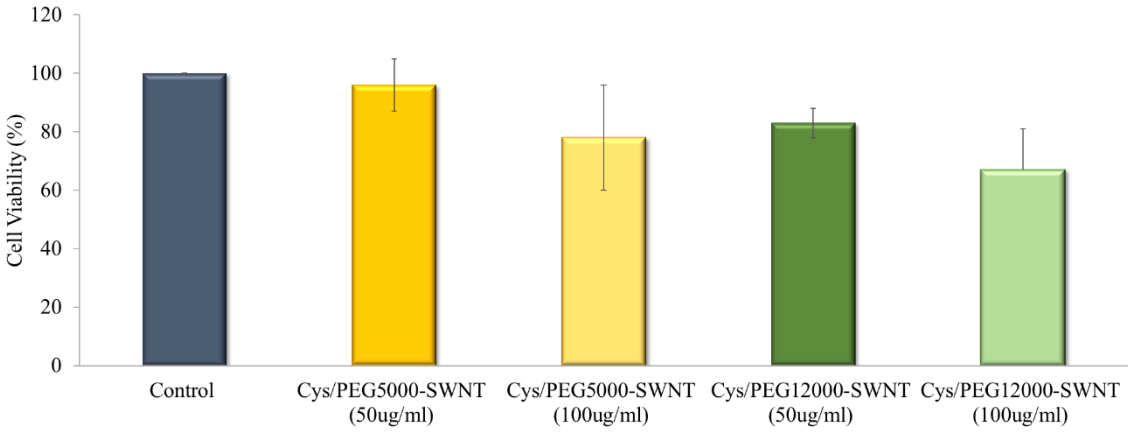

Figure 4.10 Cell viability results for Cys/PEG5000-SWNT and Cys/PEG12000-SWNT. These results are the mean of triplicate experiments. Cells were treated with two concentrations of f-SWNTs. The results were given as mean $\pm SD$.

4.6 Statistical Analysis Results

As indicated by the two-way ANOVA, a statistically significant ($p<0.05$) difference was found between the groups in view of cell viability. Also, the process included the evaluation of the material type and applied dose influence on cell viability. The most noteworthy effect on the cell viability was shaped by the applied dose of material, while material type had less impact.

We can say that the synthesized and characterized f-SWNTs have been successfully prepared as a drug delivery agent based on all the features described above. In the literature, it is seen that loading and releasing studies of various cancer drugs are carried out for similar biocompatible drug delivery systems. Similarly, drug loading and release behaviors for this system can be examined using, for example, doxorubicin, mitoxantrone, and gemcitabine [37].

In the future, besides treating cancer and infectous diseases, neuroprotection could be achieved in chronic neurological disorders using nano-drug delivery. Drug delivery across the blood-brain barrier is accomplished by the application of nanotechnology in therapeutic techniques. The usage of CNTs as a delivery method for central nervous system pathology treatment is based on CNTs structural properties, particularly CNTs improved dispersion in biological solvents by the reason of CNTs functionalization, large surface area, the ability of effortless modification by drug molecules, and biocompatibility with neural environments. As an example, acetylcholine modified SWNTs are used to treat Alzheimer's Disease.[68]

5. CONCLUSION AND RECOMMENDATIONS

In this study, an integrative approach was used to develop a nanocarrier for use as a drug delivery system. To improve the biocompatibility of CNTs and increase water solubility, non-covalent functionalization was performed. For this purpose, PEG (PEG5000, and PEG12000) carrying a Fmoc-aa were attached to the SWNT surface. Cysteine is selected as the amino acid to which the Fmoc groups used. This system was designed for drug transport and is completed in 3 steps. Primarily, to form the basis of the system, SWNT was synthesized using the chemical vapor deposition method. Thereafter, the Cys/PEG complexes were prepared. Lastly, prepared Cys/PEG complexes were mounted on the SWNT wall. Based on the literature, it is revealed that PEG-SWNTs carrying Fmoc-Cys molecules have the necessary structural strength. The stability, binding efficiency, and suspending properties of FmocPEG-coated SWNTs were investigated with the characterization studies.

