Low pressure spark plasma sintered hydroxyapatite and Bioglass® composite scaffolds for bone tissue repair

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\begin{abstract}
Hydroxyapatite (HA) based composite scaffold materials were prepared by combining it with Bioglass® (BG) to overcome the major limitations of HA such as the lack of re-osseointegration, inadequate bioactivity and bio-degradation. Earlier attempts to prepare BG reinforced HA composites via the conventional sintering process resulted excessive reactions between the constituents and/or crystallization of BG. Excessive reaction between the constituents and crystallization of BG are known to lengthen the bioactivity response. In this work, low pressure spark plasma sintering (SPS) was utilized to prepare HA-BG composite scaffold materials with BG content until 30 wt%. Compared to the conventional sintering practice, the milder processing conditions during the SPS process such as the compaction pressure, sintering time and temperature produced HA-BG composite scaffolds without the excessive reactions between the constituents and prevented the crystallization of BG. All of the developed composites were composed of calcium phosphate (HA and B-Tridicium phosphate phases) and glassy phases only. The effect of BG addition on the physical properties such as the bulk density, relative density and hardness are well in line with the XRD and FESEM analyses. The in vitro bioactivity investigation from the immersion of samples in simulated body fluid (SBF) confirmed the improved bioactivity of the composite samples with increased BG content.
\end{abstract}

1. Introduction

Bone damage resulting from trauma, infections, aging and diseases (such as: cancer, osteoporosis etc.) is one of the biggest medical challenges. This leads to excessive pain, disability and poor quality of life [1,2]. The most common strategies for bone regeneration is either by utilizing the patient’s own bone to heal the damaged bone (autograft) or by utilizing a donor bone as the substitute (allograft) [3]. However these strategies have their own demerits. Autografts not only exposes the patients to additional wounds which also require healing, but also possesses other limitations such as donor site morbidity, longer healing times and limited availability of regenerative tissues [4,5]. On the other hand, allograft carries a high risk of immune rejection and may result in infection transmission [6]. A viable alternative to these complications is the utilization of synthetic bioactive ceramic materials for bone regeneration. Calcium phosphate (CP) based ceramics, particularly hydroxyapatite (HA) (Ca\textsubscript{10} (PO\textsubscript{4})\textsubscript{6}(OH)\textsubscript{2}) has received the greatest attention from biomedical and biomaterial researchers. This is due to the chemical similarity of the mineral constituent in HA (almost 65 wt% of bone composition) [7,8] with the natural bone.

HA is an osteoconductive material, which allows bone growth at the bone-implant interface using differentiated bone cells and is a bioactive material with a tendency to bond with natural bones [9]. Thus HA exhibits very high biocompatibility, good cytocompatibility and class B bioactivity [10]. With an attractive and unique set of characteristics HA has found several applications in bone tissue engineering and dentistry [11]. In spite of that, HA also possesses some limitations as a functional biomaterial in biological environments. The insufficient bioactivity response, slow degradation rate and limited in vivo growth of porous tissues, are some of the important factors which restrict its applications as an implant material [12]. Bioactive glasses, on the other hand exhibit very high bioactivity, favorable degradation rate and advantageous reactions in biological environments [13]. Bioglass® 45S5 (46.1 mol% SiO\textsubscript{2}, 24.4 mol% Na\textsubscript{2}O, 26.9 mol% CaO and 2.6 mol% P\textsubscript{2}O\textsubscript{5}) which is

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\end{itemize}

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the first discovered bioactive glass, exhibits the highest bioactivity (tₐ = 8) among all bioceramics and forms very strong bonds with the surrounding tissues [14,15]. BG is an osteoinductive material which stimulates primitive undifferentiated cells into bone-forming cells [16]. BG could also increase the bone regeneration rate by upregulating the gene expression for human osteoblast cells. Moreover, BG dissolution products offer resistance against microbial action and enhance neo-vascularization by increasing the secretion of angiogenic growth factor [17].

