J Mater Sci: Mater Med (2011) 22:2283–2292
DOI 10.1007/s10856-011-4397-1

Mesoporous silica nanoparticle-functionalized poly(methyl methacrylate)-based bone cement for effective antibiotics delivery

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Received: 22 December 2010 / Accepted: 11 July 2011 / Published online: 24 July 2011

Abstract Poly(methyl methacrylate)-based bone cements are functionalized with mesoporous silica nanoparticles (MSN) to enable a highly efficient and sustained release of antibiotics to reduce the risk of post-operative joint infection. To overcome the limited drug release of 5% for only 1 day with the current commercial-grade bone cements, a 8 wt% MSN-formulated bone cement is able to increase the drug release efficiency by 14-fold and sustain the release for up to 80 days. The loaded MSN is suggested to build up an effective network of rod-shaped silica particles with uniformly arranged nanoporous channels, which is responsible for the effective drug diffusion and extend time-release to the external surfaces. MSN has no detrimental effect on the critical weight-bearing bending modulus and compression strength of bone cement. In vitro assay test results show a much sustained antibacterial effect and low cytotoxicity of MSN demonstrating the potential applicability of MSN-formulated bone cement.

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1 Introduction

With the fast growth in the aging population worldwide, the frequency of hip and joint replacement surgeries due to osteoarthritis and rheumatoid arthritis is set to increase rapidly. The rise in osteoporosis, another common ailment among elderly, also contributes to more cases of trauma and bone fracture. Despite advances in strict antiseptic surgical procedures, postoperative osteomyelitis remains a considerable problem in orthopedic surgery, whereby the post-operative infection rates for joint replacement still stand at a high 1–3% [1, 2]. Upon infection, the complete removal of the artificial joint and re-implantation are often necessary, which cause both a detrimental impact on the quality of life for the patient as well as a high economic cost for the public health expenditure. In order to reduce the risk of post-operative infection, the current approach is to release antibiotics at the local site by using drug-loaded implants or bone cement [3–7] where the goals of the drug delivery system are to maintain the drug in the desired therapeutic range with just a single dose, to enable localized delivery of the drug to a particular body compartment (which lowers the systemic drug level), to reduce the need for follow-up care, and to increase patient comfort and/or improve compliance.

Various drug delivery systems incorporated into bone cement have been widely investigated and reported in researches [8–11]. Currently, the commercially available antibiotics delivery systems are the antibiotics-loaded poly(methyl methacrylate) (PMMA) bone cement [12, 13]. However, there is one major problem of this polymer–ceramic composite as far as the drug release is concerned. Being utilized as a "macrophase", the drug species can only be loaded into the composite by mechanical mixing, impregnation or by adsorption directly into the matrices of

polymer–ceramic composite. Such techniques are unable to achieve a sustained drug release at site for more than a few days. The drug release profile is characterized by a sharp initial burst due to the elution of antibiotics on the surface and in the voids in the sub-layers. As the drug release is subsequently very limited, it does not allow a long-term sustained delivery of antibiotics. In addition, only a small portion of the loaded antibiotics can be released and more than 90% may still be entrapped within the PMMA matrix, which does not serve its function to protect the surrounding tissues from infection [14–16]. Recently, more attempts have been undertaken to develop techniques to enhance the antibiotic elution from acrylic bone cements by incorporation of hydroxyapatite [17], polyvinylpyrrolidone [18], xylitol and glycine [19] fillers to PMMA bone cement. However, the improved drug release was usually accompanied by a loss in mechanical strength, which is critical for weight-bearing bone cement. Overall, the addition of fillers to bone cement has been intensively investigated but the ideal filler material and amount of filler are yet to be established to enhance the elution of antibiotics while preserving the mechanical properties [14].

Mesoporous silica material has been identified with the potential to act as a convenient reservoir for various controlled drug delivery systems [20–22]. In this work, mesoporous silica nanoparticles (MSN) were employed as functional filler for the loading of antibiotics and formulated with PMMA-based bone cement for enhanced drug release. The uniformly arrayed one-dimensional pore structure could serve to build up a diffusion network inside the PMMA matrix, which facilitates the antibiotic molecules to diffuse over time from the functional bone cement matrix towards the external surface, so that good antibacterial properties can be achieved without being detrimental to the critical mechanical properties of PMMA-based bone cements.

2 Materials and methods

2.1 Materials

Mesoporous silica nanoparticles (MSN) were prepared using fluorocarbon-surfactant-mediated synthesis as reported by Han and Ying [23]. Typically, 0.5 g of Pluronic P123 and 1.4 g of FC-4 were dissolved in 80 ml of HCl solution (0.02 M), followed by the introduction of 2.0 g of TEOS under stirring. The solution was continuously stirred at 30°C for 24 h and then transferred into a polypropylene bottle and kept at 100°C for 24 h. The resultant solid was recovered by centrifuging and washed with deionized water twice, then dried at 55°C for 12 h. To remove the template molecules, the material was heated from room temperature to 550°C at a heating rate of 2°C/min and followed by calcination in air for 6 h.

