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Covalent diphenylalanine peptide nanotube conjugated to folic acid/magnetic nanoparticles for anti-cancer drug delivery

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ABSTRACT

Micro and nanotubes obtained from the self-assembly of diphenylalanine peptides (FNTs) were conjugated to folic acid/magnetic nanoparticles (FA/MNPs) and evaluated as a potential system for anti-cancer drug delivery. The conjugates were obtained by providing a covalent linkage through the amine groups on FNTs with the carboxylic groups on FA/MNPs. The anti-cancer therapeutic 5-fluorouracil (5-FU), and anti-inflammatory cargo flufenamic acid (FFA), were loaded in peptide arrays during their self-assembly in the liquid phase. AFM and CLSM analysis indicated the presence of FA aggregates on FNTs. The data revealed that the cargo 5-FU, was distributed in dendrite peptide nanotubes whereas the non-polar cargo FFA, was homogeneously embedded in the structure of large discrete micro tubes. FTIR spectra of FA-MNPs/FNTs showed peak of amide II at 1623 cm^{-1} indicating covalent interactions between amines and carboxylic groups and confirmed the successful conjugation of the nanoparticles and peptide assemblies. The results indicated that 5-FU has been released from FNTs within 4 h, and incorporation of 5-FU in FNTs hydrogels has significantly slowed the release rate within the first 2 h. Our approach offers a new pathway for cancer treatment in which several functionalities are embedded in a single carrier.

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1. Introduction

Di-phenylalanine (FF) is a dipeptide extracted from Alzheimer's polypeptide [1–6] as a core recognition motif for molecular self-assembly [3]. Formation of nanotubes from diphenylalanine (FNTs) were first reported by Reches and Gazit and since then FNTs has been used as a building block for formation of various functional nanostructures such as nanotubes, spherical vesicles and nanofibrils [7]. Typically, the self-assembly takes place by the aim of complex interaction of backbone–backbone hydrogen bonds and π···π interactions between the aromatic rings of the side-chains [8–10]. Nanotubes prepared from peptides are great alternatives over carbon nanotubes (CNT) for drug delivery purposes due to the associated risk of using CNT in human health [11,12].

The FF self-assembled structure could be designed with motifs and ligands to become smart and stimuli-responsive, therefore achieving direct and triggered drug delivery to the site of disease [13–15]. Modified nanotubes are applicable in theranostic medicine which targeted delivery together with imaging organs and tissues offers the possibility of both diagnosis and treatments effectively [16]. The resulting nanosystems, are expected to play a significant role in future of translational medicine.

Among various targeting systems, folic acid (FA) provides a useful method for delivering therapeutic or imaging agents to tumors [17, 11, 18]. It is proven that most tumors overexpress the folate receptors (FR) at advanced stage, and therefore contain increased density of FR. The overexpression of FR occurs in many cancer types such as breast cancer, lung and brain. In addition to numerous drug delivery efforts, folate-targeted technology has been successfully applied to MRI contrast agents [18], fluorescence imaging of cancer cells [19, 17], and radio-imaging of therapeutic agents [20].

Furthermore, magnetic nanoparticles (MNPs) represent a major class of nanostructures with the potential to benefit current clinical diagnostic and therapeutic techniques. Due their unique properties,

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MNPs are being actively investigated as the next generation of magnetic resonance imaging (MRI) contrast agents [21, 1] as carriers for targeted drug delivery and cancer treatments with hyperthermia [22]. Moreover, a less investigated aspect of MNPs is their potential for controlled release of cargos once exposed to an alternate magnetic field [23].

Several research groups have investigated conjugation of folic acid to nanoparticle and nanotubes. Covalent conjugations are mostly used for FA functionalization due to several advantages of covalent bindings toward non-covalent strategies such as stability at different physiological conditions [11, 24]. Castillo et al. studied the conjugation of folic acid to carbon nanotubes and demonstrated the uptake of CNT by THP-1 [24]. In another study, Zhang et al. used Gold nanoparticles (GNPs) modified with glutathione (GSH) in order to conjugate with folic acid through amino group of FA and carboxyl group of GSH [25]. Magnetic nanoparticles have been conjugated to folic acid as an effective method for the separation and detection of ovarian cancer cells. It is shown that the ability of FA to bind its receptor to allow endocytosis is not affected by covalent conjugation of small molecules [26]. Therefore, it is crucial to study the molecular structure of FF self-assembled nanostructures after binding with FA/MNPs. In this work, we combine the biocompatibility and biofunctionality of peptide nanotubes with targeting ligands of FA and MNPs and further evaluate its potential as a drug delivery vehicle.

Herein the carbodiimide/N-hydroxysuccinimide (EDC/NHS) chemistry has been utilized to covalently bond carboxyl groups of FA/MNPs to amine groups of FNTs. The conjugates were characterized using microscope and spectroscopy techniques. The results showed evidence of FA aggregates on peptide nanotube and peak of amide at 1630 cm −1 indicating a covalent conjugation. The morphology of self-assembled structures of diphenylalanine was effected by the polar and non-polar properties of the cargos which could greatly influence targeting FR receptors. We therefore argue that the synthesized peptide nanotube are suitable drug vehicles for loading 5-FU and have the potential to be used as co-delivery carriers.

