Bioactive inorganic-materials/alginate composite microspheres with controllable drug-delivery ability

Chengtie Wu,[1] Yufang Zhu,[2] Jiang Chang,[3] Yufeng Zhang,[1] Yin Xiao*[1]

1. Institute of Health & Biomedical Innovation, Queensland University of Technology, Brisbane QLD 4059, Australia;

2. ICYS-Sengen, National Institute for Materials Science, 1-2-1 Segen, Tsukuba Ibaraki 305-0047, Japan.

3. Biomaterials and Tissue Engineering Research Center, Shanghai Institute of Ceramics, Chinese Academy of Sciences, 1295 Dingxi Road, Shanghai 200050, People's Republic of China

* Correspond author: yin.xiao@qut.edu.au (Y. Xiao)
Tel: 61-7-31386240; Fax: 61-7-31386030

Abstract

Alginate microspheres are considered a promising material as a drug carrier in bone repair due to excellent biocompatibility, but their main disadvantage is low drug entrapment efficiency and non-controllable release. The aim of this study was to investigate the effect of incorporating mesoporous bioglass (MBG), non-mesoporous bioglass (BG) or hydroxyapatite (HAp) into alginate microspheres on their drug-loading and release properties. X-ray diffraction (XRD), transmission electron microscopy (TEM), scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), and atomic emission spectroscopy (AES) were used to analyse the composition, structure and dissolution of bioactive inorganic materials and their microspheres. Dexamethasone (DEX)-loading and release ability of four microspheres were tested in phosphate buffered saline with varying pHs. Results showed that the drug-loading capacity was enhanced with the incorporation of bioactive inorganic materials into alginate microspheres. The MBG/Alginate microspheres had the highest drug loading ability. DEX release from alginate microspheres correlated to the dissolution of MBG, BG and HAp in PBS, and that the pH was an efficient factor in controlling the DEX release; a high pH resulted in greater DEX release, whereas a low pH delayed DEX release. In addition, MBG/alginate, BG/alginate and HAp/alginate microspheres had varying apatite-formation and dissolution abilities, which indicate that the composites would behave differently with respect to bioactivity. The study suggests that microspheres made of a composite of bioactive inorganic materials and alginate have a bioactivity and degradation profile which greatly improves their drug delivery capacity, thus enhancing their potential applications as bioactive filler materials for bone tissue regeneration.

Key words: Drug release, Mesopore bioglass; Bioactivity Alginate

INTRODUCTION

The use of bioactive microspheres as protein/drug carriers and bone filling materials has received much attention in recent years.[1-4] Compared to traditional macroporous block scaffolds, the main advantage of microspheres is that they not only possess better drug-delivering properties, but also the ability to fill bone defects of irregular and complex shapes and sizes. However, for microspheres to become a *bone fide* application in bone-tissue regeneration there are three major issues that needs to be resolved: (i) controllability of drug-release; (ii) bioactivity; and (iii) degradability. At present, the key obstacles concerning the clinical use of both polymer[5,6] and ceramic[3,7,8] microspheres in bone-tissue regeneration, is the sub-optimal combination of these three properties.

Calcium alginate is considered one of promising material as a delivery matrix, because gel beads forms readily in aqueous solutions at room temperature, without the need for organic solvents.[2,9] An added attraction is that alginate gels dissolve under physiological conditions, and they have been demonstrated to be biocompatible.[10,11] For these reasons, alginate has been proposed as a potential bone regeneration material, when prepared as a composite with hydroxyapatite (or β-tricalcium phosphate), which greatly improves its biomechanical and bioactive properties.[12-14] However, it falls well short as a drug carrier, owing to the fact that calcium alginate microspheres have low drug entrapment efficiency and non-controllable release kinetics.[15] Mesoporous bioactive glass is a novel class of bioglass with a highly ordered mesopore channel structure and pore sizes ranging from 2-50 nm.[16] A prominent feature of MBG is its greater surface area and pore volume, greatly enhanced drug-delivery kinetics,[17] and superior bioactivity compared to non-mesoporous bioglass (BG).[16] In a previously published study we have shown that MBG dissolves at a faster rate than

BG.[18] HAp is a well known inorganic bioactive material and its rate of dissolution is slower still than that of bioactive glass. In this study we therefore set out to test whether an alginate composite with MBG, BG, or HAp, would result in materials with an improved bioactivity and controllable drug-release profile. We hypothesized that this might be the case, by virtue of their different rates of dissolution, and also given that alginate is a pH sensitive gel and that its rate of dissolution can be adjusted by pH.[9,19,20] The principal aim of this study was therefore to incorporate MBG, BG or HAp into alginate microspheres in order to control their drug-loading and release properties.