In order to verify the first step of the synthesis, TEM images were given. In the images, SWNTs synthesized with the CVD method are characterized. Afterward, to follow the synthesis of Fmoc/PEG complexes, 1H NMR spectra were used. The characteristic peaks of Fmoc-Cys(Trt)-OH and PEG in the spectra show that we successfully formed Cys/PEG complexes. Furthermore, to support 1H NMR spectra, FT-IR spectra of Fmoc-Cys(Trt)-OH, PEG, and Cys/PEG were compared. The characteristic peak of the Fmoc group and PEG observed in the spectra of the Cys/PEG complex.

In the last step of the synthesis, Cys/PEG5000 and Cys/PEG12000 complexes were mounted on SWNTs. In this step, attachment of Cys/PEG complexes on SWNTs was ensured with a fluorescence spectrophotometer, and the amount of this attachment was determined by thermogravimetric analysis. The fluorescence spectra of Fmoc/PEG complexes and Fmoc/PEG - SWNT were obtained. The damping in the spectra after binding proves the successful functionalization of SWNTs. Fmoc group was easily mounted on SWNT walls due to the presence of aromatic fluorenyl rings, and it has

been determined by TGA analyzes that cysteine is attached to SWNTs homogeneously. As a result of TGA analysis, we can say that the Cys/PEG500 complex is more effectively attached to SWNTs. Moreover, results of dispersion in water can be used in further optimization of PEG chain length. It can be deduced from its dispersion that Cys/PEG5000-SWNT has improved blood stay. It is beneficial to investigate the blood stay of the materials. Investigating the effects of f-SWNTs on fibroblast cells, MTT assay is realized. Cell viability of cells were obtained from this assay. Results show that Cys/PEG5000-SWNT has better cell viability, especially for 50 μg/ml dose cell viability is almost equal to the control. This outcome is highly coherent with characterization methods such as dispersion in water and TGA.

After all, by connecting PEG chains containing Fmoc-Cys to the CNT wall, the strength of the PEG chain and CNTs were combined; a nano-carrier, which is biocompatible, has the potential to pass through the cell wall, has a stable structure and excellent optical properties, was synthesized. The synthesized product will be a nano-carrier, which can perform targeted drug transport and release, minimizing harm/side effects in line with the need for alternative methods in cancer treatment.

Conducting in-vivo experiments is a good starting point to further optimize f-SWNTs for commercial use. Also, after completely proving biocompatibility of the f-SWNTs, drug loading and release properties must be investigated. Furthermore, other molecules, proteins or biological particles can be used to functionalize SWNTs. Besides, molecular interactions of the system should be examined through computational methods. After some further study, this material has great potential for commercial use in cancer imaging and therapy.

6. LIST OF PUBLICATIONS PRODUCED FROM THE THESIS

1. Functionalization of Carbon Nanotubes for The Use in Cancer Treatment, Ö. Z. Güner, F. S. Güner, H. Saybaşılı, 3^{rd} *Eurasian Biological and Chemical Sciences Conference* , pp. 356-364, 19-20 March, 2020.

REFERENCES

1. Siegel, R. L., K. D. Miller, and A. Jemal, "Cancer statistics, 2018," *CA: A Cancer Journal for Clinicians*, Vol. 68, no. 1, pp. 7–30, 2018.

2. Wang, A. Z., R. Langer, and O. C. Farokhzad, "Nanoparticle Delivery of Cancer Drugs," *Annual Review of Medicine*, Vol. 63, no. 1, pp. 185–198, 2012.

3. Mura, S., J. Nicolas, and P. Couvreur, "Stimuli-responsive nanocarriers for drug delivery," *Nature Materials*, Vol. 12, no. 11, pp. 991–1003, 2013.

