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Silica xerogel-chitosan nano-hybrids for use as drug eluting bone replacement

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Abstract Silica xerogel-chitosan hybrids containing vancomycin were fabricated by the sol–gel process at room temperature and their potential as a drug eluting bone replacement was evaluated in terms of their mechanical properties and drug release behaviors. Regardless of the content of chitosan, all of the prepared hybrids had a uniform mesoporous structure, which would allow the effectual loading of vancomycin. As the content of chitosan was increased, the strength, strain to failure, and work of fracture of the hybrids were significantly enhanced, while the elastic modulus was decreased. These changes in the mechanical properties were mainly attributed to the mitigation of the brittleness of the silica xerogel through its hybridization with the flexible chitosan phase. In addition, the initial burst-effect was remarkably reduced by increasing the content of chitosan. The hybrids with more than 30% chitosan could release the vancomycin for an extended period of time in a controlled manner.

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1 Introduction

Bioceramics (e.g., calcium phosphates, bioactive glasses and glass-ceramics) are well recognized as one of the attractive bone substitute materials on account of their excellent biocompatibility [1, 2]. It is well known that the process of bone healing can be significantly improved by controlling the composition and structure of the bone substitute materials [3–5]. In addition, it has recently been demonstrated that the incorporation of drugs into bone substitute materials can not only prevent the damage caused by infection, but also stimulate bone growth effectively, which would accelerate the bone healing process [6–10].

However, it is practically very difficult to combine the most widely-used bioceramics with drugs, because the heat-treatment at relatively high temperatures required for sintering the bioceramics will inevitably denature the drugs [11, 12]. Therefore, considerable effort has recently been made to explore new bioactive materials which can be processed at relatively low temperatures [13–15]. From this viewpoint, silica xerogels are recognized as one of the most promising materials, since they can be prepared using a room-temperature sol–gel process, which would be expected to allow their hybridization with drugs. Moreover, they have good bioactivity, because their atomic composition is similar to that of bioactive glasses [16–19]. These advantages make silica xerogels attractive biomaterials for use as drug eluting bone replacements.

However, silica xerogels still have some problems to be overcome before they can be used in practical bone replacement applications. They have poor mechanical properties such as high brittleness and low strength. They also react with the surrounding tissues too fast, which deteriorates their long-term stability [20–22]. In addition,

silica xerogels show a poorly controlled release profile, which limits the drug eluting effect to a short period of time [23, 24].

Therefore, in this study, we hybridized silica xerogels with flexible, biodegradable chitosan, in order to enhance their mechanical properties and control the profile of drug release [25–27]. Silica xerogel-chitosan hybrids with various chitosan contents (0, 15, 30, and 50%) were synthesized using a room-temperature sol–gel process. In addition, vancomycin was loaded into the hybrids during their synthesis process in an in situ manner, which allowed the homogenous incorporation of the drug on the nanoscale. The potential of the hybrids as a drug eluting bone replacement was evaluated in terms of their mechanical properties and drug release behaviors.

2 Materials and method

2.1 Materials and fabrication procedure

Chitosan powders with a medium molecular weight and a degree of deacetylation of 85% and the other reagents used for the synthesis of the silica xerogel were purchased from Sigma-Aldrich Chem. Co., USA. The composites were prepared in the form of membranes as follows: first, the chitosan solution (2%, w/v) was prepared by dissolving 2 g of chitosan in 100 ml of 1% acetic acid. Once the chitosan was dissolved completely, the solution was filtered for purification. The silica xerogel was synthesized as described elsewhere [14]. The composites were produced by mixing the silica xerogel with up to 50 vol.% of chitosan. The composition of the two substances was determined based on their densities. The density of chitosan was obtained from the literature and that of the xerogel was estimated from its mass and volume [28]. After stirring the xerogel-chitosan mixture until a homogeneous solution was formed, 20 mg/ml of vancomycin was added. This solution was aged in a cylindrical vial for 3 days in an incubator at 37°C and then dried for 4 days. The dried specimens were weighed using a balance and cleaned with phosphate buffered saline (PBS) solution. The possible drug loss in the PBS solution was detected by a UV spectrometer.

2.2 Characterization

The internal organized structure of the specimens was examined by transmission electron microscopy (HR-TEM, JEM-3010, JEOL, Tokyo, Japan). Specimens were ultra-thin-sectioned and attached to the grid using a FIB (SMI-3050SE, SII Nanotechnology Lnc., Japan). The prepared specimens were observed at an accelerating voltage of 300 kV. The BET surface area and pore volume of the specimens were determined by N_2 adsorption measurements. The specimens were outgassed at 100°C before the analysis.

