

Revision 2

Biodegradability of spherical mesoporous silica particles (MCM-41) in simulated body fluid (SBF)

Elena Boccardi¹, Anahí Philippart¹, Ana M. Beltrán²⁺, Jochen Schmidt³, Liliana Liverani¹,
Wolfgang Peukert³, Aldo R. Boccaccini^{1*}

¹Institute of Biomaterials, Department of Materials Science and Engineering, University of Erlangen-Nuremberg, 91058 Erlangen, Germany

² Instituto de Ciencia de Materiales de Sevilla (CSIC-Universidad de Sevilla), 41092 Seville (Spain)

⁺ Current address: Departamento de Ingeniería y Ciencia de los Materiales y del Transporte Universidad de Sevilla, 41092 Seville, Spain

³Institute of Particles Technology, Department of Chemical and Biological Engineering, University of Erlangen-Nuremberg, 91058 Erlangen, Germany

(*) Corresponding author. Email: aldo.boccaccini@ww.uni-erlangen.de

Abstract

Mesoporous silica particles of type MCM-41 (Mobile Composition of Matter No 41), exhibiting highly ordered mesoporosity (pores with diameter between 2 and 50 nm) and surface roughness, were developed and used as a functional coating on bioactive glass-based scaffolds for bone tissue engineering. In the present work, the degradability and the mesostructure stability of these novel MCM-41 particles were evaluated. The particles were immersed in simulated body fluid (SBF) for up to 28 days at 37°C, and the variation of the ordered porosity, surface characteristics, and chemical composition of the particles was assessed by SEM-EDX, HRTEM, FTIR, ICP-OES, and pH measurements. The results indicated that the MCM-41 particles were affected by immersion in SBF only during the first few days; however, the surface and the mesopore structure of the particles did not change further with increasing time in SBF. The pore channel diameter increased slightly, confirming the stability of the developed material. The release of dissolved Si-species, which reached a maximum of 260 mg SiO₂/g material, could play a key role in gene activation of osteoblast cells and in inducing new bone matrix formation.

Keywords

Mesoporous silica particles, Simulated body fluid, Biodegradability, Bone tissue engineering, Drug carriers

1. Introduction

Since 1992 (Beck et al. 1992), when silica-based mesoporous particles (type MCM-41) were developed for the first time, highly ordered mesoporous materials have attracted the attention of many scientists and in 2001 (Vallet-Regi et al. 2001) mesoporous silica particles were proposed for the first time as a potential drug delivery system. In fact, these materials are characterized by a regular pore system, high specific surface area, high pore volume, and walls terminated by silanol groups (Vallet-Regí et al. 2012; Zhao et al. 2013). These combined features confer these ordered mesoporous materials the capability to incorporate relatively high contents of drug into the mesoporous channels (Vallet-Regi et al. Zhao et al. 2013). Moreover, their silanol groups can be functionalized and their pore size modulated to achieve better control of the drug-release kinetics (Grün et al. 1997; Vallet-Regí et al. 2012a, b; Wu and Chang 2014).

MCM-41 type particles have become the most studied member of the ordered mesoporous silica materials family. In 1997, Grün et al. (Grün et al. 1997, 1999) proposed producing MCM-41 particles by modifying the well-established Stöber reaction (Stöber et al. 1968) for the synthesis of bulk silica particles.

MCM-41 particles are being increasingly investigated for biomedical applications, e.g. cancer therapy and wound healing (Izquierdo-Barba et al. 2010). In recent years, they have been proposed as a functional coating for bioactive glass-based scaffolds for bone tissue engineering (Mortera et al. 2008; Boccardi et al. 2015). The main idea was to combine the drug up-take and release capability of the MCM-41 particles with the bioactivity of 45S5 Bioglass®-based highly porous scaffolds (Mortera et al. 2008; Boccardi et al. 2015). In the past, it has been demonstrated that MCM-41 particles are not able to form hydroxyl carbonate apatite (HCA), which is one of the markers of bioactivity (Kokubo and Takadama 2006), once in contact with simulated body fluid (SBF). In addition, after 2 months exposure to SBF, it is impossible to observe the formation of a

HCA layer, presumably owing to the smaller pore size and the lower surface concentration of silanol groups ($\sim 2 \text{ mmol SiOH m}^{-2}$) compared to other silica particle types, e.g. SBA-15 and MCM-48 (Vallet-Regí et al. 2006). However, Si species released from the MCM-41 particles can play a key role in osteoblast gene activation and consequently, can induce new bone matrix formation (Hench 2006). In this sense, MCM-41 could have a double function: as drug carrier and for the release of functional ions such as soluble silica.