4. Brigger, I., C. Dubernet, and P. Couvreur, "Nanoparticles in cancer therapy and diagnosis," *Advanced Drug Delivery Reviews*, Vol. 54, no. 5, pp. 631–651, 2002.

5. Alley, S. C., N. M. Okeley, and P. D. Senter, "Antibody-drug conjugates: Targeted drug delivery for cancer," *Current Opinion in Chemical Biology*, Vol. 14, no. 4, pp. 529–537, 2010.

6. Bertrand, N., J. Wu, X. Xu, N. Kamaly, and O. C. Farokhzad, "Cancer nanotechnology: The impact of passive and active targeting in the era of modern cancer biology," *Advanced Drug Delivery Reviews*, Vol. 66, pp. 2–25, 2014.

7. Zamani, H., L. Bazgir, and A. Roomi, "Nanoparticles as drug delivery," *IIOAB Journal*, Vol. 7, pp. 342–346, 2016.

8. Wang, Jing, Li, Yiye, Nie, Guangjun, Zhao, Y., "Precise Design of Nanomedicines: Perspectives for Cancer Treatment," *National Science Review*, Vol. 6, no. 6, pp. 1107–1110, 2019.

9. Patra, J. K., G. Das, L. F. Fraceto, E. V. R. Campos, M. D. P. Rodriguez-Torres, L. S. Acosta-Torres, L. A. Diaz-Torres, R. Grillo, M. K. Swamy, S. Sharma, S. Habtemariam, and H. S. Shin, "Nano based drug delivery systems: Recent developments and future prospects 10 Technology 1007 Nanotechnology 03 Chemical Sciences 0306 Physical Chemistry (incl. Structural) 03 Chemical Sciences 0303 Macromolecular and Materials Chemistry 11 Medical and He," *Journal of Nanobiotechnology*, Vol. 16, no. 1, pp. 1–33, 2018.

10. Shubhika, K., "Nanotechnology and medicine - The upside and the downside," *International Journal of Drug Development and Research*, Vol. 5, no. 1, pp. 1–10, 2013.

11. Villegas, M., A. G. Cid, C. A. Briones, A. I. Romero, F. A. Pistán, E. E. Gonzo, J. C. Gottifredi, and J. M. Bermúdez, "Films based on the biopolymer poly(3-hydroxybutyrate) as platforms for the controlled release of dexamethasone," *Saudi Pharmaceutical Journal*, Vol. 27, no. 5, pp. 694–701, 2019.

12. Senapati, S., A. K. Mahanta, S. Kumar, and P. Maiti, "Controlled drug delivery vehicles for cancer treatment and their performance," *Signal Transduction and Targeted Therapy*, Vol. 3, no. 1, pp. 1–19, 2018.

13. Calzoni, E., A. Cesaretti, A. Polchi, A. Di Michele, B. Tancini, and C. Emiliani, "Biocompatible polymer nanoparticles for drug delivery applications in cancer and neurodegenerative disorder therapies," *Journal of Functional Biomaterials*, Vol. 10, no. 1, pp. 1–15, 2019.

14. Chauhan, A. S., "Dendrimers for Drug Delivery," *Molecules*, Vol. 23, no. 4, 2018.

15. Jogi, H., R. Maheshwari, N. Raval, K. Kuche, V. Tambe, K. K. Mak, M. R. Pichika, and R. K. Tekade, "Carbon nanotubes in the delivery of anticancer herbal drugs," *Nanomedicine*, Vol. 13, no. 10, pp. 1187–1220, 2018.

16. Manzano, M., and M. Vallet-Regí, "Mesoporous Silica Nanoparticles for Drug Delivery," *Advanced Functional Materials*, Vol. 30, no. 2, pp. 3–5, 2020.