Fourier transform infrared spectroscopy (Nicolet Magma 550 series II, Midac, USA) in the wavelength range of 4000 and 400 cm^{-1} with an average of 64 scans was used to characterize the typical functional groups of the silica network and to detect the presence of chitosan in the composite. The samples used for the FT-IR spectroscopic characterization were prepared by mixing the ground specimens with spectroscopic grade KBr powder and pressing the mixtures to form disks.

2.3 Mechanical properties

The mechanical properties of the specimens, including their compressive strength and elastic modulus, were measured by a universal testing instrument (Model 5565, Instron Corp., Danvers, MA). A compressive force was applied at an extension rate of 10 mm/min. The length to diameter ratio of the cylindrical shaped samples was about 1.5. The exact dimensions of each specimen were measured using a micrometer. The load and displacement were continuously monitored and recorded by the testing machine. From the stress-strain graph, the tensile strength and strain at break were determined. The elastic modulus was calculated from the slope of the linear portion of the stress-strain curve. The work of fracture of the specimen was estimated from the area under the whole curve of the stress-strain curve until fracture. More than eight specimens were examined for each condition.

2.4 Drug release test

In order to analyze their vancomycin release behavior, the specimens were immersed in polyethylene vials with 10 ml of PBS for 31 days. The vials were sealed tightly and incubated at 37°C without stirring. Half of the medium was withdrawn at predetermined periods of time and replaced with an equivalent amount of fresh PBS. The concentration of vancomycin released from the specimens was determined by measuring the absorbance at 280 nm using a spectrophotometer (ICP-AES; Optima-4300 DV, USA). Each absorbance value was converted to the drug concentration using a standard curve, which was drawn by measuring the optical absorbance of the vancomycin dissolved in the PBS with concentrations in the range of 0.004–0.5 mg/ml. A linear relationship between the vancomycin concentration (y) and the optical absorbance (x) was obtained ($y = 0.2396x - 0.002$). All experiments were repeated three or more times and the experimental data are expressed as means ± standard error deviation (SED).

3 Results

3.1 Characterization

Figures 1a–d show the optical images of the silica xerogel-chitosan hybrids with various contents of chitosan (0, 15, 30, and 50%). The silica xerogel was almost transparent (Fig. 1a), while the specimen became opaque with the addition of chitosan. The hybrids containing up to 50% chitosan showed excellent shape tolerance without any noticeable cracks or distortion. However, as the content of chitosan was increased, the shrinkage of the hybrids was increased considerably during drying, because a higher water content was required for the synthesis of the hybrids with a higher chitosan content. This drying shrinkage often caused severe cracks in the hybrids, when a content of chitosan more than 50% was used (data not shown).

When the specimens were observed with a scanning electron microscope (SEM), there were no features except for some cleavage lines. Therefore, the microstructure of the specimens was observed by transmission electron microscopy (TEM). The TEM images of the silica xerogel and the hybrid specimen containing 30% of chitosan are shown in Fig. 2a and b, respectively. The insets are the corresponding images with higher magnification. The silica xerogel has a disordered mesoporous structure consisting of nanometer-sized particles. Compared to the silica xerogel, the TEM image of the hybrid shows disordered silica particles and the homogeneous dispersion of nano-scale chitosan (arrowed: bright contrast in the pictures) in the silica xerogel matrix.

The surface areas of the hybrid specimens with various contents of chitosan were measured by the BET method, as shown in Fig. 3. The pure silica xerogel showed a very high BET surface area owing to its highly mesoporous structure. However, the BET surface area of the hybrids was reduced with increasing content of chitosan. The hybrid containing 50% of chitosan had a dense structure.

The chemical structures of the hybrids with various contents of chitosan were evaluated by Fourier transform infrared spectroscopy (FT-IR), as shown in Fig. 4a–d. For the purpose of comparison, a pure chitosan specimen was also examined, as shown in Fig. 4e. The pure silica xerogel showed characteristic bands at 1250–1000 cm^{-1} and 960–900 cm^{-1}, corresponding to the stretching of the Si–O–Si and Si–OH groups, respectively. A band at 500–450 cm^{-1} was also observed due to the stretching of Si–O–Si. Fig. 4b–d show the IR spectra of the hybrids containing 15, 30 and 50% chitosan, respectively. The chitosan characteristic bands are expressed by spots: C=O stretching at