2. Materials and methods

2.1. Materials

The lyophilized form of the L-diphenylalanine peptide (Code: P4126), Silicon wafers (Code: 204323), Gold coated silicon wafer (643262), Fluorouracil (5-FU), N-Hydroxysuccinimide Esters (NHS) (Code: 130672), were all obtained from Sigma–Aldrich. N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC) (code: 851007), 1,1,1,3,3,3- hexafluoro-2-propanol (HFIP) (Code: 804515) as solvent (99% purity), Iron (III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), iron (II) sulfate heptahydrate ($FeSO_4\_7H_2O$), aqueous ammonia (28%) were purchased from Merck. Deionized water (18.2 MΩ cm-1), was filtered by the Millipore Water system, and used throughout the experiment.

2.2. Synthesis of FNTs

A stock solution of FF was prepared in HFIP with concentration of 100 mg/ml. To synthesis FF self-assemble nanostructures, the stock solution was diluted to 2 mg/ml by adding deionized water and FNTs were immediately formed in solution. A drop was added to gold and silicone surfaces and left to dry. The FNTs synthesized in solution were analyzed by Zetasizer and FNTs on surfaces were imaged with AFM.

2.3. Synthesis of iron oxide MNPs modified with FA

Fe_3O_4 nanoparticles were prepared by chemical co-precipitation route. In a typical procedure, 5 g ferrous sulfate and 10 g ferric chloride and was dissolved into 25 ml of 2 M Hydrochloric acid in three-necked flask at 60 °C. Nitrogen was introduced during the synthesis to extrude the air and prevent oxidization of ferrous ions. 2 g folic acid in 2 ml water was added to reaction system to produce FA/MNPs. FA was added slowly for 30 min at 45 °C under nitrogen atmosphere and mild stirring condition [27]. Then 20 ml ammonium hydroxide (28%) was added drop wise into the iron solution under sonication and agitation for 40 min to ensure homogenous mixing. The pH was set to 9–11. After 1 h of stirring, the precipitant of Fe_3O_4 nanoparticles were collected by a permanent magnet, washed 2 times and dried in oven for 12 h.

2.4. Conjugation of FA/MNPs to FNTs with EDC

The conjugation was carried out through coupling carboxylic groups of FA to amines of FNTs with EDC. EDC is a common carbodiimide for activating carboxylic groups and binding biomolecules with carboxylic and amine groups [11]. To this aim, 20 mg FA/MNPs were put in contact with 2 mg/ml EDC in buffer pH = 5 for 1 h. The coupling reaction was carried out in the presence of sulfo-NHS to avoid formation of competing reaction by hydrolysis of the intermediate EDC-FA. 1 ml FNTs were put in contact with 0.2 ml of activated FA/MNPs for 4 h and subsequently centrifuged at 3000 rpm for 20 min and washed by distillated water.

2.5. Fluorouracil (5-FU) loadings of FNTs

The potential of FNTs as anti-cancer drug-delivery carriers were evaluated with fluorouracil. Loading 5-FU were carried out during the process of FNT self-assembly. 5-FU at concentration of 25 μg/ml was added to the mixture of FNT, leading to the spontaneous accommodation of the cargo within the tubes. The solvent was left to dry overnight at room temperature, and the assemblies were then cleaned with ultrapure water several times to eliminate residual 5-FU. The anti-inflammatory non-polar cargo flufenamic acid (FFA), were loaded within FNTs with the same approach at concentrations of 25 μg/ml.

2.6. Fluorouracil (5-FU) release from FNT hydrogels

In vitro release assays were performed using FNT hydrogels prepared in a mixture of 75% toluene and 25% ethanol. A stock solution of FNTs (100 mg/ml) were mixed with solvent (75% toluene and 25% ethanol) in a ratio of 2:100 and were left to form hydrogels for 4 h fluorouracil in concentration of 25 μg/ml were further added to hydrogel mixture and were kept for 3 h to allow efficient loading. Samples of FNT hydrogels loaded with 5-FU, were centrifuged at 6000 rpm for 20 min and loading efficiency was analyzed by UV spectrophotometer. FNT hydrogels were carefully put in contact with PBS and left for 3 h. At each intervals samples of PBS were taken and % release of 5-FU was measured with UV-VIS spectrophotometer.

2.7. Cell viability studies

MCF-7 were cultivated on 75 cm^2 flasks in DMEM supplemented with 10% FBS, 100 IU/ml penicillin, and 100 μg/mL streptomycin. Flasks were incubated at 37° C and in an atmosphere of 5% CO2. After 24 h the medium was first aspirated out, and then cells were rinsed with 3 mL of PBS and trypsinized with trypsin-EDTA to