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PII: S1751-6161(17)30032-2
DOI: http://dx.doi.org/10.1016/j.jmbbm.2017.01.022
Reference: JMBBM2193

To appear in: Journal of the Mechanical Behavior of Biomedical Materials

Received date: 28 November 2016
Revised date: 10 January 2017
Accepted date: 13 January 2017

Cite this article as: Raghad Abdulrazzaq Alhashimi, Francesco Mannocci and Salvatore Sauro, Bioactivity, cytocompatibility and thermal properties of experimental Bioglass-reinforced composites as potential root-canal filling materials, Journal of the Mechanical Behavior of Biomedical Materials http://dx.doi.org/10.1016/j.jmbbm.2017.01.022

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Bioactivity, cytocompatibility and thermal properties of experimental Bioglass-reinforced composites as potential root-canal filling materials

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ABSTRACT
To evaluate the bioactivity and the cytocompatibility of experimental Bioglass-reinforced polyethylene-based root-canal filling materials. The thermal properties of the experimental materials were also evaluated using differential scanning calorimetry, while their radiopacity was assessed using a grey-scale value (GSV) aluminium step wedge and a phosphor plate digital system.

Bioglass 45S5 (BAG), polyethylene and Strontium oxide (SrO) were used to create tailored composite fibres. The filler distribution within the composites was assessed using SEM, while their bioactivity was evaluated through infrared spectroscopy (FTIR) after storage in simulated body fluid (SBF). The radiopacity of the composite fibres and their thermal properties were determined using differential scanning calorimetry (DSC). The
cytocompatibility of two experimental BAG-reinforced polyethylene-based composites was assessed using human osteoblast-like cells and statistically analysed using the Pairwise t-test \( p < 0.05 \).

Bioglass and SrO fillers were well distributed within the resin matrix and increased both the thermal properties of polyethylene matrix and the radiopacity. The FTIR showed a clear formation of calcium-phosphates, while, MTT and AlamrBlue tests demonstrated no deleterious effects on the metabolic activity of the osteoblast-like cells.

BAG-reinforced polyethylene composites may be suitable as obturation materials for endodontic treatment. Since their low melting temperature, such innovative composites may be easily removed in retreatment cases, especially from the coronal and middle third of the root canal. Moreover, their biocompatibility and bioactivity may benefit proliferation of human osteoblast cells at the periapical area of the root.

**Keywords**

Bioactivity; Bioglass; Cytotoxicity; Polyethylene; Root filling; Thermal properties.

### 1. INTRODUCTION

The use of gutta-percha (GP), in combination with conventional root canal sealers (e.g. zinc oxide and eugenol cements), might not be able to adequately seal the root canal system; microleakage seems to be inevitable in such clinical situations (Michaud et al., 2008; Teixeira et al., 2004). This dispute is mainly related to two crucial factors: i) gutta-percha does not adhere to the canal wall; ii) several conventional root canal sealers are affected by setting shrinkage. Recently, new bioceramic root filling systems have been developed in attempt to address the shrinkage issue of sealers (Sonntag et al. 2015), and provide superior sealing ability (Antunes et al., 2016; et al., Abedi-Amin et al., 2017) and biocompatibility (Souza et al., 2015).
Marending et al., (2013) proposed a possible alternative system based on a GP prototype doped with ultrafine bioactive glass. They demonstrated that such an innovative bioactive system could self-adhere to the root canal dentine without using any sealer. The authors also hypothesised that the self-adhesive properties of such a GP (Bio-gutta) were related to the ability of bioactive glass to induce precipitation of calcium phosphates in an alkaline environment, which sealed the GP-dentine interface.

It is crucial to define that the main principle of bioactivity stands for the ability of specific ion-releasing materials to form a mineralised layer on their surface in a physiologic or slight alkaline environment (Hench et al., 2000). Based on such a concept, many other researchers have attempted to incorporate different bioactive fillers into matrix polymers, with the intention to create innovative root filling systems with improved sealing ability, in particular in the most apical regions of the root canal (Marending et al., 2013; Alani et al., 2009; Mohn et al., 2010).