MATERIALS AND METHODS

Synthesis and characterization of MBG, BG and HAp powders

Mesoporous Bioglass (MBG) powders (molar ratio: Si/Ca/P = 80/15/5) were synthesized according to our previous publication.[18,21] In a typical synthesis, 4.0 g of P123 (EO_{20}-PO_{70}-EO_{20}, Mw=5800, Sigma), 6.7 g of tetraethyl orthosilicate (TEOS), 1.4 g of $Ca(NO_3)_2{\cdot}4H_2O$, 0.73 g of triethyl phosphate (TEP, 99.8%, Sigma) and 1.0 g of 0.5M HCl were dissolved in 60 g of ethanol (Si/Ca/P = 80:15:5, molar ratio) and stirred at room temperature (RT) for 1 day. The resulting solution was introduced into a petri dish for an evaporation-induced self-assembly process, and then the dry gel was calcined at 700 ºC for 5 hr to obtain MBG powders. Non-mesoporous bioglass (BG) powders were synthesized without the addition of P123 under the same conditions and used as a control. The synthesized MBG and BG powders were ground and sieved to 230 meshes, and the inner microstructure of the powders was inspected by TEM.

The hydroxyapatite (HAp) powders were synthesized by a chemical precipitation method. Briefly, 0.1M $Ca(NO_3)_2$ water solution (100ml) was dropped into 0.06M $(NH_4)_2HPO_4$ solution

(100ml) under stirring for 12 hr and maintained a pH above 10. The resulting powders were filtered and washed three times in water and once in ethanol, then dried at 60 ºC for 24 hr and calcined at 800 ºC for 2 hr. The HAp powders were analyzed by TEM and XRD and surface area analyzed by Brunauer-Emmett-Teller (BET) method.

Preparation and characterization of DEX-loaded Alginate, MBG/Alginate, BG/Alginate and HAp/Alginate microspheres

Dexamethasone (DEX) (Sigma-Aldrich, Australia) was used for the drug loading and release kinetics studies, at a final concentration of 0.1mg/ml in water (S/L). 3.125g of MBG, BG or HAp powders were added to 40ml DEX solution and stirred for 4 hr. Alginate powder (viscosity 20-40Cp, Sigma), was dissolved in water at a 3% (w/v) concentration, and the alginate solution was then added to all of MBG, BG and HAp slurries, stirred for 1 hr and ultra-sonicating 5 min to form homogenous mixtures. The mass ratio of inorganic powders/alginate is 1.67. The mixtures were extruded drop-wise with a 0.65mm diameter needle into a 0.1 M $CaCl_2$ crosslinking solution to form spherical particles. The microspheres were hardened by 30 min immersion in the crosslinking solution, then filtered and dried at 50ºC overnight to obtain DEX-loaded composite microspheres. The $CaCl_2$ solution from the filtering step was collected for later assaying of the residual DEX content. Pure alginate microspheres were prepared without adding any inorganic bioactive powders using the same procedure as above. The surface morphology and microstructure of the dried microspheres was analyzed by SEM and their components analyzed by FTIR.

In vitro acellular tests in SBF of four types of microspheres

A short term *in vitro* bioactivity study was carried out using acellular simulated body fluids (SBF).[22] SBF has similar ion concentrations to human blood plasma. Briefly, reagent-grade $CaCl_2$,

$K_2HPO_4 \cdot 3H_2O$, NaCl, KCl, $MgCl_2 \cdot 6H_2O$, $NaHCO_3$, and Na_2SO_4 were dissolved in distilled water and pH adjusted to 7.4 with HCl and Tris. The four different microspheres species were immersed in SBF, and kept under static conditions at 37 ºC for 5 days, after which they were dried at 60ºC for 1 day and analyzed by SEM and energy dispersive spectrometer (EDS), to determine their apatite-formation abilities.

