# Bioactive SrO-$SiO_2$ glass with well-ordered mesopores: characterization, physiochemistry and biological properties

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**ABSTRACT**

For a biomaterial to be considered suitable for bone repair it should ideally be both bioactive and have a capacity for controllable drug delivery; as such, mesoporous $SiO_2$ glass has been proposed as a new class of bone regeneration material by virtue of its high drug-loading ability and generally good biocompatibility. It does, however, have less than optimum bioactivity and controllable drug delivery properties. In this study, we incorporated strontium (Sr) into mesoporous $SiO_2$ in an effort to develop a bioactive mesoporous SrO-$SiO_2$ (Sr-Si) glass with the capacity to deliver $Sr^{2+}$ ions, as well as a drug, at a controlled rate, thereby producing a material better suited for bone repair. The effect of $Sr^{2+}$ on the structure, physiochemistry, drug delivery and biological properties of mesoporous Sr-Si glass was investigated. The prepared mesoporous Sr-Si glass was found to have an excellent release profile of bioactive $Sr^{2+}$ ions and dexamethasone (DEX), and the incorporation of $Sr^{2+}$ improved structural properties, such as mesopore size, pore volume and specific surface area, as well as rate of dissolution, and protein adsorption. The mesoporous Sr-Si glass had no cytotoxic effects and its release of $Sr^{2+}$ and $SiO_4^{4-}$ ions enhanced alkaline phosphatase (ALP) activity–a marker of osteogenic cell differentiation–in human bone mesenchymal stem cells (BMSCs). Mesoporous Sr-Si glasses can be prepared to porous scaffolds which show a more sustained drug release. This study suggests that incorporating $Sr^{2+}$ into mesoporous $SiO_2$ glass produces a material with a more optimal drug delivery profile coupled with improved bioactivity, making it an excellent material for bone repair applications.

*Key words:* Mesoporous Sr-Si glass; Drug delivery; Bioactivity

## 1. Introduction

Biomaterials with properties suitable for bone regeneration need to be bioactive, and at the same time possess the capacity for controlled drug delivery [1-3]. To this end, a number of different materials,

such as inorganic bioglass [2,4,5], calcium phosphate ceramics [6] and polymers [7] have been studied for their suitability in bone tissue repair. Over the past several years, mesoporous $SiO_2$ has received a significant amount of attention. The chief advantage of mesoporous $SiO_2$ is that this material has, as the name implies, a highly ordered mesoporous structure, high surface area and pore volume–qualities that gives it an excellent drug release profile [8,9]. For bone repair applications, however, the material's bioactivity and controllable drug-delivery properties are less than optimal [2,10,11]. Vallet-Regi et al. have been able to improve the controlled drug delivery of mesoporous $SiO_2$ by manipulating the mesoporous structure, i.e. the pore size, as well as certain surface modifications [11-14]. Despite these advances there still remains room to improve both the bioactivity and controlled drug delivery by mesoporous $SiO_2$.

It is known that strontium (Sr) is an important trace element in human bone and strontium ranelate has been used for treatment of osteoporosis. $Sr^{2+}$ was found to induce osteoblast activity by stimulating bone formation and reducing bone resorption [15-17]. It has a great affinity for bone tissue and is incorporated into the bone matrix by two mechanisms: (i) surface exchange involving the incorporation of $Sr^{2+}$ into the crystal lattice of the bone mineral, and (ii) ionic substitution whereby $Sr^{2+}$ is taken up by ionic exchange with $Ca^{2+}$ [18]. For this reason, $Sr^{2+}$ has been incorporated into bioactive ceramics and traditional bioactive non-mesoporous glass to further improve their bioactivity [19-23], but there is, to the best of our knowledge, no previous reports describing the incorporation of $Sr^{2+}$ into mesoporous $SiO_2$. We hypothesized that Sr-doped mesoporous $SiO_2$ glass could deliver controlled doses of $Sr^{2+}$ into the biological medium, thereby enhancing bone cell activity. There is an earlier study that shows that the dissolution of drug carriers is one of the most important factors influencing the rate of drug release [24]. We therefore hypothesized that the incorporation of $Sr^{2+}$ into

mesoporous $SiO_2$ would also modify its structure and rate of dissolution, thus enhancing the controlled drug delivery. The aim of this study was therefore to incorporate $Sr^{2+}$ into mesoporous $SiO_2$ in order to develop a bioactive mesoporous Sr-Si glass with an improved drug delivery profile and therefore better suited for bone repair applications; here we describe the effects of $Sr^{2+}$ on mesoporous structure, physiochemistry, drug delivery and biological properties.