17. Zhou, Q., L. Zhang, T. H. Yang, and H. Wu, "Stimuli-responsive polymeric micelles for drug delivery and cancer therapy," *International Journal of Nanomedicine*, Vol. 13, pp. 2921–2942, 2018.

18. Zayed, D. G., A. S. Abdelhamid, M. S. Freag, and A. O. Elzoghby, "Hybrid quantum dot-based theranostic nanomedicines for tumor-Targeted drug delivery and cancer imaging," *Nanomedicine*, Vol. 14, no. 3, pp. 225–228, 2019.

19. Farooq, M. U., V. Novosad, E. A. Rozhkova, H. Wali, A. Ali, A. A. Fateh, P. B. Neogi, A. Neogi, and Z. Wang, "Gold Nanoparticles-enabled Efficient Dual Delivery of Anticancer Therapeutics to HeLa Cells," *Scientific Reports*, Vol. 8, no. 1, pp. 1–12, 2018.

20. Price, P. M., W. E. Mahmoud, A. A. Al-Ghamdi, and L. M. Bronstein, "Magnetic drug delivery: Where the field is going," *Frontiers in Chemistry*, Vol. 6, no. DEC, pp. 1–7, 2018.

21. Hossen, S., M. K. Hossain, M. K. Basher, M. N. Mia, M. T. Rahman, and M. J. Uddin, "Smart nanocarrier-based drug delivery systems for cancer therapy and toxicity studies: A review," *Journal of Advanced Research*, Vol. 15, pp. 1–18, 2019.

22. Bethune, D. S., C. H. Klang, M. S. De Vries, G. Gorman, R. Savoy, J. Vazquez, and R. Beyers, "Cobalt-catalysed growth of carbon nanotubes with single-atomic-layer walls," *Nature*, Vol. 363, no. 6430, pp. 605–607, 1993.

23. Iijima, S., "© 19 9 1 Nature Publishing Group," *Nature*, Vol. 354, pp. 56–58, 1991.

24. Kaur, J., G. S. Gill, and K. Jeet, *Applications of Carbon Nanotubes in Drug Delivery*, Elsevier Inc., 2019.

25. Singh, R., D. Rathore, Geetanjali, and R. Srivastava, *Carbon-based polymers for drug delivery*, Elsevier Inc., 2018.

26. Al-Qattan, M. N., P. K. Deb, and R. K. Tekade, "Molecular dynamics simulation strategies for designing carbon-nanotube-based targeted drug delivery," *Drug Discovery Today*, Vol. 23, no. 2, pp. 235–250, 2018.

27. Li, Z., A. L. B. de Barros, D. C. F. Soares, S. N. Moss, and L. Alisaraie, "Functionalized single-walled carbon nanotubes: cellular uptake, biodistribution and applications in drug delivery," *International Journal of Pharmaceutics*, Vol. 524, no. 1-2, pp. 41–54, 2017.

28. John, A. A., A. P. Subramanian, M. V. Vellayappan, A. Balaji, H. Mohandas, and S. K. Jaganathan, "Carbon nanotubes and graphene as emerging candidates in neuroregeneration and neurodrug delivery," *International Journal of Nanomedicine*, Vol. 10, pp. 4267–4277, 2015.

29. Redondo-Gómez, C., R. Leandro-Mora, D. Blanch-Bermúdez, C. Espinoza-Araya, D. Hidalgo-Barrantes, and J. Vega-Baudrit, "Recent Advances in Carbon Nanotubes for Nervous Tissue Regeneration," *Advances in Polymer Technology*, Vol. 2020, pp. 1–16, 2020.

30. Mehra, N. K., K. Jain, and N. K. Jain, "Pharmaceutical and biomedical applications of surface engineered carbon nanotubes," *Drug Discovery Today*, Vol. 20, no. 6, pp. 750–759, 2015.