For comparison, mesoporous silica submicron (SM) particles were synthesized in the presence of Pluronic P123 surfactant as described in our previous work [24]. The conventional SBA-15 with particle size in tens of micrometers was prepared according to the conventional synthesis procedure and the hydrolysis was performed under continuous stirring in the presence of P123 surfactant [25]. The mixture gel had the same composition as the submicron particles.

Commercial bone cements CMW Smart GHV (DePuy International Ltd. UK) and Simplex P (Stryker Co, UK) were used for investigation.

2.2 Preparation of antibiotic-loaded bone cements

GTMC was loaded by direct impregnation with PMMA-based bone cement powder together with MSN. Typically, 0.24 g of MSN was dispersed in 4 ml of aqueous solution containing 80 mg of GTMC. Subsequently, 1.68 g of bone cement powder was immersed into the aqueous suspension to form slurry under stirring. The wet mixture was dried under vacuum at room temperature.

The samples of antibiotic loaded bone cement listed in Table 1 were prepared by mixing the powder with the liquid monomer in a ratio of 2 g/ml in a bowl in a laminar flow hood, in accordance with the manufacturer's instruction. Monomer liquid was added to the polymer–GTMC–MSN mixture in a bowl and was stirred using a spatula until the powder was fully wetted. The soft mixture was inserted into the mold with dimensions of 6 mm in diameter and 12 mm in height. The filled mold was pressed between two glass plates for hardening overnight at room temperature. The hardened bone cement cylinders were pulled out of the mold and stored under sterile conditions at room temperature for in vitro drug release tests and compression tests. In addition, rectangular beams with dimensions of 25 × 10 × 2 mm were prepared for bending tests and antibacterial property tests.

2.3 In vitro drug release study

The drug release study was conducted by soaking three cylindrical samples of each composition in 5 ml PBS buffer (pH 7.2). The sample was put in an incubator shaker operated at 37°C and 40 rpm. The release medium was withdrawn at predetermined time intervals, and replaced with fresh PBS buffer (5 ml) each time. The accumulative amount of gentamicin released was calculated based on the initial weight of the bone cement cylinder and the drug content. The gentamicin release was followed for 80 days.

Table 1 Composition of MSN functionalized bone cements

Sample	Simplex-P Powder (g)	MSN (g)	GTMC (mg)	MMA (ml)	MSN%	Drug%
B-1	1.68	0.24	80	1.0	8.15	2.72
B-2	1.76	0.16	80	1.0	5.44	2.72
B-3	1.84	0.08	80	1.0	2.72	2.72
B-4	1.68	0.24 (SM)	80	1.0	8.15	2.72
B-5	1.68	0.24 (SBA-15)	80	1.0	8.15	2.72

An indirect method was used for measurement of the gentamicin concentration by UV–Vis spectrophotometer (Cary 50, Varian Co) because gentamicin does not absorb ultraviolet nor visible light. The *o*-phthaldialdehyde was used as a derivatizing agent to react with the amino groups of gentamicin and yield chromophoric products [26]. The reaction was carried out by making 1 ml of our problem gentamicin in solution react with 1 ml of isopropanol (to avoid the precipitation of the products formed) and 1 ml of *o*-phthaldialdehyde reagent. After full mixing, the concentration of gentamicin sulphate was determined by the UV absorbance at 332 nm.

2.4 In vitro cytotoxicity assay

3T3 mouse fibroblasts cells (3T3-Swiss albino, ATCC) were cultured in a complete growth culture medium in a 5% CO_2 incubator. The complete growth culture medium was prepared with Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum, 1 mm L-glutamine and penicillin (100 U/ml). Cell viability testing was carried out via the reduction of the MTT reagent (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide, Sigma). This assay provides a simple method for determining comparative cell viability using a standard microplate absorbance reader. The MTT assay was performed with the samples placed at the bottom of a 96-well plate following the standard procedure with minor modifications. Control experiments were carried out using the complete growth culture medium only (serving as non-toxic control) and 1% Triton X-100 (Sigma) (as toxic control). 3T3 fibroblasts in the complete growth culture medium (100 µl) were seeded at a density of 10^4 cells/well in a 5% CO_2 incubator for 24 h. The culture medium from each well was then removed and 100 µl of medium and 20 µl MTT solutions (5 mg/ml in PBS) were then added to each well. After 4 h of incubation at 37°C and 5% CO_2, the media were removed and the formazan crystals were solubilized with 100 µl dimethyl sulfoxide (DMSO, Sigma) for 15 min. The optical absorbance was then measured at 570 nm on a microplate reader (Tecan GENios). Six samples were tested for each type of bone cement.