The effect of bioactive microspheres on the proliferation of bone marrow stromal cells (BMSCs)

Isolation and culture of BMSCs was conducted following previously published protocols.[23] BMSCs ($1x10^5$/well) were seeded on 96-well template and allowed to adhere to the template for 3 hr. Then, microspheres were added to the template. The cells were incubated for 3 and 7 days, at which point the medium was replaced with 20 μL of CellTiter 96 Aqueous One Solution Reagent (Promega, Genesearch, QLD, Australia) in 100 μL PBS. After 4 hr in culture, the cell viability was determined by measuring the absorbance at 490 nm on a 96 well plate-reader.

The loading efficiency of DEX in four microspheres

To evaluate the DEX-loading efficiencies of the four microsphere species, the $CaCl_2$ solution was collected after the microspheres had been filtered, to test the residual DEX contents (R_{DEX}) by UV/VIS. The loading efficiency (LE%) of DEX was calculated using the following equation: $LE\% = ((O_{DEX} - R_{DEX})/O_{DEX}) * 100\%$, where O_{DEX} was the original DEX contents in the solution prior to preparing the microspheres.

DEX release from the microspheres in PBS with different pH value

To evaluate what effects the different bioactive inorganic materials and pH environment had on DEX release, the DEX-loaded microspheres (0.6g for alginate composite microspheres, 0.225g for pure alginate microspheres) were placed in 5ml phosphate buffered saline (PBS) (pH = 4.3, 7.4 and

8.6) at 37 ºC for 1, 3, 6 hr, and 1, 3, 7, 11 and 18 days. The PBS pH was adjusted with HCl and Tris. At each time point, the entire 5ml PBS solution was taken off and replaced with 5ml of fresh PBS. The amount of DEX released in the PBS was then assayed by UV/VIS at the wavelength of 241nm. Three samples from each microsphere species were taken for mean and standard deviation calculation.

Ions release and morphology change of microspheres in PBS with different pH value

To evaluate what effect pH had on the ions-release ability (dissolution) of microspheres in PBS, the accumulative ions release for Ca, Si and P ions in PBS at days 1, 3 and 7 were tested by inductively coupled plasma atomic emission spectroscopy (ICP-AES). The morphology change of the different microspheres, after 18 days in PBS, was analyzed by SEM.

Statistical analysis

The data was expressed as means ± standard deviation (SD) for all experiments and were analyzed using one-way ANOVA with a post hoc test, where p-value < 0.05 was considered statistically significant.

RESULTS

Characterization of MBG, BG and HAp powders

The inner structure and particle morphology of the synthesized bioactive inorganic powders were analysed by TEM, and is shown in Figure 1. The MBG powders had a highly ordered one-dimensional channel structure with a pore size of approximately 5nm (Fig. 1a). In contrast, the BG powders had a disorganized structure, lacking any discernible channels (Fig. 1b). The HAp particles, which had a size of approximately 200 nm, were composed of crystalline structures with a size ranging over several tens of nanometers (Fig. 1c).

The phase composition of the obtained HAp powders is shown in Figure 2. As expected, only the characteristic peaks of HAp (Standard cards No: JCPD 24-0033) were seen in the XRD pattern, proof that the obtained powders were indeed pure HAp.

The surface area of the MBG powder was estimated to be 400m^2/g, which was significantly greater than that of the HAp powder at 68 m^2/g, and BG powder at 57 m^2/g.

Morphology, surface microstructure and components of the four microsphere species

The morphology and surface microstructure of the microspheres is shown in Figure 3. The pure alginate microspheres had a smooth surface and slightly oblong shape, and a size of approximately 600 μm (Fig. 3a and b). The MBG/Alginate, BG/Alginate and HAp/Alginate composite microspheres all had a more uniformly round shape compared to the pure alginate microspheres (Fig. 3c, d, e, f, g and h), the size of all three composites being approximately 800μm. The surfaces of the MBG/Alginate and BG/Alginate microspheres were coarser than the surface of the pure alginate and HAp/Alginate microspheres (Fig. 3c, d, e, f, g and h).

FTIR spectra for the four species revealed the presence of silicon oxide (SiO) in the MBG/Alginate and BG/Alginate composites, and a prominent peak of phosphorous oxide (PO) in the HAp/Alginate composite. Evidence of carbon oxides (CO) and carboxylate (COO^-) from the alginate is seen in all four spectra (Fig. 4).

