Fabrication and characterization of freeze dried strontium-doped bioactive glasses/chitosan composite scaffolds for biomedical engineering

Chao-Kuang Kuo, Hsiang-Wei Huang, Liu-Gu Chen and Yu-Jen Chou

*Department of Mechanical Engineering, National Taiwan University of Science and Technology, Taipei Taiwan; ‡Department of Engineering and System Science, National Tsing Hua University, Hsinchu Taiwan

ABSTRACT
With the emerging development of bioactive glass (BG), it has been regarded as a potential candidate for bone and tissue engineering due to its superior bioactivity and osteoconductivity. In addition, chitosan is a functional material for medical applications owing to its biocompatibility and biodegradability while playing an important role in the cell attachment, proliferation, and differentiation. In the present work, preparations of Sr-BG/chitosan composite scaffolds were fabricated by freeze drying technique. The microstructure and porosity of the scaffolds were characterized by scanning electron microscopy. The swelling and degradation behaviors were examined and corresponding mechanical properties were measured using universal testing machine. In addition, in vitro bioactivity was evaluated following Kokubo’s protocol and the cytotoxicity was carried out using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. Finally, formation mechanisms of the microstructures were discussed, while the improved bioactivity and cell viability were demonstrated, indicating its potential in the field of tissue engineering.

1. Introduction
During the past few decades, bioactive materials and polymer-matrix composites has received enormous attention in the fields of tissue engineering, while studies are aiming on developing biological scaffolds for implantation and fillers in order to remodel, repair, preserve, or enhance certain functions in bone tissue [1]. However, for fabrication of the bone scaffolds, several requirements such as porosity were demanded [2,3]. First, a scaffold should possess high porosity to enable cell growth, migration and survivability [4], and studies have demonstrated that the optimal pore size for bone tissue is ranging from 100 to 350 μm [5,6]. Next, the scaffold must have the essential mechanical property to prevent structural collapse during the period of tissue remodeling. At last, all scaffolds must be biocompatible and nontoxic to human cells. In general, synthetic polymers such as poly(lactic acid) and poly(glycolic acid) have been used as scaffolds owing to their biodegradability and good mechanical properties. However, poor interactions with cells need to be overcome [7,8]. In contrast, natural polymers such as collagen, gelatin, and chitosan are capable of achieving a differentiated cell phenotype while allowing cell expansion. Yet, disadvantages of poor mechanical properties and fast degradation rate make them difficult to be considered [9]. Thus, researches have been focused on the development of bioceramic/polymer composite scaffolds [10,11].

Within the family of bioceramic, bioactive glass (BG) has attracted numerous attention in various research fields since its first report by Hench et al. in 1971 [12]. Due to its superior biomedical properties, such as bioactive, osteoconductivity and biocompatibility [13], BG has been regarded as an emerging biomaterial for bone displacement and drug delivery in human body for past few decades. During the development of BG, preparation methods of conventional glass melting and sol-gel process are commonly used. Yet disadvantages of ease to crystalize, long processing time, and irregular shapes make both process difficult to be adapted into composite scaffolds [14,15]. To overcome the above problems, Chou et al. demonstrated the use of spray drying technique for preparation of BG powders, which has been universally utilized in the field of pharmaceuticals [16]. Moreover, the development of BG is heading toward a multifunctional material, which is fabricated by controlling the release of specific metal ions (e.g. Ag, Mn, Sr, B), thus incorporating various functions such as antibacterial property, osteoblast activity, and wound healing ability, etc [17–19]. Among these functional ions, various studies have shown that strontium (Sr) ion is one of the most attractive dopants due to its role in promoting osteoconductivity and accelerate differentiation of bone cells [20–22]. All these reports indicate the importance of Sr in the fields of bone implant applications.
Polymer-wise, chitosan is a polymer produced by deacetylation of chitin, and is considered as a feasible candidate due to its excellent biological behaviors such as biocompatibility, biodegradability, antimicrobial activity, and low immunogenicity [23]. Meanwhile, the structure of chitosan is similar to the main components of glycosaminoglycan in bones and cartilage, thus it plays an important role in bone cells attachment, proliferation, and differentiation [24]. However, drawbacks of poor mechanical strength, lack of bioactivity, and shortage of biomineralization need to be overcome [25–28]. Therefore, studies have demonstrated the use of bioceramics to improve the disadvantages of pure chitosan scaffold and enhance its biological, chemical, and physical properties [29–31].

