Bioactive Glass Flakes as Innovative Fillers in Chitosan Membranes for Guided Bone Regeneration

Huijun Zhang, Jingjing Wu, Ying Wan, Stefan Romeis, Julian D. Esper, Wolfgang Peukert, Kai Zheng,* and Aldo R. Boccaccini*

Herein, novel bioactive glass flakes (BGFs) (45S5 composition), obtained by compression in the liquid phase in a stirred media mill, are combined with chitosan (CS) to develop composite membranes using solvent casting for guided bone regeneration (GBR). The incorporation of BGFs endows CS membranes with superior bioactivity as evidenced by rapid hydroxyapatite formation after 3 days of immersion in simulated body fluid (SBF). Moreover, the addition of BGFs reduces the swelling ratio of CS membranes, beneficial for GBR applications. The swelling behavior can also be modulated by tuning the added amount of BGFs. In vitro cell studies indicate the noncytotoxicity of composite membranes toward osteoblast cell line MC3T3-E1, although the presence of BGFs slightly reduces the cell viability compared to pure CS membranes. The incorporation of BGFs also improves the osteogenic differentiation of MC3T3-E1 cells as indicated by the enhanced alkaline phosphatase activity. The results show the feasibility of BGFs as anisotropic bioactive fillers in polymeric matrices. In conclusion, the developed CS-BGF composite membranes show promising potential as a novel material for GBR.

1. Introduction

Bioactive glasses (BGs) have shown great potential in a variety of biomedical applications, such as orthopedic[1–3] and dental applications,[4] because they exhibit great bone-bonding ability, biocompatibility, osteogenesis, and angiogenesis properties,[5–7] 45S5 bioactive glass (45 SiO2, 24.5 CaO, 24.5 Na2O, and 6.0 P2O5, wt%) is the first developed BG and a frequently applied bioactive material.[8] 45S5 BG powders or granules have been applied as bone defect fillers to accelerate bone repair due to their strong ability to inherently connect to soft tissue and bone.[8–10] 45S5 BG in particulate form can also be used as filler in polymers to develop mechanically strong and biocompatible composites.[11–12] To promote the reinforcing effects, 45S5 BGs have been fabricated in different morphologies, such as fibers and nanoparticles, for the application as fillers.[13–15] Glass flakes are attractive reinforcing fillers in many applications due to their relatively high aspect ratio and specific surface area. They are applied, for example, as protective coating[16] and in resin composites.[17] However, BG flakes (BGFs) have not been widely prepared and used in biomedical applications. 45S5 BGFs were successfully fabricated using a milling method in our previous study.[18,19] The flake powders showed an increased specific surface area and apatite forming ability compared with original irregularly shaped glass powders. The thickness of glass flakes can be controlled by tailoring milling parameters, such as milling time, stirrer tip speed, and grinding bead size.[20] Moreover, the biocompatibility of 45S5 BG can be retained after the milling process. Thus, the produced 45S5 BGFs are expected to be a suitable filler to enhance the properties of polymeric matrices. However, to the best of our knowledge, 45S5 BGFs have not been applied as rigid fillers in biopolymer-based composites intended for biomedical applications including wound healing and bone regeneration.

Guided bone regeneration (GBR) is a strategy that uses barrier membranes to directly induce new bone tissue regeneration at peri-implant defect sites.[21] Chitosan (CS), a natural cationic polysaccharide, has been extensively applied as a GBR membrane due to its appropriate degradation rate, low cost, superior bioactivity, antimicrobial properties, film-forming properties, and flexibility in hydrated environments.[22–23] However, CS-based membranes suffer from the deficiency of...
osseointegration and osteogenic activity, inhibiting their ability to induce effective bone regeneration.[24]

Many strategies have been applied to enhance the osseointegration and osteogenesis of CS-based membranes, including surface modification and the incorporation of bioactive fillers.[25] Due to their desirable bone-regeneration property, BGFs are attractive fillers in the GBR strategy.[23,27] Given the superior bioactivity and osteogenic effects of 45S5 BGFs, their inclusion into polymeric GBR membranes is expected to be able to significantly accelerate the formation of hydroxyapatite (an indicator of in vivo bone-bonding ability) and enhance osteogenic activities of the membranes.