Researchers have attempted to combine HA and BG to prepare composite scaffold materials, where BG is the secondary component for improved biological performance [18,19]. Moreover, the ionic substitution of Na⁺ and SiO₄²⁻ in the HA unit cell significantly improves the bioactivity of HA [20-22]. The higher temperature (1200-1350 °C) required for the conventional sintering of this composite system for 3-4 h results in the complete crystallization of BG and excessive reactions between BG and HA [23], that are suspected to delay the bioactivity response [24]. Moreover, crystallization has a deleterious effect on the sintering process which also affects the densification of HA [25]. This crystallization problem could be suppressed by either varying the chemistry of BG or by using non-conventional sintering techniques which could significantly decrease the heat exposure, thus preventing the crystallization process. Recently Bellucci et al. [26] replaced the Na₂O content in BG with K₂O which yielded some encouraging results. The crystallization tendency was suppressed without affecting the biological characteristics of the classical BG [26].

Spark plasma sintering (SPS) is a non-conventional sintering technique which consolidates samples even at low pressures and temperatures with a shorter time period, compared to the conventional processing routes [27,28]. The passage of pulsed electric current through the conductive die and sample generates faster heat, due to the Joule effect. [29]. The compressive load from the punches serves a dual purpose: i) the continuous compaction results in an improved densification and, ii) enhances the electrical connection by avoiding air pockets which is particularly important for non-conducting samples. The mild consolidation parameters during the SPS process offer several benefits such as the possibility of obtaining amorphous/nanocrystalline phases and preventing the thermal decomposition of phases [30-32]. These unique features of SPS make it an attractive consolidation technique for the preparation of BG reinforced HA composites.

More recently, Bellucci et al. [25,27] prepared SPSed HA and BGCa (a modified BG with a composition of 47.3 mol% SiO₂, 45.6 mol% CaO, 2.3 mol% K₂O, 2.3 mol% Na₂O, and 2.6 mol% P₂O₅ [33]) composites with a BGCa content until 80 wt%. Due to the shorter holding time the diffusion was limited and the formed crystalline phases were same as they would appear after SPS of individual constituents [33,34]. The HA and BGCa SPSed composites were composed of α-CaSiO₃, β-CaSiO₃, and HA [25,27].

In this study, lower temperature and pressure were utilized to prepare the classical BG reinforced HA (BG content from 0 to 30 wt%) composites via the SPS technique. This is the first report on SPS of Classical BG reinforced HA that yielded novel findings in terms of the ability to avoid excessive reactions between constituents and the devitrification of glass. Lower temperature sintering (1000 °C) was selected to prevent the devitrification of glassy phases and excessive reaction between HA and BG. A relatively longer holding time is employed to ensure proper sintering and mass transport necessary to promote ionic substitution in HA, which is necessary to stabilize the HA phase in final products and prevent devitrification of glass. The lower pressure during the SPS of the composite samples offers two benefits: i) the residual porosity in the composite samples could be maintained to favor osteointegration and ii) the cracking of samples during sintering that may result from the expansive transformation of HA phase to the β-TCP phase could be prevented by a smaller compressive load. Among the SPS parameters (compaction pressure, sintering temperature and holding time) the holding time has the minimal effect on densification rather it favors mass transport due to the applied electric field [35]. The XRD, FESEM and EDAX analyses confirmed the presence of glassy phases in the composite samples, which suggests the absence of any excessive reactions between the HA and BG. This is the first successful attempt to prepare HA and BG (45S5) composites via SPS that could avoid any severe reaction between the constituents and/or devitrification of glassy phase.

2. Materials and methods

2.1. Composite preparation

The BG 45S5 micro spheres (200 μm) were purchased from XL Sci-Tech Inc., US. These micropheres were ball milled in ZrO₂ jar with ZrO₂ balls for 6 h to obtain the BG powder. The average particle size of the milled BG powder was 5-10 μm. The commercial submicron hydroxyapatite (Ged. 21222) was procured from Sigma Aldrich (Malaysia). The HA and BG were milled together with ZrO₂ balls in a polyethylene jar with different BG contents (between 0 and 30 wt%) for 6 h. The different premixed powder compositions were designated as:

- HA > Pure HA
- HB 2.5 > 97.5 wt% HA + 2.5 wt% BG
- HB 5 > 95 wt% HA + 5 wt% BG
- HB 10 > 90 wt% HA + 10 wt% BG
- HB 15 > 85 wt% HA + 15 wt% BG
- HB 20 > 80 wt% HA + 20 wt% BG
- HB 25 > 75 wt% HA + 25 wt% BG
- HB 30 > 70 wt% HA + 30 wt% BG

2.2. Consolidation of powders

Very low pressure consolidation of the premixed powders was performed at 1000 °C through spark plasma sintering (SPS-100, SPS Syntex system) under a vacuum pressure of 5 Pa. A 30 mm diameter graphite die was filled with 8 gm premixed compositions and sintered for 30 min at a heating rate of 50 °C/min. Initially 1.83 MPa pressure was applied to ensure the current passage until the temperature reached 1000 °C. The final load reached 3.67 MPa during the sintering process. After 30 min of sintering, the samples were allowed to cool under the same load and vacuum conditions.