## 2. Materials and Methods

### *2.1. Preparation and characterization of mesoporous SrO-$SiO_2$ glasses powders*

A series of four mesoporous SrO-$SiO_2$ glass species, with different chemical compositions (Molar composition: 0SrO-100$SiO_2$, 2.5SrO-100$SiO_2$, 5SrO-100$SiO_2$ and 10SrO-100$SiO_2$, labeled as 100Si, 2.5Sr-100Si, 5Sr-100Si and 10Sr-100Si, respectively) were prepared by a modified template-induced and self-assembling method, using nonionic block copolymer EO20PO70EO20 (P123) [9]. In a typical reaction of 10Sr-100Si, 3.34 g of $Sr(NO_3)_2$ (Sigma-Aldrich) was dissolved in 30 mL dd$H_2O$ to obtain a $Sr^{2+}$ containing slurry. Twenty gram of P123 (Mw=5800; Sigma-Aldrich) was dissolved in 300 mL ethanol under stirring for 1 h, after which 33.5 g of tetraethyl orthosilicate (TEOS, 98%, Sigma-Aldrich), the aqueous $Sr(NO_3)_2$ solution, and 5.0 g of 0.5 M HCl were added to the P123-ethanol solution and stirred in a sealed beaker at room temperature for 24 h. The resulting solution was introduced into eight petri dishes for an evaporation-induced self-assembly process, and the dry gel calcined at 700ºC for 5 h resulting in the 10Sr-100Si powders. Other Sr-Si materials (100Si, 2.5Sr-100Si and 5Sr-100Si) were prepared by the same method but with different amounts of $Sr(NO_3)_2$ dissolved in 30 mL dd$H_2O$. After calcined, the Sr-Si glass was ground and sieved to 300 meshes for further testing. The particle sizes of the glass powders thus produced were less than 45μm (see Fig. 7a

inset image).

The phase composition, surface morphology, and inner microstructure of the calcined Sr-Si glass were analyzed by wide-angle X-ray diffraction (XRD), small-angle XRD, transmission electron microscopy (TEM) and scanning electron microscopy (SEM) and energy dispersive spectrometer (EDS). TEM of the samples was performed using a FEI Tecnai 10 electron microscope (FEI Company, Eindhoven, NL) with a $LaB_6$-source at 100 kV acceleration voltage. Images were recorded with a Tietz slow scan CCD F224HD TVIPS camera (2k x 2k pixels, pixel size 24 μm, digitization 16 bit) with an active area of 49 mm × 49 mm. (Tietz Video and Image Processing Systems GmbH, Gauting, Germany). Brunauer-Emmett-Teller and Barret-Joyner-Halenda analyses were used to determine the specific surface area, the nano pore size distribution and the pore volume by $N_2$ adsorption-desorption isotherms.

*2.2. Physiochemical properties of Mesoporous Sr-Si glass powders in protein-containing simulated body fluids*

To evaluate the protein adsorption and the dissolution rates of mesoporous Sr-Si (mSr-Si) glass in a biological environment, simulated body fluids (SBF) were prepared according to Kokubo [25]. Bovine serum albumin (BSA) was dissolved in the SBF solution to a final concentration of 1 mg/mL. mSr-Si glass powders were immersed in the BSA/SBF solution (0.3 g per 200 mL) and incubated at 37ºC for 1, 3, 6, 8, 24, 48 and 72 h. Protein adsorption was determined by UV spectrophotometry at 280 nm, by the difference in BSA concentration in the SBF before and after soaking (UV min-1240, Shimadzu, Japan). The release of $Sr^{2+}$ and $SiO_4^{4-}$ ions from the mSr-Si glass particles into the SBF was determined by atomic emission spectrometry (Perkin-Elmer Optima 7000DV). The pH value of SBF solution was tested by a pH meter and the microstructure of the mSr-Si glass particles after soaking

was characterized by SEM (Jeol JSM 6510). All samples were tested in triplicate.