Here, we incorporated 45S5 BGFs into CS to develop composite membranes. The formed membranes were Petri dishes and dried at room temperature (RT) for one week to characterize. Finally, the CS-BGF solutions were cast onto the CS solution. Then the mixture was mechanically homogenized (3 wt%) to form composite membranes. The precursors were homogenized in a roller mill for 1 day, 3, 7 days. The SBF solution was replaced twice per week. Then the composite membranes were taken out of the solution at certain time points, rinsed with deionized water, and dried at RT for SEM and FTIR analysis.

The swelling ratio of the samples was calculated using the following equation:

\[
\text{Swelling Ratio} = \frac{\text{Mass after soaking}}{\text{Mass before soaking}} \times \frac{\text{Volume of dried sample}}{\text{Volume of soaked sample}}
\]

2. Experimental Section

2.1. Preparation of 45S5 Bioactive Glass Flakes

45S5 BGFs were prepared as described in our previous work.[19] Briefly, an Al2O3-lined grinding chamber was loaded with \( \approx 1.8 \) kg of spherical yttria-stabilized zirconia milling beads with diameters of 2 mm (Tosoh, Tokyo, Japan). 45S5 BG in ethanol suspension (3 wt%) was used throughout all comminution experiments. For the preparation of 45S5 BG, the precursors including SiO2, CaCO3, Ca₃(PO₄)₂, and Na₂CO₃ (Sigma–Aldrich, St. Louis, MO, USA) were mixed in the corresponding molar ratios of 45S5 composition. The precursors were homogenized in a roller mill for 24 h. The powder mixture was melted to form a glass at 1450°C and kept for 1 h at this temperature before being quenched with water. The fritted glass was ground in a jaw crusher and a planetary mill to particle size of \( \approx 10 \) µm. The grinding experiments were conducted at a stirrer tip speed of 4.9 m s\(^{-1}\) and a suspension temperature of 10°C for 8 h. The particle size distribution of the obtained product was analyzed via SEM images. The particles were analyzed in terms of flake thickness and Feret diameter \( d_{ve} \) (the longest distance between two parallel lines, which restricts the particle perpendicular to the direction).

2.2. Fabrication of CS-BGF Membranes

Composite membranes were synthesized via a solvent casting method. In a typical procedure, 0.7 g CS (medium molecular weight, Sigma–Aldrich, St. Louis, MO, USA) was dissolved in 35 mL acetic acid solution (1% v/v) to form a CS solution (2% w/v). Then, specific amounts of BGFs were mixed with the CS solution. Then the mixture was mechanically homogenized for 10 min. Finally, the CS-BGF solutions were cast onto Petri dishes and dried at room temperature (RT) for one week to form composite membranes. The formed membranes were named CS-1BGF, CS-2BGF, and CS-3BGF according to the amount of BGFs (0.08, 0.175, and 0.35 g, respectively). CS membranes without BGFs were prepared using the same method and marked as the control group.

2.3. Characterization of Composite Membranes

2.3.1. Scanning Electron Microscopy (SEM)

The microstructure and morphology of CS-BGF composite membranes were characterized by SEM (Auriga, Zeiss, Germany). SEM images were taken at an accelerating voltage of 2 kV. SEM analysis was performed on a minimum of 250 particles (Gemini 55, Zeiss, Germany) to determine the particle size distribution. Energy-dispersive X-ray spectroscopy (EDS) was also executed during the SEM observation process.

2.3.2. X-Ray Diffraction (XRD)

A powder diffractometer in a Bragg-Brentano setup (D8 Advance: Bruker AXS, Karlsruhe, Germany) was used for the measurements. X-ray patterns were recorded within a 2θ range from 20° to 80°, a step width of 0.007° and a counting time of 3 s per step.

2.3.3. Fourier Transform Infrared Spectroscopy (FTIR)

The chemical structure of composite membranes was investigated by FTIR (IRAffinity-1S, Shimadzu Corporation, Japan). FTIR spectra were recorded in the region 400–4000 cm\(^{-1}\) with a resolution of 4 cm\(^{-1}\) in absorbance mode.