31. Zhu, W., H. Huang, Y. Dong, C. Han, X. Sui, and B. Jian, "$Multi-walled$ carbon $nanotube-based$ systems for improving the controlled release of insoluble drug dipyridamole," *Experimental and Therapeutic Medicine*, pp. 4610–4616, 2019.

32. Herlem, G., F. Picaud, C. Girardet, and O. Micheau, *Carbon Nanotubes: Synthesis, Characterization, and Applications in Drug-Delivery Systems*, Elsevier Inc., 2019.

33. Samadishadlou, M., M. Farshbaf, N. Annabi, T. Kavetskyy, R. Khalilov, S. Saghfi, A. Akbarzadeh, and S. Mousavi, "Magnetic carbon nanotubes: preparation, physical properties, and applications in biomedicine," *Artificial Cells, Nanomedicine and Biotechnology*, Vol. 46, no. 7, pp. 1314–1330, 2018.

34. Eatemadi, A., H. Daraee, H. Karimkhanloo, M. Kouhi, N. Zarghami, A. Akbarzadeh, M. Abasi, Y. Hanifehpour, and S. W. Joo, "Carbon nanotubes: Properties, synthesis, purification, and medical applications," *Nanoscale Research Letters*, Vol. 9, no. 1, pp. 1–13, 2014.

35. Mahajan, S., A. Patharkar, K. Kuche, R. Maheshwari, P. K. Deb, K. Kalia, and R. K. Tekade, "Functionalized carbon nanotubes as emerging delivery system for the treatment of cancer," *International Journal of Pharmaceutics*, Vol. 548, no. 1, pp. 540–558, 2018.

36. Hou, P. X., C. Liu, and H. M. Cheng, "Purification of carbon nanotubes," *Carbon*, Vol. 46, no. 15, pp. 2003–2025, 2008.

37. Beg, S., M. Rahman, A. Jain, S. Saini, M. S. Hasnain, S. Swain, S. Imam, I. Kazmi, and S. Akhter, *Emergence in the functionalized carbon nanotubes as smart nanocarriers for drug delivery applications*, Elsevier Inc., 2018.

38. Fujigaya, T., and N. Nakashima, "Non-covalent polymer wrapping of carbon nanotubes and the role of wrapped polymers as functional dispersants," *Science and Technology of Advanced Materials*, Vol. 16, no. 2, 2015.

39. Moses, J. C., A. Gangrade, and B. B. Mandal, *Carbon Nanotubes and Their Polymer Nanocomposites*, Elsevier Inc., 2018.

40. Spitalsky, Z., D. Tasis, K. Papagelis, and C. Galiotis, "Carbon nanotube-polymer composites: Chemistry, processing, mechanical and electrical properties," *Progress in Polymer Science (Oxford)*, Vol. 35, no. 3, pp. 357–401, 2010.

41. Liu, Z., S. Tabakman, K. Welsher, and H. Dai, "Carbon nanotubes in biology and medicine: In vitro and in vivo detection, imaging and drug delivery," *Nano Research*, Vol. 2, no. 2, pp. 85–120, 2009.

42. Kushwaha, S. K. S., S. Ghoshal, A. K. Rai, and S. Singh, "Carbon nanotubes as a novel drug delivery system for anticancer therapy: A review," *Brazilian Journal of Pharmaceutical Sciences*, Vol. 49, no. 4, pp. 629–643, 2013.

43. Bilalis, P., D. Katsigiannopoulos, A. Avgeropoulos, and G. Sakellariou, "Non-covalent functionalization of carbon nanotubes with polymers," *RSC Advances*, Vol. 4, no. 6, pp. 2911–2934, 2014.

44. Mohammadi, H., M. Hafezi, S. Hesaraki, and M. M. Sepantafar, "Preparation and characterization of Sr-Ti-hardystonite (Sr-Ti-HT) nanocomposite for bone repair application," *Nanomed. J*, Vol. 2, no. 23, pp. 203–210, 2015.