*2.3. Rates of drug delivery by the mesoporous Sr-Si glass powders*

Dexamethasone (DEX) was selected as the model drug in this study. The DEX was loaded into the mSr-Si glass particles by dissolving DEX in water to a final concentration of 50 μg/mL; 0.50 g of mSr-Si glass particles was soaked in 50 mL of DEX solution under stirring for 12 h. The DEX-loaded glass particles were collected by centrifugation at 4500 g for 15 min, then dried at 60ºC for 24 h. To determine the amount of DEX loading in the mSr-Si glass particles, 1.5g of DEX-loaded and non-loaded glass particles were calcined at 700ºC for 1 h at a heating rate of 5ºC/min. The amount of DEX loaded was calculated by the weight difference tested by a high precision balance between the non-loaded and DEX-loaded Sr-Si glass after heat treatment. The loading efficiency was calculated as the ratio of the loaded DEX and total amount of DEX in the aqueous solution. The assay was performed in triplicate.

To determine the DEX release from the mSr-Si glass particles, 40 mg of DEX-loaded mSr-Si glass was soaked in 4 mL of PBS at 37ºC for 1, 3, 6, 8, 10, 24, 72, 168, 336, 504 and 672 h. At each time point, 2 mL PBS solution was taken off to quantify the concentration of released DEX by UV spectrophotometry at 240 nm. The PBS solution was topped up with 2 mL of fresh PBS every time 2 mL was taken off for assaying. Three samples for each material were assayed to calculate mean and standard deviations.

*2.4. Cytotoxicity evaluation of the mesoporous Sr-Si glass powders*

The bone marrow samples ($n=5$) for isolation of human bone mesenchymal stem cells (BMSCs) were obtained from patients undergoing elective surgery at The Prince Charles Hospital, Brisbane, Australia, after informed consent had been given. BMSC cultures were carried out according to previous

publications [26]. The extracts of mSr-Si glass were prepared in culture medium according to International Standard Organization (ISO/EN) 10993-5 [27], by adding mSr-Si glass particles to serum free DMEM culture medium at a final concentration of 50 mg/mL. After incubating at 37ºC for 24 h, the mixtures were filtered and the supernatants collected. Serial dilutions of extracts (25, 12.5, 6.25 and 3.125 mg/mL) were prepared using serum-free DMEM medium (without L-glutamine or ascorbic acid). The diluted extracts were filter sterilized and used for subsequent BMSC cell culture experiments. The ionic concentrations of the extracts were measured by atomic emission spectrometer (Perkin-Elmer Optima 7000DV). BMSCs were seeded at a density of $3\times10^3$ cells/well into 96-well plate with regular DMEM medium and incubated for 24 h, after which the medium was removed and replaced by 50 μL of DMEM medium supplemented with 20% FCS and 50 μL of diluted extracts. 100 mL of culture medium supplemented with 10% FCS but without the addition of diluted extracts was used as a blank control. The corresponding concentrations of $SiO_4^{4-}$ and $Sr^{2+}$ ions of the mixture (extract and medium) for cell culture were listed in Table 2. The cells were incubated at 37ºC in 5% $CO_2$ for 7 d. To assess cell toxicity, an MTT assay was performed by adding 20 μL of 0.5 mg/mL of MTT solution (Sigma-Aldrich) to each well and incubated 37ºC to form formazan crystals. After 4 h, the media was removed and the formazan solubilized with 100 μL of dimethyl sulfoxide (DMSO). The absorbance of the formazan-DMSO solution was read at 495 nm on a plate reader. Two separate MTT assays were performed with each sample. Results were expressed as the absorbance reading from each well minus the optical density value of blank wells.

*2.5. Alkaline phosphatase (ALP) activity of BMSCs cultured with mesoporous Sr-Si glass powders*

Osteogenic differentiation was assessed by measuring a time course of alkaline phosphatase (ALP) activity of the BMSCs cultured with the extracts from the four mSr-Si glass species. BMSCs were

seeded at a density of $5\times10^4$ cells/well into a 24-well plate and incubated for 24 h. The culture medium was then removed and replaced with 500 μL of DMEM medium supplemented with 20% FCS and 500 μL of diluted extracts. Culture medium supplemented with 10% FCS without the addition of diluted extracts was used as a blank control. The cells were incubated at 37ºC in 5% $CO_2$ for 14 d and the medium changed every 3 days. On day 14 the total protein was harvested by lysing the cells with 0.5 mL of 0.2% Triton® X-100. The cell lysates were transferred into 1.5 mL tubes and sonicated, then the samples were clarified by centrifugation for 15 min in 14,000 g at 4°C and the supernatant transferred to fresh tubes. ALP activity in each sample was measured in a 96-well plate using an ALP activity assay kit (BioAssay System Com., Hayward, CA, USA). Optical density (OD) was measured at 405 nm on a plate reader. The ALP activity was expressed as $\Delta OD_{405nm}$ /min/μg protein.

