Materials Science and Engineering C 50 (2015) 107–116

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

Magnetically responsive siliceous frustules for efficient chemotherapy

Vivek S. Javalkote [a], Abhijeet P. Pandey [b], Pravin R. Puranik [a], Prashant K. Deshmukh [b,*]

[a] Department of Biotechnology, School of Life Sciences, North Maharashtra University, Jalgaon, Maharashtra, India
[b] H. R. Patel Institute of Pharmaceutical Education and Research, Shirpur, Maharashtra, India

ARTICLE INFO

Article history:
Received 14 June 2014
Received in revised form 6 December 2014
Accepted 23 January 2015
Available online 26 January 2015

Keywords:
Diatoms
Ferrofluid
Cell viability
Curcumin
Cervical cancer

ABSTRACT

In the present investigation, curcumin loaded magnetically active frustules have been reported. The diatoms were cultured and frustules were obtained by chemical and thermal processes. The frustules were rendered magnetically active by incorporation of iron oxide nanoparticle using two different methods involving ferrofluid (CMDM-F) and in situ synthesis (CMDM-I) of iron oxide nanoparticle. These CMDM prepared by two techniques were characterized using FT-IR and vibrating sample magnetometer (VSM) analyses. Particle size and potential were measured using the Malvern Zetasizer. Scanning electron microscopy (SEM) was utilized for studying the surface morphology of CMDM, and in addition to this elemental analysis was also performed for confirming the presence of iron. The cell viability assay was carried out using the HeLa cell line. SEM images showed a change in surface morphology of diatoms before and after rendering magnetic activity. Cell viability assay revealed that CMDM-F had reasonably high cytotoxicity (60.2%) compared to Curcumin (42.1%), DM (1.9%), CDM (44.8%), and CMDM-I (59.9). Both, CMDM-F and CMDM-I showed improved cytotoxicity when compared with pure curcumin. The overall study suggests that the developed CMDM could be utilized as a potential carrier to deliver cargo for efficient chemotherapy.

1. Introduction

With vast development in the field of drug delivery, many inorganic nanoparticles have been explored for them to be used as a potential drug delivery platform. Mesoporous silica based nanoparticles and microparticles have been explored widely for drug delivery purposes of enhancing solubility of water insoluble drug and providing sustained drug release [1–3]. Various mesoporous silica nanoparticles such as MCM-14 and SBA-16 have been synthesized and studied for drug delivery [1,4,5]. The process of synthesizing such particles is time-consuming and includes the use of several chemicals and solvents. Similar to mesoporous silica, diatoms have recently gained a lot of attention.

Diatoms are a unique class of eukaryotic, unicellular photosynthetic algae. They are an important constituent of the phytoplankton community of oceans. Generally diatom species ranges in size from 2 μm to 2000 μm and do have a large variety in its morphologies and possess a unique transparent rigid cell wall made up of amorphous silica which can form empty envelopes known as frustules. These diatoms have a distinct 3D structure which is made up of porous silica microshells commonly known as frustules. The distribution of pores is highly regulated and the shape varies with the species [6–8]. Considering its application in various sectors, frustules attracted the interests of the researchers. One of the most important applications of diatom silica is in the development of novel drug delivery systems [7].

The diverse characteristics of diatoms which make it a possible carrier for drug delivery include, large surface area, nanoscale porosity, biocompatibility and biodegradability of amorphous diatom silica. Apart from these, easy functionalization, protection and design for controlled drug release through nano-sized pores or by embedding in the silica further prove diatoms as a potential drug delivery carrier. Recently it has been shown that diatom frustules can be functionalized with antibodies and enzymes [7,9–11]. The diatom possesses structural, mechanical and chemical features that might assist in overcoming challenges associated with the usual delivery of therapeutic agents [11]. Diatoms can be well cultivated in great measures. Recently, a few researchers have studied the potential of diatoms as a drug delivery carrier [12]. The outcomes show the effectiveness of diatom silica for drug delivery application, with about good drug loading capacity and sustained drug release over two weeks.