Apatite-formation capacity of the four microsphere species in SBF

SEM morphology and EDS analysis of the microsphere species after 5 day incubation in SBF is shown in Figure 5. MBG/Alginate microspheres induced apatite particle deposition on their surface (Fig. 5c) and high magnification SEM revealed that apatite particles had formed a porous layer (Fig. 5d). EDS analysis showed that the ratio of Ca/P of this formed apatite was about 1.57. There was no obvious apatite particles formation on the other three microspheres (Fig. 5a, b, e, f, g

and h). The surface of the pure alginate microspheres was smooth and there was no evidence of Ca and P from the EDS analysis (Fig. 5a and b). The BG/Alginate microspheres, similarly, did not induce any apatite deposition and only Si was detected by EDS (Fig. 5e and f). After 5 days in SBF the surface of the HAp/Alginate microspheres appeared to be smoother than prior to soaking. The Ca/P ratio of the SBF exposed HAp particles was 1.67, which is the same stoichiometry as the HAp materials.

The effect of bioactive microspheres on the proliferation of bone marrow stromal cells (BMSCs)

The effect of four microspheres on BMSC proliferation was shown in Figure 6. After incorporation of bioactive MBG, BG and HAp into alginate, the proliferation of BMSC has been significantly enhanced.

DEX-loading efficiency and release of the four microsphere species

Pure alginate microspheres had the lowest DEX-loading efficiency of the four microsphere species with an estimated 39% efficiency (Table 1). Incorporating MBG, BG, or HAp into the microspheres improved their loading efficiencies. MBG/Alginate microspheres showed highest loading efficiency compared to those of HAp/Alginate and BG/Alginate microspheres (Table 1).

The results of the effect of different pH values on the release of DEX from the four materials are shown in Figure 7. The incorporation of MBG and BG into alginate microspheres significantly enhanced DEX release rate across the range of pH environments, and after 18 days of soaking in PBS the MBG/Alginate showed the greatest rate of release of the four microsphere species. Interestingly, with the HAp/Alginate microspheres the DEX release increased between pH 4.3 and 7.4, but then showed a decrease at pH 8.6 (Fig. 7c).

The effect of the pH value of PBS on DEX release from the different microspheres is shown in

Figure 8. With both the MBG/Alginate and BG/Alginate microspheres, DEX release increased with an increase of the pH value in PBS (Fig. 8b and c). There was no obvious effect on DEX release from pure alginate and HAp/Alginate microspheres when pH was between 4.3 and 7.4, however, at pH 8.6, DEX release was significantly enhanced (Fig. 8a and d).

Ions release and morphology changes of the microspheres in PBS with different pH value

The release of Si ions into PBS was greater in MBG/Alginate than in BG/Alginate microspheres and was unaffected by the pH value (Fig. 9a). MBG/Alginate microspheres also released more Ca ions than BG/Alginate microspheres (Fig. 9b). There was an obvious effect of pH on P ions release from HAp/Alginate microspheres (Fig. 9c); at a pH between 7.4 and 4.3 there was a slow increase in P ions release commensurate with soaking time, however, P ions release decreased dramatically at pH 8.6 (Fig. 9c).

The surface morphology of the four microsphere species, after 18 days incubation in PBS with different pH values, is shown in Figure 10. At low pH (4.3) the morphology and microstructure of all four microsphere species remained unchanged (Fig. 10a, c, e and f). At high pH (8.6) there was a clearly discernible change of microstructure of the microspheres. The shape of the pure alginate microspheres became irregular and there were large pores up to 300μm across on the surface these spheres (Fig. 10b). The MBG, BG and HAp composite microspheres retained their round shape (Fig. 10d, f and h). When incubated in high pH PBS, the surfaces of the composite microspheres became coarser than those incubated in low pH PBS. There was also evidence of some micro-particle deposition on the surface of the microspheres incubated in high pH PBS (Fig. 10d, f and h).

DISCUSSION

In this study we have demonstrated that the incorporation of bioactive inorganic materials with

alginate into microspheres enhances their drug-loading ability and release kinetics. The composite microspheres were developed by incorporating MBG, BG or HAp into alginate and controlling the pH environment. These composite microspheres combined better bioactivity, degradability and controllable drug delivery properties, which enhanced their potential for bone tissue regeneration applications.