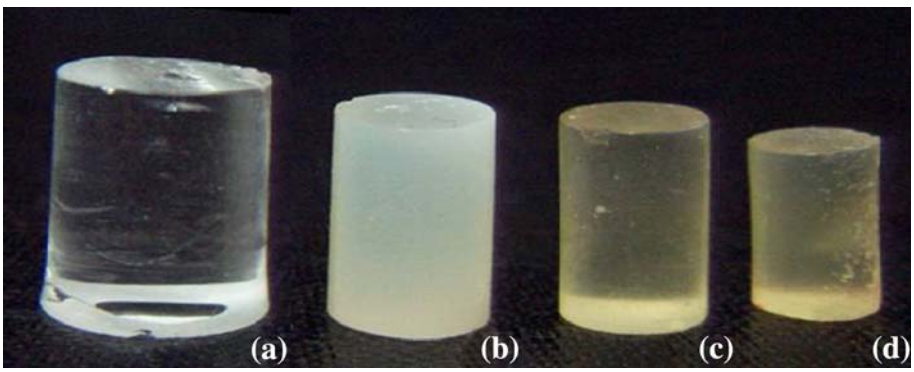

Fig. 1 Optical photographs of the hybrids with various chitosan contents of **a** 0%, **b** 15%, **c** 30% and **d** 50%

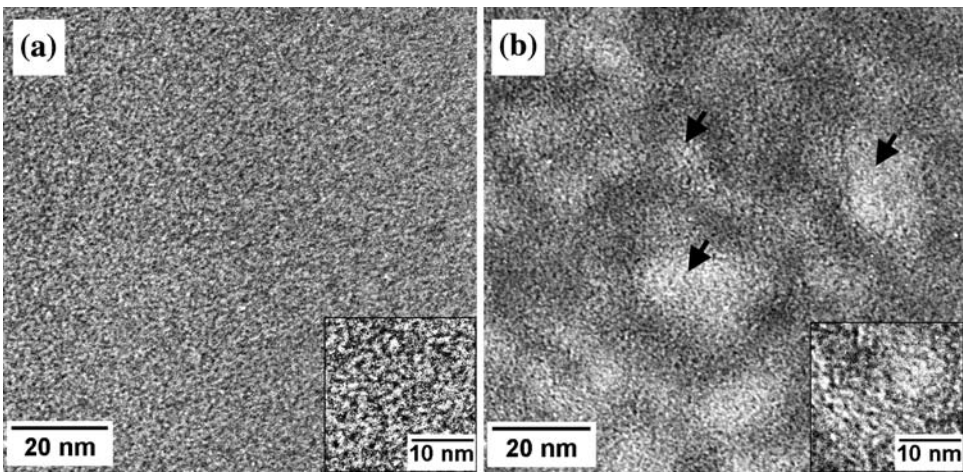

Fig. 2 TEM images of **a** the silica xerogel and **b** the hybrid with a chitosan content of 30%. The insets represent the TEM images of the samples at higher magnification