To fabricate scaffolds for tissue engineering, various methods such as electro spinning, gas forming foam, and freeze drying have been demonstrated [32,33]. Among these processes, freeze drying is commonly used because it is environmentally friendly and economical. In addition, ease of casting with different pore morphologies while avoiding dried stress and shrinkage making it suitable for fabrication of composite scaffolds [34]. Thus in this work, we prepared the Sr-doped BG (Sr-BG) powders by spray drying technique, which provides advantages of rapid fabrication, mass production and high purity. The resulting Sr-BG powders were used for fabrication of chitosan based composite scaffold using the freeze drying technique. The microstructures were characterized using scanning electron microscopy (SEM), the porosities, swelling ratios, and degradation behaviors were measured by immersion tests, and the mechanical properties were examined using universal testing machine. In addition, evaluation of in vitro bioactivity was carried out by both X-ray diffraction (XRD) and SEM, and the cytotoxicity was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenylte-trazolium bromide (MTT) assay.

2. Materials and methods

2.1. Powder synthesis

In this work, Sr-BG powder was synthesized using the spray drying technique. Composition of 58S (SiO$_2$: CaO: P$_2$O$_5$ = 60: 35: 5 (mol%)) was used in order to achieve better bioactivity. Initially, the precursor solution was prepared by adding 76.52 g tetraethyl orthosilicate (Si(OC$_2$H$_5$)$_4$, 99.9%, Showa, Japan), 50.60 g calcium nitrate tetrahydrate (Ca(NO$_3$)$_2$.4H$_2$O, 98.5%, Showa, Japan), 11.15 g triethyl phosphate ((C$_2$H$_5$)$_3$PO$_4$, 99.0%, Alfa Aesar, UK), and 3.40 g strontium nitrate (Sr(NO$_3$)$_2$, 99.0%, Sigma-Aldrich, United States) into 120.00 g ethanol as the foundation of SiO$_2$, CaO, P$_2$O$_5$, and SrO, respectively. The precursor solution was stirred at 25°C for 1 h for complete dissolution of precursors, then de-ionized water was added till 1000 mL and stirred again for 24 h to ensure solution homogeneity. For the spray drying process, the precursor solution was dispersed into small droplets with high speed rotating disc set at 20,000 rpm. A flow rate of 50 ml/min was used to spray into the spray drying machine (SD DD-03, IDTA machinery Co., Taiwan). The chamber was set at 200°C to dry and form the initial powder. The powder was then calcined at 600°C for 1 h with a heating rate of 1°C/min to form the final Sr-doped BG powder.

3. Scaffold fabrication

Initially, for the 100% chitosan scaffold, 3.00 g chitosan powder was dissolved in 100 mL 2% acetic acid solution and stirred for 24 h. The resultant solution was transferred into a cylindrical mold (diameter of 8 mm and height of 10 mm), frozen at −45°C for 3 h, and followed by lyophilization at −1 and 25°C for 53 and 7 h. For the Sr-BG/chitosan composite scaffolds, various addition of Sr-BG (0, 1, 3, 5, 10, and 20 wt%) were mixed with the chitosan powder and dissolved in the acetic acid solution. Following the same freeze drying process of molding, freezing, and lyophilization.

4. Characterization

First, the microstructures were observed using SEM (6500 F, JEOL, Japan) operating under voltage of 15 kV. The scaffolds were fixed on the stub with carbon-coated tape and coated with Pt by sputtering. In addition, the scaffold porosities were derived using liquid displacement method. Each scaffold was immersed in deionized water for 2 h until saturated and the porosity was computed following the equation below,

\[
\text{Porosity} = \left( \frac{w_2 - w_1}{\rho v} \right) \times 100\%
\]

where $w_2$ and $w_1$ represent the weight of the scaffolds before and after immersion, while $\rho$ is the density of water and $v$ is the volume of the scaffold.