2.3.4. Tensile Test

Standard uniaxial tensile test (Instron, Darmstadt, Germany) was used to evaluate the mechanical properties of the composite membranes. Sample strips (5 mm × 40 mm, \( n = 5 \)) were tested under a loading speed of 10 mm min\(^{-1}\) with a gauge length of 20 mm, and a 50 N cell load. The ultimate tensile strength, elongation at break, and Young’s modulus were determined from the stress–strain curves. Average values and standard deviations (SDs) were defined after the test of at least five specimens of each type of membranes.

2.4. Bioactivity Study

Bioactivity of the composite membranes was determined by immersing them in simulated body fluid (SBF). Membrane samples (5 mm × 10 mm × 0.01 mm) were soaked in 50 mL SBF and maintained in a shaking incubator (37°C, 90 rpm) for 1 day, 3, and 7 days. The SBF solution was replaced twice per week. Then the composite membranes were taken out of the solution at certain time points, rinsed with deionized water, and dried at RT for FTIR and SEM analysis.

2.5. In Vitro Swelling Behavior

The in vitro swelling tests of CS and CS-BGF membranes were conducted together with the bioactivity test. Dried membranes were immersed in SBF for different periods. Excess liquid was removed from the membranes before recording their mass. The swelling ratio of the samples was calculated using the following equation
where \( M_0 \) and \( M_1 \) are the masses of the samples before and after swelling, respectively.

2.6. In Vitro Cytotoxicity

MC3T3-E1 cell line (ATCC CRL 2594) was cultured and expanded in the α-MEM medium (10% fetal bovine serum, 1% penicillin/streptomycin, and 1% HEPES), in a 5% CO\(_2\) humidified atmosphere at 37 °C in subsequent cell-specific experiments. Cells were first expanded in the α-MEM medium. The Cell Counting Kit-8 (CCK-8, Dojindo, Japan) assay was employed to evaluate cell viability quantitatively. Cells were seeded in 96-well plates with 2000 cells/well and incubated for 1 day and 3 days. The optical density (OD) values were measured and recorded by a microplate reader (Model 680, Bio-Rad) for 15 min at 405 nm.

3. Results and Discussion

3.1. Morphology of Bioactive Glass Flakes

45S5 BGFs were analyzed by SEM/EDX and the gathered results are shown in Figure 1. SEM micrographs confirmed the flake-like shape of BGFs. As shown in Figure 1A, the BGFs exhibit smooth surfaces with irregular shapes and rough edges due to the breakage of larger BG powders during pre-milling.[19] Feret diameter \( d_{Fe} \) and flake thickness distributions are given in Figure 1. The \( d_{Fe,50} \) value was determined to be 3.66 μm with an \( x_{50} \) thickness of 238 nm. SEM micrographs show agglomerates of flake-like particles with dimensions well above 10 μm. The specific surface area of 45S5 BGF reported in a previous study was between 22 and 30 m\(^2\) g\(^{-1}\), which is significantly larger than that of conventional melt-derived 45S5 BG (≈1 m\(^2\) g\(^{-1}\)).[19] The shape formation process to obtain flake-like particles has been reported by Esper et al. for glasses with multicomponent compositions.[19,28] Briefly, the glass shows brittle fracture until critical particle size is reached (brittle-to-ductile transition). Below this size, the particles are deformed plastically and upon milling platelets are formed.[29,30] Due to the incorporation of glass network modifiers, the brittle-to-ductile transition particle size shifts to bigger sizes. The derived size distributions in this work are in good agreement with work reported on several other glass systems. EDS confirms the peaks of Ca, P, and Si belonging to the composition of 45S5 BGFs.