The major disadvantage of calcium alginate microspheres is large gel porosity which causes leakage of drugs, low drug entrapment efficiency, and non-controllable protein release.[15] In an effort to solve these problems, three different inorganic bioactive materials (MBG, BG and HAp) were successfully incorporated into alginate microspheres. The incorporation of these materials enhanced the drug-entrapment efficiency of alginate, and this was demonstrated by the increased DEX-loading ability of the composite microspheres. The composites had increased surface areas compared to the pure alginate microspheres. MBG/Alginate microspheres possess the highest loading ability. The greater loading efficiencies may therefore be the result of the larger surface areas containing more hydroxyl groups. A study by Xia and colleagues have shown that the drug molecules interact with the surface by hydrogen bonds.[17]

The incorporation of bioactive inorganic materials into alginate not only enhances drug-loading ability, but can also improve the drug release kinetics from microspheres. DEX release rates increased in alginate microspheres containing HAp, BG and MBG, in that order. It is known that the dissolution of materials is one of the most important factors that influence drug release.[24-26] Of the three materials MBG, BG and HAp, it was found that MBG had a more rapid ion release than both BG and HAp, which indicated that MBG had highest dissolution in PBS. It is therefore reasonable to speculate that different rates of dissolution of MBG, BG and HAp may be the main contributing factor influencing DEX release in the composite microspheres.

The pH is another important environment parameter in a drug-delivery system, given that the pH changes occurs locally or at pathological sites within the body, such as in the stomach, the intestines, endosomes, lysosomes, blood vessels, the female reproductive tract, and different tumorous extracellular sites.[27,28] A pH-sensitive drug-delivery system has therefore attracted considerable interest. In this study we found that the pH value of PBS was a crucial factor with which to control the DEX release from the four species of microspheres. Generally, the higher the pH value (alkaline environment) the greater the amount of DEX release, whereas a lower pH value (acidic environment) suppressed the release of DEX. Previous studies have shown that the dissolution of alginate is influenced by the surrounding pH and that a low pH decreases the dissolution of alginate due to the suppressing effect on the dissociation of carboxyl groups in alginate molecules.[19,20] Our results from SEM analysis of the microspheres after soaking in PBS (Fig. 10) clearly indicated that a higher PBS pH induced greater erosion and deformation of the alginate microspheres than did a lower pH value. The pH may therefore predominantly influence the dissolution of microspheres and further control DEX release. Figure 11 illustrates this hypothesis as to how the pH may influence the DEX release in the microspheres.

Interestingly, when the PBS pH was between 4.3 and 7.4, the HAp/Alginate microspheres showed an increased DEX release rate, whereas DEX release decreased at pH 8.6 (Fig. 7c). In addition, P ion release into the PBS also decreased at pH 8.6, suggesting that HAp/Alginate microspheres may absorb P ions from the PBS and form apatite particles on the surface of microspheres. Previous studies have shown that apatite does inhibit drug release due to its nanostructure.[18,29] It is therefore possible that at pH 8.6, some apatite particles may have been deposited on the surface of the HAp/Alginate microspheres, and this might have led to the decrease of DEX release compared to pure alginate microspheres. Although MBG and BG contained alginate

microspheres have higher apatite-formation ability than HAp/Alginate, however, MBG/alginate and BG/Alginate microspheres have quicker dissolution than HAp/Alignate, which results in higher DEX release from MBG/alginate and BG/Alginate microspheres than that from HAp/Alignate and pure alginater microspheres. The local pH condition encountered in bone varies. Previous studies have shown that the local pH around the ruffled border of osteoclasts is about 4.0 during bone remodelling,[30,31] and the homeostatic body fluid is about pH 7.4. Further study showed that the local environment in the initial fracture hematoma is acidic, which later becomes neutral as the healing progresses and ultimately, alkaline which helps to support differentiation-related events in the healing process.[32] Therefore, our study was carried out in PBS buffer with varying pH condition to simulate the local pH environment in bone. Our study has shown that the drug release from bioactive MBG, BG or HAp/alginate microspheres is pH sensitive. These bioactive microspheres may be used for drug carrier to control drug delivery in varying pH condition encountered by bone.