By measuring the initial weights of the dried scaffolds, denoted as $W_{\text{initial}}$, the scaffolds were then soaked in deionized water for 2 h till saturation. Then, the excess water on surface of swollen scaffold were wiped by tissue paper, and the wet weights were measured and denoted as $W_{\text{wet}}$. Finally, the swelling behaviors were represented by the ratio computed following the equation below:

\[
\text{Swelling ratio} = \left( \frac{W_{\text{wet}} - W_{\text{initial}}}{W_{\text{initial}}} \right)
\]

Following ISO standard 10,993–14, the degradation tests were performed by immersing the scaffolds into phosphate buffered saline (PBS). Initially, the scaffolds were precisely weighted before immersion which denoted as $W_{\text{initial}}$. Then, the scaffolds were immersed
into PBS solution and placed in a rotational isothermal incubator set at 37°C and 60 rpm for various durations of up to 336 h (14 d). After immersion, the PBS solutions were discarded and the scaffolds were washed repeatedly by deionized water to remove any trace of water-soluble compounds. Subsequently, the washed scaffolds were transferred into an oven held at 70°C and dried for 24 h, the dried weight were measured and denoted as \( W_{\text{dry}} \). Finally, the weight loss were computed via the following equation:

\[
\text{Weightloss(\%)} = \left( \frac{W_{\text{initial}} - W_{\text{dry}}}{W_{\text{initial}}} \right) \times 100\%
\]

The mechanical properties of the Sr-BG/chitosan composite scaffolds were investigated by a universal testing machine. The cylindrical scaffolds were placed on the stage and compression rate of 300 mm/min was used for measurements of compressive stresses and compressive strains. In addition, compressive modulus of all scaffolds were calculated by the force required to compress to 10% strain.

Next, the bioactivities of all Sr-BG/chitosan scaffolds were evaluated following Kokubo’s protocol [35]. By immersing the scaffolds into the simulated body fluid (SBF), which has a similar ion concentration to human blood plasma, with a ratio of 0.02 g/mL. All scaffolds were kept in an orbital shaker (S300R, Firstek Scientific, Taiwan) and held at 37°C for 168 h, note that SBF was changed every 24 h. The resulting powders were cleaned with both deionized water and acetone for three times, then placed in a 70°C oven to dry for 24 h. Both XRD (D2 Phaser, Bruker, Germany) and SEM were used for the evaluation of bioactivity of each specimen. The XRD patterns were acquired with wavelength of 1.54 Å using Ni-filtered Cu-Ka source between 20 and 80°, while SEM images were acquired following the above setup.

Evaluation of cytotoxicity was carried out by MTT assay. According to the standard testing protocol ISO 10,993–5, the cell viabilities of all Sr-BG/chitosan scaffolds were determined using serial extractions. First, all scaffolds were sterilized by autoclave and dispersed in minimum essential medium at various extraction concentrations of 20%, 40%, 60%, 80%, and 100%. Meanwhile, seeded cells were cultured at a density of \( 2 \times 10^4 \) cells along with the extracts on a 24-well plate and then incubated at 37°C for 24 h in a humidified atmosphere controlled at 95% air and 5% CO₂. Next, the media were removed, 300 µL MTT reagent was added into each well, and the plates were transferred into a CO₂ incubator at 37°C for 72 h. Then, the medium was aspirated, followed by addition of 200 µL dimethyl sulfoxide (DMSO) into each well. Each solution was transferred to a 96-well plate, while a microplate reader (Multiskan Go, Thermo Scientific, USA) was employed for measurements of optical density at a wavelength of 570 nm. Finally, Statistical analysis of ANOVA (Analysis of Variance) was applied to study the effects of multiple factors.