2.3. Materials characterisation

The samples were cut using a diamond cutter for further characterization. The X-ray diffraction (XRD) analysis was carried out on XRD-6100 Shimadzu with mono-chromated Cu Kα radiation (λ = 1.54056 Å). The XRD was operated at 40 kV, 30 mA, a step size of 0.02° and a scanning rate of 2° min⁻¹ (0.033 s⁻¹) over a 20 range from 10° to 80°. The microstructural analyses were performed by a high resolution FEI Quanta 200F field emission scanning electron microscopy (FESEM), Prior to the FESEM analyses, the ground and polished samples were coated with a thin layer of Pt to avoid the charging effect. The elemental composition was determined by energy dispersive X-ray analysis (EDAX) attached to the FESEM instrument. The rule of mixtures was employed to calculate the theoretical densities while the Archimedes principle was used to determine the bulk density. The hardness of the consolidated samples was measured using a Wolpert
Wilson 450 SVA Vickern macro-hardness tester. The micro hardness of the glassy phase was measured on a Schimatdu micro hardness tester HMV-G21 DT. An average of ten hardness readings was reported for improved accuracy.

2.4. In vitro biocactivity analysis

The in vitro biocactivity analysis was performed in simulated body fluid (SBF) as per protocol formulated by Kokubo and Takadama [36]. The samples from each composition were soaked in SBF for 14 days. The samples with an average surface area of 650 mm² were immersed in 65 ml SBF in polyethylene beakers in an incubator at 37 °C. The SBF was refreshed every 48 h to achieve a homogenous concentration. After 14 days of immersion, the samples were dried in an incubator at 60 °C for 2 days before further characterization. The FTIR analysis of biomimetic hydroxyapatite layer was performed to confirm the apatite formation. Before the microstructural analysis by FESEM, a Pt coating was deposited on the sample surface to avoid the charging effect. The microstructural analysis was performed on the samples using SEM Phenom ProX and FESEM Hitachi SU 8220, while the elemental composition of the deposited HA was confirmed by EDAX technique. The microstructural analyses were carried out on a vertical surface instead of a horizontal surface, which represents the apatite formation more appropriately [36].

3. Results and discussion

Mechanically stable composite disks were obtained from the SPS technique. The mechanism of pressure assisted sintering techniques (such as SPS) is based on the creep phenomenon, hence the compaction pressure and sintering temperature have the highest impact on final density [37,38]. Both these significant parameters were selected on the lower side to ensure residual porosity. No visible cracks could be observed on any of the samples. Furthermore, all samples could be easily drawn out without strong adherence to the die, suggesting that there was no chemical reaction between the graphite die and the composite.

3.1. Chemical characterization

The XRD analysis of the SPSed samples yielded some interesting findings. The optimal sintering conditions could present the excessive reactions between the HA and BG. Fig. 1 shows the absence of crystalline phases from the chemical reactions between the constituents or from the devitrification of glass. Apart from the glassy phase, the composite samples were composed of HA and β-TCP phases only. The pure unsintered HA and SPSed HA showed additional peaks for Dicalcium Phosphate anhydrous (CaHPO₄) as reported by Cuccu et al. [34] for HA procured from Sigma Aldrich (Cod. 21223). Ca₄P₂O₇ transforms to calcium pyrophosphate (CPP, Ca₃P₂O₇) upon exposure to temperatures around 450 °C [39], which is associated to favor the formation and stabilization of β-TCP [40]. Moreover, the larger decomposition of HA into β-TCP phase that may be associated with the presence of CaHPO₄ phase (when compared to other commercial HAs) during the SPS process was also observed in the same study by Cuccu et al. [34]. Very low intensity peaks of HA were observed and the product was primarily composed of the β-TCP and CaHPO₄ phases when HA was SPSed for 5 min at 900 °C [41]. In the SPS experiments at 1000 °C for 30 mins, all the HA were transformed into the β-TCP phase with a minor content of CaHPO₄. The higher temperature and longer sintering time allowed the complete transformation of HA.