45. Digge, M. S., R. S. Moon, and S. G. Gattani, "Application of carbon nanotubes in drug delivery: A review," *International Journal of PharmTech Research*, Vol. 4, no. 2, pp. 839–847, 2012.

46. Romano-Feinholz, S., A. Salazar-Ramiro, E. Muñoz-Sandoval, R. Magaña-Maldonado, N. H. Pedro, E. R. López, A. G. Aguilar, A. S. García, J. Sotelo, V. P. De La Cruz, and B. Pineda, "Cytotoxicity induced by carbon nanotubes in experimental malignant glioma," *International Journal of Nanomedicine*, Vol. 12, pp. 6005–6026, 2017.

47. Ravelli, D., D. Merli, E. Quartarone, A. Profumo, P. Mustarelli, and M. Fagnoni, "PEGylated carbon nanotubes: Preparation, properties and applications," *RSC Advances*, Vol. 3, no. 33, pp. 13569–13582, 2013.

48. Pastorin, G., "Crucial functionalizations of carbon nanotubes for improved drug delivery: A valuable option?," *Pharmaceutical Research*, Vol. 26, no. 4, pp. 746–769, 2009.

49. Meran, M., P. D. Akkus, O. Kurkcuoglu, E. Baysak, G. Hizal, E. Haciosmanoglu, A. Unlu, N. Karatepe, and F. S. Güner, "Noncovalent Pyrene-Polyethylene Glycol Coatings of Carbon Nanotubes Achieve in Vitro Biocompatibility," *Langmuir*, Vol. 34, no. 40, pp. 12071–12082, 2018.

50. Antonucci, A., J. Kupis-Rozmysłowicz, and A. A. Boghossian, "Noncovalent Protein and Peptide Functionalization of Single-Walled Carbon Nanotubes for Biodelivery and Optical Sensing Applications," *ACS Applied Materials and Interfaces*, Vol. 9, no. 13, pp. 11321–11331, 2017.

51. Sireesha, M., V. Jagadeesh Babu, A. S. Kranthi Kiran, and S. Ramakrishna, "A review on carbon nanotubes in biosensor devices and their applications in medicine," *Nanocomposites*, Vol. 4, no. 2, pp. 36–57, 2018.

52. Ray, S. S., *Springer Series in Materials Science volume 278, Processing of Nanocomposites*, 2018.

53. Bhattacharya, K., C. Sacchetti, P. M. Costa, J. Sommertune, B. D. Brandner, A. Magrini, N. Rosato, N. Bottini, M. Bottini, and B. Fadeel, "Nitric Oxide Dependent Degradation of Polyethylene Glycol-Modified Single-Walled Carbon Nanotubes: Implications for Intra-Articular Delivery," *Advanced Healthcare Materials*, Vol. 7, no. 6, pp. 1–11, 2018.

54. Umemura, K., "Hybrids of nucleic acids and carbon nanotubes for nanobiotechnology," *Nanomaterials*, Vol. 5, no. 1, pp. 321–350, 2014.

55. Hu, Z., J. Zhao, Z. Song, and C. Yang, "Filled and peptide-modified single-walled carbon nanotubes: Synthesis, characterization, and in vitro test for cancer cell targeting," *RSC Advances*, Vol. 5, no. 22, pp. 16792–16800, 2015.

56. Cordeiro, M. F., F. A. Girardi, C. O. Gonçalves, C. S. Peixoto, L. Dal Bosco, S. K. Sahoo, A. P. Santos, C. Fantini, G. E. Bruch, A. P. Horn, and D. M. Barros, "Toxicological assessment of PEGylated single-walled carbon nanotubes in early developing zebrafish," *Toxicology and Applied Pharmacology*, Vol. 347, pp. 54–59, 2018.