5. Results

The macroscopic morphology of freeze dried chitosan scaffold, and 1, 3, 5, 10, and 20 wt% Sr-BG/chitosan composite scaffolds were shown in Figure 1. Initially, Figure 1 (a) shows the 100% chitosan scaffold, the graph shows that a nominal size of 10 mm in height were observed, indicating a successful fabrication of freeze dried scaffold. For the Sr-BG/chitosan composite scaffolds, similar morphology can be found from the 1, 3, and 5 wt% Sr-BG/chitosan composite scaffolds (as

![Figure 1](image-url)
shown in Figure 1 (b), (c), and (d)). However, when the solid content of Sr-BG increased to 10 and 20 wt%, the scaffolds cannot be molded and appeared fragmented. SEM images of freeze dried chitosan scaffolds, and 1, 3, and 5 wt% Sr-BG/chitosan composite scaffolds are shown in Figure 2 (b), (c), and (d), similar porous structures were observed, while the averaged pore diameters were measured as 185.2 ± 56.2, 191.2 ± 77.1, and 192.9 ± 70.2 µm, respectively. In contrast, the 10 and 20 wt% Sr-BG/chitosan composite scaffolds tended to be agglomerated without formation of porous structure. Meanwhile, insets of high magnification SEM images showed the distributed Sr-BG particles within each scaffold, indicating the successful synthesis of Sr-BG/chitosan composite scaffolds. In brief, the results show that freeze dried pure chitosan scaffolds, and 1,
3, and 5 wt% Sr-BG/chitosan composite scaffolds exhibited porous structures which satisfied the ideal pore size of the bone tissue scaffold ranging between 100 and 350 μm [5,6]. Note that since the 10 and 20 wt% Sr-BG/chitosan composite scaffolds cannot be formed, further characterization will be carried out excluding both 10 and 20 wt% Sr-BG/chitosan composite scaffolds.

The porosity of freeze dried chitosan scaffold, and 1, 3, and 5 wt% Sr-BG/chitosan composite scaffolds as shown in Figure 3, which were computed as 87.16 ± 1.68, 82.56 ± 3.85, 70.83 ± 4.22, and 66.47 ± 5.33%. Based on Chong et al., high porosity greater than 70% is needed for cell seeding and ingrowth [36], indicating that freeze dried chitosan scaffold, and 1 and 3 wt% Sr-BG/chitosan composite scaffolds were suitable as a scaffold for bone regeneration.

In tissue engineering, the swelling behavior aids the supply of oxygen and nutrients, while uncontrolled swelling ratio could be harmful for certain applications [37]. Figure 4 shows the swelling ratio of freeze dried chitosan scaffold and 1, 3, and 5 wt% Sr-BG/chitosan composite scaffolds. It can be seen from the figure that pure chitosan scaffold has the highest swelling ratio of 16.67 ± 0.40 among all specimens. Meanwhile, the results suggested that by increase of Sr-BG powders the swelling ratio decreased. Resulting in 11.91 ± 0.56, 6.4 ± 0.56, and 5.17 ± 0.42 for 1, 3, and 5 wt% Sr-BG/chitosan scaffolds, respectively. In addition, the degradation behaviors of all scaffolds were evaluated by PBS immersion for 672 h. The results showed that the weight loss of freeze dried chitosan scaffold increased rapidly till 57% in the first 24 h. After immersion for 24 h, the dissolution rates began to slow down and reached 68%, 84%, and 85% and for the periods of 72 h (3 d), 168 h (7 d), and 336 h (14 d), respectively. Meanwhile, similar trends can be observed from the Sr-BG/chitosan composites scaffolds with percentages of weight loss reached 63%, 56%, and 28% in the first 24 h, while ending up with 68%, 59%, and 31% after 336 h of immersion for 1, 3, and 5 wt% Sr-BG/chitosan scaffolds, respectively. In brief, the results showed that with the increasing addition of Sr-BG powder, the weight loss decreased significantly. Showing that the order of degradation rate is pure chitosan scaffold > 1 wt% Sr-BG/chitosan composite scaffold > 3 wt% Sr-BG/chitosan composite scaffold > 5 wt% Sr-BG/chitosan composite scaffold.

The mechanical properties of the Sr-BG/chitosan composite scaffolds were investigated by universal testing machine. A maximum stress of 0.57 MPa can be observed in 5 wt% Sr-BG/chitosan composite scaffold, followed by 0.27 MPa for 3 wt% Sr-BG/chitosan composite scaffold, 0.14 MPa for 1 wt% Sr-BG/chitosan composite scaffold, and 0.10 MPa for pure chitosan scaffold under the same strain condition. In addition, the corresponding compressive modulus were computed at 10% of strain due to the consistency of linear slope and the results are shown in Figure 5. It can be seen from the graph that with addition of Sr-BG powder into chitosan scaffold, the compressive modulus can be improved. As compared to pure chitosan scaffold, the compressive modulus of 1, 3, and 5 wt% Sr-BG/chitosan scaffold can be increased by 2.8, 6.2, and 8.1 times, respectively.