The first attempt to create an endodontic material with bioactive properties was performed by Alani et al., (2009), who incorporated a phosphate-rich bioactive glass into a polycaprolactone polymer matrix. Gubler et al., (2008) showed little antimicrobial activity with such materials containing bioactive glass, and they concluded that this approach would not be so clinically relevance in root canal treatments. Furthermore, polycaprolactone is a biodegradable material; this can lead to reducing the survival rate of treatment. Nevertheless, all these studies have created an important scenario for further studies to create and evaluate the effect of incorporating nano-particles of bioactive glass such as Bioglass 45S5 (BAG) into a matrix of polyisoprene (Gutta Percha) (Mohn et al., 2010). It is has been extensively demonstrated that BAG are able to induce hydroxycarbonate apatite
formation (HCA) when immersed in physiological fluids (e.g. blood and saliva). Moreover, such bioceramics produce a unique biological response at interfaces that causes the formation of a bioactive bond between material and adjacent tissues (Hench et al., 2000; Nganga et al., 2012). The bioactive bond is established on the material surface via the nucleation and growth of an apatite-like phase, which creates the so called “material-tissue integration” (Arcos et al., 2009), and promotes dentine remineralisation (Sauro et al., 2011).

Moreover, polyethylene-based composites reinforced with BAG have been advocated for several biomedical applications, as they combine the high bioactivity of BAG and the mechanical properties (e.g. toughness and stiffness) of polyethylene. Additionally, BAG-doped composites have been shown to inhibit matrix metallo proteinases (MMPs)-mediated collagen degradation (Osorio et al., 2012). BAG also have specific antibacterial properties, even when embedded in a polymeric composite; this is mainly due to the high alkalinity of the glass phase (Allan et al., 2001; Koller et al., 2008). Thus, BAG-reinforced composites, with a bio-stable polymer such as polyethylene, might represent a potential innovative material for root canal obturation.

The aim of the present study was to perform a series of in vitro tests to evaluate the bioactivity and the cytocompatibility of two experimental Bioglass (BAG)-reinforced polyethylene-based composites developed as potential root-canal filling materials. Moreover, the thermal properties of the experimental materials were evaluated using differential scanning calorimetry, while their radiopacity was assessed via a grey-scale value (GSV) aluminium step wedge and a phosphor plate digital system.
2. MATERIALS AND METHODS

2.1. Preparation and SEM characterisation of the experimental composites

The experimental composites tested in this study were created as described in previous studies (Alhashimi et al., 2012; Alhashimi et al., 2014). In brief, micro-particles of BAG (Bioglass 45S5, Sylc, Osspray Ltd. London, UK), with a particle size distribution of 45-80 µm, and SrO (Sigma–Aldrich, Gillingham, UK) were incorporated at different concentrations into a polyethylene matrix (Sigma–Aldrich, UK) in order to generate two composite fibres: i) 20 wt% BAG + 30 wt% SrO (LDPEBAGSR20/30); ii) 30 wt% BAG + 20wt% SrO (LDPEBAGSR30/20), (Table 1). These were fabricated and optimised using a single screw extruder at a processing temperature of 150 °C, and rotary speed around 20-25 rpm. As the total inorganic content was maintained constant, the extrusion parameters to create the two experimental composites required no changes (Alhashimi et al., 2014). The homogeneity and the dispersion of fillers within the polymer matrix of the two experimental composite fibres were evaluated in three specimens for each composite (2mm long and 1.5mm thick), through SEM (Hitachi S-3500N, Hitachi High Technologies, Tokyo, Japan) at different magnifications (X500 and X900). The field of micrograph vision was 0.50 µm.

