Colloids and Surfaces B: Biointerfaces 155 (2017) 182–192

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Full Length Article

Dendrimer-conjugated iron oxide nanoparticles as stimuli-responsive drug carriers for thermally-activated chemotherapy of cancer

Saumya Nigam[a,b,c], Dhirendra Bahadur[c,*]

[a] IITB-Monash Research Academy, Indian Institute of Technology Bombay, Mumbai, India
[b] Department of Materials Engineering, Monash University, Clayton, Melbourne, Australia
[c] Department of Metallurgical Engineering and Materials Science, Indian Institute of Technology Bombay, Mumbai, India

ARTICLE INFO

Article history:
Received 20 January 2017
Received in revised form 5 April 2017
Accepted 9 April 2017
Available online 13 April 2017

Keywords:
Cancer
Combination therapy
Dendrimers
Doxorubicin
Drug delivery
Iron oxide nanoparticles
Magnetic hyperthermia
PAMAM

ABSTRACT

In recent years, functional nanomaterials have found an appreciable place in the understanding and treatment of cancer. This work demonstrates the fabrication and characterization of a new class of cationic, biocompatible, peptide dendrimers, which were then used for stabilizing and functionalizing magnetite nanoparticles for combinatorial therapy of cancer. The synthesized peptide dendrimers have an edge over the widely used PAMAM dendrimers due to better biocompatibility and negligible cytotoxicity of their degradation products. The surface engineering efficacy of the peptide dendrimers and their potential use as drug carriers were compared with their PAMAM counterparts. The peptide dendrimer was found to be as efficient as PAMAM dendrimers in its drug-carrying capacity, while its drug release profiles substantially exceeded those of PAMAM's. A dose-dependent study was carried out to assess their half maximal inhibitory concentration (IC_{50}) *in vitro* with various cancer cell lines. A cervical cancer cell line that was incubated with these dendritic nanoparticles was exposed to alternating current magnetic field (ACMF) to investigate the effect of elevated temperatures on the live cell population. The DOX-loaded formulations, in combination with the ACMF, were also assessed for their synergistic effects on the cancer cells for combinatorial therapy. The results established the peptide dendrimer as an efficient alternative to PAMAM, which can be used successfully in biomedical applications.

1. Introduction

In the past few years, superparamagnetic iron oxide nanoparticles (SPIONs) have been established as excellent candidates in biomedical applications, when compared with a vast variety of magnetic nanoparticles. These SPIONs have paved their way into various applications such as drug delivery [1,2], magnetic resonance imaging [1,3,4], gene delivery [1,5,6], biosensing [7,8], and many others [9,10]. The decrease in the size of the particles from bulk to nanoscale results in a relatively large surface area-to-volume ratio. This in turn leads to high surface energy of the nanoparticles and a tendency to aggregate due to *van der* Waals forces and magnetic dipole–dipole interactions. This necessitates the development of newer and better synthetic strategies to stabilize these SPIONs [11]. For different biomedical applications, a wide variety of molecules, polymers, inorganic systems and macromolecules have been studied for surface engineering of the SPIONs, which in turn also facilitates the attachment of bioactive molecules [12,13]. There are two primary strategies to engineer the surface of nanoparticles: embedding the SPIONs in polymer matrices [10,14,15] or encapsulating the SPIONs within macromolecules [16,17]. The first approach of embedding the nanoparticles in polymeric matrices has been successful in reducing the aggregation of nanoparticles in aqueous suspensions, but falls short of generating the required density of surface functional groups for further conjugation with a therapeutic payload or other molecules. Therefore, encapsulating these SPIONs by dendrimeric macromolecules presents itself as a promising alternative since it not only limits aggregation but also provides uncompromised and multiple surface functionalities.

Among a long list of promising molecules, dendritic macromolecules have proven themselves better than other polymeric macromolecules by providing tailorable multiple functional groups on their surface, and an internal cavity that could also be used to accommodate guest molecules [18]. Due to the presence of these dual sites for cargo attachment, the search for newer aqueous colloidal and biocompatible dendrimer-based systems is of

* Corresponding author at: Department of Metallurgical Engineering and Materials Science, Indian Institute of Technology Bombay, Powai, Mumbai, 400076, India.
E-mail address: dhirenb@iitb.ac.in (D. Bahadur).

http://dx.doi.org/10.1016/j.colsurfb.2017.04.025
0927-7765/

considerable interest. Dendrimers are three-dimensional macromolecules that have radial symmetry and originates from a central core molecule, which results in a spherical morphology [19]. The repeating branching units produce a hyper-branched structure and govern the chemical properties of the internal cavities as well as of the free functional groups on their exteriors. The branching of the monomers usually follows a geometric progression, thereby, generating a large number of functional groups on the surface as opposed to classical polymers. These functional groups, in turn, are responsible for their enhanced reactivity. The freedom of choice of core and branching molecules opens up a wide variety of possible chemical compositions, internal chemical environments, tailorable surface groups, structural properties and architecture of these dendrimers. Multi-functionalized dendrimers have also been successfully synthesized, which offers adjustments in physical and chemical properties as required, mainly by modifying the terminal functional groups [20,21].

