Materials Science and Engineering C 63 (2016) 393–410

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

Curcumin-loaded silica-based mesoporous materials: Synthesis, characterization and cytotoxic properties against cancer cells

Vishnu Sravan Bollu [a,1], Ayan Kumar Barui [a,1], Sujan Kumar Mondal [a,1], Sanjiv Prashar [b], Mariano Fajardo [b], David Briones [c], Antonio Rodríguez-Diéguez [c], Chitta Ranjan Patra [a,*,1], Santiago Gómez-Ruiz [b,*]

[a] Biomaterials Group, CSIR-Indian Institute of Chemical Technology, Uppal Road, Tarnaka, Hyderabad 500007, India
[b] Departamento de Biología y Geología, Física y Química Inorgánica, E.S.C.E.T., Universidad Rey Juan Carlos, Calle Tulipán s/n, 28933, Móstoles, Madrid, Spain
[c] Departamento de Química Inorgánica, Universidad de Granada, Facultad de Ciencias, Fuente Nueva s/n 18071, Granada, Spain

ARTICLE INFO

Article history:
Received 29 October 2015
Received in revised form 28 February 2016
Accepted 1 March 2016
Available online 4 March 2016

Keywords:
Mesoporous silica materials
Cytotoxicity
Anti-cancer
Curcumin
Nanomedicine

ABSTRACT

Two different silica based (MSU-2 and MCM-41) curcumin loaded mesoporous materials **V3** and **V6** were synthesized and characterized by several physico-chemical techniques. Release kinetic study revealed the slow and sustained release of curcumin from those materials in blood simulated fluid (pH: 7.4). The materials **V3** and **V6** were found to be biocompatible in non-cancerous CHO cell line while exhibiting significant cytotoxicity in different cancer cells (human lung carcinoma cells: A549, human breast cancer cells: MCF-7, mouse melanoma cells: B16F10) compared to pristine curcumin indicating the efficacy of the mesoporous silica materials based drug delivery systems (DDSs). The generation of intracellular reactive oxygen species (ROS) and down regulation of anti-apoptotic protein leading to the induction of apoptosis were found to be the plausible mechanisms behind the anti-cancer activity of these DDSs. These results suggest that curcumin-loaded drug delivery system may be successfully employed as an alternative treatment strategy for cancer therapeutics through a nanomedicine approach in near future.

1. Introduction

In Asian countries, especially India and China, *Curcuma longa* is well-known as a food additive, conserving agent and additionally as a therapeutic agent for several diseases such as liver dysfunctions, and diabetes [1,2]. Therefore, biological activity and pharmacological properties of *Curcuma longa* and its extracts have been studied in detail during the past 20 years [3]. Its bioactive component: curcumin, exhibits growth inhibition of microbes, reduces blood glucose levels, inhibits formation of free radicals and prevents tumour growth [4]. The anti-inflammatory property of curcumin has also been tested in clinical trials [5]. The most exhilarating property of curcumin is its high therapeutic potential against cancer cells through the induction of apoptosis following different molecular mechanistic pathways [6–8]. Curcumin has also been found to be biocompatible in non-cancerous cells even at high doses making it a very good candidate for anticancer therapy. However, there is a major problem to use curcumin in cancer therapy derived from its reduced bioavailability due to its low solubility and stability in water [9,10]. Therefore the scientific community has focussed on finding novel strategies for the improvement of bioavailability of curcumin, for example the encapsulation of curcumin with phospholipids, polymeric nanoparticles, and metal or non-metal nanoparticles [11–13]. Amongst these strategies, the encapsulations of curcumin with nanoparticles have been found to be the most prominent approach so far. Polymeric nanoparticles have extensively been used as encapsulators but the biological properties of novel curcumin-based nanosystems have not been evaluated in detail [14,15]. Therefore, several investigations are still required in order to assess the toxicity of the nanoparticles along with their efficacy as drug delivery vehicle with slow and sustained release of drugs [16–19].