3.2. Morphology and Structure of the Composite Membranes

To study the morphology of the CS-BGF composite membranes, SEM images at two magnifications were taken and shown in Figure 2. The pure CS membranes show smooth and plane surfaces (Figure 2A,B). The overall surface of CS-BGF composite is homogeneous but rougher than the surface of pure CS. Some asperities were observed on the surfaces recognized as BGFs (Figure 2C–H). As the BGFs content increases in the composite, more aggregates could be observed (Figure 3C–H) being well distributed on the whole surface. EDS mapping results further confirmed that BGFs distribute homogeneously on the surface of CS-BGF membranes, indicated by the homogeneous appearance of Si belonging to 45S5 BG flake (Figure 3).

3.3. FTIR Spectra and Swelling Behavior

The broadband in the 3000–3500 cm\(^{-1}\) region corresponds to the vibration of water molecules (Figure 4A). The new peaks in the 793–800 and 926 cm\(^{-1}\) regions can be mainly associated with Si–O and Si–O–Si vibration modes, which indicates the successful addition of BGFs into the composites. The glass incorporation results in the increased absorption band intensity at 1096 cm\(^{-1}\) (C–O stretching vibrations) due to the interaction between the amide groups and the glass surface.[19] A new peak at 1638 cm\(^{-1}\) can be assigned to the binding between bioactive glass and CS as reported by Maji et al.[32] In summary, FTIR results demonstrated the successful incorporation of BGFs into CS.
As shown in Figure 4B, the composite CS-BGF membranes showed a lower swelling ratio compared to pure CS membranes in a period of 276 h. It is imperative to control the swelling ratio of membranes for GBR application. A low swelling rate could lower wound pressure and avoid inflammation. Moreover, membranes with a high swelling ratio may occupy too much space and allow a more significant cell infiltration. The CS-BG membranes gradually exhibited a decreased swelling ratio compared to the pure CS membranes in a period of 276 h. While this behavior may be due to BGFs absorbing less water compared to CS under the same conditions, our results suggest that it is possible to control the swelling behavior of CS membranes by adding different amounts of BG flakes.

3.4. Composite Membranes Bioactivity

To test the bioactivity of composite membranes for GBR applications, the formation of hydroxyapatite (HA) in SBF was observed. After incubation in SBF for 3 and 7 days, the HA forming ability of CS-BGF composite membranes was analyzed by FTIR measurements and SEM observations. As shown in Figure 5A–D, two separate bands at 570 and 600 cm\(^{-1}\) (P-O bending vibrations) confirm the formation of the HA layer after immersion in SBF for 3 and 7 days.

After 3 days immersion in SBF, the surface morphology of CS-BGF composite membranes was observed by SEM (Figure 5E–H). SEM images show the cauliflower-like structure, a typical morphology of HA formed on BG-containing biomaterials after immersion in SBF. As the content of BG increases, the HA layer seems to become denser. However, the difference is not significant. In summary, BGF enhanced the bioactivity of CS. The superior bioactivity of composite CS-BGF membranes indicates their bone bonding ability, which makes them favorable candidates for GBR applications.

3.5. Mechanical Properties of the Composite Membranes

We carried out tensile tests for the composite membranes to investigate the effects of BGF incorporation on the mechanical properties.

![Figure 1. A–C) SEM images of 45S5 BGFs at different magnifications D) EDS spectrum, E) Feret diameter size distribution, and F) flake thickness size distribution.](image-url)
properties. Table 1 summarizes the tensile strength, elongation at break, and Young’s modulus of CS, CS-1BGF, CS-2BGF, and CS-3BGF, while Figure 6 displays representative stress–strain curves. The elongation at break increased after the incorporation of BGF confirming the toughening effect of BGF. However, the tensile strength and Young’s modulus of the membranes decreased after the BGF incorporation, which could be induced by the mismatch in Young’s modulus between BGF fillers and the CS matrix resulting in the stress concentrations. Similar results have been observed in a previous study when BG particles and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) microspheres were added to CS membranes. \[23\]

Overall, the membranes became more flexible when BGF were incorporated, although the membranes were less strong. The results indicate that the composite membranes exhibit favorable mechanical properties, which makes the membranes easier to handle and to be cutted during surgical procedures.