A wide variety of dendrimers such as polyamidoamine (PAMAM), polyethylene imine (PEI), polypropylene imine (PPI) has found its way into biomedical applications such as drug delivery [17,22,23], gene delivery [24,25], bioseparation [26], biosensing [8] and magnetic resonance imaging [27,28]. The world of biomedical applications of dendrimers is currently dominated by the PAMAM dendrimers. PAMAM surpasses other dendritic systems due to the ease of its preparation, desirable chemical and physical properties, surface functional groups, and comparatively lower toxicity to other dendrimers. In spite of showing lower cytotoxicity in comparison with other dendrimers and dendritic systems, the degradation products of PAMAM show significant toxicity, which limits their use in biological systems [29,30]. However, when various factors (such as biocompatibility, haemocompatibility, immunogenicity, and biodistribution) are taken into consideration, cytotoxicity remains the most critical factor for successful use of any dendritic system in any living system. The cytotoxicity of these dendrimers is not only attributed to the charges of the surface functional groups but also to the number of generations, and has been well reported [29–32].

Thus, the primary aim of the current work was to fabricate dendrimers with enhanced biocompatibility without compromising their chemical composition, internal and external environment, physical and chemical properties and efficacy in biomedical applications. The structure of the dendrimer was tailored by the use of ethylene diamine as the core molecule along with cationic amino acids as its branching monomers. Amino acids were chosen as the branching units because, being nutrients, they could be utilized by the cells for their metabolism after degradation of the dendrimer, thereby reducing the toxicity. The amide bond established between these amino acids is similar to the peptide bonds seen in protein molecules; thus, these dendrimers can also be treated as pseudo-peptides and categorized as peptide dendrimers. Upon stabilizing and functionalizing the SPIONs, the peptide dendrimer-coated magnetic nanoparticles were examined for their efficacy in chemotherapy and combinatorial thermo-chemotherapy against the widely used PAMAM dendrimer-coated magnetic nanoparticles.

2. Experimental

2.1. Synthesis of peptide dendrimer

The synthesis of the peptide dendrimer was undertaken as described elsewhere, with minor modifications [33]. For a typical synthesis, 1 mmol of EDA and 4 mmol of ester-activated di-Boc-L-lysine were dissolved in DMF. The reaction was maintained at 0–5 °C for 24 h under constant stirring and a nitrogen atmosphere. The synthesis scheme of peptide dendrimer is elaborately depicted in Scheme 1. Compound **1** precipitates at the end of the coupling reaction and is washed with 15% NaCl, 5% aqueous CA, 5% $NaHCO_3$ solution, and milli-q water successively, and subsequently dried under high vacuum. It is then purified by column chromatography using 3% ethyl acetate in PET ether as the eluent, and subjected to deprotection to remove the boc- groups in order to yield compound **2** (generation 1: 72.1% yield). For the synthesis of the second generation dendrimer, 8 mmol of di-Fmoc-L-Arginine was dissolved in anhydrous DCM and activated in the presence of EDC and NHS for 45 min. Simultaneously, 1 mmol of compound **2** was dissolved in anhydrous DCM and stirred under a nitrogen atmosphere in an ice bath. The activated arginine solution was added to the solution under continuous stirring, under a nitrogen atmosphere, and maintained between 0 and 5 °C for 24 h. After completion of the reaction, a white compound **3** was precipitated, dried under reduced pressure, and washed thoroughly in a manner similar to compound **1**. Compound **3** was then purified by column chromatography with 7% ethyl acetate in PET ether as the eluent, and subjected to deprotection to remove the Fmoc- groups in order to yield compound **4** (EDA-KR_2 dendrimer) (generation 2: 61.7% yield).

2.2. Synthesis of dendrimer-coated magnetic nanoparticles

Iron oxide nanoparticles were synthesized by the conventional co-precipitation method and stabilized by glutamic acid, as described in our earlier published work [22]. The surface of the glutamic acid-modified iron oxide nanoparticles (Glu-IO) was further modified with commercially available PAMAM dendrimers (generation 2) and as-synthesized EDA-KR_2 to yield PAMAM-modified iron oxide (PAMAM-IO) and EDA-KR_2-modified iron oxide (KR_2-IO) nanoparticles, respectively (Supplementary Information).

2.3. Drug loading and release studies

The carrier efficiency of the PAMAM-IO and KR_2-IO nanoparticles was evaluated using DOX, which is a widely used anti-cancer therapeutic agent. The drug loading efficiency of both the carrier systems was investigated by recording the fluorescence spectrum (λ_{ex} = 490 nm and λ_{em} = 560 nm) of DOX. Each experiment was performed in triplicates and the standard deviation was calculated. The binding interactions of DOX molecules with both the carrier systems were further studied and understood using a modified Stern–Volmer plot. To perform the drug release experiments, the DOX-loaded dendritic nanoparticles were quantified according to their loading efficiencies. The release behavior of DOX was assessed under reservoir-sink conditions using low pH as a stimulus. (Supplementary Information)

2.4. Cancer chemotherapy

The toxicity of functional nanomaterials toward a living system is an important factor that limits their use in biomedical applications. To meet this end, PAMAM, EDA-KR_2, PAMAM-IO and KR_2-IO were assessed for their biocompatibility with murine fibroblast (L929), human cervical cancer (HeLa), human oral carcinoma (KB), human breast adenocarcinoma (MCF-7), and human prostate cancer (PC-3) cell lines (Supplementary Information). The therapeutic efficacy of the DOX-loaded nanoparticles was evaluated with different cancer cell lines (HeLa, KB, MCF-7 and PC-3). The concentration of the DOX-loaded nanoparticles that reduce the cell population by 50% was referred to as the inhibitory concentration (IC_{50}) values of the said formulation, and was calculated by a dose-responsive sigmoidal curve fitting from Origin 8.0 software. The live cell population was determined by the sulforhodamine-B colorimetric assay