Materials Chemistry and Physics 228 (2019) 201–209

Contents lists available at ScienceDirect

Materials Chemistry and Physics

journal homepage: www.elsevier.com/locate/matchemphys

Characterization and cytotoxicity evaluation of bio-inspired bioactive Glass/Collagen/Magnesium composites

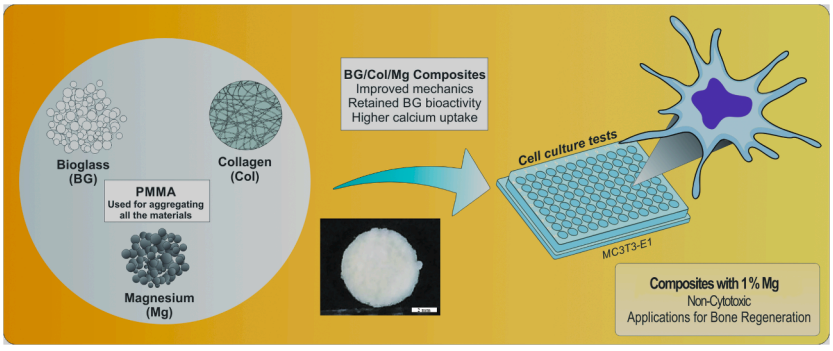
P.R. Gabbai-Armelin[a,*], K.R. Fernandes[a], A.M.P. Magri[a], A.C. Da Silva[b], C.A. Fortulan[c], A.C.M. Renno[a]

[a] Laboratory of Biomaterials and Tissue Engineering, Department of Biosciences, Federal University of São Paulo (UNIFESP), Silva Jardim, 136, Santos, SP, 11015-020, Brazil
[b] Nuclear and Energy Research Institute (IPEN), São Paulo, SP, 05508-000, Brazil
[c] Department of Mechanical Engineering, University of São Paulo (USP), Trabalhador São Carlense, 400, São Carlos, SP, 13566-590, Brazil

HIGHLIGHTS

- Magnesium (Mg) was successfully introduced into Bioglass (BG) and Bioglass/Collagen (BG/Col) composites.
- Mg improved mineralization and mechanical properties of BG and BG/Col.
- Composites containing 1% Mg were non-cytotoxic and biocompatible, and they are promising for forward works.

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Bioactive glass
Collagen
Magnesium
Composites
Bone repair

ABSTRACT

Bone fractures are a common clinical event related to trauma, aging or diseases. Since bone repair is complex, abnormal consolidation may occur or, even, non-union. Biomaterials have a key role in this context, since they can stimulate bone cell differentiation, accelerating the healing process. Bioactive glasses (BG) represent a promising class of biomaterials due to its high bioactivity and osteogenic potential. Nevertheless, the osteoconductive properties of BG may not be enough to stimulate consolidation in critical situations. Thus, it was hypothesized that enriching BG with other materials such as collagen (Col) and magnesium (Mg), trying to make a composite with similar properties to bone tissue, would constitute a more suitable graft for tissue engineering. This work aimed at obtaining BG/Col/Mg composites and evaluating their physicochemical features. Moreover, L929 and MC3T3-E1 cell culture studies were done to investigate the cytotoxicity of the composites. The results showed that Mg could be successfully introduced, at different percentages (1, 3 and 5%), into BG and BG/Col composites, improving mechanical properties and retaining the bioactivity of BG. Ca assay measurements demonstrated that reactions in the Mg/solution interface, i.e. reactions between Mg and the ions in the simulated body fluid (SBF) have led to an increased Ca uptake for composites-containing 3 and 5% Mg compared to plain BG and BG/Col. *In vitro* studies showed that BG and BG/Col containing 1% of Mg were non-cytotoxic and biocompatible. This percentage of Mg is promising for forward works. Our data on the present BG/Mg and BG/Col/Mg-based composites are encouraging and may lead to further molecular and cell culture studies, and *in vivo* investigations to clarify the biological performance of these new biomaterials.

* Corresponding author.
E-mail address: paulogabbai@gmail.com (P.R. Gabbai-Armelin).

https://doi.org/10.1016/j.matchemphys.2019.02.072
Received 1 November 2017; Received in revised form 4 January 2019; Accepted 18 February 2019
Available online 19 February 2019

1. Introduction

Bone fracture is a common clinical event, frequently found in the medical practice, related mainly to traumas, aging or diseases [1]. Although bone consolidation is a complex process, in most cases, bone tissue is capable of healing by itself, restoring its usual architecture and mechanical properties [2–4]. However, in specific critical situations such as deficient blood supply, mechanical instability or large bone defects, complete recovery may not occur, culminating in an abnormal consolidation or, even, in non-union fractures [3].

In this context, there is a great need of developing therapeutic interventions able of stimulating bone tissue and producing fracture consolidation. Biomaterials based bone grafts have a key role in this field, being able of stimulating bone cell differentiation and newly formed bone deposition, accelerating the process of healing [4–7]. One of the most promising class of biomaterials is the bioactive glass (BG), mainly due to its high bioactivity index and osteogenic potential [6,8]. It is well known that BGs, when in contact with biological tissues and body fluids, form a hydroxycarbonate apatite (HCA) layer, stablishing an extremely strong chemical bonding between tissue and implant, which mimics the interface formed naturally in the recovery of the bone tissue [6,9,10]. Many authors have demonstrated that BG stimulates osteoprogenitor cell proliferation, which differentiate into matrix-producing osteoblasts and accelerate the rate of newly bone deposition and bone ingrowth [9,11–13].

Despite the excellent osteogenic potential, the osteoconductive properties of BG may not be sufficient to stimulate consolidation in critical situations [9,10]. Consequently, composite materials (presenting characteristics from different biomaterials) has been emerging as a feasible alternative [11–13]. Thus, the association of a mineral part, such as BG, with an organic part (such as the introduction of collagen (Col)), may mimic better the composition and structure of the human bone tissue [14,15], constituting a bone graft with improved biological properties.

Col-based scaffolds are able of upregulating the expression of bone morphogenetic proteins (BMPs), bone sialoprotein (BSP), osteopontin, stimulating bone cell proliferation and differentiation in experimental models of bone fractures [16]. Wheeler et al. (2013), in an *in vitro* study, verified that elastin/BG/Col composites increased the activity of alkaline phosphatase of osteoblastic cell [17]. Investigations conducted by Sun et al. (2013) demonstrated that Col scaffolds promoted a higher expression of RUNX2 and osteocalcin in osteoblastic lineage cultures, accelerated endochondral ossification and increased bone quality in an *in vivo* study [18].