2.5 Viability of bacteria on bone cement surface

The rectangular bone cement beams with dimensions of 25 × 10 × 2 mm were immersed in SBF buffer for 2 weeks prior to antibacterial tests. The viability of bacteria on the surface of the bone cements was investigated by staining with a combination dye (LIVE/DEAD *Bac*light bacteria viability kits, Molecular Probes, L13152). After immersion in the bacteria suspension of 10^8 cells/ml in broth at 37°C for 3 h, the substrates were washed with water and stained using 50 µl of the combination dye (propidium iodide (PI) and SYTO 9) and subsequently analyzed with a Leica DMLM microscope with a 100 W Hg lamp. The viable and non-viable cells can be distinguished under the fluorescence microscope since the viable cells appear green under the light microscope while non-viable or membrane compromised cells appear red.

2.6 Testing of mechanical property

Three point bending tests were performed on the Instron universal materials testing machine (Model 5544). According to the standard test method of ASTM D790-3, the span length was 20 mm and loading rate was 1 mm/min. The bending modulus (E_B) was calculated according to the following equation: $E_B = L^3m/4bd^3$, where L is the support span (mm), b is width of beam tested (mm), d is depth of beam tested (mm), and m is slope of the tangent to the initial straight-line portion of load–deflection curve (N/mm).

The compression tests were carried out on the bone cement cylinders with same dimensions as that for drug release investigation [27]. The compression force was applied along the axis using a crosshead speed of 5 mm/min. The compression strength was calculated from the obtained load-deformation curves. The compression strength (CS) was calculated using following equations

$$CS = F/A$$

where F is the applied load (N) at the highest point of the load–deflection curve and A is the cross-section area of the sample tested.

2.7 Absorption study

The absorption of simulated body fluid (SBF) was studied for samples with varied MSN content without the loading of GTMC in the bone cement matrix, in order to avoid the weight loss due to drug release. SBF was prepared to have a composition very similar to the human plasma (mixed solution of: 136.8 mM of NaCl, 4.2 mM of $NaHCO_3$, 3 mM of KCl, 1.0 mM of $K_2HPO_4{\cdot}3H_2O$, 1.5 mM of $MgCl_2{\cdot}6H_2O$, 2.5 mM of $CaCl_2{\cdot}H_2O$ and 0.5 mM of Na_2SO_4). The solution was buffered at pH 7.4 with 50 mM of tri(hydroxymethyl)aminomethane). The bone cement samples were weighed precisely before being soaked in 5 ml of SBF at 37°C. At certain intervals, the samples were taken out from the SBF solution and its surface was wiped with paper to remove excess liquid. The samples were weighed and re-immersed in the SBF solution until the next data reading point. The absorption measurements were performed for 168 h and the percentage of SBF absorbed was calculated as $M_t/M_0 \times 100$ (%), where M_t is the mass of SBF uptake at time t and M_0 is the initial mass prior to soaking in SBF solution.

2.8 Characterization

The external and fracture surface of the bone cements were examined by high resolution scanning field emission electron microscope (SEM, JSM-6700F, JEOL, Japan) operating at 5 kV. Prior to analysis, the samples were sputter coated with platinum for 1 min by a sputter coater (Cressington Sputter Coater 208HR, UK). High resolution transmission electron microscopy (TEM) images were taken by a TECNAI F20 (G^2) (FEI, Philips Electron Optics, Holland) electron microscope operating at 200 kV. Nitrogen adsorption/desorption isotherms were measured by using an Autosorb-6B gas adsorption analyzer (Quantachrome) at a temperature of −196°C. Before nitrogen adsorption–desorption measurements, each sample was heated at 300°C under vacuum for 3 h. The specific surface areas of the samples were determined from the linear portion of the Brumauer–Emmett–Teller (BET) plots. The total pore volume, V_T, was estimated from the amount adsorbed at a relative pressure of 0.95.

3 Results and discussion

3.1 Characterization of MSN and drug loading

Figure 1 illustrates the morphology of MSN revealed by TEM and SEM measurement. The well dispersed mesoporous silica exhibits a rod-like morphology and the particle size is about 100–400 nm in length and 100 nm in diameter. The TEM image shows the mesoporous pore channels uniformly arranged along the axial direction of the rod-like nanoparticles. The pure MSN shows a high capacity for N_2 adsorption as well as a large pore volume of 1.01 cm^3/g as well as a high surface area of 734 m^2/g. As the original PMMA-based bone cement powder is a non-porous material with low pore volumes (0.010 cm^3/g) and low surface areas (3.49 m^2/g), most of the GTMC would be entrapped into the nanoporous structure of the MSN after the impregnation of GTMC together with MSN and bone cement powder mixture. As compared with directly mixing GTMC crystal particles with PMMA powder, the MSN loaded GTMC formulated with bone cement powder can achieve a higher homogeneity of drug distribution in the bone cement matrix.