The BG addition in HA under these conditions stabilized the HA and retarded its decomposition into β-TCP [42]. HA is not a stoichiometric compound and could incorporate several substitutions with a general formula of M₂[PO₄]₂X₂ (Where M is Ca, Mg, Na, Sr etc., and X is P, Si, CO₂ etc. and X is OH, CO₂, F etc.) [43]. The crystallographic formula for Si substituted HA is Ca₀.₆(P₂O₄)₂SiO₃(OH)₂₉ (where x = 0.05–1.38) and is negatively charged due to the substitution of phosphate ions by silicate ions which is adjusted by the creation of OH vacancies. [44]. These defects enhance the dissolution rate of HA, hence the bioactivity [45–47]. Furthermore, Si substitutions of less than 1 wt% stabilize the PHA phase against transformation into β-TCP [48]. Similarly, Na can substitute Ca in HA lattice and this substitution is also reported to enhance the thermal stability of HA up to 1200 °C [49].

The BG addition (< 5 wt%) is merely considered a sintering aid for HA, by decomposing it into β-TCP. The HA decomposition into β-TCP phase is an expansive transformation (transformation involving lattice expansion) that results in decreased porosity and improved mechanical properties [20]. The β-TCP phase formation upon sintering of HA and its composites enhances the biodegradation rate and bioactivity due to the relatively lower Ca/P ratio i.e. 1.5 [50]. The combination of HA and β-TCP is called Biphasic Calcium phosphate (BCP) which provides an opportunity to control the bioactivity, biodegradation behavior and mechanical stability respective to HA and β-TCP contents [1]. Moreover osteoinductive nature of BCP was confirmed by several in vivo analyses [50].

In the present study, the BG addition has retarded the HA decomposition without causing excessive reactions between the HA and BG. The diffusion of BG elements (Na and Si) into the HA matrix was observed in the EDAX analyses. These ionic substitutions at low temperature assisted by the electric field effect of SPS, stabilized the HA against the decomposition into β-TCP phase [43]. The stabilization of HA phase against decomposition with the addition of classical BG, was never reported earlier. A BG addition of more than 5 wt% usually promotes the excessive reaction between BG and HA, resulting in the formation of new crystalline phases [18,19,23]. Santos et al. [51] observed a small percentage of calcium silicate even with a BG addition as low as 2.5 wt%, while a complete transformation of HA occurred with only 5 wt% of BG addition [51]. Demirkiran et al. [18,23] observed a combination of Ca₃(PO₄)₂SiO₃ and β-TCP phases when sintered with 10 wt% BG reinforced HA composite by conventional sintering at 1200 °C for 4 h. While Goller et al. [19] found a tremendous reaction between HA and BG under similar sintering conditions, producing the Ca₃(PO₄)₂SiO₃, Na₂HPO₄·7H₂O and Ca₃P₂O₇·4H₂O phases, with a complete removal of HA or β-TCP phases [19]. In the present study, the formation of new crystalline phases was avoided with a BG addition of 10 wt% (and even until 30 wt%) with SPS technique. The peak intensity for HA improved with increased BG content, however β-TCP still remains the major phase. The FESEM analysis reveals the presence of glassy phases while EDAX confirms the elemental composition of the
Table 1
Comparison of the product phases with the addition of BG.

<table>
<thead>
<tr>
<th>Composition</th>
<th>Phases observed in this study</th>
<th>Phases reported in literature by conventional sintering for 3-4 h</th>
</tr>
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<tbody>
<tr>
<td>HA</td>
<td>(\beta)-TCP, HA and CaBF(_4) ((\beta)-TCP dominant)</td>
<td>HA</td>
</tr>
<tr>
<td>HA 2.5 BG</td>
<td>(\beta)-TCP and HA ((\beta)-TCP dominant)</td>
<td>HA + (\beta)-TCP (HA dominant) \cite{18}, HA and Calcium silicate \cite{51}</td>
</tr>
<tr>
<td>HA 5 BG</td>
<td>(\beta)-TCP and HA ((\beta)-TCP dominant)</td>
<td>HA + (\beta)-TCP ((\beta)-TCP dominant) \cite{18}, Calcium silicate \cite{51}</td>
</tr>
<tr>
<td>HA 10 BG</td>
<td>(\beta)-TCP and HA ((\beta)-TCP dominant)</td>
<td>(\beta)-TCP, Na(_2)HPO(_4)(\cdot)8H(_2)O, Calcium phosphate silicate, Ca(_2)P(_2)O(_7)(\cdot)3H(_2)O \cite{19}</td>
</tr>
<tr>
<td>HA 15 BG</td>
<td>(\beta)-TCP and HA ((\beta)-TCP dominant)</td>
<td>HA, (\text{Na}_2\text{Ca}_2\text{Si}_2\text{O}_7\cdot\text{O}_2) \cite{20}, (\text{NaCaPO}_4), (\text{Na}_2\text{Ca}_2\text{Si}_2\text{O}_7) \cite{20}</td>
</tr>
<tr>
<td>HA 20 BG</td>
<td>(\beta)-TCP and HA ((\beta)-TCP dominant)</td>
<td>Sodium calcium phosphate \cite{21}</td>
</tr>
<tr>
<td>HA 25 BG</td>
<td>(\beta)-TCP and HA ((\beta)-TCP dominant)</td>
<td>(\beta)-TCP, Na(_2)Ca(_2)Si(_2)O(_7) \cite{20}</td>
</tr>
</tbody>
</table>