In spite of the encouraging effects of BG and Col based composites in stimulating bone tissue, their mechanical properties needs to be improved especially for using as bone grafts in load-bearing bones [19]. To overcome this limitation, the addition of a metallic component (such as magnesium) would improve the mechanical properties of bioactive bone substitutes. Mg is the fourth cation more abundant in the human body and have been a promising component for producing biomaterials composites, with improved biomechanical properties [20]. Some studies which investigated Mg/Calcium alloys [20] and BG/Mg composites [21] showed improved biomechanical properties, increased biodegradation and low corrosion, and that these materials can stimulate the differentiation and proliferation of osteoblastic cell lines [20,21]. Later investigations on novel injectable magnesium/calcium sulfate hemihydrate (Mg/CSH) composites indicated enhanced mechanics, and higher degrees of cell attachment, proliferation and osteogenic differentiation compared to CSH. Moreover, *in vivo* studies, utilizing these Mg/CSH composites in canine tibial defects, showed elevated bone mineral density and bone area fraction [22]. Other *in vivo* work on Mg-enriched hydroxyapatite (Mg-e HA) implanted in post-dental extraction site in 20 patients showed that Mg-e HA allows the complete healing of the alveolar pocket with mineralized and well-organized bone tissue around the residual biomaterial particles [23].

In view of the growing interest in developing more suitable composites for bone substitutes, it was hypothesized that the introduction of Mg into BG/Col composites would improve material mechanical properties, retaining the bioactivity of BG. Thereby, this work aimed at obtaining BG/Col/Mg composites and investigating the physicochemical and morphological properties of such biomaterials (mechanical properties, degradation, mineralization and pH). Additionally, preliminary cell culture studies were done to investigate the cytotoxicity of these new composites.

2. Materials and methods

2.1. Materials

BG, belonging to the system SiO_2-CaO-Na_2O-P_2O_5 [10,11], was produced and provided by Nuclear and Energy Research Institute (IPEN, São Paulo, Brazil). Briefly, for the BG obtainment, mineral Silica 98.0 wt% powder was purified by attacking with hot hydrochloric acid (Merck, P.A.) followed by filtration (Whatmman 40) and 30 washings with boiling distilled water for removal of impurities (R_2O_3). Additionally, the following reagent analytical-grade were used: Sodium Hydroxide (NaOH 97.0 wt%, heavy metals ≤ 0.003 wt%, Cl- ≤ 0.005 wt%, Fe ≤ 0.001 wt%, Hg ≤ 0.1 ppm, K ≤ 0.02%, Na_2CO_3 ≤ 1.0 wt%, NH_4OH ≤ 0.02 wt%, Ni ≤ 0.001 wt%, PO_4^{3-} ≤ 0.001 wt%, SO_4^{2-} ≤ 0.003 wt%, absorbed water ≤ 2.0 wt%; Nuclear, São Paulo, Brazil), Calcium Oxide (CaO 97.0 wt%, heavy metals ≤ 0.005 wt%, Cl- ≤ 0.05 wt%, SO_4^{2-} ≤ 0.5 wt%, Fe ≤ 0.5 wt%, insolubles ≤ 0.01 wt%, absorbed water ≤ 2.0 wt%; Química Moderna, São Paulo, Brazil), Sodium Phosphate (Na_3PO_4 99.0 wt%, heavy metals ≤ 5 ppm, insolubles ≤ 0.01 wt%, SiO_4 0.005 wt%, PO_4^{3-} ≤ 0.001 wt%, Fe ≤ 5 ppm, Na_2CO_3 ≤ 0.02 wt%, NH_4OH ≤ 0.01 wt%, Ca and Mg ≤ 0.01 wt%, SO_4^{2-} ≤ 0.004 wt%, Cl^- ≤ 0.1 ppm; Química Moderna, São Paulo, Brazil). The compounds were weighed and mixed in a polyethylene bottle for 30 min. Pre-mixed batches were melted in an alumina crucible at 1500 °C (Lindberg Blue vertical super kanthal furnace – USA). The melting time was standardized as 2 h. Samples were quenched in deionized water and milled to powder grain (particle size: 125–250 μm).

Table 1
Experimental formulations of PMMA control and BG/Col/Mg composites.

Groups	PMMA (wt%)	MMA (wt%)	Bioglass (wt%)	Collagen (wt%)	Magnesium (wt%)	CMC (wt%)	Water (wt%)
PMMA	23.32	46.63	0.00	0.00	0.00	2.12	27.93
BG100	13.14	26.28	26.72	0.00	0.00	2.39	31.47
BG/Mg1	13.19	26.37	25.48	0.00	0.97	2.40	31.59
BG/Mg3	13.28	26.57	22.97	0.00	2.94	2.42	31.83
BG/Mg5	13.38	26.77	20.42	0.00	4.93	2.43	32.07
BG/Col	13.64	27.28	19.42	4.51	0.00	2.48	32.68
BG/Col/Mg1	13.69	27.38	18.10	4.53	1.01	2.49	32.80
BG/Col/Mg3	13.80	27.59	15.43	4.56	3.05	2.51	33.05
BG/Col/Mg5	13.91	27.81	12.73	4.60	5.12	2.53	33.31

Tendon bovine collagen type I was provided by United States Biological (particle size: < 500 μm; US Biological Life Sciences, Massachusetts, USA) and magnesium powder (particle size of 74–105 μm), by Alfa Aesar (purity: 99.6%; Massachusetts, USA).

Poly (methyl methacrylate) (PMMA, particle size: 15 μm) and methyl methacrylate (MMA, purity: 99.09%) were provided by VIPI Dental Products (Pirassununga, São Paulo, Brazil). Carboxymethyl cellulose (CMC), density 1.59 g/cm^3, was provided by Sigma Aldrich (Missouri, USA). Both polymer and monomer were utilized exclusively to aggregate all the tested materials, i.e., BG, Col and Mg. It is well stablished that PMMA is biocompatible and inert [24,25].

2.2. Preparation of BG/Col/Mg composites

For manufacturing BG/Col/Mg composites, the materials (i.e., PMMA, MMA, BG, Col, Mg, CMC and distilled H_2O) were added at different proportions according to each group (Table 1). Additionally, the amounts of BG, Col and Mg in the BG/Col/Mg-based composites, considering only the weight (wt%) of these components, are presented in Table 2.

PMMA scaffolds were manufactured and used as control for comparison purposes. CMC was utilized as the porogenic agent [26,27]. The amount of PMMA utilized was the lowest one (determined by tests) feasible to aggregate the materials. Briefly, all the materials, in powder form, were weighed and mixed in a silicone container using a spatula. After that, water was added, and the combination was mixed again. Finally, the MMA monomer was added and mixed to start the crosslink. Then, the mixture was rapidly transferred to a silicon mold of 6 mm diameter x 2 mm height (for mechanical tests, cylinders of 5 mm × 10 mm were prepared using different molds). Subsequently, the molds were sealed and submitted to a pressure air chamber at 0.6 MPa for 30 min. Afterwards, the unsealed molds were vacuum dried (10^{-3} Torr) for 15 min and the composites were set to dry at room temperature.

2.3. Structural morphology by scanning electron microscopy (SEM)

After drying, the samples were mounted on stubs with carbon tape and sputter coated using gold (System Bal-Tec Med 020; Balzers, Liechtenstein). SEM (Zeiss Leo 440; Cambridge, England) was performed to analyze the structural morphology of the composites.