In 2015 (Boccardi et al. 2015), a novel synthesis method for MCM-41 particles was proposed. The resulting particles are characterized by spherical shape, highly ordered mesoporous structure and a highly porous surface. Moreover, the total amount of particles produced per single batch was found to increase by 60% compared to other methods described in the literature. The aim of the present work was to study the degradability of these novel MCM-41 particles by monitoring the Si release, the surface modification and the variations of ordered mesoporosity and pore size during immersion in SBF up to 28 days. Comparable analyses were also performed on another MCM-41 particle type, produced with a different synthesis procedure already available in literature (Cai et al. 2001), in order to compare the behavior of both types of MCM-41 particles (Boccardi 2016).

2. Materials and Methods

2.1 MCM-41 particle synthesis

The MCM-41 particles were produced following a recent method developed by our group (Boccardi et al. 2015). This method comprises a combination of the standard pathway to produce mesoporous silica particles and modified Stöber reaction (Stöber et al. 1968) to prepare non-porous silica spheres as proposed by Grün et al. (Grün et al. 1999). Briefly, 18 mL of ethanol (EtOH, 96%) and 18.5 mL of ammonia (NH₃, 28–30 wt.%) were mixed with 11 mL of deionized water. 0.2 g of *n*-hexadecyltrimethylammonium bromide (CTAB) were added under continuous stirring for 20 min. Once the solution became clear, 1 mL of tetraethyl orthosilicate (TEOS) was added (rate 0.25 mL

min^{-1}). All synthesis steps were carried out at room temperature. After stirring for 2 hours, the resulting dispersion was centrifuged and washed once with deionized water and twice with EtOH to remove every trace of NH_3 . The precipitate was then collected in a ceramic crucible, dried, and calcined in air. The thermal treatment was carried out at 60°C (2°C min^{-1}) for 12 h and at 550°C (2°C min^{-1}) for 6 h. These particles are labeled MCM-41 throughout the text (Boccardi et al. 2015).

As a comparison, another type of MCM-41 particles was synthesized following one of the most used protocols known in literature (Cai et al. 2001). A solution of 480 mL of deionized water and 3.5 mL of NaOH (2M) was heated to 80°C in an oil bath. Once the temperature had stabilized, 1g of CTAB was added and the solution was stirred until complete dissolution of the surfactant. Then, 5 mL of TEOS were added (0.25 mL min^{-1}). After 2 hours of stirring, the solution was centrifuged and the precipitates were washed once with water and twice with EtOH. The surfactant was removed overnight using a surfactant extracting solution made with 25 mL deionized water, 475 mL of ethanol and 5 g of ammonium nitrate. These particles will be named MCM-41_Ref throughout the text.

2.2 Degradation in simulated body fluid (SBF)

Simulated body fluid (SBF) was prepared by dissolving reagent grade 8.035 g L^{-1} NaCl, 0.355 g L^{-1} NaHCO_3 , 0.225 g L^{-1} KCl, 0.231 g L^{-1} $\text{K}_2\text{HPO}_4 (3\text{H}_2\text{O})$, 0.311 g L^{-1} $\text{MgCl}_2 (6\text{H}_2\text{O})$, 0.292 g L^{-1} CaCl_2 , and 0.072 g L^{-1} Na_2SO_4 in deionized water and buffered at pH 7.4 at 36.5°C with 6.118 g L^{-1} tris(hydroxymethyl) aminomethane ($(\text{CH}_2\text{OH})_3\text{CNH}_2$) and 1M HCl, as previously reported by Kokubo and Takadama (Kokubo and Takadama 2006). MCM-41 particles were immersed in SBF at a 1.5 g L^{-1} ratio (Cerruti et al. 2005; Maçon et al. 2015). The specimens were kept in a polypropylene vial at 37°C in an incubator on an oscillating tray for up to 28 days. The solution was not renewed (Maçon et al. 2015) and a falcon tube containing SBF as a control was also used for the entire period of the experiment to control the stability of the testing solution. At each time point (0, 1, 3, 7, 14 and 28 days), the particles were centrifuged and washed with deionized water and