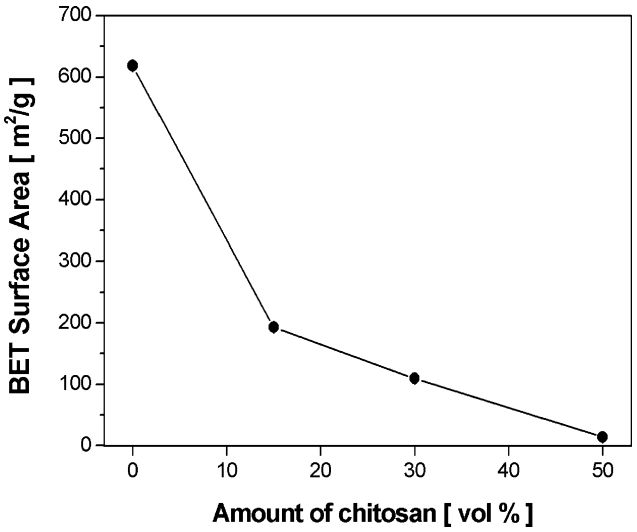

Fig. 3 BET surface areas of the hybrids as a function of the content of chitosan

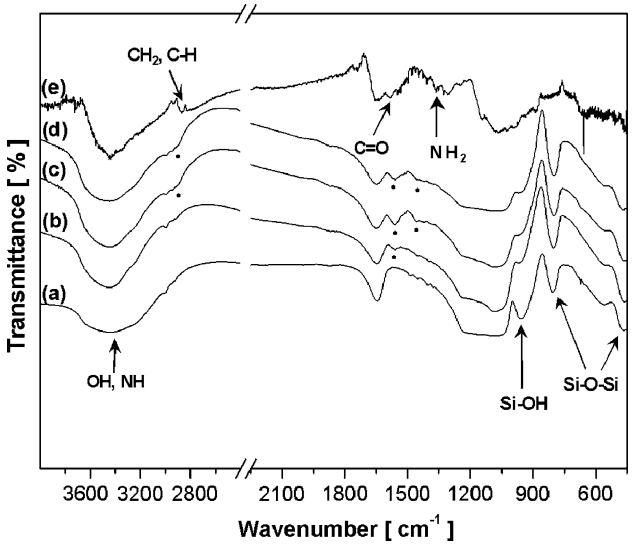

Fig. 4 FT-IR spectra of the hybrids with various chitosan contents of *a* 0%, *b* 15%, *c* 30%, *d* 50% and *e* 100% (pure chitosan)

1600–1700 cm^{-1} for amide I, NH deformation at 1500–1550 cm^{-1} for amide II, C–H stretching at 2890–2940 cm^{-1}, and C–O stretching at 750–1150 cm^{-1}. The broad band centered at 3400 cm^{-1} was assigned to the NH stretching, which overlaps the OH stretching in the same region. All of the hybrids have IR patterns corresponding to a mixture of pure chitosan and the silica xerogel, with only the relative intensity of the characteristic bands being changed as the chitosan content is varied. In the case of the specimens containing vancomycin, their IR patterns are similar to those of the samples without vancomycin. This means that the silica xerogel-chitosan hybrids were well prepared and that the incorporation of chitosan and vancomycin did not have any discernible influence on their chemical structure.

3.2 Mechanical properties

The mechanical properties of the hybrids were evaluated by conducting compression tests on cylindrical specimens. The typical stress versus strain responses of the hybrids are shown in Fig. 5. All of the prepared specimens showed brittle fracture behavior. However, it should be noted that, as the content of chitosan was increased, the compressive strength and strain to failure were improved remarkably, while the compressive modulus was decreased.

The compressive strength, modulus, strain to failure, and work of fracture of the hybrids were calculated by considering their strain versus strain responses, as shown in Fig. 6a–d. The compressive strength of the hybrids was remarkably improved from 8.9 ± 5.4 to 67.0 ± 8.5 MPa as the content of chitosan was increased from 0 to 50%, as shown in Fig. 6a. More importantly, the addition of chitosan resulted in the large reduction the compressive modulus of the hybrids, as shown in Fig. 6b. These changes in the compressive strength and modulus were attributed to the hybridization of the strong, rigid silica xerogel with the flexible chitosan. In addition, it was observed that the strain to failure and work of fracture were considerably enhanced by increasing the content of chitosan, as shown in Fig. 6c and d, respectively. These results indicate that the

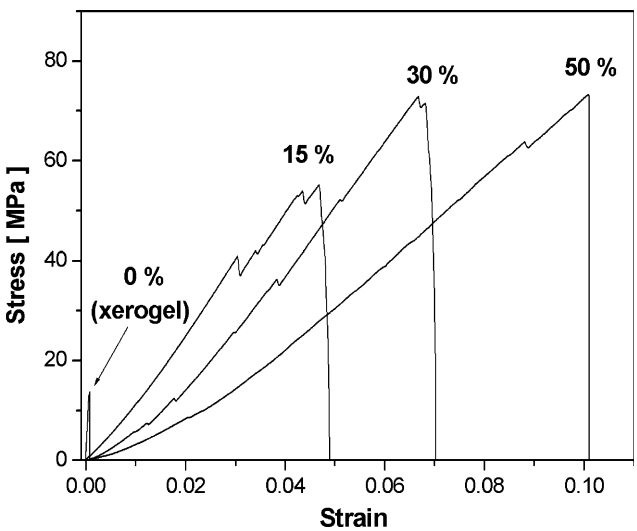

Fig. 5 The typical stress versus strain responses of the hybrids with various chitosan contents during the compressive strength tests