A further addition of BG beyond 10 wt% significantly stabilized the HA phase without any substantial reactions between the BG and HA. The ionic substitutions of Na\(^+\) with Ca\(^{2+}\) and SiO\(_2\) with PO\(_4\)\(^{2-}\) are responsible for the enhanced stability of HA phase \cite{42,43}. The peak intensity for HA improved with the BG addition, resulting in the stabilization of HA and leaving \(\beta\)-TCP as the minor phase in samples with higher BG content (until 30 wt%). Demirkiran et al. \cite{18,22} observed a single crystalline \(\text{NaCa}_2(\text{PO}_4)_2\) phase with 25 wt% BG addition. On the other hand, Bellucci et al. \cite{12} reported two crystalline phases (Na-CaPO\(_4\) and CaSiO\(_3\)) when HA composite was sintered with 40 wt% BG at 1150 °C for 3 h. The increase in the BG content beyond 10 wt% resulted in the loss of the original matrix phase (HA or the decomposition product TCP). This effect was due to the excessive reactions between

![Fig. 2. EDX spectra of pure non-sintered HA 2a(i) and BG 2a(ii), composite samples HB 10 (2b), HB 20 (2c) and HB 25 (2d).](image-url)
the HA and BG from the extreme sintering conditions. A comparison of product phases with the addition of BG as reported in several studies for HA-classical BG is presented in Table 1.

The EDAX analysis confirms the diffusion of Na and Si from the BG into the matrix, while Ca diffused from the HA into the glassy phase. The primary driving forces for this diffusion are prolonged holding time and concentration gradient between the constituents [52]. The diffusion of Na out from the glassy phase and its replacement from the diffusion of Ca from the CaP based phase makes the BG less vulnerable towards crystallization [33,53]. The crystallization tendency of BG was successfully decreased by other researchers with the replacement of Na2O in classical BG by K2O or CaO [54-56].
A small concentration of Si was also detected in EDA analysis which could be due to the contamination during the polishing stage. The diffusion of Ca from HA and outward movement of Na from the glassy phase could be easily perceived by comparing the EDAX analysis of the as-received materials, as shown in Fig. 2a (i, ii). Fig. 2b presents the chemical composition of different CaP rich sections in the HB 10 sample. The composition of different phases are very similar to the chemical composition of HA powder as shown in Fig. 2a (i). The slight incorporation of Na and Si (at some spots) in the CaP phase could be detected due to the addition of BG.

With further addition of BG, glassy phase richer in Si appears more abundantly. This phase is richer in Ca and P content compared to the parent BG, which could have resulted due to the diffusion of Ca and P from HA. The formation of a relatively larger number of glassy pockets is visible in Fig. 2c with a further increase in BG content. The glassy phases with different contrasts could be observed in Fig. 3d. The darker glassy phase in HB2.5 is richer in Si compared to the other glassy phases. Despite the different compositions of glassy phases at different points, the devitrification was prevented with the current SPS conditions.