dried at 60°C overnight. The microstructural changes were investigated by scanning electron microscopy (SEM-EDS) (Auriga 0750, ZEISS). The ordered mesoporous structure before and after immersion in SBF was checked with high-resolution transmission electron microscopy (HRTEM) in a FEI Tecnai G2F30 S-Twin microscope (0.2 nm point resolution) operated at 300 kV. For TEM observation, particles were homogeneously dispersed in ethanol by ultrasonic treatment and dropped onto a carbon film. The pore diameter analysis of the ordered mesoporous particles was conducted on HRTEM images with ImageJ analysis software (Schneider et al. 2012). The SBF was retained for analysis of the concentration of aqueous Si species by inductively-coupled plasma optical emission spectroscopy (ICP-OES Optima, Perkin Elmer USA) and for pH analysis. For the ICP-OES analysis, six point calibrations (100, 50, 25, 10, 5 and 1 ppm) were performed by diluting certified standards. For ICP-OES and pH measurements, the samples were measured in triplicate and mean values with standard deviation calculated. The eventual nucleation of HCA was assessed by FTIR (Nicolet 6700, Thermo Scientific, Germany), using KBr pellets and 32 scans at a resolution of 4 cm⁻¹, which were repeated over the wavenumber range of 4000 – 400 cm⁻¹.

3. Results and Discussion

Particles in as-synthesized conditions and after immersion in SBF were analyzed by SEM to evaluate how the surface of the materials was affected by the interaction with simulated body fluid (SBF). The ordered mesoporous silica particles synthesized with the new method (MCM-41) were spherically shaped with an average diameter of 300 nm (Fig. 1a) and a highly porous surface (pores in a range 10-30 nm). The particles were larger compared to the MCM-41_Ref reference particles shown in Figure 1d, that, however, were not homogeneously size-distributed. It should be noted that the MCM-41 particles were agglomerated, whereas the MCM-41_Ref appeared to be well dispersed. The particles were linked together by small “arms” visible in Figure 1d. After immersion in SBF for one day, a substantial modification of the particle surfaces was evident (Fig. 1 b,e). In case of MCM-41 particles (Fig. 1b), the surface porosity increased and some particles, in particular

the smaller ones, broke. MCM-41_Ref particles (Fig.1d) are characterized at the beginning of the test by a smooth surface. After immersion in SBF, they developed a highly porous surface and agglomerated. The links connecting the particles to each other degraded. After 3 days, no further changes of the surface topography were detected.

SEM-EDX analyses (Fig. 2) were performed to detect possible precipitation of hydroxycarbonate apatite (HCA) during immersion in SBF. No variation in the chemical composition of the surface of the particles occurred, even after 28 days exposure. It only was possible to detect Si and O, but no P or Ca ions, which would indicate a CaP-rich phase.

The ordering of the mesoporous structure of the synthesized particles was assessed by HRTEM analysis (Fig. 3). Both types of particle are characterized by highly ordered mesoporosity (Fig. 3 a,e). After immersion in SBF, the ordered structure of the materials was still in evidence even after 28 days exposure (Fig. 3 b-d, f-h), although a clear degradation of ordering, in particular of the external surfaces, was detected over time.

The profile plots indicating the distance between pore channels before and after immersion in SBF, obtained from HRTEM images with ImageJ plug-in software, are reported in Figure 4. After immersion in SBF, the distance between pores increased due to the degradation of the material and loss of order of the pore arrangement. The MCM-41 particles revealed a pore size of 3.2 nm (SD +/- 0.1 nm), which increased up to 4 nm (SD +/-1.3 nm) already after 3 days of testing and then stabilized at this value. The pore size of the MCM-41_Ref particles was more affected by degradation. The initial pore size of the MCM-41_Ref was 3.6 nm (SD +/- 0.2 nm) and after immersion in SBF increased to 7.1 nm (SD +/-1.8 nm) after 28 days exposure.

Figure 5 reports FTIR analyses of MCM-41 particles before and after immersion in SBF for up to 28 days. All the principal vibrations of the Si-O bonds were detected and they did not change with immersion time. Typical bands centered at 560 and 600 cm^{-1} , ascribable to the P-O asymmetric

bending vibrations (Zheng et al. 2015), related to the formation of a Ca-P rich layer (amorphous calcium phosphate (ACP) or crystalline HCA), were not detected in the analyzed samples at any time point (Peitl et al. 2001; Vallet-Regí et al. 2006).

The pH values of the SBF solutions were monitored during the entire test (Fig. 6a). The starting pH values of the SBF was around 7.4; due to the degradation of the silica particles, the pH increased up to a maximum value of 7.65 for the MCM-41 and 7.60 for the MCM-41_Ref materials. In addition, the pH value of the SBF control increased with time almost up to 7.65, most probably due to the aging of the solution, which is supposed to be stable at 4°C for ~ 1 month. The Si species release is reported in Figure 6b. The MCM-41 particles showed a burst release of Si ions during the first day of immersion in SBF. The MCM-41_Ref showed a slower release kinetic: the highest release of Si ions was reached after 2 days. Both materials, although characterized by different particle sizes, pore sizes and surface porosity, released the identical amounts of 400 mg L⁻¹ of soluble silica species corresponding to ~ 260 mg SiO₂/g material.