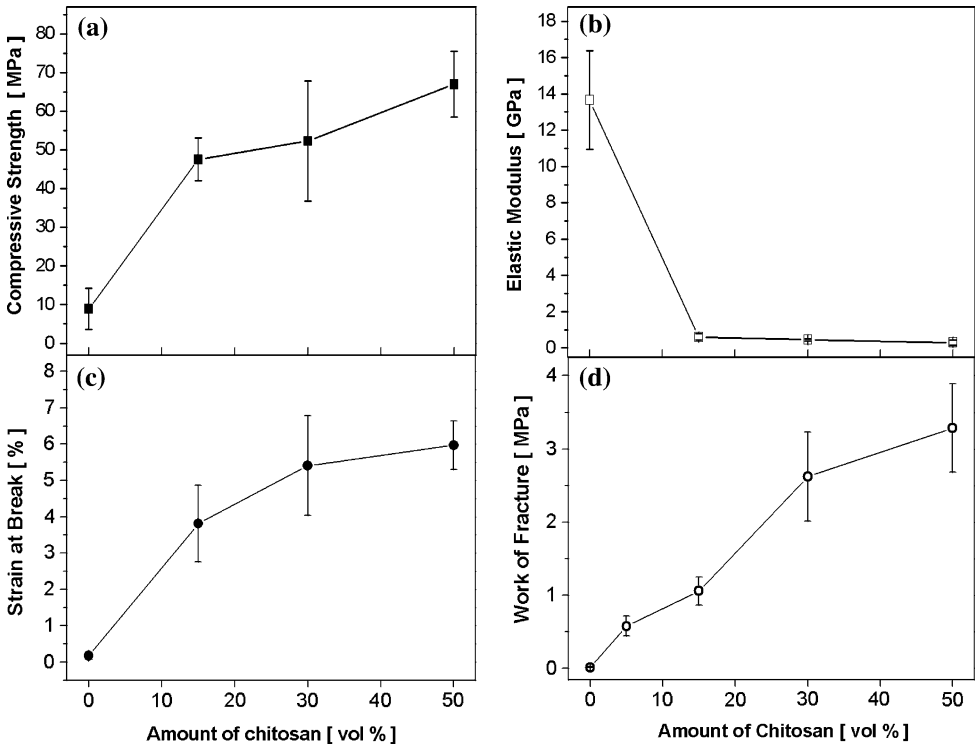

Fig. 6 **a** Compressive strength, **b** compressive modulus, **c** strain to break, and **d** work of fracture of the hybrids with various contents of chitosan

hybrids provided superior mechanical stability compared to the pure silica xerogel, when used as a bone replacement.

3.3 Vancomycin release assay

The effect of the chitosan content on the profile of vancomycin release from the hybrids was evaluated for up to 31 days. Regardless of the content of chitosan, all of the prepared samples were loaded with an equal amount of vancomycin of 20 mg. However, during the washing process, approximately 0.2% of the drug was lost in the case of the hybrids with chitosan contents of 30 and 50%. The cumulative amounts of vancomycin released from the specimens with different compositions were expressed as a function of time. Figure 7 shows the amount of vancomycin released relative to the amount of the drug initially loaded. Despite the fact that vancomycin was released for an extended period of time for all of the compositions, their initial drug release behaviors were quite different. A large amount of vancomycin was released rapidly from the pure silica xerogel at the initial stage. After 31 days, 99, 94, 78 and 49% of the vancomycin were released from the silica xerogel (0%) and 15, 30, and 50% hybrids, respectively, as shown in Fig. 7. It is of note that only 49% of the vancomycin was released from the 50% hybrids after 31 days, indicating that about half of the initial amount of vancomycin was to be released after 31 days.

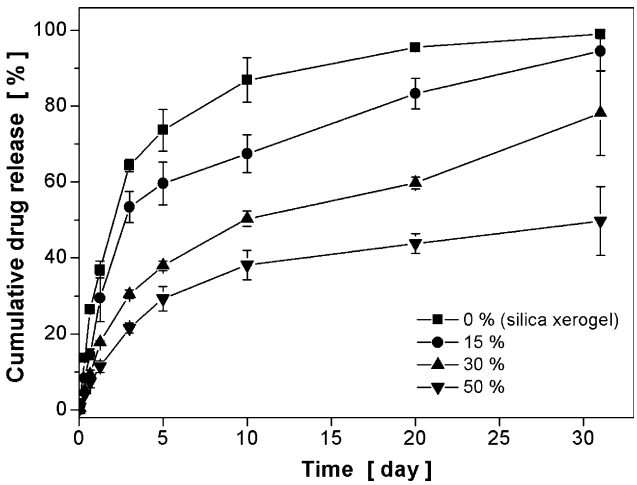

Fig. 7 Cumulative release of vancomycin from the specimens with various contents of chitosan, plotted as a function of the release time. The error bars represent the standard deviation. ($n = 3$)

4 Discussion

In the present study, the silica xerogel-chitosan hybrid materials used as drug eluting bone replacements were fabricated by the sol–gel process and their characteristics were evaluated, with the aim of improving their mechanical properties and function for controlled drug release. The controlled release of therapeutic agents such as drug is one of the major issues in orthopedic surgery for effective bone

healing. The ideal implantable controlled release materials should provide an adequate amount of the drug at the target site with a constant release profile, and they also need to have good osteoconductivity and mechanical properties for hard tissue engineering.