Figure 4. Swelling ratio of freeze dried chitosan scaffold, and 1, 3, and 5 wt% Sr-BG/chitosan composite scaffolds under continuous compression.
For the in vitro bioactivity, Figure 6 shows the XRD patterns of freeze dried chitosan scaffold, and 1, 3, and 5 wt% Sr-BG/chitosan composite scaffolds after immersing into SBF for 7 d. Initially, it can be seen from Figure 6 that no distinct peaks were found in pure chitosan scaffolds, suggesting that the structure is amorphous. In contrary, with the addition of Sr-BG powders, 002 and 112 diffractions could be observed around 26° and 32° for all composite scaffolds, which indicate the formation of hydroxyapatite (Ca$_5$(PO$_4$)$_3$(OH), JCPDF 84–1998).

Moreover, Figure 7 shows the SEM images of freeze dried chitosan scaffold, and 1, 3, and 5 wt% Sr-BG/chitosan composite scaffolds after the in vitro bioactivity test. It can be seen from Figure 7 (a) that the surface of pure chitosan scaffold remain smooth. In contrast, needle-shaped HA structures can be observed on the surface of Sr-BG/chitosan composite scaffolds as shown in Figure 7 (b), (c) and (d). In summary, the SEM images showed a good agreement with the XRD patterns, and both characterizations confirmed that all Sr-BG/chitosan composite scaffolds are bioactive.
Evaluation of cytotoxicity of all freeze dried scaffolds were examined by MTT assay, and the resulting cell viability are shown in Figure 8. Following ISO protocol, cell viability was determined by computing the percentage of living cells against the control specimen, and a standard level of 70% (shown in dashed line) were recommended for consideration. As shown in the figure, all freeze dried scaffolds conformed the standard level under extraction concentrations of 20%, 40%, 60%, and 80%. Yet, at 100% extraction concentration, the cell viability of pure chitosan scaffold decreased to 70.2 ± 6.5% nearly passed the standard level. In addition, p values were computed against the pure chitosan scaffold, the results suggested that

Figure 7. Low and high magnification SEM micrographs of freeze dried (a) chitosan scaffold, and (b) 1, (c) 3, and (d) 5 wt% Sr-BG/chitosan composite scaffold after immersing in SBF for 7 d.

Figure 8. Cell viability of freeze dried chitosan scaffold and 1, 3, and 5 wt% Sr-BG/chitosan composite scaffolds derived from MC3T3-E1 cells after incubation for 3d. (*: p value < 0.05).
p values computed from 1 wt% Sr-BG/chitosan composite scaffold at all extraction concentrations were greater than 0.05, indicating the difference was not significant compared to the pure chitosan scaffold. In contrary, p values computed using 3 wt% and 5 wt% Sr-BG/chitosan composite scaffolds were lower than 0.05 at various extraction concentrations, showing a significant difference against the pure chitosan scaffold. In brief, the results indicated that the addition of Sr-BG powders were able to improve the cell viability and all Sr-BG/chitosan scaffolds were nontoxic at every extraction concentrations from 20% to 100%.

6. Discussion

First, the formability of the freeze dried scaffolds was discussed. Based on the SEM images shown in Figure 2, the particle dispersed evenly for 1, 3, and 5 wt% Sr-BG/chitosan composite scaffolds (Figure 2 (b), (c), and (d)). In contrast, aggregations were observed from both 10 and 20 wt% Sr-BG/chitosan composite scaffolds (Figure 2 (e) and (f)) which cannot form the layered porous structure. Herein, the reasons causing the agglomeration phenomenon could be categorized into pH value and the effect of bubbles. Owing to the property of chitosan, it is only soluble in acid solution with pH value less than 5. However, BG has an alkaline property during its dissolution. This will results in acid-base neutralization and cause the rise of pH value. The increased pH value will decrease the solubility of chitosan and result in prior precipitation. Furthermore, water, which cannot dissolve chitosan, will be formed from the combination of hydroxide ion and oxygen ion owing to the neutralize reaction. This will result in increased viscosity and poor dispersibility, thus leading to agglomeration. In addition, bubbles are produced during the steam sublimation stage of the freeze drying process. However, as the solid contents of Sr-BG increased, the steam in bottom of the mold might be trapped by the excessive powder and could not be sublimated completely. Hence, the trapped steam will lead to collapse of scaffold during the freeze drying process. In brief, with increased addition of Sr-BG powder, the formability of the chitosan scaffold is decreased.