2.4. Mechanical test

After preparing the different composites, the compressive strength (CS) of BG/Col/Mg cylinders was measured, in the longitudinal direction of the specimens, at a loading rate of 0.5 mm/min utilizing a testing bench machine with a 1 kN load (3340 Series Single Column Systems, Instron, Norwood, MA, USA), using three cylinders per experimental group (n = 3).

2.5. Mass measurements

Mass measurements were performed to check the *in vitro* degradation behavior of the composites. For this purpose, the scaffolds (n = 4) were placed in 2.0 ml of PBS and incubated for 3, 7, 10 and 14 days at 37 °C. After each time, the scaffolds were retrieved from the solution and weighed. The mass variation was calculated using the formula:

$$\text{Mass } \% = [(W_t - W_0)/W_0] \text{ x } 100\%$$

where W_0 is the weight of the sample before immersion in PBS and W_t is the weight of the sample after immersion time (t) in PBS. Measurements were performed in quadruplicate.

2.6. Ca assay in simulated body fluid (SBF)

The mineralization behavior of BG within BG/Col/Mg composites was assessed *in vitro* by following the methods described by Kokubo and Takadama [28]. SBF having the same ionic composition as blood serum was prepared under laminar flow to prevent contamination. PMMA and BG/Col/Mg composites (1 scaffold; n = 4) were placed in glass vials containing 5 ml of SBF at 37 °C on a shaker table (70 Hz) for up to 14 days, with refreshment on days 3, 7 and 10 [29]. At each refreshment, the solution of the previous period was saved for analysis of the calcium content in SBF using the orthocresolphtalein complexone (OCPC) assay [30]. Briefly, the solutions were incubated overnight in 1 ml of 0.5 N acetic acid on a shaker table. For analysis, 300 μl working reagent was added to 10 μl sample or standard in a 96-well plate. The plate was incubated for 10 min at room temperature. The absorbance of each well was measured on a microplate spectrophotometer at 570 nm (Bio-Tek Instruments, Winooski, VT, USA). The standards were prepared using a $CaCl_2$ stock solution. Data were obtained from triplicate samples and measured in duplo. The depletion of Ca was plotted cumulatively, by measuring the difference between the Ca concentration in the sample-free SBF control solutions and the Ca concentration of SBF solution in the presence of BG/Col/Mg composites.

2.7. pH measurements

At each time point during incubation in SBF, the pH was monitored (n = 4) by using a pH electrode (Orion Star A211, Thermo Scientific, Massachusetts, USA).

2.8. Cell culture studies

The cytotoxicity of the new formulations was evaluated by alamarBlue®, via an indirect assay [31] using extracts of the materials. For this purpose, after preparation followed by sterilization using ethylene oxide (Acecil, Campinas, Brazil), all scaffolds (n = 4) were put in contact with 2 ml of cell culture medium (Dulbecco's Modified Eagle's medium; DMEM or alpha Minimal Essential Medium without ascorbic acid; α-MEM; Vitrocell, Campinas, Brazil) supplemented with 10% fetal bovine serum (FBS; Vitrocell) and 1% p/s (penicillin/streptomycin; Vitrocell) for 7 days. Controls were constituted by four empty wells filled with the same amount of each medium.

L929 murine fibroblastic cells (ATCC CCL-1, passage 8; Banco de Células do Rio de Janeiro, BCRJ, RJ, Brazil) and MC3T3-E1 murine pre-osteoblastic cells (ATCC CRL-2594, passage 10; BCRJ, RJ, Brazil) were cultured in proliferation medium containing DMEM and α-MEM (Vitrocell) respectively supplemented with 10% FBS (Vitrocell) and 50 μl/ml gentamicin (Vitrocell) in a humidified incubator set at 37 °C and 5% CO_2. Upon 80% confluency, cells were detached using trypsin and seeded at a density of 1×10^4 cells per cm^2 in 48-well plates containing 500 μl of composites extracts, that was previously collected, and the cells were incubated for 1, 3 and 6 days. Afterwards, the alamarBlue® assay (Bio-Rad AbD Serotec GmbH, Puchheim, Germany)

Table 2
Amounts of BG, Col and Mg in the BG/Col/Mg-based composites, considering only the wt% of these components.

Groups	Bioglass (wt%)	Collagen (wt%)	Magnesium (wt%)
BG100	100	0	0
BG/Mg1	96	0	4
BG/Mg3	89	0	11
BG/Mg5	81	0	19
BG/Col	81	19	0
BG/Col/Mg1	77	19	4
BG/Col/Mg3	67	20	13
BG/Col/Mg5	57	20	23

was used on all samples, at each time point, to evaluate cell viability. For this analysis, 50 µl of alamarBlue® solution was added to each well, and the plate was stored in the dark for 4 h at 37 °C in a cell culture incubator. After this period, 200 µl of the samples were transferred to a 96-well plate. Measurements were performed using a microplate reader (Bio-Tek Instruments, Inc.) at 570 nm in duplo.

2.9. Statistical analysis

Data were expressed as mean ± standard deviation. Statistical analysis was performed using GraphPad Prism 6 (GraphPad Software, San Diego, CA, USA). Shapiro-Wilk normality test was used to check distribution. Kruskal-Wallis test and Dunn post hoc were used for nonparametric data. One-way analysis of variance (ANOVA) and Tukey multiple comparisons post-tests were used for parametric data. Differences were considered significant at $p \leq 0.05$.

3. Results

3.1. SEM

SEM representative micrographs of the samples are depicted in Fig. 1. In all samples, PMMA particles (used to aggregate the materials) could be observed. After combining BG with the polymer, particles of the bioactive material could be noticed (Fig. 1B). Also, in the other groups, BG particles were observed (Fig. 1C–E) and Col particles were noticed for collagen-containing groups (Fig. 1D and E). Col particles presented an aspect of fibers with smoother surfaces and they could be found among the BG particles. Additionally, Fig. 1F represents a higher magnification (1000x) of the BG/Col/Mg3 composites in order to more clearly depict the different components in the SEM images. Mg particles could not be visualized by SEM analysis.

3.2. Mechanical test

The mechanical tests (Fig. 2) indicated for BG/Mg groups, a tendency in increasing CS for BG/Mg-based composites compared to plain BG. The values for compressive strength were statistically higher for BG/Mg1 (52.443 ± 3.872 MPa), BG/Mg3 (51.029 ± 10.084 MPa) and BG/Mg5 (45.895 ± 0.850 MPa) compared to plain BG (29.883 ± 1.323 MPa). Also, statistical differences were observed comparing PMMA (76.350 ± 4.560 MPa) to all other groups ($0.00009 < p < 0.0013$; Fig. 2A).