3.2 In-vitro release of GTMC from MSN incorporated bone cement

Figure 2 displays the effect of MSN content on GTMC release profiles of the bone cement samples and compared with commercial GTMC formulated bone cement, Smartset GHV. It is noticed that the commercial Smartset GHV exhibits a very limited drug release. Only about 5% of GTMC is observed to be released in the first day of

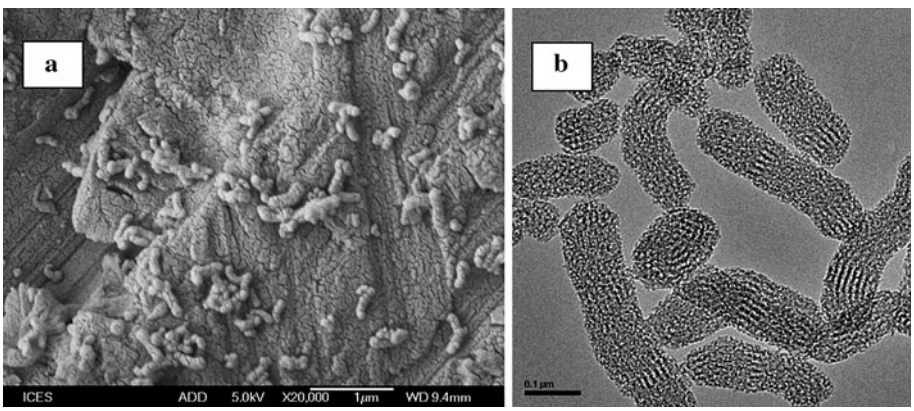

Fig. 1 Morphology of MSN particles: **a** FESEM image and **b** TEM image

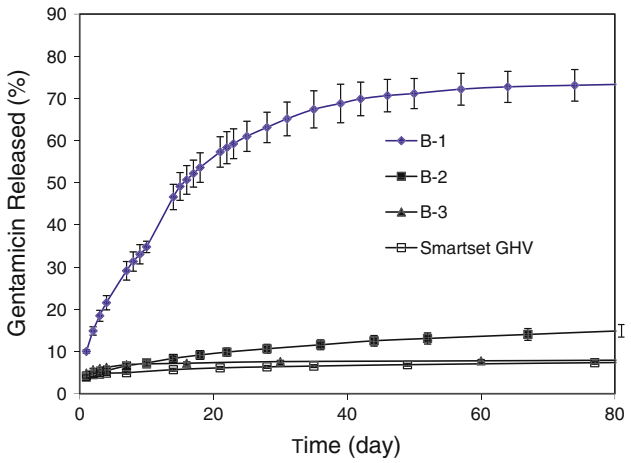

Fig. 2 Effect of MSN content on GTMC release profile and compared with commercial antibiotic Smartset GHV bone cement

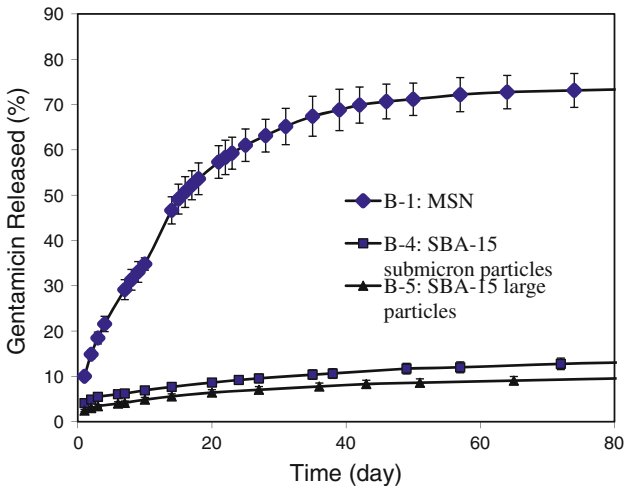

Fig. 3 Effect of particle size of mesoporous silica on release profiles of bone cement

immersion in PBS solution. No GTMC release was detectable in the following 80 days of investigation. When the sample is formulated with MSN filler, the release from the MSN–bone cement composite can be enhanced. However, the release rate is obviously affected by the content of MSN in the final composite. The sample B-3 does not exhibit an obvious improvement on the release of GTMC although it contains 2.72 wt% of MSN in the bone cement. With content of MSN increased to 5.44 wt%, the total release of GTMC is slightly increased to about 15% in 80 days. When the MSN content is increased to 8.15 wt% in the formulation, the release of GTMC from the sample B-1 is significantly enhanced. After 10% of release in first day, sustained release of GTMC from the MSN incorporated bone cement is observed in 80 days and reaches about 70% release. The MSN incorporated in the bone cement is believed to form a nano-network path for GTMC diffusion from the composite to the surface and released to the medium. For GTMC formulated bone cement in the absence of MSN, GTMC particles are embedded inside the PMMA matrix during polymerization and cannot diffuse to the surface of the bone cement for release. For the GTMC-MSN loaded bone cement composite, most of the GTMC was entrapped inside the mesoporous channels of the rod-like MSN. A critical content of MSN inside the bone cement is required to build up the network for GTMC to diffuse from matrix to medium. When the content of MSN is below 6 wt%, most of the GTMC loaded MSN are isolated and also embedded in the bone cement matrix. The release of GTMC from bone cement composite cannot then be obviously improved. Only GTMC loaded MSN on shallow surface of bone cement can contribute to drug release. Once the MSN content is increased to 8 wt% or above, a nano-network is built up through "particle–particle" contact and allows GTMC molecules to continuously diffuse from the PMMA-based matrix to the surface and be release into medium, thus the release profile of GTMC is obviously improved. It should be noted that because the diffusion rate is also limited by the nano sized pore channels in network, the drug release is well controlled and achieves a sustained release of GTMC from MSN functionalized antibiotic bone cement over 80 days. This long term sustained release has not been achieved by using calcium-phosphate bone substitutes [28, 29] or bioactive ceramic-polymer composites [30], which allowed GTMC to release much faster and complete most of its release in the first few days or several hours.