Next, the correlations between the porosity, swelling behavior, degradation behavior, and mechanical property were discussed. First, by comparing the porosity, swelling ratio, and degradation rate, similar trends can be observed following the Sr-BG concentration, i.e. with the increase of Sr-BG concentration, the porosity, swelling ratio, and degradation rate decreased. Following Pouraghagouy et al. [38], the main reason of the change of porosity is due to the absence of chitosan wall pores after addition of Sr-BG powders, which could be seen from the SEM images as shown in Figure 2. Then, for the swelling ratio, since both chitosan and Sr-BG powders possess hydrophilic property, these hydrophilic groups were engaged in the interaction. Thus would cause reduction in the number of free hydrophilic groups and result in decrease of swelling ratio of the scaffolds [39]. At last, the decreased porosities will result in lower specific surface areas, which leads to slower degradation rates. This showed that the addition of Sr-BG powder significantly affects the ability of both swelling and degradation behaviors of the scaffolds. In contrast, comparing the porosity against the compressive modulus, an inverse relationship was observed, i.e. with the increased of porosity, the compressive modulus decreased. Note that higher mechanical properties were examined from the 5 wt% Sr-BG/chitosan composite scaffold as compared to previous studies [40,41]. Briefly, these results indicate that the performance of the scaffold could be controlled by adjusting the composition of scaffold, and the amount of Sr-BG added in could be modified targeting different requirements.

At last, we discuss the bioactivity and cell viability of all freeze dried scaffolds. As shown in the XRD patterns (Figure 6) and SEM images (Figure 7), all Sr-BG/chitosan composite scaffolds were confirmed to be bioactive, and since higher peak area corresponds to higher crystallinity; the order of bioactivity can be computed as 5 wt% Sr-BG/chitosan composite scaffold > 3 wt% Sr-BG/chitosan composite scaffold > 1 wt% Sr-BG/chitosan composite scaffold, indicating a direct correlation that the bioactivity was contributed by the addition of Sr-BG powders. In addition, regarding the cell viabilities of all scaffolds, studies have shown that Sr dopant is capable of enhancing the activity of alkaline phosphatase, stimulate the formation of bone cells and osteocalcin, without affecting the mineralization of bone matrix [42,43]. This effect of Sr dopant can be seen from the cytotoxicity results as shown in Figure 8. By culturing with osteoblast cells for 3 d, all Sr-BG/chitosan composite scaffolds exhibit higher cell viability compared to the pure chitosan scaffold. This is due to the release of Sr ions during dissolution which helps the proliferation of osteoblast cells. Note that in certain extraction concentrations, the cell viabilities of Sr-BG/chitosan composite scaffolds reached more than 100%, indicating its ability to promote cell culture and

| Table 1. Two-way ANOVA analysis for evaluation of cytotoxicity. |
|-------------------|----|--------------|----------------|----------------|
| Source of variances | D  | Sum of square | Mean sum of square | Fadj* |
| Sr-BG addition    | 3  | 1503.40      | 501.12           | 6.93  |
| MTT extraction concentration | 4  | 2314.20      | 578.54           | 8.00  |
| Residual          | 12 | 867.60       | 72.30            |       |
| Total             | 19 | 4685.10      |                  |       |

(Significant level at 5%, F0.05, 3, 12 = 3.49, F0.05, 4, 12 = 3.26)
7. Conclusions

In this study, spray dried Sr-doped BG powder was prepared and Sr-BG/chitosan composite scaffolds were successfully fabricated using freeze drying technique. The resulting morphologies, porosities, and mechanical properties of all scaffolds were characterized and the corresponding formability were discussed. In addition, the swelling and degradation behaviors were examined, suggesting that all Sr-BG/chitosan composite scaffolds are bioactive and non-toxic to osteoblast cells. At last, enhanced bioactivity and cell viability was reported, which demonstrated that Sr-BG/chitosan composite scaffolds could be considered as a potential candidate in fields of medical applications.