There are two main sources of diatom biosilicas, diatomaceous earth (diatomite) and living diatom cells. After the death of diatoms the internal organic matter get decayed and disintegrated but the biosilica shells persist longer and become important constituents of naturally formed biosilica rich rocks known as diatomite or diatomaceous earth. Biosilica derived from diatomite have various disadvantages, such as lot of impurities of different metal oxides, disintegration of the native structure of the diatom shell as it remains buried under the earth for thousands of years and the presence of different species leads to the lack of uniformity in biosilica structures [7,8]. So, for application like drug delivery

* Corresponding author at: Department of Pharmaceutics, H. R. Patel Institute of Pharmaceutical Education and Research, Karwand Naka, Shirpur, Dist-Dhule-425 405, Maharashtra, India.
E-mail address: pkdesh@rediffmail.com (P.K. Deshmukh).

http://dx.doi.org/10.1016/j.msec.2015.01.079
0928-4931/

biogenic silica derived from living diatoms could be more advantageous. Work has been done on diatom for drug delivery, but very little work has been reported on the use of magnetically active diatoms for the delivery of drug for cancer therapy. The use of magnetically active diatom frustules can be very helpful in targeting the drug to the tumor site. Iron oxide nanoparticles possess unique properties including the generation of heat in alternating magnetic fields or an ability to be pointed to a specific tissue or organ under the influence of external magnetic field. The power generation of heat under alternating magnetic field can be used in cancer therapy. Such a combination of silica based natural microparticles with iron oxide nanoparticle can be helpful.

The present investigation explores the use of magnetically active diatom frustules as an efficient drug carrier. Curcumin was selected as a model water insoluble drug, it is a phenolic compound extracted from *Curcuma longa* L., well known as a chemopreventive agent. Various studies have demonstrated that curcumin modulates angiogenesis, proliferation, invasion, and tumor progress in various types of cancer including cervical cancer [13–15]. The molecular targets and mechanisms of curcumin involved in treatment of cancers have been well documented [16–18]. Although curcumin possesses pleiotropic effects on the various cancer cells through the modulation of cellular signaling pathways including cell cycle, apoptosis, proliferation, invasion, angiogenesis, and metastasis in addition to its influences in numerous biochemical and molecular cascades which eventually inhibit the growth of cancer cells, the use of curcumin is currently limited in clinical utility owing to its low solubility [19]. This study demonstrates that diatoms can be used as an efficient carrier for the loading and delivery of water insoluble anticancer drug. The present work, involves the comparative study of magnetically active diatom microshells fabricated using in situ method (CMDM-I) and ferrofluid (CMDM-F) method. The use of ferrofluid for fabricating CMDM is simple, efficient and less time consuming. To the best of our knowledge, we are the first to utilize ferrofluid for fabricating CMDM and successfully load curcumin on prepared CMDM for the treatment of cervical cancer. Instead of using diatomaceous earth, we have cultured diatoms and treated it for emptying the contents of frustules to make space for the loading of cargo. Two methods have been used for treating cultured diatoms i.e. ethanol extraction and thermal treatment. The present work provides a new technique for using cultured diatoms and making them magnetically responsive drug delivery platform.

2. Experimental

2.1. Materials

Curcumin was purchased from Sigma Aldrich, India. Ferrofluid was generously gifted by Ferrotec Corporation, USA. Ferric Chloride hexahydrate, Ferrous Chloride tetrahydrate and ammonium hydroxide were purchased from Sigma Aldrich, India. All other chemicals and reagents used were of analytical grade.