The earlier reported literature illustrate the unique properties of silica based mesoporous materials in drug delivery system viz., tunable particle size, easy surface functionalization, uniform and tunable pore size, high surface area, large pore volume and more biocompatible nature compared to other carriers such as polymers or phospholipids [20,21]. Furthermore, numerous reports demonstrated the accumulation, clearance, metabolic fate and toxicity study of silica-based mesoporous materials/nanoparticles in animal model [22–26]. Most of the literature supported the biodegradability, low toxicity and rapid clearance of mesoporous silica materials, observed in animal model. Considering the outstanding feasible nature of mesoporous silica and its resourceful functionalization ability, two mesoporous silica materials MSU-2 and MCM-41 were selected as vectors for the delivery of anti-cancer drug curcumin in present study. In our earlier published article, MSU-2 and MCM-41 materials were functionalized with simple monoamino ligand (3-aminopropyltriethoxysilane) followed by functionalization with curcumin (MSU-2: **S4**; MCM-41: **S6**) [27]. The

* Corresponding authors.
E-mail addresses: crpatra@iict.res.in, patra.chitta@gmail.com (C.R. Patra), santiago.gomez@urjc.es (S. Gómez-Ruiz).
[1] Academy of Scientific and Innovative Research (AcSIR), Taramani, Chennai 600113, India.

http://dx.doi.org/10.1016/j.msec.2016.03.011
0928-4931/

new hypothesis of the present work is that the incorporation of a polyamino ligand (tris-(2-aminoethyl)amine) onto the mesoporous material would lead to a higher amine functionalization as well as higher loading of anti-cancer drug than materials functionalized with a simple monoamino ligand (3-aminopropyltriethoxysilane) [27], even though similar silica based materials were employed in both studies. Thus, the proof of concept is that the higher the amine functionalization, the higher would be the loading of curcumin as observed in the present study compared to our last work [27]. It is well established that the therapeutic efficacies or biological activities depend on the loading of chemotherapeutic drug (here curcumin) conjugated with mesoporous silica-based materials. Accordingly, we have observed that the newly functionalized materials loaded with curcumin show better anti-cancer activity compared to our previous report [27].

In the present study, the two silica based materials MSU-2 and MCM-41 were synthesized followed by chloro functionalization using 3-chloropropyltriethoxysilane leading to the formation of chloro functionalized materials **V1** and **V4**, respectively. Then, **V1** and **V4** were again treated with tris(2-aminoethyl)amine to give rise to amine functionalized materials **V2** & **V5** respectively. The surface and pores of the mesoporous silica were functionalized with poly-amino ligands such as tris (2-aminoethyl) amine in order to increase the loading content through the formation of weak intermolecular forces between amine functionalized materials and the polar groups of drugs, such as carbonyl, ether and alcohol groups in curcumin [28–31]. Further, we developed **V2** & **V5** based drug delivery systems by conjugating them with curcumin, namely **V3** & **V6** respectively. All the materials were thoroughly characterized by several analytical techniques such as XRD, TEM, SEM, TG, DLS, MAS NMR, FT-IR, BET isotherm etc. The *in vitro* data showed that the silica based curcumin loaded materials (**V3** and **V6**) were biocompatible in normal cells while significantly inhibiting cancer cell proliferation (anti-cancer activity) compared to pristine curcumin indicating the efficacy of the drug delivery systems (DDSs). Release kinetic study with the materials **V3** and **V6** exhibited slow and sustained release of curcumin in blood simulated fluid (pH: 7.4) which could be beneficial for cancer therapy suggesting the future potential application of the DDSs. The generation of intracellular reactive oxygen species (ROS: O_2^-) and down regulation of anti-apoptotic proteins EGFR (epidermal growth factor receptor) and BCL-2 (B-cell lymphoma 2) were found to be the probable mechanisms for the anticancer activity of those materials. This study may put forward the foundation for future advancement of alternative treatment strategies for cancer therapeutics by developing appropriate drug delivery system through nanomedicine approach.

2. Experimental

2.1. Materials and methods

All manipulations were performed under dry nitrogen gas using standard Schlenk techniques and dry box. Solvents (all purchased from SDS) were distilled from the appropriate drying agents and degassed before use. Tetraethylorthosilicate (TEOS) 98% (MW = 208.33, d = 0.934 g mL^{-1}), dodecylamine (DDA) 98% (M = 185.36), Tergitol® NP-9 (MW = 616.82) and NaF (extra pure) were purchased from Sigma-Aldrich and used without further purification. Water (resistance 18.2 MΩ cm) used in the preparation of materials was obtained from a Millipore Milli-Q-System (Billerica, MA, USA). Curcumin (95% purity) was purchased from Alfa Aesar and used without further purification.