4. Discussion

Since their development in 1992 (Beck et al. 1992), ordered mesoporous materials have gained increasing research interest for different applications in the biological field. Mesoporous particles of MCM-41 type are the most investigated members of the ordered mesoporous silicas. They have been proposed for a number of biomedical applications, including as drug delivery system for cancer therapy, wound healing (Izquierdo-Barba et al. 2010; M Vallet-Regí et al. 2012b) and as a functional coating of bioactive glass-based scaffolds for bone tissue engineering (Mortera et al. 2008; Boccardi et al. 2015).

In the present work, MCM-41 particles developed via a novel synthesis method were prepared and characterized after immersion in SBF for up to 28 days. The idea was to evaluate the behavior of

these material once in contact with SBF to assess the Si ion release and the stability of the ordered mesoporous structure.

The novel procedure adopted here for ~~the production of~~ producing MCM-41 particles was a combination of the standard pathway for ~~the production of~~ synthesizing mesoporous silica and a modification of the Stöber reaction (Stöber et al. 1968) ~~for preparation of~~ to prepare non-porous silica spheres as proposed by Grün et al. (Grün et al. 1999). The resulting particles are characterized by a well-defined spherical shape, highly ordered mesoporous structure, and high surface porosity. After immersion in SBF, the porous surface of the MCM-41 particles increased in roughness even more and some of the particles broke. The main effects on the surface topography were observed during the first day of testing and no other significant changes were identified ~~with the progress of the test~~ afterwards. The same behavior was observed for the MCM-41_Ref particles used as a reference. The smooth surface that characterized the MCM-41_Ref particles was affected by the SBF and the surface became porous and extremely rough. Moreover, the MCM-41_Ref particles were seen to lose their ~~feature of being homogeneously dispersed~~ homogeneous dispersion immediately after 1 day in SBF, i.e. the material connecting the particles dissolved and the particles ~~agglomerated~~ were able to agglomerate. A simple explanation of this behavior is that “arms” helped particles to keep the distance from each other, but once these arms dissolved there was no physical obstacle for the particles to move closer.

The ordered mesoporous structure of the MCM-41 particles was not significantly affected by the immersion in SBF even after 28 days of exposure to SBF. The measure of the MCM-41 particles revealed an average pore size of 3.2 nm (SD +/-0.1nm), which increased up to 4 nm (SD +/-1.3nm) after 28 days in SBF. The difference in pore size between before and after the bioactivity test is very small and it is not considered to be significant. The pore size of the MCM-41_Ref particles was affected by degradation to a higher extent than that of the other particles. The initial average pore size of the MCM-41_Ref was 3.5nm (SD +/-0.2nm) and after immersion in SBF for up to 28

days increased up to 7.1 nm (SD +/-1.8nm). The higher standard deviation associated to the pore diameter after immersion in SBF compared to the standard deviation of the as synthesized particles can be explained by the non homogeneous effect of SBF on the pores. No changes in the chemical composition were observed for both types of particles and no precipitation of HCA was identified. The results are in agreement with the already available literature (Vallet-Regí et al. 2006).

The highest Si ion release took place during the first 24 hours for the MCM-41 particles and after 48 hours for the reference particles. In both cases, the concentration of released Si ions was the same (~ 260 mg SiO₂/g material). This value was slightly higher than the one reported by Izquierdo-Barba et al. (Izquierdo-Barba et al. 2010) for SBA-15 particles in SBF.

5. Implications

The results of this study have implications in relation to the biomedical applications of MCM-41 particles. For example, novel MCM-41 particles developed in this study are suitable as a functional coating of bioactive glass-based scaffolds for bone tissue engineering. In particular, surface roughness will likely enhance protein and cell adhesion, the Si ion release will have a positive effect on osteoblast gene activation (Hench 2006), and the stable mesoporous structure suggests the possibility to use the particles as drug carrier (Vallet-Regí et al. 2006).

Acknowledgements

Liliana Liverani acknowledges funding from the European Union's Horizon 2020 Research and Innovation Programme under the Marie Skłodowska-Curie grant agreement No 657264. Ana M. Beltrán thanks Talent-Hub Program funded by the Junta de Andalucía and the European Commission under the Co-funding of the 7th Framework Program in the People Program (Marie Curie Special Action). The authors also acknowledge the Laboratory for Nanoscopies and Spectroscopies (LANE) at the ICMS (Consejo Superior de Investigaciones Científicas) for use of their TEM facilities.