*2.6. Preparation, characterization and drug delivery of mesoporous Sr-Si glass scaffolds*

We have found that 10Sr-100Si mesoporous glasses have improved physochemistry and drug delivery property. Therefore, 10Sr-100Si mesoporous glasses were selected to prepare scaffolds. 100Si (no Sr) scaffolds was prepared for the control material. The scaffolds were prepared using polyurethane template method according to our previous publications [1], in which mesoporous glasses were coated on the polyurethane foam to duplicate the porous structure. Then, the polyurethane foam was burned out to obtain porous mesoporous glass scaffolds. The scaffold morphology and pore structure were observed by optical microscopy and SEM. The porosity of the obtained scaffolds was tested by Archimedean methods.

To investigate the drug delivery of the scaffolds, DEX loading and release from the scaffolds were tested using similar method described in the section of "*2.3. Rates of drug delivery by the mesoporous Sr-Si glass powders"*.

*2.7. Statistical analysis*

The data is expressed as means ± standard deviation (SD) for all experiments and were analyzed using One-Way ANOVA with a Post Hoc test. A $p$-value<0.05 was considered statistically significant.

## 3. Results

*3.1. Characterization of mesoporous Sr-Si glass powders*

Wide angle XRD analysis showed that the four Sr-Si glass species had no sharp diffraction peaks (Fig. 1). The diffraction patterns of 100Si, 2.5Sr-100Si and 5Sr-100Si, had a wide $SiO_2$ peak with low intensity, but this peak was not obvious for 10Sr-100Si. Two minor α-$SrSiO_3$ peaks appeared in the pattern of 5Sr-100Si (Fig. 1). TEM analysis showed that the Sr-Si glass particles had well-ordered mesoporous channel structures (Fig. 2). The 10Sr-100Si (Fig. 2c and d) appeared to have a more mesoporous structure than did the Sr-Si glass particles with lower $Sr^{2+}$ content and pure $SiO_2$ glasses (Figs. 2a and b). The periodicity of the lattice is approximately 7 nm (Fig. 2c) with a pore size of approximately 4 nm in the case of lamellar ordering (Fig. 2d). Small angle XRD results are listed in Figure 3. There is a weak peak at 2θ 2.4 degree for pure $SiO_2$ (Fig. 3a). With the increase of Sr content, the diffraction peak moves towards a lower angle position (Figs. 3b and c). 10Sr-100Si having a more pronounced diffraction peak at 2θ 1.25 deg (Figs. 3c), compared to the other materials. Similarly, with increased Sr content in the composite glass, the average pore size increases, with 10Sr-100Si having significantly higher surface area and pore volume than does the other three species (Table 1). The results for $N_2$ adsorption–desorption analysis of 10Sr-100Si showed a type of IV isotherm and pore distribution in the 3–5 nm range, a typical characteristic of a mesoporous structure (Fig. 4).

*3.2. Physiochemical properties of mesoporous Sr-Si glass powders in protein containing SBF*

BSA adsorption rates for the mSr-Si glass particles increased with the length of soaking time up to the first 24 h. The 10Sr-100Si glass had the greatest amount of absorbed BSA of the four mSr-Si species (Fig. 5). Generally, with increased Sr content, more $Sr^{2+}$ and $SiO_4^{4-}$ ions were releases from the mSr-Si glass particles (Fig. 6a and b) and the pH value of the SBF increased slightly, maintaining a range of 7.3–7.6 (Fig. 6c). After 3 days of soaking in SBF, the concentration of $PO_4^{3-}$ of SBF for 10Sr-100Si decreased from 31ppm to 26ppm. EDS analysis shows the rate of Sr/Si in four powders was 0/100, 2.06/100, 4.06/100 and 8.20/100, respectively, which is quite close the theoretical component (Fig. 7). SEM analyses revealed no significant difference between the surface microstructure of 100Si and 2.5Sr-100Si before and after soaking in SBF (Fig. 7a, b and Fig. 8a, b); however, Due to the quicker dissolution for 5Sr-100Si and 10Sr-100Si, there are some microparticles and fiber-like particles deposited on the surface of materials in simulated body fluids (Fig. 8c and d, see arrows). EDS shows that there are Ca and P peaks after soaking 10Sr-100Si in SBF, indicating some Ca-P containing particles deposited on the surface of 10Sr-100Si glass (Fig. 8e). The ratio of Ca/P/Sr/Si is 1.87/3.19/0.55/75.92.