A further disadvantage with using pure alginate for microspheres is the material's failure to retain its original shape.[13] It has been shown that after desiccation, the shape of the pure alginate microspheres could not be maintained due to their low intrinsic strength. However, after incorporating MBG, BG or HAp into the alginate, the native round shape of the microspheres was retained, indicating that the inorganic materials had a reinforcing effect on the microspheres.

There is an unmet demand for bioactive materials, with a range of degradation rates and bioactivities, for applications in bone repair and bone tissue engineering.[33,34] This is why microspheres made from bioactive inorganic materials and alginate composites, such as those described in this study, would be a useful contribution from the point of view of clinical applications. Our previous studies have shown that MBG has a better bioactivity profile than BG,

due to the former's special nano-channel structure.[21] In this study, our results show that MBG/Alginate has better *in vitro* apatite-formation abilities than the three other types of microspheres tested. These results further suggest that MBG containing microspheres may have a greater *in vivo* potential for bone-formation than the other three microspheres species. In addition to this, our results showed that ion release from the MBG containing microspheres was greater than BG and HAp microspheres, which strongly suggest that MBG/Alginate composites may have a more rapid rate of *in vivo* degradation than do BG and HAp microsphere. In addition, the incorporation of bioactive inorganic materials into alginate significantly enhanced BMSCs proliferation. The potential reason may be relative to the stimulatory effect of the released bioactive ions from composites microspheres. Therefore, considering their obvious difference in the drug-delivery properties, bioactivity and degradation of four kinds of alginate microspheres, they may have potential to be used for different clinical applications as bone filling materials. Further *in vivo* experiments will be needed to confirm whether this is indeed the case.

CONCLUSIONS

Bioactive inorganic materials/Alginate composite microspheres were successfully prepared using the method of alginate crosslinking to $CaCl_2$ solution by incorporating MBG, BG or HAp into alginate. The incorporation of bioactive inorganic materials into alginate microspheres showed enhanced drug-loading efficiency. The drug release from alginate microspheres could be controlled by altering the three inorganic materials. In addition to this, the pH was shown to be another efficient tool to control the protein release from the composite microspheres. Therefore, the drug-delivery ability of bioactive inorganic materials/Alginate composite microspheres has been controlled by varying three bioactive inorganic materials and pH environment. MBG/Alginate,

BG/Alginate and HAp/Alginate microspheres possess different bioactivity and degradation ability, indicating their potential application as bioactive filler materials for bone tissue regeneration.

Acknowledgements

The authors would like to acknowledge the Queensland University of Technology Vice-chancellor's Research Fellowship 241402-0120 (Dr Wu) for funding this research. We would also like to thank Dr Tim Dargaville for his technical support in using FTIR, and Mr Thor Friis for proof reading of the manuscript.

References

1. Chen X, Ooi C. Hydrolytic degradation and drug release properties of ganciclovir-loaded biodegradable microspheres. Acta Biomater 2008;4(4):1046-1056.
2. Jay SM, Saltzman WM. Controlled delivery of VEGF via modulation of alginate microparticle ionic crosslinking. J Control Release 2009;134(1):26-34.
3. Mateus AY, Barrias CC, Ribeiro C, Ferraz MP, Monteiro FJ. Comparative study of nanohydroxyapatite microspheres for medical applications. J Biomed Mater Res A 2008;86(2):483-493.
4. Abdel-Fattah W, Jiang T, El-Bassyouni G, Laurencin C. Synthesis, characterization of chitosans and fabrication of sintered chitosan microspheres matrices for bone tissue engineering. Acta Biomater 2007;3(4):503-514.
5. Kang SW, Yang HS, Seo SW, Han DK, Kim BS. Apatite-coated poly(lactic-co-glycolic acid) microspheres as an injectable scaffold for bone tissue engineering. J Biomed Mater Res A 2008;85(3):747-756.
6. Li H, Chang J. Preparation, characterization and in vitro release of gentamicin from PHBV/wollastonite composite microspheres. J Control Release 2005;107(3):463-473.
7. Ribeiro CC, Barrias CC, Barbosa MA. Preparation and characterisation of calcium-phosphate porous microspheres with a uniform size for biomedical applications. J Mater Sci Mater Med 2006;17(5):455-463.
8. Barrias CC, Ribeiro CC, Lamghari M, Miranda CS, Barbosa MA. Proliferation, activity, and osteogenic differentiation of bone marrow stromal cells cultured on calcium titanium phosphate microspheres. J Biomed Mater Res A 2005;72(1):57-66.
9. Kikuchi A, Kawabuchi M, Watanabe A, Sugihara M, Sakurai Y, Okano T. Effect of Ca2+-alginate gel dissolution on release of dextran with different molecular weights. J Control Release 1999;58(1):21-28.
10. Park K, Shalaby WS, Park H. Biodegradable hydrogels for drug delivery. Technomic, Lancaster, 1993;Ch.5:99-140.
11. Kibat PG, Igari Y, Wheatley MA, Eisen HN, Langer R. Enzymatically activated microencapsulated liposomes can provide pulsatile drug release. Faseb J 1990;4:2533-2239.
12. Hosoya K, Ohtsuki C, Kawai T, Kamitakahara M, Ogata S, Miyazaki T, et al. A novel covalently crosslinked gel of alginate and silane with the ability to form bone-like apatite. J Biomed Mater Res A 2004;71(4):596-601.
13. Turco G, Marsich E, Bellomo F, Semeraro S, Donati I, Brun F, et al. Alginate/Hydroxyapatite Biocomposite For