57. Dündar-Tekkaya, E., and N. Karatepe, "Effect of reaction time, weight ratio, and type of catalyst on the yield of single-wall carbon nanotubes synthesized by chemical vapor deposition of acetylene," *Fullerenes Nanotubes and Carbon Nanostructures*, Vol. 23, no. 6, pp. 535–541, 2015.

58. Karatepe, N., and N. Yuca, "Hydrogen adsorption on carbon nanotubes purified by different methods," *International Journal of Hydrogen Energy*, Vol. 36, no. 17, pp. 11467–11473, 2011.

59. Zhang, P., J. Lu, Y. Huang, W. Zhao, Y. Zhang, X. Zhang, J. Li, R. Venkataramanan, X. Gao, and S. Li, "Design and evaluation of a pegylated lipopeptide equipped with drug-interactive motifs as an improved drug carrier," *AAPS Journal*, Vol. 16, no. 1, pp. 114–124, 2014.

60. Turecek, P. L., M. J. Bossard, F. Schoetens, and I. A. Ivens, "PEGylation of Biopharmaceuticals: A Review of Chemistry and Nonclinical Safety Information of Approved Drugs," *Journal of Pharmaceutical Sciences*, Vol. 105, no. 2, pp. 460–475, 2016.

61. Zeng, S., F. Wu, B. Li, X. Song, Y. Zheng, G. He, C. Peng, and W. Huang, "Synthesis, characterization, and evaluation of a novel amphiphilic polymer RGD-PEG-chol for target drug delivery system," *The Scientific World Journal*, Vol. 2014, 2014.

62. Singh, V., K. Snigdha, C. Singh, N. Sinha, and A. K. Thakur, "Understanding the self-assembly of Fmoc-phenylalanine to hydrogel formation," *Soft Matter*, Vol. 11, no. 26, pp. 5353–5364, 2015.

63. Samaritoni, J. G., J. G. Martynow, M. J. O'Donnell, and W. L. Scott, "Preparation and Use of a General Solid-Phase Intermediate to Biomimetic Scaffolds and Peptide Condensations," *Molecules (Basel, Switzerland)*, Vol. 23, no. 7, 2018.

64. Dust, J. M., Z. H. Fang, and J. M. Harris, "Proton NMR Characterization of Poly(ethylene Glycols) and Derivatives," *Macromolecules*, Vol. 23, no. 16, pp. 3742–3746, 1990.

65. Cousins, B. G., A. K. Das, R. Sharma, Y. Li, J. P. McNamara, I. H. Hillier, I. A. Kinloch, and R. V. Ulijn, "Enzyme-activated surfactants for dispersion of carbon nanotubes," *Small*, Vol. 5, no. 5, pp. 587–590, 2009.

66. Biale, C., V. Mussi, U. Valbusa, S. Visentin, G. Viscardi, N. Barbero, N. Pedemonte, and L. Galietta, *Carbon nanotubes for targeted drug delivery*, Elsevier Inc., 2009.

67. Dr. Nicolas Rodondi, MD, MAS, Ms. Wendy P. J. den Elzen, MSc, Dr. Douglas C. Bauer, MD, Dr. Anne R. Cappola, MD, ScM, Dr. Salman Razvi, MD, FRCP, Dr. John P. Walsh, MBBS, FRACP, PhD, Dr. Bjørn O. Åsvold, MD, PhD, Dr. Giorgio Iervasi, MD, Dr. Misa Imaizumi, P., "PEG Branched Polymer for Functionalization of Nanomaterials with Ultralong Blood Circulation," *Bone*, Vol. 23, no. 1, pp. 1–7, 2010.

68. Yang, Z., Y. Zhang, Y. Yang, L. Sun, D. Han, H. Li, and C. Wang, "Pharmacological and toxicological target organelles and safe use of single-walled carbon nanotubes as drug carriers in treating Alzheimer disease," *Nanomedicine: Nanotechnology, Biology, and Medicine*, Vol. 6, no. 3, pp. 427–441, 2010.