Acknowledgments

The authors acknowledge the financial support from Ministry of Science and Technology of Taiwan (Grant number of MOST 108-2218-E-011-035).

Disclosure statement

© The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

© The authors declare the following financial interests/personal relationships which may be considered as potential competing interests.

Funding

This work was supported by the Ministry of Science and Technology, Taiwan [MOST 108-2218-E-011-035].

ORCID

Chao-Kuang Kuo https://orcid.org/0000-0002-4273-2782

References

[1] Vacanti JP, Langer R. Tissue engineering: The design and fabrication of living replacement devices for surgical reconstruction and transplantation. Lancet. 1999;354:532–534.

[2] Madihally SV, Matthew HW. Porous chitosan scaffolds for tissue engineering. Biomaterials. 1999;20(12):1133–1142.

[3] Suh J-KF, Matthew HW. Application of chitosan-based polysaccharide biomaterials in cartilage tissue engineering: a review. Biomaterials. 2000;21(24):2589–2598.

[4] Murphy CM, Haugh MG, O’Brien FJ. The effect of mean pore size on cell attachment, proliferation and migration in collagen-glycosaminoglycan scaffolds for bone tissue engineering. Biomaterials. 2010;31(3):461–466.

[5] Kramschuster A, Turng L-S. Fabrication of tissue engineering scaffolds. Handbook of Biopolymers and Biodegradable Plastics. 2012;427–446.

[6] Bose S, Roy M, Bandyopadhyay A. Recent advances in bone tissue engineering scaffolds. Trends Biotechnol. 2012;30(10):546–554.

[7] Chang C-H, Liu H-C, Lin -C-C, et al. Gelatin–chondroitin–hyaluronan tri-copolymer scaffold for cartilage tissue engineering. Biomaterials. 2003;24(26):4853–4858.

[8] Sato T, Chen G, Ushida T, et al. Tissue-engineered cartilage by in vivo culturing of chondrocytes in collagen/poly(lactic-co-glycolic) acid nanoparticles-loaded chitosan/bioactive glass scaffolds as a localized delivery system in the bone defects. Biomater Int. 2014(2014):898930.

[9] Hench LL, Splinter RJ, Allen WC, et al. Bonding mechanisms at the interface of ceramic prosthetic materials. J Biomater Appl. 1991;5(6):117–141.

[10] Hench LL. Bioceramics: from concept to clinic. J Am Ceram Soc. 1994;77(7):1487–1510.

[11] Leite AJ, Mano J. Biomedical applications of natural-based polymers combined with bioactive glass nanoparticles. J Mat Chem B. 2017;5(24):4555–4568.

[12] Tseng C-F, Fei Y-C, Chou Y-J. Investigation of in vitro bioactivity and antibacterial activity of manganese-doped spray pyrolyzed bioactive glasses. J Non-Cryst Solids. 2020;549:120336.

[13] Naseri S, Lepry WC, Nazhat SN. Bioactive glasses in wound healing: Hope or hype? J Mat Chem B. 2017;5(31):6167–6174.

[14] Amudha S, Ramya JR, Arul KT, et al. Enhanced mechanical and biocompatible properties of strontium ions doped mesoporous bioactive glass. Compos Part B Eng. 2020;196:108099.
[21] Gentleman E, Fredholm YC, Jell G, et al. The effects of strontium-substituted bioactive glasses on osteoblasts and osteoclasts in vitro. Biomaterials. 2010;31(14):3949–3956.

[22] Strobel L, Hild N, Mohn D, et al. Novel strontium-doped bioactive glass nanoparticles enhance proliferation and osteogenic differentiation of human bone marrow stromal cells. J Nanopart Res. 2013;15(7):1–9.

[23] Jayakumar R, Menon D, Manzoor K, et al. Biomedical applications of chitin and chitosan based nanomaterials—a short review, Carbohydr. Polym 2010;82:227–232.