2.2. Purification of frustules

The frustules obtained from *Nitzschia* sp. in aqueous solution was used after the organic matter has been removed using various treatment methods. In the first method, the organic matter was removed using ethanol. The frustules along with organic matter were mixed with ethanol. After shaking for a particular time period, the mixture was centrifuged to remove the organic matter which was dissolved in the supernatant and the sediment contained silica frustules. In the second method, the organic matter was removed using thermal treatment in a muffle furnace. The aqueous suspension of frustules was kept in a muffle furnace at 700 °C for 5 h. The suspension was removed after 5 h. The samples of diatom frustules obtained from both the abovementioned procedure were treated with organic acids for the removal of various oxide impurities such as Al_2O_3, Fe_2O_3, $CaCO_3$, CaO, P_2O_5, K_2O, Na_2O, and MgO. For the removal of oxide impurities, the diatoms were treated with nitric acid (70%, V/V) for 1 h, then washed in distilled water, and treated by concentrated sulphuric acid (97%) and washed again. The detailed procedure of the washing of diatom frustules can be found in the previously published literature [20,21]. The washed samples were refluxed in 30% H_2O_2 (50 v/v %), at 90 °C for up to 4 h and dried, in order to increase available hydroxyl groups on frustules.

2.3. Preparation of FITC loaded diatom microshells

The diatom microshells (DM) were explored for their cargo loading ability. FITC was loaded on diatoms for qualitative determination of drug loading on diatoms. Before performing the cargo loading experiment, pure DM (0.1 g) were mixed with ethanol (2 mL) and sonicated for 2 h to facilitate efficient pore wetting and improve drug encapsulation inside the pores as reported previously [22]. After 2 h, the DM were left for drying. For loading of FITC, a 1 mg/mL of FITC solution was prepared in distilled water and was added to the dried sample of DM in a drop-wise manner. FITC molecules permeate through the pores of DM into the hollow center of diatom structure. The sample was vacuum dried to obtain FITC loaded diatom microparticle.

2.4. Preparation of curcumin loaded diatom microshells

For loading curcumin on diatom microshells (CDM), curcumin was first dissolved in acetone (10 mg/mL). The preconditioned DM were added to the solution of curcumin and were stirred (for 1 h) to facilitate the permeation of curcumin inside the pores of diatoms till the whole solvent evaporates. The dry CDM were washed thrice with distilled water to remove surface adsorbed curcumin. The washed sample of CDM was vacuum dried to obtain CDM.

2.5. Preparation of curcumin loaded magnetic diatom microshells (ferrofluid technique)

Ferrofluid was utilized for the preparation of curcumin loaded magnetic diatom microshells (CMDM-F) according to a previously reported method by our group with slight modification [23]. Before performing the loading experiment, pure DM (0.1 g) were preconditioned by mixing with ethanol (2 mL) and sonicated for 2 h to facilitate efficient pore wetting and improve drug encapsulation inside the pores. Curcumin was dissolved in acetone as described before. The dried preconditioned DM were then mixed with curcumin solution with continuous stirring for 30 min. Ferrofluid was added to the curcumin solution and stirring was continued for 2 h to facilitate the permeation of both curcumin and iron oxide nanoparticles. The stirring was further continued until all the solvent evaporated. The curcumin loaded magnetically active DM were then dispersed in deionized water and sonicated for removing surface adsorbed iron oxide nanoparticle and curcumin. The dispersed DM were centrifuged at 6000 rpm for 15 min to obtain CMDM. The obtained microshells were vacuum dried.

2.6. Preparation of curcumin loaded magnetic diatom microshells (in situ technique)

The in situ preparation of CMDM (CMDM-I) was done using a previously reported method with slight modification [24]. Prior to the loading experiment, pure DM (0.1 g) were preconditioned by mixing with ethanol (2 mL) and sonicated for 2 h to facilitate efficient pore wetting and improve encapsulation inside the pores. The preconditioned DM were dispersed in water and sonicated. A separate solution of $FeCl_3 \cdot 6H_2O$ (87.5 mg) and $FeCl_2 \cdot 4H_2O$ (35 mg) was prepared in 200 mL Milli-Q water. An appropriate quantity (40 mL) of the above prepared solution was added to the suspension of DM. The mixture was sonicated for 30 min to facilitate the entry of magnetic solution