2.2. Thermal properties evolution (DSC) of the experimental composites

Differential scanning calorimetry (DSC, Perkin Elmer, Waltham, MA, USA) was employed to measure the thermal properties of the experimental BAG-reinforced and polyethylene filler-free composites as function of their glass transition (T_g), melting (T_m), and crystallisation (T_c) temperature (Alhashimi et al., 2016). Each experimental material was placed in an aluminium vessel, whilst no material was placed inside the pan as a control. Both the experimental and control specimens were maintained at the same temperature over the
entire assessment. Two heating and two cooling cycles were recorded to determine the thermal properties described above. By observing the difference in heat flow between the experimental specimens and control reference, it was possible to measure through DSC scans the amount of heat absorbed (endothermic) or released (exothermic) during the phase transitions.

2.3. Radiopacity evaluation

Five acrylic plates (1 mm in depth and 5 mm diameter) were fabricated as described by Carvalho et al., (2007). The radiopacity of the tested materials was assessed using a dental radiography equipment (Planmeca Oy, Helsinki, Finland) in combination with a phosphor plate digital system and a grey scale value aluminium step wedge, which represents the different shades between black and white varying from 0 to 255 pixels, where 0 represents black and 255 represents white. The grey scale measurement was then converted into mm equivalents of aluminium. The exposure parameters were 70 kV, 8 mA and 0.2 s. The object-to-focus distance was 30 cm. The degree of radiopacity was determined using an aluminium washer, 1 mm thick and an internal diameter of 10 mm, filled with each tested material and radiographed together with the graduated aluminium step wedge, with thickness varying from 1 mm to 10 mm in steps of 1 mm each (ANSI/ADA 2000 specification number 57) (ANSI/ADA 2000). The radiopacity of the filling material inside the washer was compared with the steps of the aluminium step wedge on a computer screen using the Digora radiographic software (Soredex Orion Corporation, Helsinki, Finland) (Hwang et al., 2009).
2.4. **ATR-FTIR spectroscopy – bioactivity evaluation**

Three specimens with standard dimension (5 mm x 1 mm x 1 mm) were first created for each experimental BAG-reinforced composites and the filler-free polyethylene, and then heated at 160°C and reassembled into small discs (3 mm diameter and 1 mm thick). The specimens were immediately submitted to ATR-FTIR spectroscopy in order to characterise their chemical composition and confirm the incorporation of BAG fillers within the polyethylene. These specimens were then immersed in simulated body fluid solution (SBF) for 72 h and then analysed to assess their potential bioactivity (i.e. formation of calcium-phosphates).

A Spectrometer (ATR-FTIR: Perkin-Elmer Spectrum One; Perkin-Elmer, Beaconsfield, UK) was used in the region of 650–4000 cm\(^{-1}\) with a resolution of 4 cm\(^{-1}\) and 64 number of scans for each spectrum. The ATR area had a 2 mm diameter and the IR radiation penetration was approximately 3–5 μm. In order to place the specimens in good contact with the ATR crystal, a moderate pressure was applied (5 psi) during the measurement to reduce the background noise and attain high quality spectra.

2.5. **Cytocompatibility of the polyethylene-bioglass composites**

The cytocompatibility of the experimental composites created in this study was assessed using human osteoblast-like cells (HOB) obtained using a protocol previously described by Camilleri et al., (2005). The cell culture was performed at 37 °C in Dulbecco’s modified eagles medium (DMEM) and in a humidified atmosphere with 5% CO\(_2\). Discs of each tested experimental composite with a diameter of 12 mm were created as previously described. Three discs for each material were used for the indirect test, while five discs were used for
the direct test. The experimental composites were assessed for the *in vitro* biocompatibility according to ISO 10993-Part 5, 1992. The cytotoxicity of the eluent was also evaluated. The experimental composites were placed into labelled containers filled with DMEM, supplemented with 10% foetal calf serum (FCS), seeded with HOB cells (1 x 10^4 cells mL^{-1}) and incubated for 1 day to allow cell confluence. The containers were sealed and placed onto a roller mixer (Luckham 4RT, Burgess Hill, UK). Methyltetrazolium (MTT) assay was performed to measure the cell metabolic function; the plates were removed from the incubator, and 100 uL of media was removed from the wells and submitted to elution assessment at 24 h and 72 h cell exposure time (1 and 3 days). Standard culture medium was used as a negative nontoxic control and 10% ethanol diluted in media as a positive control.