To build up an effective diffusion network for the sustained release of GTMC from bone cement matrix, the particle size of mesoporous silica is also found to be important in the formulation. As shown in Fig. 3, sample B-1 formulated using MSN with the smallest particle size exhibits the highest release rate. As a comparison, samples of B-4 and B-5 are formulated with mesoporous silica with particle size at submicron (0.5–0.8 μm) and tens of micrometers, respectively [24]. Although the content of silica (wt%) is the same as B-1, samples of B-4 and B-5 exhibit only 13% and 9% GTMC release in 80 days. The results imply that the larger particles could not build up an effective diffusion network for effective release of GTMC. Due to the larger particle size of mesoporous silica at submicron and tens of micrometer scales, the number of particles is much less than that of MSN with the same content of silica by weight. The fewer mesoporous silica particles and less homogeneity of distribution of those larger particles fails to build up an effective diffusion network by particle–particle contact, thus the release profiles of GTMC is far below that of antibiotic bone cement functionalized by MSN fillers.

Due to the nano-sized network of MSN functionalized bone cement, the GTMC release kinetics are different from the reported results of GTMC formulated PMMA-based bone cement with hydroxyapatite content of up to 40%, where GTMC could reach 25% of release in the first 10 h [17]. In this study, a more controllable drug release could be achieved due to the smaller nano-network in the bone cement matrix controlling the diffusion rate. Only up to 10% of drug is released in the first 24 h of immersion, and a sustained release in the later stages is more important to perform antibacterial activity in the long term. As the drug release kinetics is mostly controlled by diffusion, thus the release could be expressed as: [31]

$$\frac{M_t}{M_{tot}} = 1 - \sum_{n=1}^{\infty} \frac{4}{a^2 \alpha_X^2} \exp\left(-D\alpha_n^2 t\right) \sum_{m=0}^{\infty} \frac{8}{l^2 \beta_m} \exp\left(-D\beta_m^2 t\right) \tag{1}$$

where M_t is the GTMC mass released at time t, M_{tot} is total GTMC mass in the sample, a is the radius and l is the height of cylinder. The terms β_m are defined as: $\beta_m = (2m+1)\,\pi/l$ and the terms α_n are the positive roots of $J_0(a\,\alpha_n) = 0$. Here J_0 is the zero-order Bessel function of the first kind and $a\alpha_n$ are the zeros of that function.

For sustained and controlled release at long times, only the first term of each sum will contribute in Eq. 1, thus:

$$\frac{M_t}{M_\infty} = 1 - \frac{32}{a^2 l^2 \alpha_1^2 \beta_0^2} \exp[-D(\alpha_1^2 + \beta_0^2)t] \tag{2}$$

and Eq. 2 can be expressed as

$$\ln\left(1 - \frac{M_t}{M_\infty}\right) \propto -D\left(-\alpha_1^2 + \beta_0^2\right)t \tag{3}$$

when $\ln(1-M_t/M_{tot})$ is plotted as a function of time, it is observed that MSN formulated samples show good linear fit in this plot. The diffusion coefficients can be calculated by plotting $\ln(1-M_t/M_{tot})$ as a function of time:

$$D_{B-1} = 6.2 \times 10^{-13}\mathrm{m}^2/\mathrm{g};\ D_{\mathrm{smartset}} = 4.9 \times 10^{-15}\mathrm{m}^2/\mathrm{s}$$

$$D_{B-2} = 1.9 \times 10^{-14}\mathrm{m}^2/\mathrm{s};\ D_{B-3} = 3.2 \times 10^{-15}\mathrm{m}^2/\mathrm{s}$$

The results indicate that the sample B-1 possesses the highest diffusion coefficient among these MSN functionalized bone cement and commercial antibiotic bone cement Smartset GHV. The high diffusion coefficient is attributed to the effective diffusion network structure formed in the presence of MSN in the composite bone cement matrix. The diffusion coefficient is smaller than that reported in hydroxyapatite formulated PMMA-based bone cement [17], as the nano-network in MSN functionalized antibiotic bone cements can control the diffusion rate of the antibiotic molecules.