BG/Col/Mg groups showed also a tendency of increasing CS for BG/Col/Mg-based composites compared to BG/Col (Fig. 2B). Statistical differences were found for BG/Col/Mg3 (58.272 ± 0.923 MPa) compared to BG/Col (40.905 ± 3.319 MPa, $p = 0.0137$). Moreover, statistically higher values were observed for BG/Col/Mg5 (84.967 ± 5.740 MPa) compared to BG/Col (40.905 ± 3.319 MPa, $p < 0.0001$), BG/Col/Mg1 (52.465 ± 10.211 MPa, $p = 0.0002$) and BG/Col/Mg3 (58.272 ± 0.923 MPa, $p = 0.0009$). PMMA value for CS was also statistically higher than BG/Col, BG/Col/Mg1 and BG/Col/Mg3 ($0.00009 < p < 0.0164$; Fig. 2B).

3.3. Mass measurements

After 3 days of immersion in PBS, a slight decrease in the initial mass was observed for PMMA and BG100, reaching values of ~97 and 98%, respectively, which were kept until the last time point (Fig. 3A). On the same experimental period (3 days), BG/Mg1 and BG/Mg3 presented some weight gain in their original mass (~102 and 103%), and these values increasing to 105% at day 14 for both groups. BG/Mg5 showed a more evident mass gain at day 3 (~107%) compared to the other groups, and this phenomenon was accentuated at days 7 (~112%) and 10 (~122%), reaching ~124% at day 14. Statistical differences were found for BG/Mg5 compared to PMMA and BG100 at all time points ($0.008 < p < 0.021$).

Mass measurements for BG/Col and BG/Col/Mg groups are depicted in Fig. 3B. PMMA and BG/Col revealed some loss in their initial mass, 3 days after incubation, reaching ~97 and 98% of the initial mass, respectively. Still at day 3, statistical higher values were found for BG/Col/Mg3 ($p = 0.032$) and BG/Col/Mg5 ($p = 0.022$) compared to PMMA. At day 7, BG/Col/Mg, in the different compositions (1, 3 and 5%) presented some weight gain compared to their initial mass. Furthermore, the value presented for BG/Col/Mg5 (~104%) was statistically different compared to BG/Col (~97%; $p = 0.039$). The weight gain continued, after 10 days, for all BG/Col/Mg groups, being more evident for BG/Col/Mg3 (~104%) compared to PMMA and BG/Col (~97%; $p < 0.045$). Likewise, 14 days post-incubation, BG/Col/Mg5 mass (~109%) was statistically higher than PMMA (~97%; $p = 0.003$) and BG/Col (~100%; $p = 0.026$).

3.4. Ca assay

On day 3, BG100 and BG/Mg1 presented Ca release which continued until day 7, reaching ~290 and 220 µg respectively (Fig. 4A). After 7 days, a plateau was reached for both groups, keeping the same above-mentioned values until the last time point. Differently, the groups BG/Mg3 and BG/Mg5 showed Ca uptake which was more evident after 7 days of incubation (~110 and 275 µg respectively) compared to the previous time point. The Ca mineralization continued for BG/Mg5 until day 14, reaching ~320 µg; Fig. 4A). Statistical differences were observed for BG/Mg5 compared to BG100 at all time points ($0.010 < p < 0.02$). No statistical difference was found among other groups at all time points ($p > 0.05$).

BG/Col and BG/Col/Mg1 released Ca in the solution, especially after 3 days of experiments, with the values of ~420 and 315 µg, respectively, on the last experimental period (Fig. 4B). In contrast, BG/Col/Mg3 and BG/Col/Mg5 mineralized Ca overtime and this fact was more evident for BG/Col/Mg5 which presented a continuous uptake of the ion, reaching 615 µg at the last time point (Fig. 4B). At all

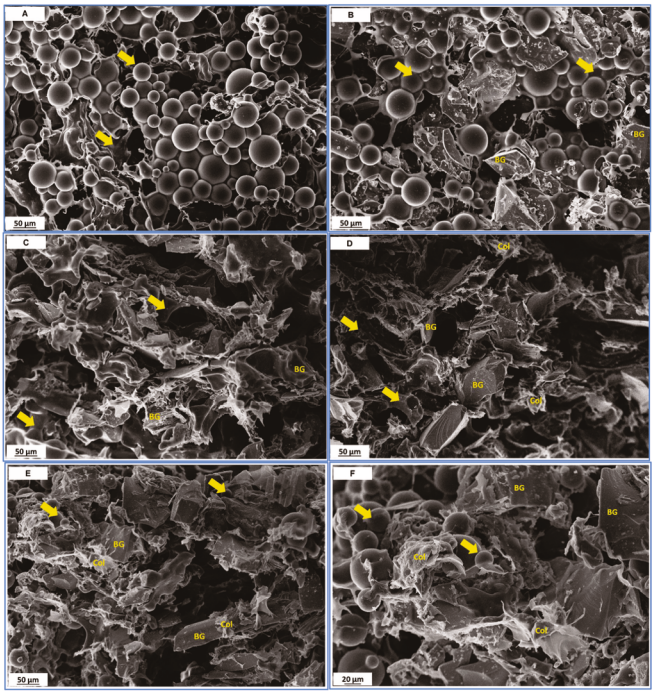


Fig. 1. SEM micrographs of the BG/Col/Mg composites. 500x magnification: [A] PMMA, [B] BG100, [C] BG/Mg3, [D] BG/Col and [E] BG/Col/Mg3. 1000x magnification: [F] BG/Col/Mg3. Arrows indicate PMMA polymer, BG bioactive glass and Col collagen.

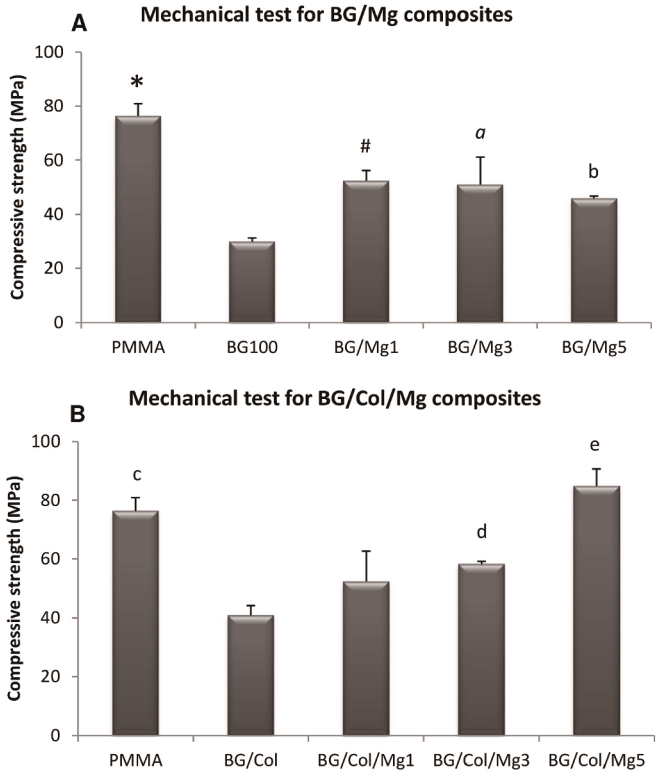


Fig. 2. Mechanical tests (compressive strength) for (A) BG/Mg and (B) BG/Col/Mg composites. *PMMA compared to BG100 ($p < 0.0001$), BG/Mg1 ($p = 0.0018$), BG/Mg3 ($p = 0.0012$) and BG/Mg5 ($p = 0.0003$); # BG/Mg1 compared to BG100 ($p = 0.0027$); a BG/Mg3 compared to BG100 ($p = 0.0043$); b BG/Mg5 compared to BG100 ($p = 0.0027$); c PMMA compared to BG/Col ($p < 0.0001$), BG/Col/Mg1 ($p = 0.0022$) and BG/Col/Mg3 ($p = 0.0163$); d BG/Col/Mg3 compared to BG/Col ($p = 0.0137$) and e BG/Col/Mg5 compared to BG/Col ($p < 0.0001$), BG/Col/Mg1 ($p = 0.0002$) and BG/Col/Mg3 ($p = 0.0009$).