Moreover, at selected time-points 3, 7, 14, 21 and 28 days, 100 uL media was mixed with 1 mL of Alamar Blue™ (diluted 1 : 10 in phenol red-free) and incubated for 4 h at 37 ºC at 5% CO₂. Wells without any cells were used as the blank control, whilst Thermanox™ was used as nontoxic negative control. Subsequent to the incubation period, 100 uL aliquots from each well were taken and transferred to a 96-well plate. Absorbance was measured on a fluorescent plate reader (excitation wavelength 540 nm; emission wavelength of 570 nm). Statistical analysis was performed using the Pairwise t-test (α = 0.05).

3. RESULTS

3.1. **SEM characterisation of the experimental composites**

The BAG fillers in LDPEBAGSRO 20/30 and LDPEBAGSRO 30/20 were evenly distributed and dispersed within the low density polyethylene matrix; the presence of BAG increased with
its content within the formulation of the two different experimental composites (Figure 1A-1D).

3.2. Thermal properties evolution (DSC) of the experimental composites

The incorporation of BAG fillers increases the thermal properties of polyethylene matrix (LDPE - filler free). However, the two experimental composites (LDPEBAGSRO 20/30 and LDPEBAGSRO 30/20) showed similar thermal properties in terms of melting and crystallisation temperatures (Table 2).

3.3. Radiopacity evaluation

The degree of radiopacity of LDPEBAGSRO 20/30 and LDPEBAGSRO 30/20 are shown in figures 2A and 2B, respectively. The mean density of LDPEBAGSRO 20/30 composite was 140 which is equivalent to ~4.1 mm of aluminium step-wedge. On the other hand, LDPEBAGSRO 30/20 exhibited a radiopacity 3 mm of aluminium step-wedge, which was lower than that of LDPEBAGSRO 20/30. The pure LDPE (filler free) showed no radiopacity.

3.4. ATR-FTIR spectroscopy – bioactivity evaluation

The FTIR spectra of the LDPEBAGSRO 20/30 and LDPEBAGSRO 30/20 composites before and after 2 days of storage in SBF were obtained to characterise the bioactivity of the composites. The pure LDPE (filler free), LDPEBAGSRO 20/30 and LDPEBAGSRO 30/20 composites before soaking in SBF showed a band at 1465 cm\(^{-1}\) (stretching vibration of CH\(_2\)), and two sharp peaks at 2848 cm\(^{-1}\) and 2916 cm\(^{-1}\), which represented the –CH\(_2\) stretching and –CH\(_2\) deformation in the polyethylene segments, respectively (Figures 3A, 3B, 3D). LDPEBAGSRO 20/30 and LDPEBAGSRO 30/20 composites showed absorption band in the range 1040-900 cm\(^{-1}\) (stretching vibrations of Si-O), which confirmed the presence of BAG
within the organic matrix (Figures 3B & 3D). The specimens of LDPEBAGSRO 20/30 stored in SBF for 2 days at 37°C showed no Si-O vibration, but a new broad phosphate peak, P-O emerged at 1037 cm\(^{-1}\). The formation of this peak is related to the calcium phosphate formation. A small peak at 871 cm\(^{-1}\) inditing the presence of carbonate precipitation was also observed. It was also noted a farther broad peak of carbonate appeared at 1621 cm\(^{-1}\); this indicated the incorporation of carbonate groups in the structure of the calcium phosphates formed within the structure of the two experimental composites (Figure 3C). The CH\(_2\) vibration peaks were observed in the region of 1600-1400 cm\(^{-1}\), which superimposed with other carbonate peaks in the same region; thus, it was difficult to confirm that the peak was related to carbonate or CH\(_2\) vibration modes.