[24] Brandenberg G, Leibrock LG, Shuman R, et al. Chitosan: a new topical hemostatic agent for diffuse capillary bleeding in brain tissue. Neurosurgery. 1984;15(1):9–13.

[25] Aljawish A, Chevalot I, Jasniewski J, et al. Enzymatic synthesis of chitosan derivatives and their potential applications. J Mol Catal B. 2015;112:25–39.

[26] Kanatt SR, Chander R, Sharma A. Chitosan and mint mixture: a new preservative for meat and meat products. Food Chem. 2008;107(2):845–852.

[27] Zhang Y, Zhang M. Synthesis and characterization of macroporous chitosan/calcium phosphate composite scaffolds for tissue engineering. J Biomed Mater Res. 2001;55(3):304–312.

[28] LogithKumar R, KeshavNarayan A, Dhiya S, et al. A review of chitosan and its derivatives in bone tissue engineering, Carbohydr. Polym 2016;151:172–188.

[29] Correia CO, Leite AJ, Mano JF. Chitosan/bioactive glass nanoparticles scaffolds with shape memory properties, Carbohydr. Polym 2015;123:39–45.

[30] Jayakumar R, Ramachandran R, Divyarani VV, et al. Fabrication of chitin–chitosan/nano TiO2 composite scaffolds for tissue engineering applications. Int J Biol Macromol. 2011;48(2):336–344.

[31] Sivashankari PR, Prabaharan M. Three-dimensional porous scaffolds based on agarose/chitosan/graphene oxide composite for tissue engineering. Int J Biol Macromol. 2020;146:222–231.

[32] Liang D, Hsiao BS, Chu B. Functional electrospun nanofibrous scaffolds for biomedical applications. Adv Drug Deliver. Rev. 2007;59(14):1392–1412.

[33] Turnbull G, Clarke J, Picard F, et al. 3d bioactive composite scaffolds for bone tissue engineering. Bioact Mater. 2018;3:278–314.

[34] Wu X, Liu Y, Li X, et al. Preparation of aligned porous gelatin scaffolds by unidirectional freeze-drying method. Acta Biomater. 2010;6(3):1167–1177.

[35] Kokubo T, Kushitani H, Ohtsuki C, et al. Chemical reaction of bioactive glass and glass-ceramics with a simulated body fluid. J Mater Sci Mater Med. 1992;3:79–83.

[36] Chong EJ, Phan TT, Lim UJ, et al. Evaluation of electrospun pcl/gelatin nanofibrous scaffold for wound healing and layered dermal reconstitution. Acta Biomater. 2007;3(3):321–330.

[37] Peter M, Binulal NS, Soumya S, et al. Nanocomposite scaffolds of bioactive glass ceramic nanoparticles disseminated chitosan matrix for tissue engineering applications, Carbohydr. Polym 2010;79:284–289.

[38] Pourhaghgouy M, Zamanian A, Shahrezaee M, et al. Physicochemical properties and bioactivity of freeze-cast chitosan nanocomposite scaffolds reinforced with bioactive glass. Mater Sci Eng C. 2016;58:180–186.

[39] Saravanan S, Leena RS, Selvamurugan N. Chitosan-based biocomposite scaffolds for bone tissue engineering, Int J Biomacromol. 2016;93:1354–1365.

[40] Li X, Yin H-M, Su K, et al. Polydopamine-assisted anchor of chitosan onto porous composite scaffolds for accelerating bone regeneration. ACS Biomater Sci Eng. 2019;5(6):2998–3006.

[41] El-Sayed SA, Mabrouk M, Khalil ME, et al. Antibacterial, drug delivery, and osteoinduction abilities of bioglass/chitosan scaffolds for dental applications, J Drug Deliv Sci Technol. 2020;57:101757.

[42] Verberckmooes SC, De Broe ME, D’Haese PC. Dose-dependent effects of strontium on osteoblast function and mineralization. Kidney Int. 2003;64(2):534–543.

[43] Dahl SG, Allain P, Marie PJ, et al. Incorporation and distribution of strontium in bone. Bone. 2001;28(4):446–453.