Figure 4 compares the morphology of the original Simplex-P and MSN functionalized bone cements. Both external and fracture surfaces were examined by SEM measurement. It is observed that both the external and fracture surfaces of the original Simplex-P bone cement are smooth and condensed. As a comparison, a large number of micropores are formed on the external surface of MSN functionalized bone cement and a microporous structure is also observed on the fracture surface. The pore size is smaller than 1 μm. It was reported that the pore size of the bone cement containing a large portion of hydroxyapatite ranged between 3 and 45 μm [17], thus the larger pore size resulted in a faster drug release than the MSN functionalized bone cement. The microstructure created by the incorporation of MSN into the PMMA matrix is believed to facilitate the release of antibiotics compared with the original PMMA bone cement and the small pore size also contributed the controlled sustained release. As seen in Fig. 4e and f, after the 80 days of drug release tests in PBS buffer, the basic framework of the MSN formulated bone cement was not changed although some calcium species could be deposited on the surface or internal structure after being dried. The microporous structures were still observed on the fracture surface. As compared with the SEM taken before drug release, no larger size voids were formed after drug release.

Figure 5 shows the percentage of SBF uptake as a function of time for samples with varied MSN contents and particle size. It is noticed that sample B-1 exhibited the most outstanding absorbance of SBF among all tested samples. The enhanced capacity of SBF uptake of MSN formulated bone cement B-1 indicated the efficient diffusion paths created by MSN incorporation. The SBF absorbance for sample B-1 achieved equilibrium at 2.7%. By comparison, the samples (B-2, B-3) with MSN content below 6.0 wt%, or the sample with larger mesoporous silica particles (B-5) only achieved equilibrium of 1.2–1.3% SBF uptake, as their diffusion network was not efficiently built up. The release of antibiotics from the PMMA-based bone cement was to a great extent dependent on the penetration of dissolution fluid into the polymeric matrix to dissolve drugs, followed by diffusion from cement to medium [32]. The efficient network built up by MSN incorporation in the bone cement matrix facilitates penetration of fluid and diffusion of antibiotics, thus corresponding to a significant enhancement in the drug release profile of sample B-1.

3.3 Mechanical properties of GTMC-MSN loaded bone cements

The mechanical properties of the acrylic bone cement are crucial factors to determine its application in orthopedic