These requirements were easily obtained through the sol–gel process at room temperature in this study. The sol–gel process provided a uniform distribution of the drug without its decomposition and the mesoporous structure required for its encapsulation, as presented in Figs. 1 and 2. This low temperature process also elicited the easy hybridization of chitosan with the xerogel [29–31]. The silica xerogel was hybridized with chitosan in order to enhance its properties for use as a drug eluting bone replacement.

First, the hybridization of the silica xerogel with flexible chitosan was expected to enhance its mechanical properties. According to the result of the mechanical test, it was confirmed that the mechanical properties of the hybrids were greatly improved compared to those of the silica xerogel (Fig. 6). The poor mechanical properties of the silica xerogel are an obstacle to its use as a bone replacement, but the incorporation of chitosan increased its strength by a factor of 6. This value is similar to the strength of the cortical bone (about 100 MPa). The work of fracture of the hybrid, which means the amount of energy required to fracture it, is significantly higher than that of the silica xerogel, due to the increase of its strain as well as the improvement of its strength. This indicates that the silica xerogel-chitosan hybrids can react more flexibly in a high-load environment compared to the silica xerogel.

Second, the incorporation of the chitosan also had an effect on the drug release of the hybrids. It was observed that increasing the chitosan content caused a decrease in their drug release rate (Fig. 7). This result was attributed to the addition of chitosan, which has a slow degradation rate and led to the reduction of their pore volume. Vancomycin, which was employed as a drug in this study, is one of the most commonly used antibiotics that inhibit damage caused by bacteria like staphylococcus aureus. Prior studies reported that the minimum inhibitory concentration (MIC) of vancomycin for staphylococcus aureus is 1.5–3.12 μg/ml [32]. In this study, in spite of the decrease in vancomycin release rate by addition of the chitosan, the concentrations of vancomycin released from the hybrid specimens during the entire elution time were higher than the MIC.

The drug release process depends on various factors, such as the crystallinity and degradation rate of the material, as well as its geometry, such as its porosity and morphology. In the case of mesoporous materials such as the silica xerogel studied herein, the release of the drug from the material is significantly dependent on the latter's structural character and is largely terminated in the initial period [23, 24], because the drug is entrapped in the pores and released by diffusion based on the Higuchi model [33]. Accordingly, changing the porous structure of the silica xerogel by hybridizing it with chitosan can influence the drug release profile. On the other hand, organic substances such as chitosan embed bioactive agents into their matrix, so that they can be released only by the degradation (dissolution) of the former [34–36]. The degradation rate of organic materials greatly affects the drug release rate, and organic materials normally exhibit a slow drug release pattern without a burst effect. Therefore, the release of a drug dispersed in the silica xerogel-chitosan hybrids can occur via a combined process consisting of diffusion through the solvent-filled capillarity channels and the dissolution of the material. This suggests that the drug release behavior of the hybrids could be controlled by adjusting the chitosan content. The drug release test revealed that the shortcomings of the silica xerogel, namely its burst-effect (zero-order release), were overcome and that the long-term release of drug was achieved by adding chitosan (Fig. 7). Hence, the silica xerogel-chitosan hybrids can be considered to be a suitable material for controlled drug release.

Over the past few decades, attempts have been made to improve the drug release from silica xerogels, because they tend to show a poorly controlled release profile. Many of these studies focused on the modification of their mesoporous structure. They mainly attempted to regulate the material's porous structure by changing the molar ratio of water to the reagent by using different reagents or other methods [23, 24, 37, 38]. However, no significant change in the release pattern was achieved in these experiments. By contrast, in the present study, we successfully realized silica xerogels not only with effectively controllable drug release, but also with improved mechanical properties, by hybridizing them with chitosan. These experimental findings suggest that silica xerogel-chitosan hybrids have the potential to be used as controlled drug eluting bone replacements. Moreover, the water-based hybrid materials are easy to mold at low temperature, thus they are applicable as a drug deliverying vehicle in various forms, such as microsphere, porous scaffold and coating [39–41].