3.2. Physical and mechanical characterisation

The physical characteristics of the composite samples are in good agreement with the chemical analyses. As BG possesses lower density compared to HA, the bulk density of the composite samples generally shows a downward trend with the increase in BG content. The highest bulk density was observed for the purely sintered HA, which is primarily composed of β-TCP phase. HA → β-TCP transformation is an expansive transformation that tends to close the pores leading to enhanced hardness. As the BG content increases, the bulk density of the samples decreases due to the increasing porosity. The increased BG content promotes the formation of higher density HA (i.e. 3.15 g cm⁻³). This leads to the formation of open pores in the samples as the HA lattice is more compact compared to β-TCP, at constant SPS pressure. On the other hand, a significant increase (> 15 wt%) in the BG content considerably enhances the formation of the glassy phase. The glassy phase is normally pore free and has lower density (2.45 g cm⁻³), hence decreases the overall porosity of the composite samples. The same effect was also observed on the relative density (RD). The density and hardness of the samples are presented in Table 2. For a better understanding of the bulk density, hardness and the RD behavior, a graph is plotted as a function of BG content in Fig. 3.

The hardness of the SPSed samples follow a similar pattern. The porosity serves as the driving feature for hardness. Pure HA (actually pure β-TCP) exhibits the highest hardness due to the lowest porosity. As the BG content and porosity increases, the hardness of the samples decreases significantly until 15 wt% of BG content. Another mechanism comes into effect beyond this threshold composition. An increased number of glassy pockets offer significant resistance against deformation from the penetration of the Vickers indenter through the CaP rich phase, which leads to increased hardness. The hardness of the glassy phase rises to very high values as shown in Fig. 3, hence improving the overall hardness index. The hardness of the sintered BG is around 458 HV [19]. The average hardness of the glassy phase is quite close to the reported value. The image of the micro hardness measurement of the glassy phase in the HB 30 sample gives the highest hardness value as presented in Fig. 4.

Table 2

<table>
<thead>
<tr>
<th>S.No</th>
<th>Sample code</th>
<th>Bulk density</th>
<th>RD%</th>
<th>Thickness mm</th>
<th>Average Vickers hardness HV2</th>
<th>Average Microhardness of glassy phase HV</th>
</tr>
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<tbody>
<tr>
<td>01</td>
<td>HB</td>
<td>2.91</td>
<td>62.2</td>
<td>4.35</td>
<td>183.30</td>
<td></td>
</tr>
<tr>
<td>02</td>
<td>HB 2.5</td>
<td>2.81</td>
<td>90.5</td>
<td>4.97</td>
<td>83.43</td>
<td></td>
</tr>
<tr>
<td>03</td>
<td>HB 5</td>
<td>2.73</td>
<td>88.6</td>
<td>5.88</td>
<td>29.30</td>
<td></td>
</tr>
<tr>
<td>04</td>
<td>HB 10</td>
<td>2.70</td>
<td>88.2</td>
<td>5.91</td>
<td>26.56</td>
<td></td>
</tr>
<tr>
<td>05</td>
<td>HB 15</td>
<td>2.60</td>
<td>86.1</td>
<td>6.05</td>
<td>15.01</td>
<td></td>
</tr>
<tr>
<td>06</td>
<td>HB 20</td>
<td>2.71</td>
<td>90.75</td>
<td>5.8</td>
<td>34.40</td>
<td></td>
</tr>
<tr>
<td>07</td>
<td>HB 25</td>
<td>2.74</td>
<td>93.3</td>
<td>5.65</td>
<td>43.60</td>
<td></td>
</tr>
<tr>
<td>08</td>
<td>HB 30</td>
<td>2.72</td>
<td>93.9</td>
<td>5.63</td>
<td>40.43</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 3. Graphical representation of the effect of BG content on RD, bulk density and average hardness.
3.3. Microstructural characterisation

The microstructural analysis confirmed the presence of glassy phase and porosity in the SPsed samples. The pure HA SPsed sample shows the lowest porosity with homogeneous microstructure. The black region is homogenously distributed on the surface which suggests the presence of porosity in the samples. The microstructure remains almost unchanged with the addition of 2.5 wt% BG but the increase of black
region suggests an increase in the porosity of the samples. This porosity is directly related to the decrease in density and hardness of the samples, as mentioned in the earlier section. A well-distributed glassy phase appears in the samples with further increase in the BG content beyond 5 wt%. The glassy phase usually assumes a circular morphology, as marked with black circles in Fig. 5(e, d and f). It can be observed that the porosity in the glassy phase is much lower compared to the porosity in the CaP phase. Moreover, a higher porosity is observed in the CaP matrix of HB 10 sample as shown in Fig. 5c. The glassy pockets appear more abundantly with a further increase in the BG content. HB 20 sample also shows porosity which is much lower than HB 10 (as explained in earlier section) samples, as shown in Fig. 5d. Some huge glassy phase pockets are also formed which resulted in an increased local brittleness and cracking, as presented in Fig. 5d for HB 25 sample. The CaP rich section of HB 25 is presented in Fig. 5d. Due to the greater hardness of the glassy phase compared to the CaP rich phase, the surface of the polished samples exhibits an undulating pattern. The material wear rate during grinding and polishing of the CaP rich phase was higher compared to the glassy phase. The difference in the surface straightness can be observed clearly in Figs. 2c and 5f. The relatively lower porosity of the CaP rich phase in HB 25 and HB 30 samples (Fig. 5e & f) could be easily observed compared to the HB 10 sample (Fig. 5d).