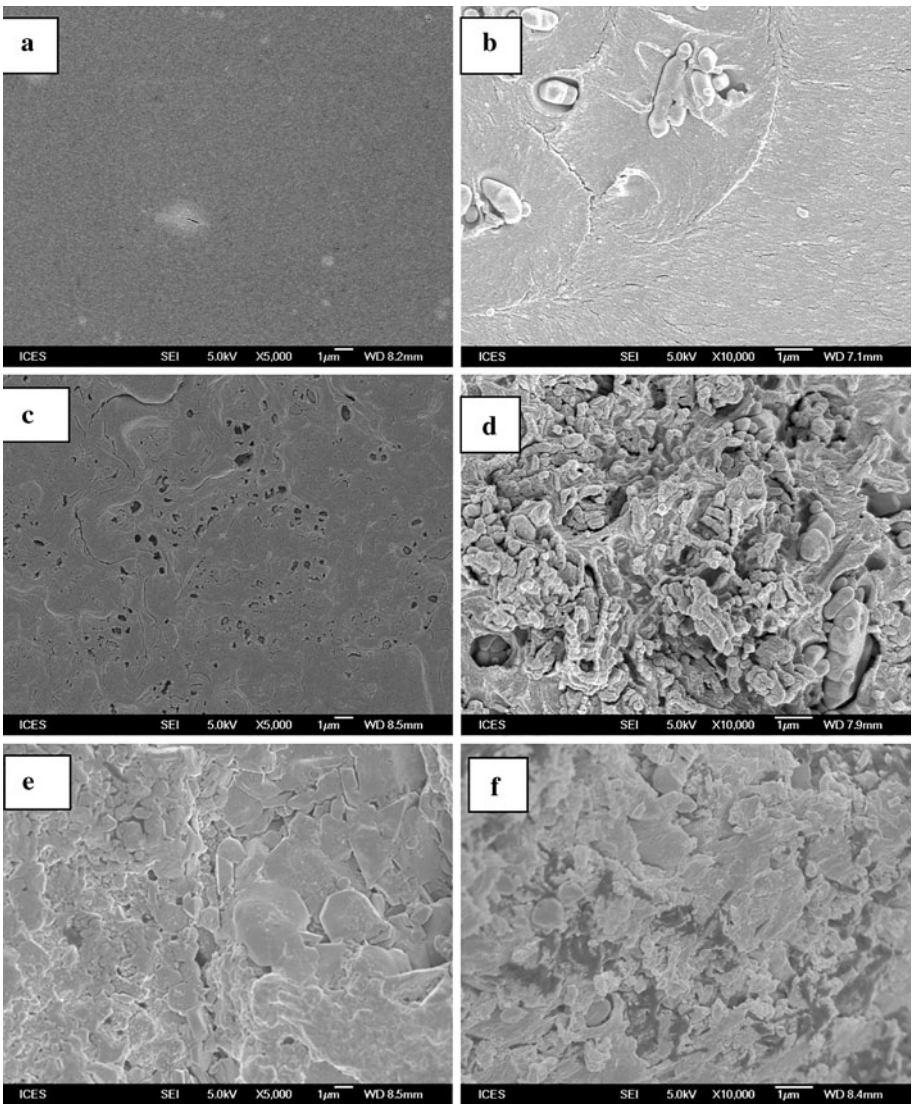

Fig. 4 SEM images of: **a** external surface and **b** fracture surface of Simplex P bone cement; **c** external surface and **d** fracture surface of sample B-1; **e** external surface and **f** fracture surface of sample B-1 after drug release for 80 days

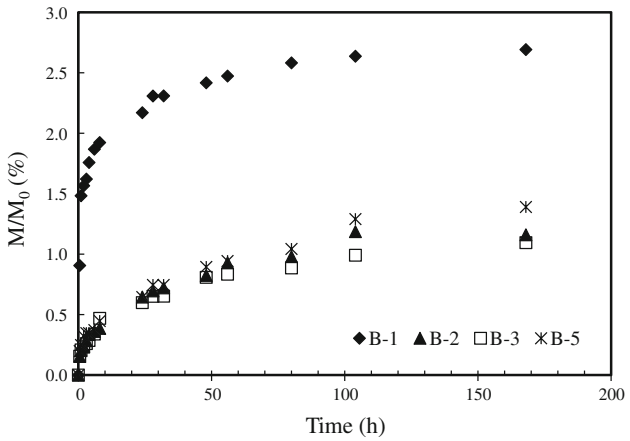

Fig. 5 Percentage of SBF uptake as a function of time

surgery for successful long-term stability of prosthesis. It is found that the bending modulus is slightly affected by the incorporation of MSN in bone cement matrix. For sample B-1 with 8.15 wt% of MSN, the bending modulus is about 90% of the corresponding properties of the original bone cement while sample B-1 exhibits significantly improved drug release profiles. It is also observed that the compression strength is negligibly affected by the incorporation of MSN into antibiotic bone cement. The compression strength of the original bone cement is 93.3 ± 1.5 MPa, while sample B-1 shows 93.5 ± 6.1 MPa, which is above the ASTM F541 and ISO 5833 minimum of 70 MPa. Although some forms of antibiotic loaded bone cement were reported to exhibit excellent drug delivery profiles, they failed to be used in clinical practice because of their negative influence on the mechanical properties of PMMA [33]. On other hand, it was found that the drug release rate could be significantly enhanced by the incorporation of a large amount of hydroxyapatite (up to 35 wt%) [17, 34], however, the mechanical properties of the formulated bone cement were not reported. We tested the mechanical

properties of the hydroxyapatite formulated bone cement and found that its compression strength decreased to about 50% of the original PMMA-based bone cement when 32 wt% of hydroxyapatite was incorporated. The larger pore size of the bone cement containing large portions of hydroxyapatite at 3–45 μm [17] could contribute to its weaker mechanical properties. In this study, as seen in Fig. 4, the microporous (<1 μm) structure created by incorporation of MSN in the PMMA-based bone cement matrix does not cause observable detriment on the strong mechanical properties while the functional fillers of MSN built up a diffusion network for effective local delivery of antibiotics. After drug release in PBS medium for 80 days, the compression strength of B-1 is 84.0 ± 2.7 MPa, which is acceptable for application. The drug was loaded inside the hollow nanostructures of the MSN. After drug release through nano-channel diffusion, the MSN still remains in the matrix of the bone cement and supports the structures, thus its mechanical properties could be well maintained. This formulation overcomes the disadvantages of formulation by high drug loading or liquid form antibiotic formulations [35–37], where the drug release resulted in large portions of voids in the bone cement and the mechanical properties were negatively affected. As seen in Fig. 4e, f, the microstructure is not substantially changed after drug release. The drug release in this study does not create extra voids outside the MSN and thus the mechanical properties of the bone cement can be well preserved after drug release.