*3.3. Dexamethasone delivery of mesoporous Sr-Si glass powders*

The weight difference of DEX-loaded and unloaded particles after heat-treatment was 2.07±0.08, 1.40±0.05, 0.89±0.03 and 2.93±0.13 mg for 100Si, 2.5Sr-100Si, 5Sr-100Si and 10Sr-100Si, respectively. The 10Sr-100Si composite glass had greater DEX loading efficiency (39%) than the other three mSr-Si glass species (100Si = 27.6%; 2.5Sr-100Si = 18.6%, and 5Sr-100Si = 11.8%). All four mSr-Si composites had a sustained DEX-release profile. When the Sr content was less than 5%, the DEX release kinetics increased proportionally with the increase of Sr content. The 10Sr-100Si glass particles had the slowest release rate over the full time period (Fig. 9a and b). The relationship

of the release rate of DEX in mesoporous Sr-Si glass system over the square root of time is shown in Figure 9c.

*3.4. Cytotoxicity and ALP activity of BMSCs cultured with mesoporous Sr-Si glass powders*

The cell viability assays showed there was no discernible cytotoxicity associated with the mSr-Si glass extracts, by the fact that all the experimental groups had values greater than 90% of the controls. The viability of BMSCs cultured with the concentration of mSr-Si glass extracts (ranging from 3.125 to 50 mg/mL) was comparable to that of the controls, with the exception of the 10Sr-100Si group at 12.5 and 50mg/mL (Fig. 10a). The ALP activity of the BMSCs cultured with the mSr-Si glass extracts is shown in Figure 10b. At the highest concentration (50 mg/mL), mSr-Si glass clearly has an inhibitory effect on the ALP activity of BMSCs. However, at the lower concentrations (3.125–25 mg/mL), ALP activity of BMSCs cultured with mSr-Si glass extract was comparable to that of the controls. At 12.5 mg/mL concentration, ALP activity of BMSCs was enhanced in 10Sr-100Si extract compared to 100Si extract (Fig. 10b). The corresponding ion concentrations of the mixture for cell culture is listed in Table 2, showing of $SiO_4^{4-}$ and $Sr^{2+}$ ion concentrations ranging from 2.4 – 102 ppm and 1.7 – 37.5 ppm, respectively.

*3.5. Characterization and drug delivery of Sr-Si mesoporous scaffolds*

10Sr-100Si and 100Si mesoporous glass scaffolds were successfully prepared. They have similar pore structure and the large pore size is about 400μm (Fig. 11a, b, and c). The porosity of the obtained scaffolds is about 90%. Further study has shown that 10Sr-100Si mesoporous glass scaffolds have a slower release of DEX than those of pure 100Si scaffolds (Fig. 11d).

**4. Discussion**

We have successfully prepared mesoporous $SrO$-$SiO_2$ glass particles with a well-ordered structure, and

investigated what effects of Sr has on mesopore structure, physiochemistry, drug delivery and biological properties. It was found that incorporating of $Sr^{2+}$ into mesoporous $SiO_2$ glass improved the mesoporous structures (pore size, volume and surface area) and rates of dissolution, as well as protein adsorption. The incorporation of $Sr^{2+}$ at differing amounts, into the mesoporous $SiO_2$ glass, resulted in a more controlled and sustainable drug release profile. These novel mesoporous Sr-Si glass particles had no cytotoxic effects and at a certain concentrations of $Sr^{2+}$ and $SiO_4^{4-}$ from the mSr-Si glass particles actually enhanced ALP activity in BMSCs. This study indicates that incorporating $Sr^{2+}$ into mesoporous $SiO_2$ glass is a feasible method to improve this material's drug delivery and bioactivity for potential drug carrier and bone tissue repair applications.

We found that a certain amount of water was required to dissolve $Sr(NO_3)_2$ in order to obtain a uniform $SiO_2$ gel with which to prepare mesoporous glass with a well-ordered structure. If water was not used to dissolve the $Sr(NO_3)_2$, it would become unevenly distributed within the $SiO_2$ gel. Interestingly, at low concentrations of Sr (0, 2.5 and 5%), the Sr-Si glasses had fewer mesopores and smaller pore size and volume than those with higher Sr content (10%: 10Sr-100Si). Previous studies have shown that reaction condition, such as water and inorganic salts, and the crystallinity of the mesoprous materials are two of important factors to influence the mesopore structure [28-30]. We can think of two causes which best explain the phenomena seen in this study. Number one is that by adding water to the reaction the condensation degree may decrease, thereby destroying the balance of self-assembly conditions in the low Sr containing mesoporous glass. However, in the 10Sr-100Si mesoporous glass, which had the highest Sr content, the addition of $Sr(NO_3)_2$ might in fact enhance the cross-linkage of the silica monolith and enhance the self-assembly reaction. This type of phenomenon has previously been reported by Zhong *et al*. They found that adding inorganic salts (NaCl, $NaNO_3$ or