The FTIR spectra of LDPEBAGSRO 30/20 composite after immersion in SBF was compared to that of LDPEBAGSRO 20/30 in terms of availability of PO\(_4\) and CO\(_3\) peaks. The main difference was the intensity of the phosphate peak, which was slightly higher in LDPEBAGSRO 30/20 than that of LDPEBAGSRO 20/30 (Figures 3E).

### 3.5. Cytocompatibility of the polyethylene-bioglass composites

The amount of BAG filler in LDPEBAGSRO 20/30 and LDPEBAGSRO 30/20 composites had no deleterious effects on the metabolic activity of cells compared to the negative non-toxic control group (p > 0.05) (Figures 4). In particular, the tested materials showed no significant drop (p > 0.05) in metabolic activity at 24 h and 72 h of 1-day cell exposure (Figure 4A). After 3-day cell exposure, a good biocompatibility was also observed for both LDPEBAGSRO composites (Figure 4B).
The Alamar Blue assay results regarding the biological response of human osteoblast-like cells on the BAG-reinforced polyethylene composites for 28 days compared to Thermanox™ (control) are shown in (Figure 4C). Cell proliferation on the LDPEBAGSRO 20/30 and LDPEBAGSRO 30/20 composites showed no significant difference ($p > 0.05$) in comparison to the non-toxic control Thermanox during 28 days.

**DISCUSSION**

One of the latest trends in dental research is about the development of innovative root canal filling materials able to bond to dentine providing a reliable seal at the interface, and at the same time, reinforce the tooth against fracture (Bueno et al., 2016). Accordingly, bioactive materials such as mineral trioxide aggregate (MTA) as well as further bioceramics have extended their applications in such a field (Atmeh et al., 2012; Grotra et al., 2012). These materials showed remarkable clinical results when used in root canal perforations and as filling materials for retrograde obturations (Katsamakis et al., 2013; Saunders et al., 2008), MTA cements may also be used as core root canal filling material to fill the root canal space (Bogen & Kuttler 2009). Such cements release an important concentration of ions that favour mineral precipitation as well as formation of more complex agglomerations such as calcium carbonates; in presence of phosphate ions the latter may transform into calcium phosphates, which may fully turn into carbonate-substituted hydroxyapatite (CHA) in specific environment (i.e. alkaline pH) (Atmeh et al., 2012).

Wang et al., (1998) showed that bioactive glass particles could be incorporated in high density polyethylene matrix by using a twin screw extruder, in order to attain
bioactive/biocompatible composites for several biomedical applications. The present study aimed at generating two experimental root-canal filling materials made of polyethylene matrix reinforced with Bioglass 45S5 (BAG) particles and SrO. These were assessed for their thermal properties, radiopacity, bioactivity and cytocompatibility. Bioglass 45S5 is approved by the U.S. Food and Drug Administration for certain clinical applications in orthopaedic and maxillofacial surgery (Nganga et al., 2012), so it was chosen to be used as biologically active micro-filler for the experimental root-canal filling materials tested in this study.

The particle size of the BAG filler incorporated into the experimental composites tested in this study was pre-determined in a pilot study. Accordingly, the polyethylene matrix composition was maintained constant at 50 wt% in both the experimental composites, while the amount of BAG was 20 wt% or 30 wt%, and 20 wt% or 30 wt% SrO as radiopacifier (Alhashimi et al., 2012; Alhashimi et al., 2014).