3.6. Cell Viability

Cytotoxicity results of the composite membranes toward MC3T3-E1 cells is shown in Figure 7. MC3T3-E1 cells cultured on pure CS membranes were the control group. A significant absorbance increase was observed on CS-1BGF \((P < 0.05)\), CS-2BGF \((P < 0.01)\), and CS-3BGF \((P < 0.0001)\) membranes compared to the pure CS group on day 1. This result indicates that the incorporation of 45S5 BGF into the CS membranes (regardless of the incorporated amount) did not exert any cytotoxic effects. On day 3, the OD values of CS, CS-1BGF, and CS-2BGF increased in comparison to those on day 1, which indicates that these membranes did not reduce the proliferation of MC3T3-E1 cells. However, CS-3BGF showed a significant decrease in cell viability after 3 days of culture compared to the control, CS-1BGF, and CS-2BGF groups, which could be attributed to the
pH rise induced by 45S5 BGF. It is well known that 45S5 BG can increase the local pH due to the release of a relatively large amount of Ca and Na ions when immersed in physiological fluids. Such a pH increase could cause cytotoxicity.

A live/dead assay was also employed to evaluate the viability of MC3T3-E1 cells cultured on CS, CS-1BGF, CS-2BGF, and CS-3BGF composite membranes (Figure 8). In general, more live cells (green) were observed on CS-BGF composite membranes than CS membranes on day 1. After 3 days of cultivation, MC3T3-E1 cells showed proliferation on all membranes, indicating the adhesion of cells on the membranes. However, with increasing concentration of BGFs incorporated in the membranes, no significant increase in cell proliferation could be observed, which is consistent with the results of the CCK-8 tests (Figure 7). The results thus demonstrate that proper concentrations of 45S5 BGFs in CS membranes were not cytotoxic against MC3T3-E1 cells and could support their proliferation.

3.7. Alkaline Phosphatase Activity

Alkaline phosphatase activity (ALP) was measured on the CS, CS-1BGF, CS-2BGF, and CS-3BGF membranes after culture for 7 days (Figure 9) as ALP is one of the most widely recognized biochemical markers in early interim osteoblast activity. MC3T3-E1 cells cultured on pure CS membranes were the control group. MC3T3-E1 cells show slightly higher ALP activities on the CS-BGF membranes. The slightly enhanced ALP activity could be induced by ions released from BGFs in the composite membranes, which is known to improve osteoblast activity.

In summary, CS-BGF composite membranes have no significant toxic effect on MC3T3-E1 cells. At the same time, the incorporation of 45S5 BGFs can slightly increase MC3T3-E1 cell attachment and proliferation with the extension of cultivation time. This result indicates that CS-BGF composite membranes are potentially able to stimulate and guide new bone growth, which is one of the most important steps in GBR.
Figure 5. SEM images of A) CS, B) CS-1BGF, C) CS-2BGF, and D) CS-3BGF membranes after 3 days immersion in SBF. FTIR spectra of E) CS, F) CS-1BGF, G) CS-2BGF, and H) CS-3BGF membranes before and after 3 days immersion in SBF.
4. Conclusion

CS and CS-BGF composite membranes intended for guided bone tissue engineering were prepared successfully by the solvent casting method. BGFs were homogeneously distributed in the composite membranes. HA formation on CS-BGF membranes after 1 day of immersion in SBF indicates the superior bioactivity of composite membranes compared to CS membranes. The in vitro cytotoxicity test and the live/dead assay revealed that BGFs incorporation had a positive effect on the biocompatibility of CS membranes. ALP activity measurement indicated that the composite membranes could induce interim osteoblast activity. These results suggest that developed CS-BGF membranes are a promising composite system for GBR applications. Further studies should investigate in more detail in vitro cell behavior and antibacterial effects before evaluation in bone defect animal models.

### Table 1. Mechanical properties of CS, CS-1BGF, CS-2BGF, and CS-3BGF composite membranes.

| Sample      | Elongation at break [%] | Tensile Strength [MPa] | Young’s Modulus [MPa] |
|-------------|-------------------------|------------------------|-----------------------|
| CS          | 6.1 ± 1.1               | 10.2 ± 1.6             | 189 ± 23              |
| CS-1BGF     | 6.7 ± 1.2               | 8.9 ± 3.7              | 132 ± 15              |
| CS-2BGF     | 7.2 ± 0.6               | 7.2 ± 0.6              | 111 ± 38              |
| CS-3BGF     | 9.0 ± 2.0               | 5.8 ± 0.8              | 47 ± 20               |

Figure 6. Representative stress–strain curves of CS-BGF composite membranes.