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Figure 1 SEM micrographs of MCM-41 particles as synthesized (a), after immersion in SBF for 1 day (b) and 3 days (c); SEM micrographs of MCM-41_Ref particles as synthesized (d), after immersion in SBF for 1 day (e) and 3 days (f).

Figure 2 SEM-EDX analyses of MCM-41 particles before (a) and after (b) immersion in SBF for 28 days; SEM-EDX analyses of MCM-41_Ref particles before (c) and after (d) immersion in SBF for 28 days.

Figure 3 HRTEM images of as-synthesized MCM-41 particles (a) and particles after immersion in SBF for 3 days (b), 14 days (c) and 28 days (d); HRTEM images of as-synthesized MCM-41_Ref (e) and particles after immersion in SBF for 3 days (f), 14 days (g) and 28 days (h).

Figure 4 Plots of the distance between the pore channel diameters of the MCM-41 and MCM-41_Ref particles before and after immersion in SBF up to 28 days.

Figure 5 FTIR spectra of MCM-41 particles before and after immersion in SBF for up to 28 days.

Figure 6 pH variation of the SBF containing the silica particles and the control for up to 28 days of test (a); ICP analysis of the Si ion release up to 7 days (b).

Figure 1

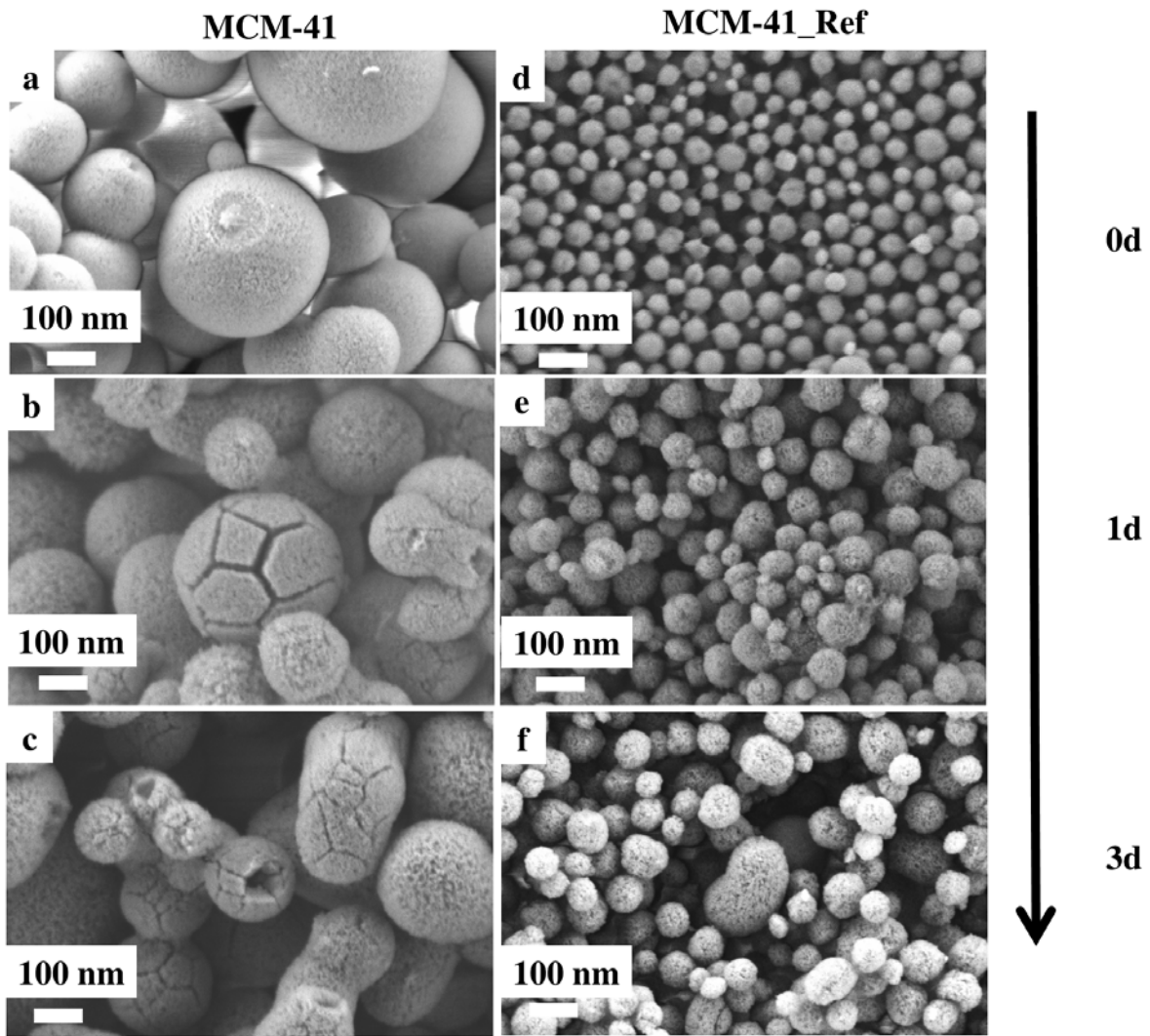


Figure 2

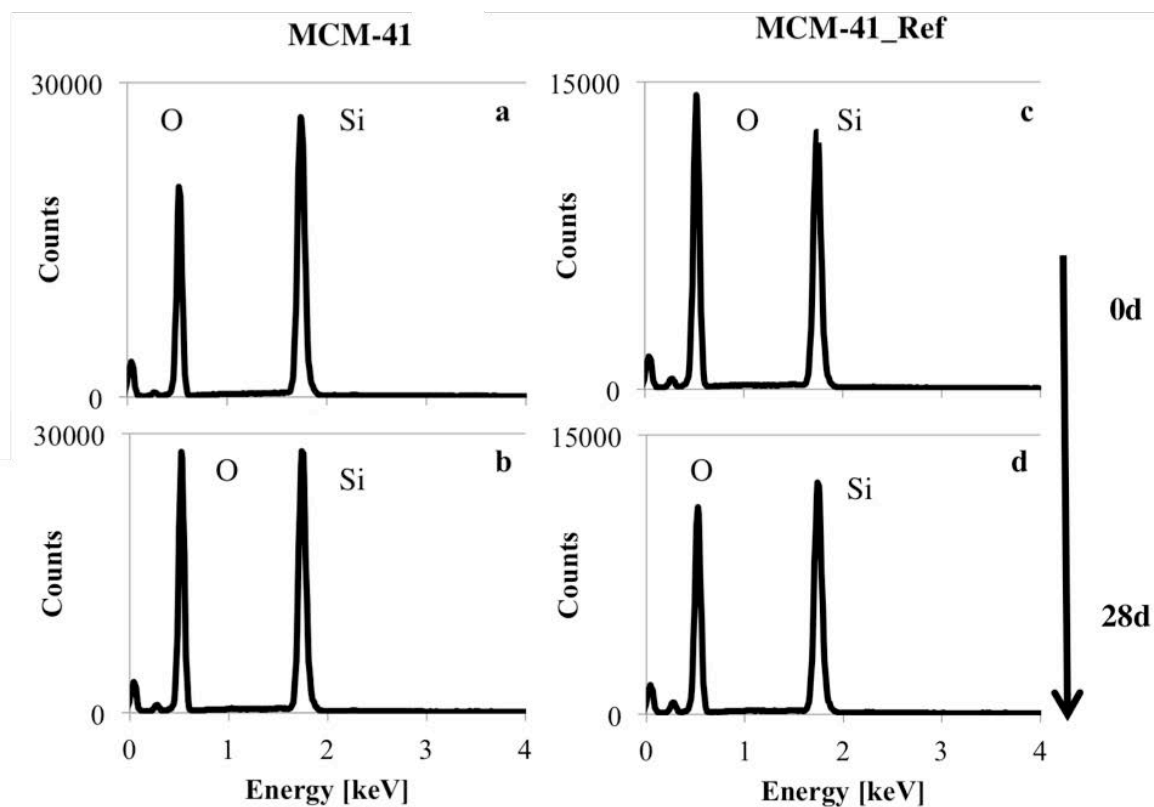


Figure 3

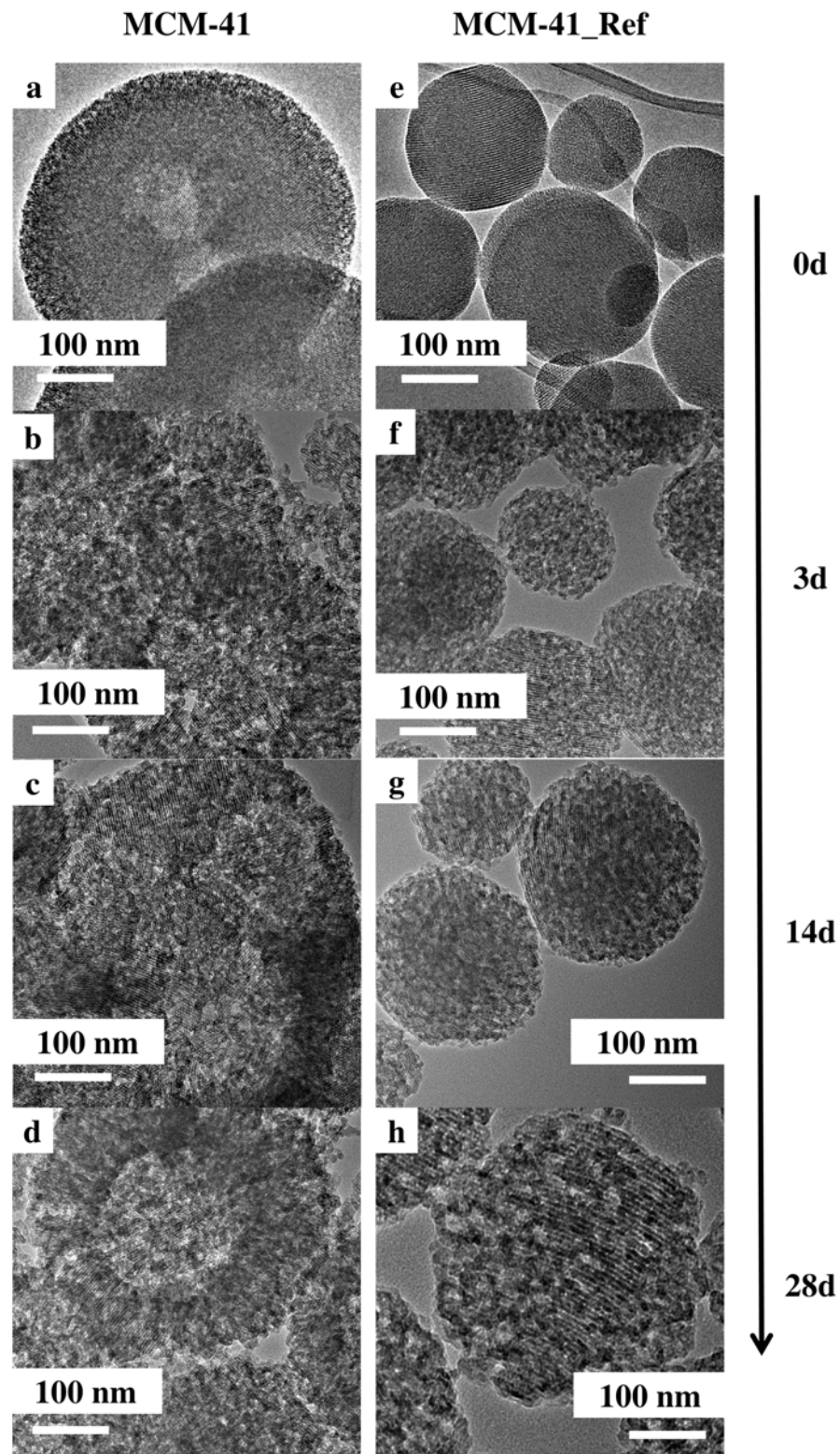


Figure 4

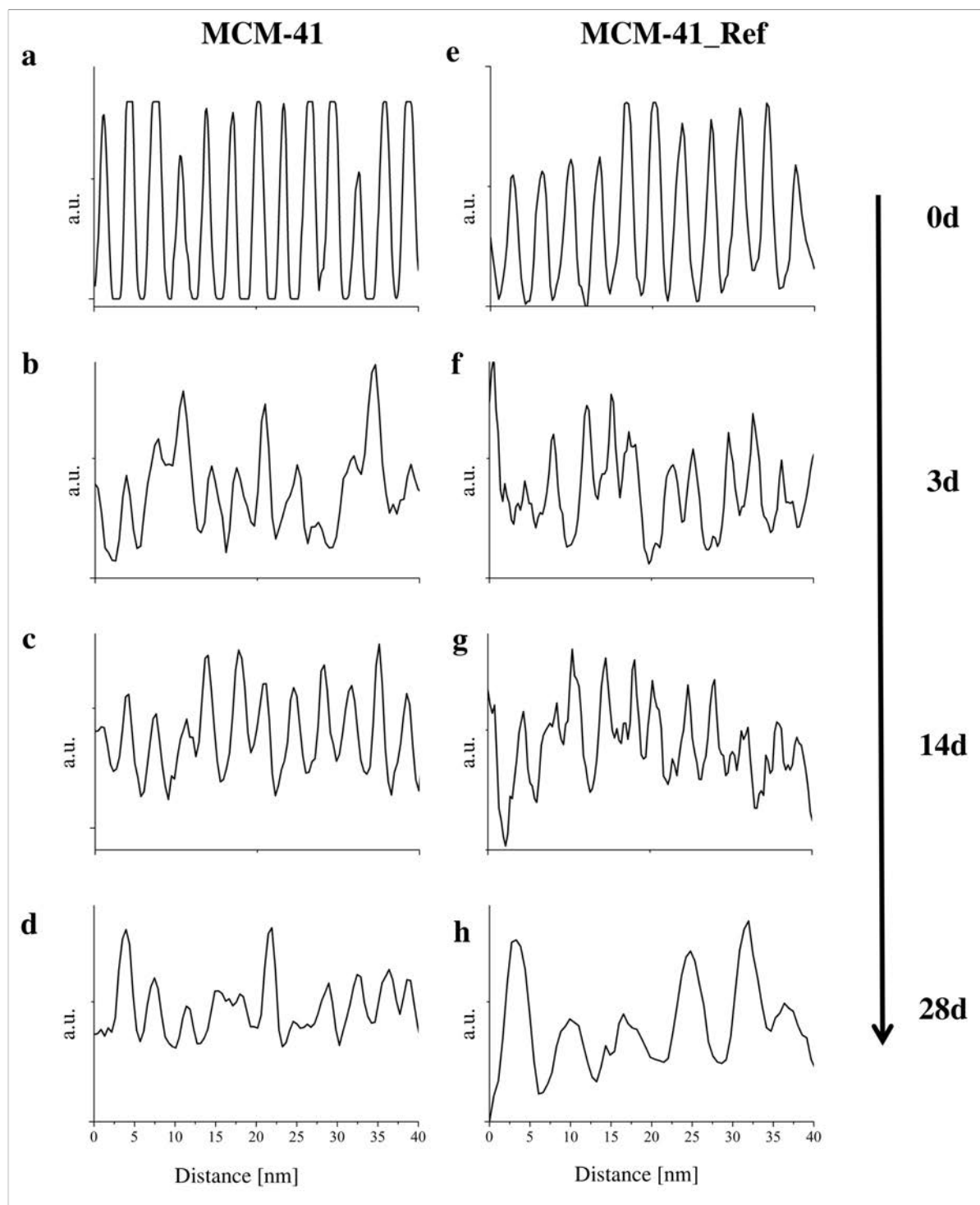


Figure 5

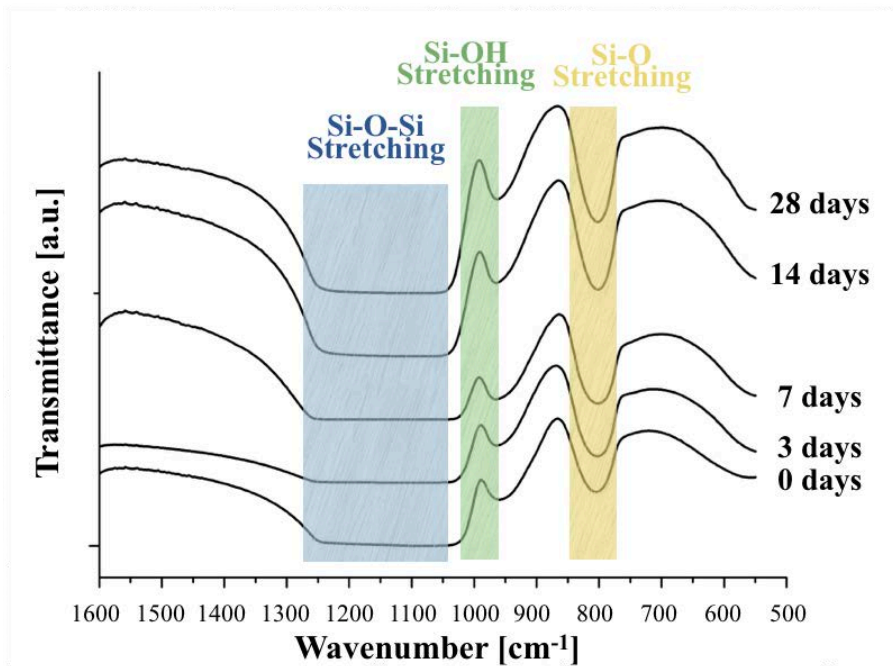


Figure 6