5 Conclusions

Silica xerogel-chitosan hybrids were fabricated by the sol–gel process for use as a bone replacement containing a drug. The hybrids have a mesoporous structure which enables a drug to be loaded within them and could be changed by adjusting the amount of chitosan. The drug release from the hybrid specimens decreased with increasing chitosan content and ultimately became moderate. Namely, a large amount of chitosan elicited the steady long term release of the drug. Moreover, the mechanical

properties, which are a weakness of sol–gel derived silica xerogels, were improved by their hybridization with chitosan. These results indicate that silica xerogel-chitosan hybrid materials have the potential to be used as a drug eluting bone replacement.

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References

1. Aza PND, Guititi F, Aza SD. Bioeutectic: a new ceramic material for human bone replacement. Biomaterials. 1997;18:1285–91.
2. Lemons JE. Ceramics: past, present, and future. Bone. 1996;19:1219–88.
3. Vassilis K, David K. Porosity of 3D biomaterial scaffolds and osteogenesis (Review). Biomaterials. 2005;26:5474–91.
4. Coombes AGA, Meikle MC. Resorbable synthetic polymers as replacements for bone graft. Clin Mater. 1994;17:35–67.
5. Hing AK. Bone repair in the twenty-first century: biology, chemistry or engineering. Phil Trans R Soc Lond A. 2004;362: 2821–50.
6. Teller M, Gopp U, Neumann HG, Kuhn KD. Release of gentamicin from bone regenerative materials: an in vitro study. J Biomed Mater Res B. 2007;81:23–9.
7. Le Ray AM, Chiffoleau S, Iooss P, Grimandi G, Gouyette A, Daculsi G, et al. Vancomycin encapsulation in biodegradable poly(ε-caprolactone) microparticles for bone implantation. Influence of the formulation process on size, drug loading, in vitro release and cytocompatibility. Biomaterials. 2003;24:443–9.
8. Pedro GR, Clement S. Functional hybrid materials. : Wiley-VCH Verlag GmbH & Co. KGaA; 2004.
9. Radin S, Ducheyne P, Kamplain T, Tan BH. Silica sol–gel for the controlled release of antibiotics. I. Synthesis, characterization, and in vitro release. J Biomed Mater Res. 2001;57:313–20.
10. Meseguer-Olmo L, Ros-Nicolas MJ, Clavel-Sainz M, Vicente-Ortega V, Alcaraz-Banos M, Lax-Perez A, et al. Biocompatibility and in vivo gentamicin release from bioactive sol–gel glass implants. J Biomed Mater Res. 2002;61:458–65.
11. Choi D, Marra KG, Kumta PN. Chemical synthesis of hydroxyapatite/poly(e-caprolactone) composites. Mater Res Bull. 2004;39:417–32.
12. Reis RL, Cunha AM, Oliveira MJ, Campos AR, Bevis MJ. Relationship between processing and mechanical properties of injection molded high molecular mass polyethylene + hydroxyapatite composites. Mat Res Innovat. 2001;4:263–72.
13. Kim HW, Knowles JC, Kim HE. Hydroxyapatite/poly(ε-caprolactone) composite coatings on hydroxyapatite porous bone scaffold for drug delivery. Biomaterials. 2004;25:1279–87.
14. Laurencin CT, Attawi MA, Lu LQ, Borden MD, Lu HH, Gorum WJ, et al. Poly(lactide-co-glycolide)/hydroxyapatite delivery of BMP-2-producing cells: A regional gene therapy approach to bone regeneration. Biomaterials. 2001;22:1271–7.
15. Komlev VS, Barinova SM, Koplik EV. A method to fabricate porous spherical hydroxyapatite granules intended for time-controlled drug release. Biomaterials. 2002;23:3449–54.
16. Lee EJ, Shin DS, Kim HE, Kim HW, Koh YH, Jang JH. Membrane of hybrid chitosan–silica xerogel for guided bone regeneration. Biomaterial. 2009;30:743–50.
17. Hamadouche M, Meunier A, Greenspan DC, Blanchat C, Zhong JP, La Torre GP, et al. Long-term in vivo bioactivity and degradability of bulk sol–gel bioactive glasses. J Biomed Mater Res. 2001;54:560–6.
18. Li P, Ye X, Kangasniemi I, de Blieck-Hogervorst JMA, Klein CPAT, de Groot K. In vivo calcium phosphate formation induced by sol–gel-prepared silica. J Biomed Mater Res A. 1995;29: 325–8.