3.4 Antibacterial assay

After immersion in PBS solution for 2 weeks, the antibacterial property of formulated antibiotics bone cement was compared with commercial antibiotic bone cement treated under the same conditions. The distribution of viable and dead bacteria which adhered on the surface of the substrates after immersion in the bacterial suspension of 10^8 cells/ml for 3 h at 37°C was observed via staining with the combination dye. As shown in Fig. 6, the fluorescence microscopy images of sample B-1 is compared with Smartset-GHV bone cements after exposure to *Staphylococcus aureus*. The presence of a large portion of viable cells (stained green) can be observed on the Smartset–GHV bone cement surface, while at the same time, there are very few dead cells (stained red). As a comparison, the number of live cells on sample B-1 is significantly decreased and a large portion of dead cells can be seen. The stronger antibiotic property of sample B-1 is attributed to its enhanced drug delivery capability. The drug release profile of sample B-1 indicated that gentamicin could sustain its release after 2 weeks of drug release investigation. The antibiotic gentamicin formulated in MSN functionalized bone cement diffuses from bulk to the surface and reduces the viable rate of bacterial cells adhered on the surface. However, as indicated in Fig. 2, the commercial Smartset–GHV bone cement almost stops the release of antibiotics on the second day of immersion in PBS buffer. The antibiotics embedded in the bone cement cannot diffuse to the surface of the bone cement, and thus more bacterial cells adhered on the surface of the bone cement could be viable. In this study, the antibiotics diffused to the external surface of the bone cement effectively reduced the survival of bacteria adhered. Once MSN functionalized bone cement is applied for fixing metal implantations, sustained local release of antibiotics to body fluid can protect the surrounding tissues from infection. It has been reported that chitosan nanoparticles formulated bone cement could effectively prevent viable bacteria surviving on the surface of the bone cement [2]; however, the natural polymer of chitosan could not be released to the body fluid to protect surrounding tissues from postoperative infection.

3.5 Cytotoxicity assay

As the ultimate purpose of this work is to develop antibacterial bone cement for implanted materials, the cytotoxicity of the modified bone cement is essential to evaluate the materials for clinical applications. The MTT cytotoxicity of sample B-1 is assayed using 3T3 mouse fibroblasts and the

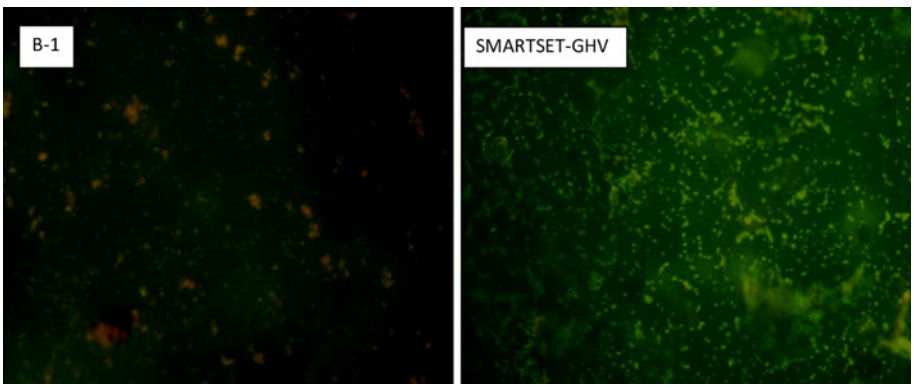

Fig. 6 Fluorescence microscopy images of B-1 and Smartset GHV bone cements after immersion in bacterial suspension (10^8 *Staphylococcus aureus* cells/ml, for 3 h at 37°C)

measurement is compared with the PMMA-based bone cement without modification. The 3T3 cells are selected for this assay as their viable rates are substrate-dependent and they are non-specific cell lines. The MSN formulated sample B-1, which exhibits the highest drug release rate, are not significantly different from that of the non-toxic control (growth culture medium) and commercial PMMA bone cement. It is observed that 91% of 3T3 cells can be viable on the pure MSN material. With 8.15 wt% MSN in bone cement formulation, a 96% of cell viability rate is observed on sample B-1, while the PMMA bone cement without MSN in formulation shows a 98% cell viability rate.

4 Conclusions

An innovative formulation based on functionalizing PMMA-based bone cement using mesoporous silica nanoparticles (MSN) as drug carrier and novel filler is developed to enable a highly efficient, sustained and localized delivery of antibiotics. MSN performs as drug carriers and novel fillers for the bone cement to form an effective diffusion network in the PMMA-based matrix. The effective diffusion network built up in the PMMA-based matrix significantly enhanced the release profiles of GTMC and an extended release is achieved. At 8.15 wt% MSN content, more than 70% of the total GTMC loaded can be released in a well controlled manner for 80 days. Unlike other fillers reported, it may be worthwhile to note that the microstructure and nano-network formed by MSN fillers does not result in an obvious detrimental effect on the critical weight-bearing mechanical properties, such as the bending modulus and compression strength of bone cement. The MSN-formulated bone cements also exhibit low cytotoxicity to 3T3 fibroblast cells as well as a sustained and enhanced antibacterial effect, which suggested its suitability to be considered for preclinical investigations.

Acknowledgment This work was generously supported by Institute of Chemical Engineering and Science, Agency of Science Technology and Research, Singapore.

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