$Na_2SO_4$) into silica stimulated the formation of mesopore structures [28]. Reason number two is that Sr atoms, at high concentration (10Sr-100Si), actually inhibits the crystallization of Sr-Si glass (as seen in Fig. 1), which may benefit the formation of an ordered mesopore structure [30].

Protein adsorption is an important factor when evaluating the potential of biomaterials for tissue repair. Most mammalian cells are anchorage dependent and need a biocompatible, protein rich surface for attachment, differentiation and migration to form new tissue [31]. In the present study, we chose to use BSA as a model protein since it is a stable molecule, and also the most abundant protein in blood, albumin accounting for nearly 60% of the total serum proteins. BSA is also inexpensive and is commonly used in studies to simulate human albumin, given the close structural homology of the two [32]. Our data showed quite convincingly that Sr was an important factor affecting BSA adsorption to mSr-Si glass particles. We attribute this to the capacity of Sr to affect the pore size and volume of the mSr-Si glass, and enhanced protein adsorption ability was especially seen in 10Sr-100Si. We compared the protein adsorption by normalizing to surface area between 10Sr-100Si (0.047mg protein/specific surface) and pure 100Si mesoporous bioglass (0.22mg protein/specific surface). It is clear that at the same surface area, 100Si has higher protein absorption ability than 10Sr-100Si. We therefore reasoned that the greater surface area of 10Sr-100Si is the chief contributor to the improved protein adsorption. There are two potential reasons to explain the peak value of BSA absorption for 10Sr-100Si. One reason is that there is a dynamic balance between the protein absorption and desorption to materials; the other reason is that 10Sr-100Si has higher specific area and dissolution rate than other three kinds of materials. At the early stage, 10Sr-100Si will absorb BSA obviously to reach a maximum value; however, with the increase of time, 10Sr-100Si glass will dissolve by releasing of Si and Sr ions (see Fig. 6), which will decrease the BSA absorption.

Moreover, incorporating Sr into the mesoporous glass enhances its dissolution, resulting in a controllable release of bioactive ions ($Sr^{2+}$ and $SiO_4^{4-}$). The incorporation of $Sr^{2+}$ into meospore $SiO_2$ glass may produce some defects in the atomic array, such as atomic vacancies or dislocations. These defects will result in the improved dissolution of the materials, which has been described in a previous study [33]. Although the higher solubility led to slightly higher pH values in SBF, in our study the pH stayed within a narrow physiological range (7.3–7.6), which is conducive to cell growth. In addition, our study has shown that the incorporation of Sr into mesopours $SiO_2$ glass enhanced the deposition of Ca-P containing particles, which may indicate the improved bioactivity according to previous studies [25,34].

Mesoporous $SiO_2$ glass has been widely studied as a potential drug delivery system, since its nano-channel structure allows it to maintain a sustained release; however, controlled drug delivery using mesoporous $SiO_2$ still remains a challenging issue. Until recent, in most studies the reaction conditions (solvent, surfactant etc.) and surface modifications to control the pore and surface structure were manipulated in order to better control drug release in mesoporous $SiO_2$ [12-14]. In our study, dexamethasone was chosen as a model drug since it is already used as an anti-inflammatory agent to treat rheumatoid arthritis [35]. It was found that incorporating $Sr^{2+}$ into mesoporous $SiO_2$ yielded a more controllable drug release and the mSr-Si glass particles maintained a sustained release of DEX. At the low end of Sr content (5% and less), the faster dissolution rate of the mSr-Si glass most likely also contributed to the faster rate of DEX release. At the high end of Sr contents (10%), 10Sr-100Si, despite having the fastest rate of dissolution, also had a significantly enhanced mesopore structure which appeared to contribute to its slower DEX release rate. The release rate of DEX in mSr-Si glass system was not a simple linear one, but rather appeared to be a function of the square root of time.

This indicates that DEX release in this system is not only affected by diffusion [36], but also by the dissolution of the glass itself.