3.4. In vitro bioactivity characterisation

The *in vitro* immersion in SBF is one of the most reliable techniques to mimic the behavior of materials in human physiological environments. The ability to deposit a carbonate apatite (bone-like apatite layer) on the surface is one of the first indicators of a material which could bond with natural bones in physiological environments [57, 58]. All of the developed scaffold materials formed a biomimetic hydroxapatite layer on the surface, forming a characteristic cauliflower appearance [59]. Furthermore, the phase confirmation of the deposited layer was performed by FTIR analysis which resembles the characteristic pattern of biomimetic hydroxyapatite [60], as shown in Fig. 6. The FTIR spectra of the samples confirmed the characteristic \( V_2\ O_4^3^- \) bend around 550 and \( 660 \text{ cm}^{-1} \) and \( V_2\ PO_4^3^- \) bend around \( 1050 \text{ cm}^{-1} \). The C-O bending vibration is visible around \( 870 \text{ cm}^{-1} \), while the \( V_2\ O-H \) vibration is visible around \( 1630 \text{ cm}^{-1} \). Fig. 6 presents the FTIR spectra of HA, HB 5, HB 20 and HB 30 samples.

Samples with lower BG content (<5 wt%) exhibited somewhat limited bioactivity resulting in the precipitation of NaCl crystals at discrete spots as visible in Fig. 7a. Whereas, the samples with more than 5 wt% BG content developed thick and homogeneous apatite layer throughout the surface. The developed apatite layer on all the samples exhibited similar morphology. The SEM image presented in Fig. 7b indicates a cauliflower appearance of the apatite layer on HB 15 sample which is further confirmed by ESEM images presented in Fig. 7c for sample HB 30. An increased SiO2 content with higher BG content seems to be the primary reason for enhanced bioactivity [47]. The formation of apatite layer on the samples showed some cracks and pores that could have resulted during the drying of the samples, after removal from the SBF solution [61]. The EDAX analyses confirmed the formation of the CaP phase on the samples with minute content of Na, Mg and O. The Ca/P ratio of the developed layer is almost 1.65, which
4. Conclusion

The SPS technique employed for the preparation of HA-BG composite scaffold materials yielded some extremely encouraging and new findings, in terms of the development of phases and ability to prevent devitrification and excessive reactions between the constituents. Earlier results reported undesirable and unintended excessive reactions between the constituents which produced crystalline phases that were totally different from the starting constituents and devitrified glass. The composite scaffolds prepared in this study were composed only of HA, β-TCP and glassy phase. No other crystalline phases such as Ca₆(PO₄)₂SiO₄, Na₂Ca₆(PO₄)₃, NaCaPO₄ and Ca₃SiO₄Na₃HPO₄·7H₂O and Ca₅P₂O₇·4H₂O were observed. This finding is in contrast to the presence of at least one or several of these phases in the sintered BG reinforced HA composites as reported in earlier studies. The tendency to avoid the excessive reactions and crystallization is critical, as the unique characteristics of BG (high bioactivity and extremely high osteointegration) and HA (similarity with the mineralized phase of the bone) will be lost or significantly deteriorate as a result of excessive reaction and crystallization. The incorporation of BG stabilized the HA phase in the composite scaffolds by promoting ionic substitutions in the HA lattice. The β-TCP was the dominant phase at zero or low BG content in the composite scaffolds. On the other hand, the HA phase began to dominate at higher BG content. The developed scaffold


[56] D. Beltrán, V. Casellas, A. Soló, Calcium and potassium additive to facilitate the sintering of bioactive glasses, Mater. Lett. 65 (2011) 1025-1027.