Figure 7. Cell viability of MC3T3-E1 cells cultured on CS and CS-BGF membranes for 1 day (*P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001).

Figure 8. Live/dead assay of MC3T3-E1 cells cultured on CS, CS-1BGF membranes, and CS-2BGF and CS-3BGF composite membranes for 1 and 3 days (scale Bar: 200 μm).

Figure 9. ALP activity of MC3T3-E1 cells on CS, CS-1BGF, CS-2BGF, and CS-3BGF composite membranes after 1 week.
Acknowledgements
The authors acknowledge funding from DFG (German Science Foundation) through the Cluster of Excellence “Engineering of Advanced Materials (EAM),” project EXC 315, at the University of Erlangen-Nuremberg.

Open access funding enabled and organized by Projekt DEAL.

Conflict of Interest
The authors declare no conflict of interest.

Author Contributions
H.Z.: contributed to the preparation of the original draft, and involved in the investigation and formal analysis of the data. J.W.: was involved in the investigation and formal analysis of the data. Y.W.: was involved in the writing process by critically reviewing and editing the manuscript. S.R.: contributed to the formal analysis of the data and was involved in the writing process by critically reviewing and editing the manuscript. J.D.E.: contributed to the investigation of the data. W.P.: contributed in the acquisition of the funding and supervised the data analysis. A.R.B.: contributed to the acquisition of the funding, supervised the manuscript, and supervised the methodology of research. In addition, A.R.B. was involved in the writing process by critically reviewing and editing the manuscript. All authors have read and provided input to the published version of the manuscript.

Data Availability Statement
The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords
bioactive fillers, bioactive glass flakes, chitosan, composites, guided bone regeneration

Received: August 11, 2021
Revised: September 9, 2021
Published online: September 28, 2021