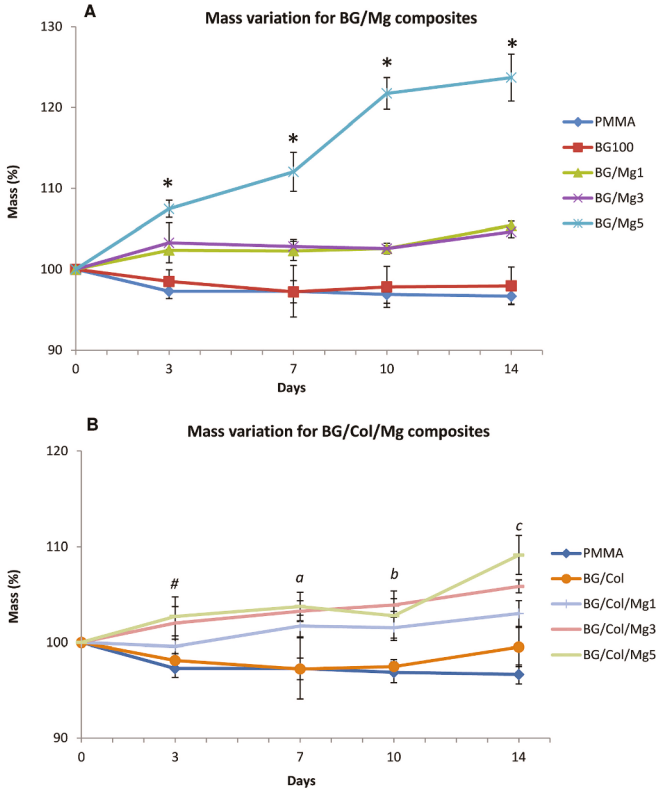


Fig. 3. Mass variation for (A) BG/Mg and (B) BG/Col/Mg composites for up to 14 days of immersion in PBS. * BG/Mg5 compared to PMMA and BG100 at all time points ($0.008 < p < 0.021$); # BG/Col/Mg3 ($p = 0.032$) and BG/Col/Mg5 ($p = 0.022$) compared to PMMA; *a* BG/Col/Mg5 compared to BG/Col ($p = 0.039$); b BG/Col/Mg3 compared to PMMA and BG/Col ($p = 0.035$ and 0.044 respectively); c BG/Col/Mg5 compared to PMMA and BG/Col ($p = 0.003$ and 0.026 respectively).

experimental periods, mineralization was statistically higher for BG/Col/Mg5 compared to BG/Col ($0.013 < p < 0.027$).

3.5. pH measurements

The pH measurements indicated that PMMA and BG100 formulations showed relatively constant pH values over time (variations between 7.4 and 7.8). It was observed an increased pH at day 3 for groups BG/Mg1, BG/Mg3 and BG/Mg5 reaching values between 8.5 and 9.0 (Fig. 5A). After that, these composites presented a pH decrease, with values ranging from 7.7 to 8.0 at day 21 post-incubation. Statistically higher values were found for BG/Mg5 compared to PMMA at days 3, 7 and 10 ($p = 0.0160$, 0.0102 and 0.0183 respectively). No statistical difference was found among other groups at all time points ($p > 0.05$).

Similarly, PMMA and BG/Col compositions presented relatively constant pH values over time (variations between 7.4 and 7.8). On day 3, an increased pH was observed for BG/Col/Mg1, BG/Col/Mg3 and BG/Col/Mg5, with values of 8.6, 8.6 and 8.8 respectively (Fig. 5B). After this time point, the composite BG/Col/Mg1 had a sharp decrease, reaching a pH value of ~7.7 at day 7. At this same experimental period, BG/Col/Mg3 and BG/Col/Mg5 composites also revealed a pH decrease, reaching values of 8.2 and 8.4 respectively. After this experimental period, a plateau was reached for BG/Col/Mg1 (pH value = 7.7) and, on the other hand, the pH for BG/Col/Mg3 and BG/Col/Mg5 continued decreasing over time, with values between 7.8 and 8.0 after 21 days of incubation for both groups. Statistically higher values were observed for BG/Col/Mg5 compared to PMMA at days 3, 7 and 10 ($p = 0.0101$, 0.0192 and 0.0189 respectively). No statistical difference was found among other groups at all time points ($p > 0.05$).

3.6. Cell culture studies

Investigation of the cytotoxicity on L929 cells by alamarBlue® showed that, at day 1, cell proliferation was lower for BG/Mg5 compared to PMMA ($p = 0.0479$) and BG100 (0.0018). After 3 and 6 days of culture, statistical differences were also found for BG/Mg5 compared to Control and BG100 ($0.005 < p < 0.025$; Fig. 6A).

Similarly, BG/Col/Mg5 presented statistical lower values compared to PMMA ($p = 0.0479$) and BG/Col ($p = 0.0018$) at day 1. In addition, at days 3 and 6, statistical differences were observed for BG/Col/Mg5 compared to Control and BG/Col ($0.006 < p < 0.025$). Interestingly, BG/Col/Mg3 presented similar values for cell proliferation compared to the Control, especially after 6 days of culture (71.31 ± 2.03 and 86.11 ± 8.32; Fig. 6B). No other statistical difference was found among the groups at all time points ($p > 0.05$).

Cell culture studies using MC3T3-E1 indicated a lower proliferation for BG/Mg5 compared to Control ($p = 0.0479$) and BG/Mg1 ($p = 0.0246$; Fig. 7A) at day 1. At day 3, statistically higher values for Control ($p = 0.0174$) and BG100 ($p = 0.0040$) were also found compared to BG/Mg5. After 6 days of culture, similarly to the first period, proliferation was higher for Control ($p = 0.0479$) and BG/Mg1 ($p = 0.0012$) compared to BG/Mg5. Interestingly, at the last time point, values were statistically different comparing BG/Mg1 and BG/Mg3 ($p = 0.0246$; Fig. 7A).