2.2. Preparation of the non-functionalized materials

2.2.1. Preparation of MSU-2

Mesoporous silica of the MSU-X family (MSU-2-type) was prepared using the previously reported synthetic protocol [32]. MSU-2 was synthesized by a two-step process: firstly TEOS was added to a stirring solution of Tergitol® NP-9 (0.08 M, pH 4.8) in Milli-Q water at room temperature to obtain a milky suspension (TEOS/surfactant solution molar ratio of 8/1). The resulting suspension was then aged without agitation for 20 h to obtain a clear solution. In the second step, sodium fluoride solution (0.24 M) was added drop wise with stirring to the TEOS/surfactant suspension to obtain a NaF/TEOS molar ratio of 0.025/1. The solutions were placed in a bath with agitation at 55 °C for 48 h. The final product was filtered off, washed with Milli-Q water and air-dried at 100 °C for 4 h. Finally, the surfactant was removed by calcination in air at 600 °C for 12 h. The surface was then dehydrated under a vacuum (10^{-2} mm Hg) for 16 h at 250 °C, cooled and stored under dry nitrogen. Textural properties: BET surface (S_{BET}): 843 $m^2\ g^{-1}$. Pore volume (Vp): 0.97 $cm^3\ g^{-1}$. Pore diameter (dp): 51.1 Å.

2.2.2. Preparation of MCM-41

MCM-41 was prepared according to the reported protocol of Landau et al. using hydrothermal crystallization [33]. The surface was dehydrated under a vacuum (10^{-2} mm Hg) for 16 h at 200 °C, cooled and stored under dry nitrogen. Textural properties: BET surface (S_{BET}): 1117 $m^2\ g^{-1}$. Pore volume (Vp): 1.12 $cm^3\ g^{-1}$. Pore diameter (dp): 29.6 Å.

2.3. Preparation of the functionalized materials

We have already demonstrated the functionalization of silica-based materials (MSU-2 and MCM-41) with monoamino ligand (3-aminopropyltriethoxysilane) for loading of curcumin [27]. However, in the present study in order to enhance the loading efficiency of curcumin onto the surface of silica materials, we have performed a two step functionalization of MSU-2 and MCM-41. Here, silica based materials (MSU-2 and MCM-41) were initially functionalized with 3-chloropropyltriethoxysilane followed by amine functionalization with polyamino ligand (tris (2-aminoethyl)amine).

2.3.1. Preparation of V1 (chloro functionalized MSU-2: MSU-2-CP)

A solution of 3-chloropropyltriethoxysilane (CP: 5.50 mL, 20.7 mmol) in toluene (100 mL) was added to dehydrated MSU-2 (5.00 g) and the mixture was stirred for 48 h at 110 °C. The slurry was filtered through fritted discs and the solid residue was washed with toluene (5 × 200 mL), ethanol (5 × 200 mL), methanol (5 × 200 mL) and diethylether (5 × 200 mL). The resultant solid was dried under a vacuum at room temperature for 24 h giving a white free flowing powder. Textural properties: BET surface (S_{BET}): 729 $m^2\ g^{-1}$. Pore volume (Vp): 0.93 $cm^3\ g^{-1}$. Pore diameter (dp): 49.3 Å.

2.3.2. Preparation of V2 (amine functionalized-V1: MSU-2-CP-TR)

A solution of tris(2-aminoethyl)amine (TR: 3.58 mL, 23.9 mmol) in toluene (50 mL) was added to a suspension of **V1** (3.50 g) in toluene (50 mL) and the mixture was stirred for 48 h at 110 °C. The slurry was filtered through fritted discs and the solid residue was washed with toluene (5 × 200 mL), ethanol (5 × 200 mL), methanol (5 × 200 mL) and diethylether (5 × 200 mL). The resultant solid was dried under a vacuum at room temperature for 24 h giving a white free flowing powder. Textural properties: BET surface (S_{BET}): 304 $m^2\ g^{-1}$. Pore volume (Vp): 0.52 $cm^3\ g^{-1}$. Pore diameter (dp): 48.3 Å.

2.3.3. Preparation of V3 (curcumin loaded-V2: MSU-2-CP-TR-Curc)

A solution of curcumin (Curc: 0.63 g, 2.40 mmol) (theoretical 20% curcumin/**V2**) in ethanol (100 mL) was added to **V2** (2.50 g) and the mixture was stirred under reflux for 48 h. The slurry was filtered through fritted discs and the solid residue was dried under a vacuum for 24 h at room temperature yielding a pale orange free flowing powder. Textural properties: BET surface (S_{BET}): 266 $m^2\ g^{-1}$. Pore volume (Vp): 0.40 $cm^3\ g^{-1}$. Pore diameter (dp): 47.0 Å.