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L-Trp adsorption into silica mesoporous materials to promote bone formation

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Abstract

The properties of ordered mesoporous silicas as bioactive materials, able to induce bone-tissue regeneration, have been combined with their abilities to host and release specific biomolecules in a controlled fashion. The possibility of locally deliver peptides and proteins is of great scientific importance because it opens new paths for the design of implantable biomaterials than can promote bone formation where needed. These biomaterials can host such biofactors, and their adsorption can be enhanced by chemically modifying the silica surface, with the aim of encouraging host–guest interaction and, thereby, increasing the loading capacity of the biomaterial matrix. *L*-Tryptophan (*L*-Trp) is a hydrophobic amino acid present in the three-dimensional structure of numerous proteins, and it is used here as model system to predict peptide delivery systems. Unmodified, silanol-rich, bioactive SBA-15 ordered mesoporous silica has been found to be incapable of confining *L*-Trp in its mesopores due to the hydrophobic character of this molecule. Organically modifying SBA-15 with quaternary amines results in approximately two-thirds of the silica surface being functionalized, increases the surface hydrophobicity allowing an increased *L*-Trp loading, and also induces different release kinetics. The control of the *L*-Trp release is the first step in controlled and localized protein delivery technologies, and opens novel perspectives for designing bioactive silica-based devices suitable for bone-healing applications.

Keywords: Bioactive silica; Bone-healing; Peptides; Adsorption; Controlled release

1. Introduction

Since the synthesis of the M41S mesoporous materials by Mobil Oil researchers in 1992 [1,2] a growing number of publications from research groups worldwide have detailed the synthesis and applications of these materials [3]. Ordered mesoporous silicas represent a new generation of materials that provide uniform porous structures with high surface area and tunable morphology. Among them, SBA-15 is characterized by a hexagonal packing of cylindrical pores with diameters between 6 and 10 nm [4,5].

The development of ordered mesoporous materials as drug delivery devices has opened a wide research field in biomedicine in recent years [6,7]. The possibility of combining the improved bone-bonding abilities of silica-based materials [8] with the drug release capacity observed for mesoporous matrices [9] offers the prospect of more specific systems for clinical applications. To date, many kinds of drugs have been loaded into ordered mesoporous materials with, essentially, hexagonal arrays of cylindrical nanometric channels, and all of them have been investigated in terms of molecular adsorption into the mesopore channels. An organic modification of the pore walls with suitable chemical groups has led to better control of the adsorption and delivery of several kinds of drugs and molecules [10–12], and is a common technique for current applications of ordered mesoporous matrices.

Additionally, two of the main features of this type of material, bone-bonding and drug-delivery abilities, suggest that it can be used for osseous regeneration technologies. SBA 15 materials could be applied in clinical situations as

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bone-filling materials for small osseous defects [13,14]. In fact, certain proteins with osteogenic capabilities can be confined and subsequently released in a sustained fashion using ordered mesoporous materials as host matrices. In the past, different proteins have been immobilized into mesoporous systems in the so-called bioreactors [15,16], but our scientific approach differs from them in the possibility of locally releasing proteins where they are needed into the living body. A well-known protein with osteogenic capability is the parathyroid hormone-related protein (PTH-*r*P), which exhibits a wide range of roles in normal human physiology [17]. The C-terminal (107–139) region of PTH-*r*P seems to inhibit the osteoclastic bone resorption and to affect the osteoblastic growth and differentiation. Within the C-terminal region, the 107–111 sequence (Thr-Arg-Ser-Ala-Trp) shows the highest anti-resorptive activity, while the remainder of the fragment appears simply to act as a largely globular carrier [18]. Therefore, the potential delivery of this pentapeptide with osteogenic ability seems to be interesting in bone regeneration technologies. Using large mesopore matrices like SBA-15 [4,5] would overcome possible pore size limitations [19]. Prior to adsorption of the whole pentapeptide, a model system using *L*-tryptophan (*L*-Trp) as the confined molecule is the first step of this investigation. *L*-Trp was selected because it is located at the 111 position, far from the 107 (Thr) position, which preliminary studies indicate is responsible for the link that produces osteoclastic inhibition [20]. Thus, the active end of the pentapeptide would not be affected by the interaction with the matrix walls. *L*-Trp is a well-known hydrophobic amino acid present in the three-dimensional structure of proteins. The aromatic indole ring of *L*-Trp makes necessary to modify the silanol-rich walls of mesoporous materials. Otherwise, very low *L*-Trp adsorption is achieved due to the difference in host–guest hydrophobicity when using unmodified SBA-15 materials.