Regarding BG/Col/Mg composites, values for BG/Col/Mg5 were statistically lower compared to Control ($p = 0.0345$) and BG/Col/Mg1 (p = 0.0146; Fig. 7B) at day 1. After 3 days of cell seeding, proliferation

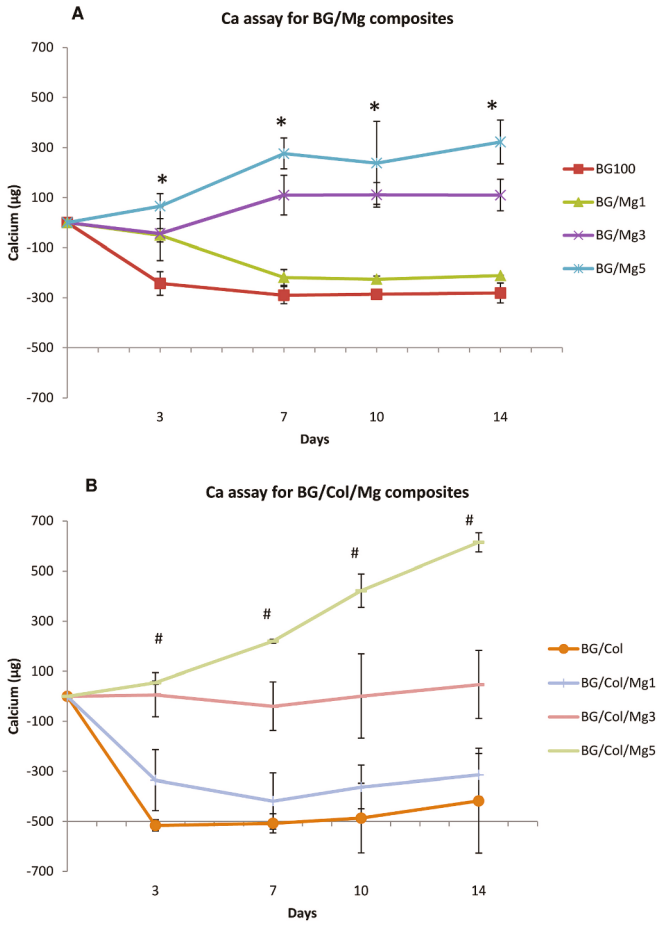


Fig. 4. Ca release/uptake for (A) BG/Mg and (B) BG/Col/Mg composites for up to 14 days of immersion in SBF. * BG/Mg5 compared to BG100 at all time points ($0.010 < p < 0.02$); # BG/Col/Mg5 compared to BG/Col at all experimental periods ($0.013 < p < 0.027$).

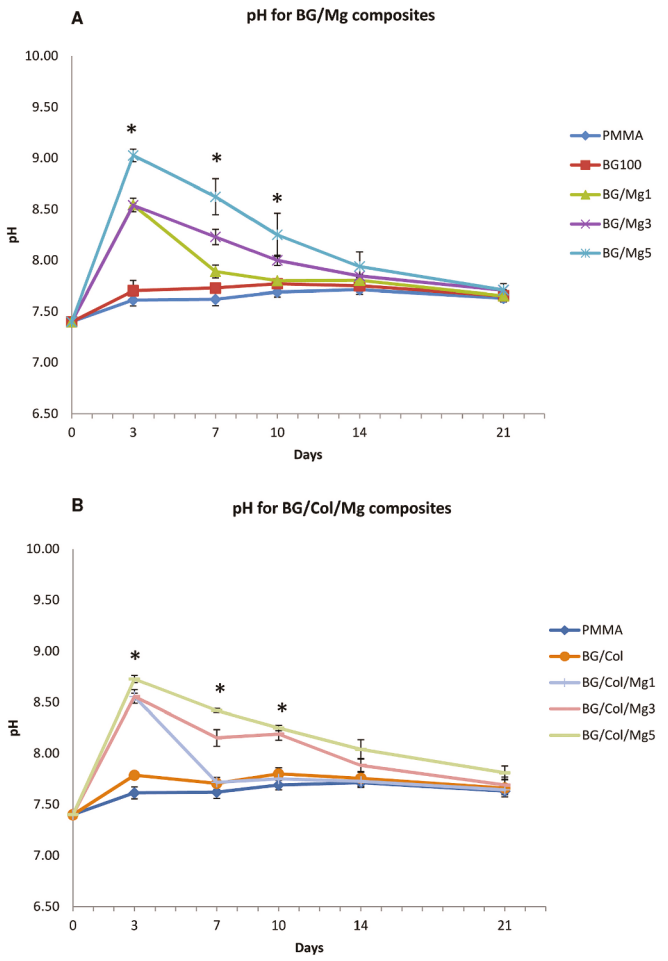


Fig. 5. pH of SBF solution in contact with (A) BG/Mg and (B) BG/Col/Mg composites for up to 21 days. * BG/Mg5 compared to PMMA ($p = 0.0160$, 0.0102 and 0.0183 at days 3, 7 and 10 respectively); # BG/Col/Mg5 compared to PMMA ($p = 0.0101$, 0.0192 and 0.0189 respectively at days 3, 7 and 10 respectively).

was higher for Control compared to BG/Col/Mg3 ($p = 0.0344$) and BG/Col/Mg5 ($p = 0.0018$). At days 6, statistical differences were found only between BG/Col/Mg1 and BG/Col/Mg5 ($p = 0.0030$; Fig. 7B). No other statistical difference was observed among the groups at all time points ($p > 0.05$).

4. Discussion

The present study investigated Mg incorporation, at different percentages, into BG and BG/Col composites, with the aim of improving the physic-chemical features, and *in vitro* biological performance of above-mentioned materials. The results showed that Mg could be successfully introduced into BG and BG/Col composites. A tendency in increasing CS for BG/Mg (with samples-containing all percentages of Mg), and for BG/Col/Mg (in composites-containing 3 and 5% Mg) was observed. A significant increase in mass samples was detected for BG/Mg5, BG/Col/Mg3 and BG/Col/Mg5 after incubation in PBS for up to 14 days. Furthermore, Ca assay measurements demonstrated a higher calcium uptake for BG/Mg3, BG/Mg5, BG/Col/Mg3 and BG/Col/Mg5 compared to BG and BG/Col. The pH measurements indicated increased values for BG/Mg5 and BG/Col/Mg5, but all the groups reached similar pH values at the end of the experiment. A lower L929 and MC3T3-E1 cell viability was observed only for BG/Mg5 and BG/Col/Mg5. Interestingly, BG/Mg1 and BG/Col/Mg1 presented similar values for cell viability compared to Control, demonstrating non-cytotoxicity, especially to MC3T3-E1 lineage.

The successfully obtained BG/Col/Mg composites presented cohesion [32] upon incubation in different solutions, as PBS and SBF, and these materials were easy to handle. The planned volume and 60% porosity [33,34] used for formulation of the composites were bio-inspired, mimicking natural bone composition [35–37]. The resulting wt % for BG, Col and Mg in each composition were higher than the planned bio-inspired ones, nevertheless, these greater amounts of the biomaterials may be structural, not functional [38–40]. Additionally, SEM demonstrated that the components of the materials were well distributed, and BG and Col particles could be noticed and differentiated in each formulation. Mg particles could not be visualized by SEM analysis, since Mg has an atomic number (Z) close to the ones of BG elements and the electron image depends on the Z [10,41].