Sr and Si are both important trace elements in human bone tissues. Previous studies have shown that high concentrations of $Sr^{2+}$ and $SiO_4^{4-}$ ions can result in excessive cytotoxicity [21,34]. In our study, $Sr^{2+}$ and $SiO_4^{4-}$ ion concentrations from mSr-Si glass reached as high as 34.5 and 102 ppm, respectively. These levels were not cytotoxic to the BMSCs, but there was a slight inhibitory effect on ALP activity (see Fig. 10b and Table 2) when the $Sr^{2+}$ concentration was greater than 26.5 ppm; below this level ALP activity was comparable to that of the controls. The BMSC ALP activity was greater in cells grown in medium with the 10Sr-100Si extract compared to 100Si extract, when the extract concentration was 12.5 mg/mL. This could be attributed to Sr somehow compensating for the negative effect of high concentration of $Si^{2+}$. $Sr^{2+}$ and $SiO_4^{4-}$ ions at an optimum concentration clearly have the ability to enhance the ALP activity of BMSCs by a synergistic effect of the two ions.

Our further study has shown that mesoporous Sr-Si glasses can be prepared to porous scaffolds with a highly porous structure and large pore size which will benefit cell growth in. The incorporation of Sr into mesoporous $SiO_2$ scaffolds maintained a more sustained release, which indicates that the obtained 10Sr-100Si mesoporous glass, in the form of scaffolds, can be used for drug delivery and bone tissue engineering. Further study will be conducted to investigate the effect of controllable DEX release from mesoporous Sr-Si glass scaffolds on the proliferation and differentiation of BMSCs.

## 5. Conclusion

A novel bioactive SrO-$SiO_2$ glass with a well-ordered mesopore structure has been successfully prepared by the incorporation of $Sr^{2+}$ into $SiO_2$. At 10% Sr content, mesoporous Sr-Si glass has an improved mesopore structure, dissolution and protein adsorption compared to mesoporous $SiO_2$. The

release profile of bioactive $Sr^{2+}$ ions and a model drug (DEX) can be controlled by altering the Sr content in mesoporous Sr-Si glass particles. No cytotoxicity was associated with these compounds, and the released $Sr^{2+}$ and $SiO_4^{4-}$ ions, at an optimum concentration, enhanced the ALP activity of BMSCs. Mesoporous Sr-Si glasses can be prepared to porous scaffolds which show a more sustained drug release. These results indicate that incorporating $Sr^{2+}$ into mesoporous $SiO_2$ glass may yield a bioactive material with improved drug release properties for potential drug carrier and bone repair application.

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## Captions for Figures

**Figure 1.** Wide-angle XRD patterns for the mesoporous Sr-Si glasses powders with different Sr contents.

**Figure 2.** TEM images for 100Si (a), 5Sr-100Si (b), 10Sr-100Si (c) and (d) powders. (c) is showing a high regular rhombohedral ordering with a lattice parameter of about 7 nm as indicated by the fast Fourier transform (FFT) inset at the left bottom. (d) is a higher magnification image with a lattice periodicity of 7.6 nm (pore and wall) and lamellar pore size (width) of ~4.2 nm. The obtained Sr-Si glass containing well ordered mesopore channel structures.

**Figure 3.** Small-angle XRD patterns for the mesoporous Sr-Si glasses powders with different Sr contents. (a) 100Si, (b) 5Sr-100Si, and (c) 10Sr-100Si. 10Sr-100Si has most obvious peaks at 2θ 1.25 deg. The diffraction peaks were pointed by arrows.

**Figure 4.** Nitrogen adsorption-desorption isotherm and pore size distribution of 10Sr-100Si mesoporous glass powders.

**Figure 5.** The protein (BSA) absorbance for Sr-Si mesoporous glass powders with different Sr contents in BSA-containing simulated body fluids.

**Figure 6**. Sr (a) and Si (b) ions release for the mesoporous Sr-Si glass powders with different Sr contents, and pH change for simulated body fluids (c).

**Figure 7.** SEM images for 100Si (a), 2.5Sr-100Si (b), 5Sr-100Si (c) and10Sr-100Si (d) powders before soaking in simulated body fluids. The inset image in Figure (a) shows the particle size of the obtained mesopore powders. EDS analysis shows the rate of Sr/Si in four powders was 0/100, 2.06/100, 4.06/100 and 8.20/100, respectively, which is quite close the theoretical component.