The present work concerns the surface modification on the well-known ordered mesoporous silica SBA-15 by post-synthesis grafting of two quaternary organic amines displaying two different alkyl lengths. These organic modifications have two aims: to promote the electrostatic interaction of the carboxylic group in the amino acid with amine groups covering the mesoporous matrix; and to favor the interaction of the hydrophobic indole ring from the *L*-Trp with the hydrophobic alkyl chains [21]. The functionalization procedure carried out for the *L*-Trp loading modifies the mesoporous framework of the hexagonal arrays.

The hypothesis of this work was based on establishing an adsorption and release model system for *L*-Trp that could be then applied to more specific proteins, since *L*-Trp is an essential amino acid present in numerous proteins.

2. Materials and methods

2.1. Reagents

Tetraethyl orthosilicate (TEOS) (Aldrich), *N*-trimethoxysilylpropyl-*N,N,N*-trimethyl ammonium chloride ($C_9H_{24}ClNO_3Si$, 50% in MeOH; TMOS–$C_3N^+Me_3Cl$, ABCR GmbH) and octadecyldimethyl(3-trimethoxysilylpropyl) ammonium chloride ($C_{26}H_{58}ClNO_3Si$, 60% in MeOH; TMOS–$C_3N^+Me_2C_{18}Cl$, ABCR GmbH) were used as received. Reagent-grade hydrochloric acid (HCl 37 wt.%, Panreac), sodium hydroxide (NaOH, 98%, Panreac), sodium chloride (NaCl, 98%, Panreac) and tris(hydroxymethyl)aminomethane ($(HOCH_2)_3CNH_2$, 99.9%, Aldrich) were used without further purification. Polyoxyethylene–polyoxypropylene block copolymer (PEO_{20}–PPO_{70}–PEO_{20}, Pluronic® P123) was kindly supplied by BASF Co. (New Jersey, USA). *L*-Trp (99.0%) was purchased from Fluka. Chromasolv® acetonitrile and water for HPLC (gradient grade) were obtained from Sigma–Aldrich.

2.2. Synthesis of SBA-15

SBA-15 ordered mesoporous materials were synthesized according to the procedure described by Zhao et al. [5] using TEOS as the silica source. In every case, template phases were prepared using Pluronic® P123. HCl 37 wt.% was also employed to catalyze the templating process reaction. The template solution was prepared by dissolving 4 g of P123 in 104 ml of deionized water and 20 ml of HCl 37 wt.% under magnetic stirring. When the surfactant was completely dissolved, 9.16 ml of TEOS were added to the template solution to yield a molar composition of 1.0TEOS:0.017P123:6.03HCl:145H_2O. The resulting mixture was kept under magnetic stirring for 12 h at room temperature in a sealed Teflon container and subsequently aged at 100 °C for 24 h. Solid silica particles were then filtered, washed with deionized water and dried at 60 °C for 12 h. Finally, dried powders were thermally treated at 550 °C at a heating rate of 5° min^{-1} under nitrogen flow for 2 h and then under atmospheric air for further 2 h, leading to the removal of the surfactant.

2.3. Functionalization of SBA-15

A mass of 500 mg of template-free mesoporous material was refluxed with 2.5 mmol of TMOS–$C_3N^+Me_3Cl$ or TMOS–$C_3N^+Me_2C_{18}Cl$ in acetonitrile for 16 h at 80 °C. The whole system was purged with argon for 2 h before the addition of reagents so that an inert atmosphere was achieved within the system. Final products were filtered, washed of unreacted and self-condensed reagents with a mixture of toluene and diethyl ether (1:1), and finally dried at 60 °C for 12 h in air. The different degrees of organic modification were confirmed by thermal analysis and quantified by X-ray photoelectron spectroscopy (XPS).

2.4. Material characterization

The obtained materials were characterized by thermal analysis with a Perkin Elmer 2400 CHN analyzer. Thermal analyses (thermogravimetric (TG) and differential thermal

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