Mechanical tests showed a trend in increasing the CS for BG/Mg and BG/Col/Mg-based composites. Apparently from the discussed literature, the reinforcing Mg enhanced the mechanical properties of the composites, mainly in the ones containing the biopolymer Col [42], being suitable for repairing load-bearing bones [19]. This fact may be most due to the incorporation of Mg, since PMMA was also utilized for producing BG and BG/Col, and these groups presented lower CS values compared to the respective Mg-based groups. Previous work by Staiger et al. (2006) reported the advantages of magnesium as biodegradable material and, also, mentioned that its mechanical properties make this biomaterial attractive as orthopedic implants [43]. Studies by Khandaker and Tarantini (2012) found that the interface strength for bone-PMMA is lower than the interface strengths for bone-PMMA with MgO particles [44].

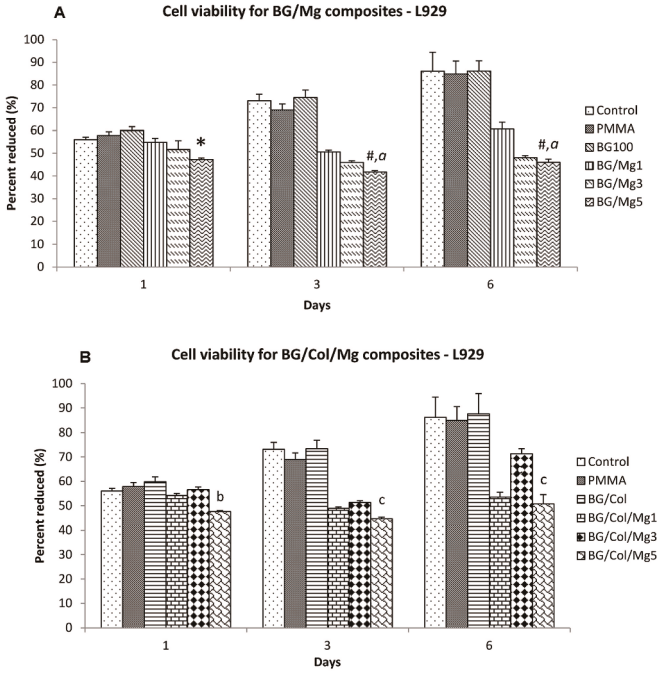


Fig. 6. Cell viability by alamarBlue for L929 cells in contact with pre-conditioned medium obtained after 1, 3 and 6 days. (A) and (B) for BG/Mg and BG/Col/Mg respectively in contact with L929. * BG/Mg5 compared to PMMA (p = 0.0479) and BG100 (p = 00018); # BG/Mg5 compared to Control at days 3 and 6 (p = 0.007 and 0.0246 respectively); *a* BG/Mg5 compared to BG100 at days 3 and 6 (p = 0.0058 and 0.0174 respectively); b BG/Col/Mg5 compared to PMMA (p = 0.0479) and BG/Col (p = 00018); c BG/Col/Mg5 compared to Control and BG/Col at days 3 and 6 (p = 0.007 and 0.02 respectively).

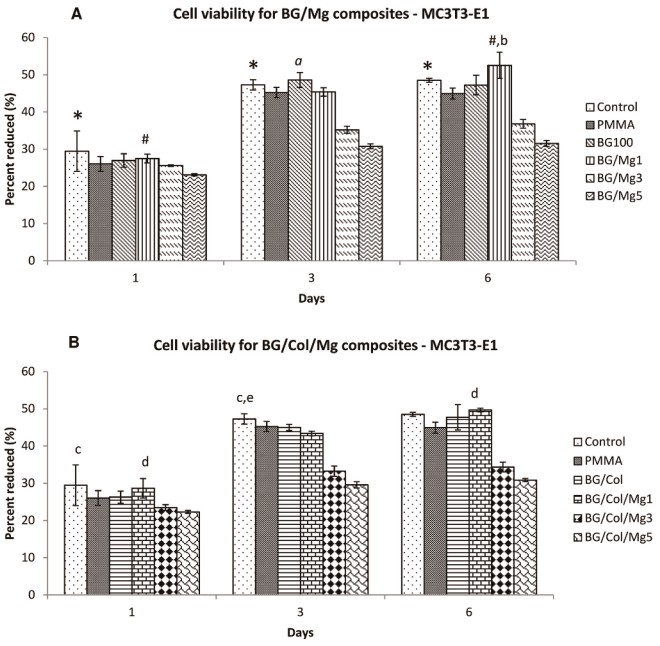


Fig. 7. Cell viability by alamarBlue for MC3T3-E1 cells in contact with pre-conditioned medium obtained after 1, 3 and 6 days. (A) and (B) for BG/Mg and BG/Col/Mg respectively in contact with MC3T3-E1. * Control compared to BG/Mg5 at days 1, 3 and 6 (p = 0.0479, 0.0174 and 0.0479 respectively); # BG/Mg1 compared to BG/Mg5 at days 1 and 6 (p = 0.0246 and 0.0012 respectively); *a* BG100 compared to BG/Mg5 (p = 0.0040); b BG/Mg1 compared to BG/Mg3 (p = 0.0246); c Control compared to BG/Col/Mg5 at days 1 and 3 (p = 0.0345 and 0.0018 respectively); d BG/Col/Mg1 compared to BG/Col/Mg5 at days 1 and 6 (p = 0.0146 and 0.0030 respectively); e Control compared to BG/Col/Mg3 (p = 0.0344).

Mass loss measurements showed that BG and BG/Col basically presented a constant sample mass during the experimental periods and the introduction of Mg produced an increase of mass. It is known that the rate of BG dissolution is low [10,11]. This fact may explain the constance of BG sample mass. Also, the introduction of collagen did not have any statistical influence in the material dissolution, possibly because the used amount of this component was not sufficient to provoke any difference on this matter. On the other, most probably the samples with Mg gained weight through the faster precipitation of phosphate on their surface compared to the plain ones. Similarly, Xu et al. (2008) showed that Mg alloys-containing phosphate immersed in SBF presented weight gain after 48 h [45]. Moreover, reactions in the Mg/solution interface, i.e. reactions between Mg and the ions in the SBF have led to an increased Ca uptake in the samples-containing higher percentage of Mg (5%), both with BG and BG/Col, probably due to the phosphate precipitation and formation of the CaP layer on the surface of these composites [45]. Following this line, Witte et al. (2005) demonstrated, using implantation of Mg alloys rods into the femora of guinea pigs, high mineral apposition rates on these Mg rods related to the CaP layer [46].