Bone Ingrowth: A Trabecular Structure With High And Isotropic Connectivity. Biomacromolecules 2009.
14. Matsuno T, Hashimoto Y, Adachi S, Omata K, Yoshitaka Y, Ozeki Y, et al. Preparation of injectable 3D-formed beta-tricalcium phosphate bead/alginate composite for bone tissue engineering. Dent Mater J 2008;27(6):827-834.
15. Halder A, Mukherjee S, Sa B. Development and evaluation of polyethyleneimine-treated calcium alginate beads for sustained release of diltiazem. J Microencapsul 2005;22(1):67-80.
16. Yan X, Yu C, Zhou X, Tang J, Zhao D. Highly ordered mesoporous bioactive glasses with superior in vitro bone-forming bioactivities. Angew Chem Int Ed Engl 2004;43(44):5980-5984.
17. Xia W, Chang J. Well-ordered mesoporous bioactive glasses (MBG): a promising bioactive drug delivery system. J Control Release 2006;110(3):522-530.
18. Wu C, Ramaswamy Y, Zhu Y, Zheng R, Appleyard R, Howard A, et al. The effect of mesoporous bioactive glass on the physiochemical, biological and drug-release properties of poly(DL-lactide-co-glycolide) films. Biomaterials 2009;30(12):2199-2208.
19. Shiraishi S, Imai T, Otagiri M. Controlled-release preparation of indomethacin using calcium alginate gel. Biol Pharm Bull 1993;16(11):1164-1168.
20. Yotsuyanagi T, Yoshioka I, Segi N, Ikeda K. Effects of monovalent metal ions and propranolol on the calcium association in calcium-induced alginate gel beads. Chem Pharm Bull 1990;38(11):3124-3126.
21. Zhu Y, Wu C, Ramaswamy Y, Kockrick E, Simon P, Kaskel S, et al. Preparation, characterization and in vitro bioactivity of mesoporous bioactive glasses (MBGs) scaffolds for bone tissue engineering. Micropor Mesopor Mat 2008;112(1-3):494-503.
22. Wu C, Ramaswamy Y, Kwik D, Zreiqat H. The effect of strontium incorporation into CaSiO3 ceramics on their physical and biological properties. Biomaterials 2007;28(21):3171-3181.
23. Li J, Mareddy S, Tan DM, Crawford R, Long X, Miao X, et al. A minimal common osteochondrocytic differentiation medium for the osteogenic and chondrogenic differentiation of bone marrow stromal cells in the construction of osteochondral graft. Tissue Eng Part A 2009;15(9):2481-2490.
24. Alleso M, Chieng N, Rehder S, Rantanen J, Rades T, Aaltonen J. Enhanced dissolution rate and synchronized release of drugs in binary systems through formulation: Amorphous naproxen-cimetidine mixtures prepared by mechanical activation. J Control Release 2009;136(1):45-53.
25. Gupta V, Gupta M, Madan AK. Development of modified dosage form for enhancement of dissolution rate through amalgamation of solid dispersion and cube sugar or sintering technology using famotidine as a model drug. PDA J Pharm Sci Technol 2009;63(1):58-70.
26. Koo H, Jin GW, Kang H, Lee Y, Nam HY, Jang HS, et al. A new biodegradable crosslinked polyethylene oxide sulfide (PEOS) hydrogel for controlled drug release. Int J Pharm 2009;374(1-2):58-65.
27. He C, Kim SW, Lee DS. In situ gelling stimuli-sensitive block copolymer hydrogels for drug delivery. J Control Release 2008;127(3):189-207.
28. Huynh DP, Im GJ, Chae SY, Lee KC, Lee DS. Controlled release of insulin from pH/temperature-sensitive injectable pentablock copolymer hydrogel. J Control Release 2009.
29. Jongpaiboonkit L, Franklin-Ford T, Murphy W. Mineral-Coated Polymer Microspheres for Controlled Protein Binding and Release. Adv Mater 2009;21(19):1960-1963.
30. Baron R, Neff L, Louvard D, Courtoy P. Cell-mediated extracellular acidification and bone bone resolution. J Cell Biol 1985;101:2210-2222.
31. Baron R. Molecular mechanism of bone resolution by osteoclast. Anat Rec 1989;224:317-324.
32. Hollinger J, Wong M. The integrated process of hard tissue regeneration with special emphasis on fracture healing. Oral Surg Oral Med Pathol Oral Radiol Endod 1996;82:594-606.
33. Wu C, Chang J. Degradation, bioactivity, and cytocompatibility of diopside, akermanite, and bredigite ceramics. J Biomed Mater Res B Appl Biomater 2007;83(1):153-160.
34. Wu C, Chang J, Zhai W, Ni S, Wang J. Porous akermanite scaffolds for bone tissue engineering: preparation,