Radiopacity is one of the essential requirements for intra-oral dental materials. According to ISO (ISO 6876) standard, root canal obturation materials need to have appropriate radiopacity to allow clinicians to identify the interface between the material and the adjacent anatomical structure (e.g. root dentine). Moreover, it is also important to assess the quality of the endodontic obturation in post-operative radiography. The results of this study showed that LDPE/BAG 20 and LDPE/BAG 30 had a radiopacity higher than 3 mm of aluminium, while LDPE/BAG 20 showed greater radiopacity due to its higher amount of SrO. This clearly indicates that LDPE/BAG 20 and LDPE/BAG 30 may be suitable root-canal filling materials for clinical application, although further investigation is required to assess their sealing ability when applied in a root canal system with or without endodontic sealer.
The incorporation of BAG particles into low density polyethylene matrix had no effect on the melting temperature of the composite, which was in the range of 111-112°C for both the experimental LDPE/BAG 20 and LDPE/BAG 30. This indicates that such innovative materials may be easy removed in cases of retreatment when using heat application, with no complication and/or risk of injury of the surrounding periodontal ligament (Alhashimi et al., 2016). It is well known that during endodontic retreatment clinician should attempt to re-establish healthy periapical tissues. Hence, it is required to regain access through removal of the root canal filling materials, along with cleaning, shaping and re-obturation of the entire root canal system. (Stabholz and Friedman 1988). Removal of the coronal portion of gutta-percha and its carrier can be performed using several techniques such as rotary files, ultrasonic instruments, and hand files in combination with heat or chemicals. However, the apical portion of the root canal system as well as small, under-prepared and curved canals need negotiation during re-treatments and the use of solvents along with small K-type files seems to be a suitable solution rather than using hand files in combination with heat (Wong R. 2004).

The bioactivity of the experimental composites tested in this study was determined by soaking the specimens in SBF at physiological temperature (Kokubo et al., 2003). The FTIR results showed that the BAG filler contained in the two experimental composites was able to evoke the formation of calcium-phosphates and carbonates. It has been demonstrated that the precipitation of calcium-phosphates may occur on the surface of various bioactive polymer-based materials when immersed in supersaturated solution such as SBF (Abe et al., 1990; Kokubo et al., 1991). Apatite nucleation on polymers is evoked by an increase of supersaturation of simulated body fluid solutions (SBF) by silica and calcium ion release.
from BAG (Kokubo et al., 1991). The initial step of the precipitation is characterised by heterogeneous mineral nucleation induced by a local pH of the medium higher than the isoelectric point of the solid surface. Subsequently, Ca\(^{2+}\) ions are adsorbed on the negatively charged surface, which is then followed by the attraction of HPO\(_4\)^{2−} (Zhang et al., 2010). After that, ion concentrations in SBF are appropriate to initiate spontaneous growth of amorphous calcium-phosphate on a silica-rich layer, and subsequent crystallisation into hydroxyapatite (Hench and Andersson, 1993). However, in vivo apatite formation may be slower compared to that occurring in simulated in vitro conditions due to presence of serum proteins and to the considerable buffer effect of body fluids (e.g. Blood and Saliva) (Bohner and Lemaitre, 2009). Indeed, Tuusa et al., (2007) observed in animal studies, performed using glass-fibre-reinforced composite implanted in calvarias bone defects in rabbits, no apatite formation on the polymer surfaces after a 12 weeks study period; however, bioactive polymer-based materials presented advanced osseointegration in both in vitro and in vivo conditions (Ballo et al., 2008; Zhao et al., 2009).

The results of our study are also in accordance with those of previous studies (Vallet-Regi et al., 1999; Juhasz et al., 2003), wherein they pointed out the formation of an apatite-like layer on the polyethylene matrices, indicating the excellent bioactivity of such types of composites (in vitro). This bioactivity may be particularly advantageous in the apical region of the root canal space where the BAG-reinforced composites may come in contact with blood at the periapical tissues. Indeed, this might favour the regeneration of the damaged apical tissues such as periodontal ligament, cementum and periapical bone (Gupta et al., 2010; Jung et al., 2010).
It is well known that an ideal endodontic filling material should be able to ensure impervious sealing of the entire length of the treated root canal space. If this is not achieved, the risk of failures related to coronal-to-apical leakage through gaps and porosities may be quite high. A further beneficial response from bioactive glasses is related to their high alkalinity, which may elicit an antibacterial effect, and thus decrease bacterial colonisation within the root canal space (Allan et al., 2001). Moreover, the alkalinising properties of bioactive glasses may play a crucial part during healing processes at the periapical region, as well as in the stimulation of the release of alkaline phosphatase, which is involved in the processes of bone formation (Reilly et al., 2007; Jell et al., 2008).