[1] S. Lopez-Esteban, E. Saiz, S. Fujino, T. Oku, K. Suganuma, A. P. Tom sia, J. Eur. Ceram. Soc. 2003, 23, 2921.
[2] J. Ajita, S. Saravanam, N. Selvamurugan, Mater. Sci. Eng. C 2015, 53, 142.
[3] J. Wu, K. Zheng, X. Huang, J. Liu, H. Liu, A. R. Boccaccini, Y. Wan, X. Guo, Z. Shao, Acta Biomater. 2019, 91, 60.
[4] X. Chatzistavrou, O. Tsigkou, H. D. Amin, K. M. Paraskevopoulos, V. Salih, A. R. Boccaccini, J. Eur. Ceram. Soc. 2012, 32, 3051.
[5] T. Kokubo, Biomaterials 1991, 12, 155.
[6] L. L. Hench, R. J. Splinter, W. C. Allen, T. K. Greenlee, J. Biomed. Mater. Res. 1971, 5, 117.
[7] L. L. Hench, H. A. Paschall, J. Biomed. Mater. Res. 1973, 7, 25.
[8] L. L. Hench, J. Mater. Sci.: Mater. Med. 2006, 17, 967.
[9] I. D. Xynos, M. V. J. Hukkanen, J. J. Batten, L. D. Buttery, L. L. Hench, J. M. Polak, Calciﬁc Tissue Int. 2000, 67, 321.
[10] A. Hoppe, N. S. Guldal, A. R. Boccaccini, Biomaterials 2011, 32, 2757.
[11] V. Maquet, A. R. Boccaccini, L. Pravata, I. Notingher, R. Jérôme, Biomaterials 2004, 25, 4185.
[12] I. B. Leonor, R. A. Sousa, A. M. Cunha, R. L. Reis, Z. P. Zhong, D. Greenspan, J. Mater. Sci. Mater. Med. 2002, 13, 939.
[13] H. Arstila, M. Tukiaisen, L. Hupa, H. O. Ylönen, M. Kellomäki, M. Hupa, Adv. Sci. Technol. 2006, 49, 246.
[14] K. Zheng, A. R. Boccaccini, Adv. Colloid Interface Sci. 2017, 249, 363.
[15] M. Par, N. Spanovic, T. T. Tauböck, T. Attin, Z. Tarle, Sci. Rep. 2019, 9, 1.
[16] G. Yan, M. Wang, T. Sun, X. Li, G. Wang, W. Yin, Materials 2019, 12, 2082.
[17] M. Ehsan, H. A. Khonakdar, A. Ghadami, Prog. Org. Coat. 2013, 76, 238.
[18] A. Dörf er, R. Detsch, S. Romeis, J. Schmidt, C. Eisermann, W. Peukert, A. R. Boccaccini, J. Biomed. Mater. Res., Part B 2014, 102, 952.
[19] S. Romeis, A. Hoppe, C. Eisermann, N. Schneider, A. R. Boccaccini, J. Schmidt, W. Peukert, J. Am. Ceram. Soc. 2014, 97, 150.
[20] J. D. Esper, L. Liu, J. Willnauer, A. Strobel, J. Schwenger, S. Romeis, W. Peukert, Adv. Powder Technol. 2020, 11, 4145.
[21] P. Gentile, V. Chiono, C. Tonda-Turo, A. M. Ferreira, G. Ciardelli, Biotechnol. J. 2011, 6, 1187.
[22] C. Xu, C. Lei, L. Meng, C. Wang, Y. Song, J. Biomed. Mater. Res., Part B 2012, 100, 1435.
[23] W. Li, Y. Ding, S. Yu, Q. Yao, A. R. Boccaccini, ACS Appl. Mater. Interfaces 2015, 7, 20845.
[24] T. Zhou, X. Liu, B. Sui, C. Liu, X. Mo, J. Sun, Biomed. Mater. 2017, 12, 055004.
[25] W. Florjanski, S. Orzeszko, A. Olchowy, N. Grychowska, W. Wieckiewicz, A. Malysa, J. Smardz, M. Wieckiewicz, Polymers 2019, 11, 1.
[26] R. Moonesi Rad, D. Atila, Z. Evis, D. Keskin, A. Tezcaner, J. Tissue Eng. Regener. Med. 2019, 13, 1331.
[27] K. Zhang, Y. Wang, M. A. Hillmyer, L. F. Francis, Biomaterials 2004, 25, 2489.
[28] J. D. Esper, Y. Zhuo, M. K. S. Barr, T. Yokosawa, E. Spiecker, D. de Ligny, J. Bachmann, W. Peukert, S. Romeis, Powder Technol. 2020, 363, 218.
[29] S. Romeis, J. Schmidt, W. Peukert, Int. J. Miner. Process. 2016, 156, 24.
[30] J. Paul, S. Romeis, M. Matko víc, V. R. R. Mthatha, P. Herre, T. Przybilla, M. Hartmann, E. Spiecker, J. Schmidt, W. Peukert, Powder Technol. 2015, 270, 337.
[31] X. Bui, H. Oudadesse, Y. Le Gal, A. Mostafa, G. Cathelineau, Recent Researches in Modern Medicine, Cambridge, United Kingdom 2011, 359.
[32] K. Maji, S. Dasgupta, K. Pramanik, A. Bissoyi, Int. J. Biomater. 2016, 1, 1.
[33] Y. Wang, M. Ma, J. Wang, W. Zhang, W. Lu, Y. Gao, B. Zhang, Y. Guo, Materials 2018, 11, 6.
[34] E. Fiume, J. Barberi, E. Verné, F. Biano, J. Funct. Biomater. 2018, 9, 24.
[35] F. E. Ciraldo, E. Boccardi, V. Melli, F. Westhauser, A. R. Boccaccini, Acta Biomater. 2018, 75, 3.
[36] I. D. Xynos, A. J. Edgar, L. D. K. Buttery, L. L. Hench, J. M. Polak, J. Biomed. Mater. Res. 2001, 55, 151.