characterization, and in vitro studies. J Biomed Mater Res B Appl Biomater 2006;78(1):47-55.

Figure captions

Figure 1. TEM microstructure of MBG **(a)**, BG **(b)** and HAp **(c)** powders.

Figure 2. XRD analysis of HAp powders synthesized by chemical precipitation.

Figure 3. SEM morphology of different microspheres. Alginate **(a)** and **(b)**; MBG/Alginate **(c)** and **(d)**; BG/Alginate **(e)** and **(f)**; HAp/Alginate **(g)** and **(h)**. **(b)**, **(d)**, **(f)** and **(h)** are higher magnification SEM.

Figure 4. FTIR spectra of Alginate, MBG/Alginate, BG/Alginate and HAp/Alginate microspheres.

Figure 5. SEM morphology and EDS analysis of different microspheres in SBF for 5 days. Alginate **(a)** and **(b)**; MBG/Alginate **(c)** and **(d)**; BG/Alginate **(e)** and **(f)**; HAp/Alginate **(g)** and **(h)**. **(b)**, **(d)**, **(f)** and **(h)** are higher magnification SEM. The ratio of Ca/P of the formed apatite on MBG/Alginate microspheres is 1.57 **(d)**. The ratio of Ca/P for the HAp/Alginate is 1.67 **(h)**. There is no obvious apatite formation on pure Alginate, BG/Alginate and HAp/Alginate microspheres after soaking in SBF for 5 day.

Figure 6. The effect of microspheres on BMSCs proliferation.

Figure 7. The effect of different materials on DEX release in PBS with different pH values **(a)** 4.3, **(b)** 7.4 and **(c)** 8.6.

Figure 8. The effect of pH value of PBS on DEX release for different microspheres **(a)** Alginate, **(b)** MBG/Alginate, **(c)** BG/Alginate and **(d)** HAp/Alginate.

Figure 9. The accumulative release of Ca, Si and P ions from different microspheres after DEX release in PBS.

Figure 10. SEM morphology of different microspheres after DEX release in PBS for 18 days. Alginate **(a)** and **(b)**; MBG/Alginate **(c)** and **(d)**; BG/Alginate **(e)** and **(f)**; HAp/Alginate **(g)** and **(h)**.

(a), **(c)**, **(e)** and **(g)** in PBS with the pH value 4.3. **(b)**, **(d)**, **(f)** and **(h)** in PBS with the pH value 8.6.

Figure 11.The schematic illustration of pH-controlled DEX release from composite microspheres.