In this study a primary human osteoblast-like cell culture model along with MTT and alamarBlue assays were employed to characterise the biological response of the two experimental BAG-reinforced polyethylene composites. The MTT and the alamarBlue assay showed no deleterious or cytotoxic effects on the viability and metabolic functions of osteoblast-like cells. This suggests a good biological response with vital tissue with no leachable toxic ions released; conversely, these latter may have the ability to stimulated some kind of cellular adhesion and proliferation (Hench et al., 1993; Hench et al., 2006). The initial lowering of MTT activity at 1 day exposure expressed on cells may be attributed to the increase in the local pH of BAG-reinforced polyethylene composites. Sauro et al., (2013) demonstrated that resin based materials containing BAG particles may have a substantial alkalinising ability due to due to a rapid release of Na$^+$ or K$^+$ and the incorporation of H$^+$ or H$_3$O$^+$ into the bioglass particles. However, Bioglass 45S5 has a stimulatory effect, also when incorporated in a polyethylene composite, on the cellular activity. This is mainly due to the establishment of favourable sites for cell adhesion and growth and to the release of Si ions,
which are essential for bone formation (Hench et al., 2006). Huang et al., (1997) showed that high density polyethylene doped with bioactive glass particles evoke an excellent biological response as they significantly increase the metabolic activity of human osteoblast cells. Furthermore, it is important to highlight that the inclusion of SrO as a radiopacifier into the composites caused no change in the bioactivity and biocompatibility of the experimental BAG-reinforced materials. Massera et al., (2015) showed that the addition of SrO in both silicate and phosphate glasses was assumed beneficial for proliferation and growth of human gingival fibroblasts. This was hypothesised to be due to Sr incorporation in the reaction layer at the glass surface, which is subsequently released within the cell culture medium.

In conclusion, within the limitations of this *in vitro* study, it is possible to affirm that the two BAG-reinforced/Sr-doped polyethylene composites tested in this study might be considered suitable alternative obturation materials for endodontic treatment due to their low melting temperature; hence they are expected to allow easy removal in retreatment cases. Moreover, these types of bioactive composites have good biocompatibility and bioactivity, which are beneficial healing properties on the proliferation of human osteoblast cells, especially at the periapical area of the root. However, further *in vivo* and *in vitro* investigation is required to assess their sealing ability when applied in a root canal system.

**Disclosure Statement**

All authors declare no conflicts of interest or financial support from public or private companies.
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FIGURES CAPTION

FIGURE 1. A and B: Scanning electron micrographs of the polyethylene Bioglass (20%)-reinforced composite at different magnifications, where it is possible to see the distribution of the filler within the polymer matrix, C and D: Scanning electron micrographs of the polyethylene Bioglass (30%)-reinforced composite at different magnifications. In this case, it is possible to note the higher presence of the Bioglass fillers within the polymer matrix.

FIGURE 2 A: Degree of radiopacity of the LDPEBAGSRO 20/30 was 140 ~4.1 mm of aluminium stepwedge, B: Degree of radiopacity of the LDPEBAGSRO 20/30 was 3 mm of aluminium stepwedge.