19. Radina S, El-Bassyounia G, Vresilovicb EJ, Schepersc E, Ducheyne P. In vivo tissue response to resorbable silica xerogels as controlled-release materials. Biomaterials. 2005;26:1043–52.
20. Nilsen E, Einarsrud MA, Scherer GW. Effect of precursor and hydrolysis conditions on drying shrinkage. J Non-Cryst Solids. 1997;221:135–43.
21. Avnir D, Coradin T, Lev O, Livage J. Recent bio-applications of sol–gel materials. J Mater Chem. 2006;16:1013–30.
22. Mosquera MJ, de los Santos DM, Valdez-Castro L, Esquivias L. New route for producing crack-free xerogels: obtaining uniform pore size. J Non-Cryst Solids. 2008;354:645–50.
23. Radin S, Falaizea S, Lee MH, Ducheyne P. In vitro bioactivity and degradation behavior of silica xerogels intended as controlled release materials. Biomaterials. 2002;23:3113–22.
24. Aughenbaugh W, Radin S, Ducheyne P. Silica sol–gel for the controlled release of antibiotics. II. The effect of synthesis parameters on the in vitro release kinetics of vancomycin. J Biomed Mater Res. 2001;57:321–6.
25. Martino AD, Sittinger M, Risbud MV. Chitosan: a versatile biopolymer for orthopaedic tissue-engineering. Biomaterials. 2005;26:5983–90.
26. Tunney MM, Brady AJ, Buchanan F, Newe C, Dunne NJ. Incorporation of chitosan in acrylic bone cement: Effect on antibiotic release, bacterial biofilm formation and mechanical properties. J Mater Sci: Mater Med. 2008;19:1609–15.
27. Ueno H, Yamada H, Tanaka I, Kaba N, Matsuura M, Okumura M, et al. Accelerating effects of chitosan for healing at early phase of experimental open wound in dogs. Biomaterials. 1999;20:1407–14.
28. Trung TS, Thein-Han WW, Qui NT, Ng CH, Stevens WF. Functional characteristics of shrimp chitosan and its membranes as affected by the degree of deacetylation. Biores Tech. 2006;97:659–63.
29. Silva SS, Ferreira RAS, Fu L, Carlos LD, Mano JF, Reis RL, et al. Functional nanostructured chitosan–siloxane hybrids. J Mater Chem. 2005;15:3952–61.
30. Bandyopadhyay A, Bhowmick AK, Sarkar MD. Synthesis and characterization of acrylic rubber/silica hybrid composites prepared by sol–gel technique. J Appl Polym Sci. 2004;93:2579–89.
31. Chen X, Jia J, Dong S. Organically modified sol–gel/chitosan composite based glucose biosensor. Electroanalysis. 2003;15: 608–12.
32. Blouin RA, Bauer LA, Miller DD, Record KE, Griffen WO Jr. Vancomycin pharmacokinetics in normal and morbidly obese subjects. Antimicrob Agents Chemother. 1982;21:575–80.
33. Costa P, Lobo JMS. Modeling and comparison of dissolution profiles. Eur J Pharm Sci. 2001;13:123–33.
34. Acharya G, Park K. Mechanisms of controlled drug release from drug-eluting stents. Adv Drug Deliv Rev. 2006;58:387–401.
35. Rothstein SN, Federspiel WJ, Little SR. A unified mathematical model for the prediction of controlled release from surface and bulk eroding polymer matrices. Biomaterials. 2009;30:1657–64.
36. Patterson J, Stayton PS, Li X. In situ characterization of the degradation of PLGA microspheres in hyaluronic acid hydrogels by optical coherence tomography. IEEE Trans Med Imaging. 2009;28:74–81.
37. Ahola MS, Sailynoja ES, Raitavuo MH, Vaahtio MM, Salonen JI, Yli-Urpo AUO. In vitro release of heparin from silica xerogels. Biomaterials. 2001;22:2163–70.

38. Morpurgo M, Teoli D, Palazzo B, Bergamin E, Realdon N, Guglielmi M. Influence of synthesis and processing conditions on the release behavior and stability of sol–gel derived silica xerogels embedded with bioactive compounds. Farmaco. 2005;60:675–83.
39. Pancholi K, Ahras N, Stride E, Edirisinghe M. Novel electrohydrodynamic preparation of porous chitosan particles for drug delivery. J Mater Sci: Mater Med. 2009;20:917–23.
40. Zhao L, Chang J, Zhai WY. Preparation and HL-7702 cell functionality of titania/chitosan composite scaffolds. J Mater Sci: Mater Med. 2009;20:949–57.
41. Lu X, Wang Y, Liu Y, Wang J, Qu S, Feng B, et al. Preparation of HA/chitosan composite coatings on alkali treated titanium surfaces through sol–gel techniques. Mater Lett. 2007;61:3970–3.