**Figure 8.** SEM images for 100Si (a), 2.5Sr-100Si (b), 5Sr-100Si (c) and 10Sr-100Si (d) powders after soaking in simulated body fluids. Due to the quicker dissolution for 5Sr-100Si and 10Sr-100Si, there are some microparticles deposited on the surface of 5Sr-100Si and fiber-like particles deposited on the surface of 10Sr-100Si in simulated body fluids (see arrows). EDS shows that there are Ca and P peaks (e) after soaking 10Sr-100Si in SBF, indicating some Ca-P containing particles deposited on the surface of 10Sr-100Si glass. The ratio of Ca/P/Sr/Si is 1.87/3.19/0.55/75.92.

**Figure 9.** Drug (Dexamethasone) release behavior for the mesoporous Sr-Si glass powders with different Sr contents. (a) full time period, (b) early stage and (c) the relation of drug release rate and the square root of time ($h^{1/2}$).

**Figure 10**. (a) The viability of BMSCs cultured with the extracts with different concentrations from mesoporous Sr-Si glass powders for 7 days. Sr-Si mesopre glasses have no toxicity. * compared to blank control (p=0.0336); ** compared to blank control (p=0.00675). (b) The ALP activity of BMSCs cultured with the extracts with different concentrations from mesoporous Sr-Si glass powders for 14 days. The corresponding ion concentrations of the mixture for cell culture were listed in Table 2. At the high concentration (50 mg/ml), 2.5Sr, 5Sr and 10Sr-100Si mesoporous glass shows an inhibitory effect for ALP activity of BMSCs. At the extract concentration (12.5 mg/ml),

10Sr-100Si has improved ALP activity of BMSCs compared to 100Si, indicating Sr and Si ions at a proper concentration from mesoporous Sr-Si glass has ability to enhance ALP activity of BMSCs.

**Figure 11.** Figure 11. (a): The morphology of the prepared 0Sr-100Si (left) and 10Sr-100Si (right) mesopore-glass scaffolds; (b) and (c): SEM images for 0Sr-100Si and 10Sr-100Si mesopore-glass scaffolds, respectively; (d): Drug (Dexamethasone) release behavior for 0Sr-100Si and 10Sr-100Si mesopore-glass scaffolds.

Table 1. Pore size, volume and specific surface area for the mesoporous Sr-Si glasses with different Sr contents.

| Materials | Average pore size (nm) | Specific Surface ($m^2/g$) | Total Pore Volume ($cm^3/g$) |
|---|---|---|---|
| 100Si | 2.8 | 6.8 | 0.01 |
| 2.5Sr-100Si | 4.0 | 7.0 | 0.01 |
| 5.0Sr-100Si | 4.8 | 6.0 | 0.01 |
| 10Sr-100Si | 5.4 | 209 | 0.30 |

Table 2. The corresponding ion concentrations for the final mixture of extracts and medium for cell culture.

| **Materials** | **The extract concentrations (mg/mL)** | **Ion concentrations of final mixture for cell culture (ppm)** | |
|---|---|---|---|
| | | **$SiO_4^{4-}$** | **$Sr^{2+}$** |
| | Blank control (culture medium) | 0 | 0 |
| **100Si** | 3.125 | 2.4±0.048 | 0 |
| | 6.25 | 4.7±0.096 | 0 |
| | 12.5 | 9.4±0.188 | 0 |
| | 25 | 18.8±0.376 | 0 |
| | 50 | 37.5±0.75 | 0 |
| **2.5Sr-100Si** | 3.125 | 2.9±0.058 | 1.7±0.1649 |
| | 6.25 | 5.8±0.116 | 3.3±0.3201 |
| | 12.5 | 11.7±0.234 | 6.6±0.6402 |
| | 25 | 23.3±0.466 | 13.3±1.2901 |
| | 50 | 46.5±0.93 | 26.5±2.5705 |
| **5.0Sr-100Si** | 3.125 | 6.5±0.13 | 2.2±0.2134 |
| | 6.25 | 13±0.26 | 4.3±0.4171 |
| | 12.5 | 25.5±0.51 | 8.6±0.8342 |
| | 25 | 51±1.02 | 17.3±1.6781 |
| | 50 | 102±2.04 | 34.5±3.3465 |
| **10Sr-100Si** | 3.125 | 5.7±0.114 | 2.4±0.2328 |
| | 6.25 | 11.3±0.226 | 4.7±0.4559 |
| | 12.5 | 22.6±0.452 | 9.4±0.9118 |
| | 25 | 45.2±0.904 | 18.8±1.8236 |
| | 50 | 90.5±1.81 | 37.5±3.6375 |