FIGURE 3 A: FTIR spectra of the pure LDPE (filler free) characterised by band at 1465 cm⁻¹ (stretching vibration of CH₂), and two sharp peaks at 2848 cm⁻¹ and 2916 cm⁻¹ (CH₂ stretching and –CH₂ deformation), B: The FTIR spectra of LDPEBAGSRO 30/20 showed sharp peaks at 1465 cm⁻¹, 2848 cm⁻¹ and 2916 cm⁻¹ as observed in the pure LDPE and an absorption band at a range of 1040-
900 cm\(^{-1}\) attributed to the stretching vibrations of Si-O from bioglass, C: The FTIR spectra of LDPEBAGSRO 30/20 after immersion in SBF for 2 days at 37\(^{\circ}\)C is characterised by the conversion of the Si-O into a clear phosphate peak (vibration P-O) at 1037 cm\(^{-1}\). Moreover, a further peak at 871 cm\(^{-1}\) was also observed, indicating the presence of carbonate precipitation, as well as at 1621 cm\(^{-1}\); this latter indicated the presence of carbonates within the structure of the newly formed calcium phosphates, D: The FTIR spectra of LDPEBAGSRO 20/30 also showed the CH\(_2\) peaks at 1465 cm\(^{-1}\), 2848 cm\(^{-1}\) and 2916 cm\(^{-1}\) and the peak of Si-O at a range of 1040-900 cm\(^{-1}\), E: The FTIR spectra of LDPEBAGSRO 30/20 after immersion in SBF for 2 days also showed a clear phosphate peak (vibration P-O) at 1037 cm\(^{-1}\) and the carbonates’ peaks at 871 cm\(^{-1}\) and 1621 cm\(^{-1}\). The main difference was the LDPEBAGSRO 20/30 showed a slightly higher intensity phosphate peak compared to LDPEBAGSRO 30/20.

**Figure 4**

**A:** Viability of human osteoblast-like cells (HOB) following exposure for 1 day eluted media from the experimental composites, expressed as mean absorbance ± SD. No significant drop in metabolic activity at 24 h and 72 h was observed for the two experimental BAG/SrO-reinforced composites. While these latter had a significantly higher biocompatibility compared to the positive control group. **B:** Viability of HOB cells following exposure for 3 day eluted media
from the experimental composites, expressed as mean absorbance ± SD. Also in this case, no significant drop in metabolic activity at 24 h and 72 h was observed for the two experimental BAG/SrO-reinforced composites. While the experimental composites showed a significantly higher biocompatibility compared to the positive control group. C: Metabolic activity of HOB cells as indicated by Alamar Blue™ assay in direct contact with the experimental composites at 1, 3, 7, 14, 21 and 28 days. A good biological response of human osteoblast-like cells with the experimental BAG-reinforced polyethylene composites compared to Thermanox™ (control) for 28 days. LDPEBAGSRO 20/30 and LDPEBAGSRO 30/20 composites showed no significant difference in comparison to the non-toxic control Thermanox during the 28 days of evaluation, but a slight cell proliferation in LDPEBAGSRO 20/30 between day 7 and 14.
Table 1. Experimental composites tested in this study

<table>
<thead>
<tr>
<th>Composite</th>
<th>Composition (wt%)</th>
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<tbody>
<tr>
<td>LDPEBAGSrO 20/30</td>
<td>Low density polyethylene 50% + bioactive glass 20% + strontium oxide 30%</td>
</tr>
<tr>
<td>LDPEBAGSrO 30/20</td>
<td>Low density polyethylene 50% + bioactive glass 30% + strontium oxide 20%</td>
</tr>
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Table 2. The melting and crystallization temperatures of the experimental composites

<table>
<thead>
<tr>
<th>Composite</th>
<th>Tm/°C</th>
<th>Tg/°C</th>
<th>Tc/°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low density Polyethylene (No BAG)</td>
<td>110.5</td>
<td>-65</td>
<td>95</td>
</tr>
<tr>
<td>LDPEBAGSRO20/30</td>
<td>111.1</td>
<td>-68</td>
<td>93.1</td>
</tr>
<tr>
<td>LDPEBAGSRO30/20</td>
<td>112.6</td>
<td>-70</td>
<td>92.5</td>
</tr>
</tbody>
</table>

HIGHLIGHTS

- BAG/SrO-reinforced composites may be suitable materials for endodontic treatment
- BAG/SrO-reinforced composites have good bioactivity and biocompatibility
- Due to their low melting temperature, BAG/SrO composites may be easily removed in retreatment cases