The pH measurements confirmed that incorporation of Mg into BG and BG/Col resulted in alkalization of the immersion medium mainly in the first experimental periods. Probably, the protective buffering effect of an organism body may overcome these drawbacks and may allow bone formation [47]. The alkaline degradation of magnesium has already been reported which may contribute to the detected phenomenon in the present study [48]. This might be a new way to introduce alkaline filler in order to improve the properties of some acidic polymers [49]. No relevant influence of Col was observed. Magri et al. (2017) also showed that the incorporation of Col in BG-based materials had no effect in the pH measurements, with values close to the physiological one [50].

Cell culture studies indicated deleterious effect on cell viability in composites-containing the higher percentage of Mg (5%), and a tendency for that in materials-containing 3% Mg for both BG and BG/Col, especially for L929 lineage. This fact may probably be explained by the corrosion of Mg and its alloys, limiting further clinical applications for composites with increased amounts of this element in their composition (dose-dependent cell growth inhibition) [51]. Interestingly, no significant differences were found for cell viability of BG and BG/Col groups composed by the percentage of 1% Mg when comparing to Control group, mainly for MC3T3-E1 lineage, showing that these Mg-based composites were not harmful to these cells. Moreover, it worth highlighting the MC3T3-E1 non-cytotoxicity and cell viability in contact with extracts of BG and BG/Col 1% Mg, with values very close to the Control group, indicating that these materials are safe for this lineage. Likewise, BG and BG/Col groups presented values for cell viability similar to Control group. Earlier studies, using MC3T3-E1 pre-osteoblastic cells and BMSCs, also confirmed that Ca-Mg silicate foamed scaffolds and Mg/CSH composites, respectively, were biocompatible [52]. Additionally, previous studies already established that BG and Col are beneficial for osteoblastic cell growing and *in vivo* bone formation [8,11,50,53,54]. More specifically, other recent animal investigations showed that Mg-containing composites were beneficial for new tissue formation and healing of bone defects [22,23].

Summarily, our data on BG/Mg and BG/Col/Mg-based composites are very encouraging and may lead to further molecular and cell culture studies, and *in vivo* investigations to elucidate their osteogenic potential and biological performance for bone tissue engineering.

5. Conclusions

Based on our investigations of BG/Mg and BG/Col/Mg, it can be concluded that the mentioned composites were successfully obtained

with improved mechanical properties, retaining the bioactivity of the BG. Preliminary cell culture investigations showed that BG and BG/Col containing 1% of Mg were non-cytotoxic and biocompatible. This percentage of Mg is promising and safe to be used as constitute part of composites for bone tissue engineering purposes. Further studies should be performed to investigate the effect of Mg introduction into BG/Col samples in *in vivo* models.

Conflicts of interest

No benefit of any kind will be received either directly or indirectly by the authors.

Acknowledgments

PRGA thanks MSc. Luis A. B. Ferreira for supporting the production of the graphical abstract and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) for the scholarship (grant no. 2015/20704-8).

References

[1] E. Tsiridis, N. Upadhyay, P. Giannoudis, Molecular aspects of fracture healing: which are the important molecules? Injury 38 (Suppl 1) (2007) S11–S25.
[2] A. Schindeler, M.M. McDonald, P. Bokko, D.G. Little, Bone remodeling during fracture repair: the cellular picture, Semin. Cell Dev. Biol. 19 (2008) 459–466.
[3] G.M. Calori, W. Albisetti, A. Agus, S. Iori, L. Tagliabue, Risk factors contributing to fracture non-unions, Injury 38 (Suppl 2) (2007) S11–S18.
[4] R.K. Aaron, D.M. Ciombor, B.J. Simon, Treatment of Nonunions with Electric and Electromagnetic Fields, Clinical orthopaedics and related research, 2004, pp. 21–29.
[5] A. Wiese, H.C. Pape, Bone defects caused by high-energy injuries, bone loss, infected nonunions, and nonunions, Orthop. Clin. N. Am. 41 (2010) 1–4.
[6] L.L. Hench, J.M. Polak, Third-generation biomedical materials, Science 295 (2002) 1014–1017.
[7] J. Moura, L.N. Teixeira, C. Ravagnani, O. Peitl, E.D. Zanotto, M.M. Beloti, H. Panzeri, A.L. Rosa, P.T. de Oliveira, In vitro osteogenesis on a highly bioactive glass-ceramic (Biosilicate), J. Biomed. Mater. Res. 82 (2007) 545–557.
[8] L.L. Hench, J. Wilson, An Introduction to Bioceramics, second ed., Imperial College Press, London, U.K, 2013.
[9] I.D. Xynos, A.J. Edgar, L.D. Buttery, L.L. Hench, J.M. Polak, Ionic products of bioactive glass dissolution increase proliferation of human osteoblasts and induce insulin-like growth factor II mRNA expression and protein synthesis, Biochem. Biophys. Res. Commun. 276 (2000) 461–465.
[10] L.L. Hench, second ed., Introduction to Bioceramics vol 620, Imperial College Press, London, 2013 978–1-908977–15–1.
[11] L.L. Hench, The story of Bioglass, Journal of materials science, Mater. Med. 17 (2006) 967–978.
[12] M. Navarro, S. del Valle, S. Martinez, S. Zeppetelli, L. Ambrosio, J.A. Planell, M.P. Ginebra, New macroporous calcium phosphate glass ceramic for guided bone regeneration, Biomaterials 25 (2004) 4233–4241.
[13] Y. Fujishiro, L.L. Hench, H. Oonishi, Quantitative rates of in vivo bone generation for Bioglass and hydroxyapatite particles as bone graft substitute, J. Mater. Sci. Mater. Med. 8 (1997) 649–652.
[14] L.L. Hench, D. Greenspan, Interactions between bioactive glass and collagen: a review and new perspectives, 49 (2013) 1–40.
[15] F. Baino, M. Ferraris, Learning from Nature: using bioinspired approaches and natural materials to make porous bioceramics, Int. J. Appl. Ceram. Technol. 14 (2017) 507–520.
[16] J.K. Kim, J.S. Lee, H.J. Jung, J.H. Cho, J.I. Heo, Y.H. Chang, Preparation and properties of collagen/modified hyaluronic acid hydrogel for biomedical application, J. Nanosci. Nanotechnol. 7 (2007) 3852–3856.
[17] T.S. Wheeler, N.D. Sbravati, A.V. Janorkar, Mechanical & cell culture properties of elastin-like polypeptide, collagen, bioglass, and carbon nanosphere composites, Ann. Biomed. Eng. 41 (2013) 2042–2055.
[18] H. Sun, F. Zhu, Q. Hu, P.H. Krebsbach, Controlling stem cell-mediated bone regeneration through tailored mechanical properties of collagen scaffolds, Biomaterials 35 (2014) 1176–1184.
[19] Q. Chen, J.A. Roether, A.R. Boccaccini, Tissue engineering scaffolds from bioactive glass and composite materials, in: R.R. N Ashammakhi, F. Chiellini (Eds.), Topics in Tissue Engineering, 2008.
[20] H.S. Han, Y. Minghui, H.K. Seok, J.Y. Byun, P.R. Cha, S.J. Yang, Y.C. Kim, The modification of microstructure to improve the biodegradation and mechanical properties of a biodegradable Mg alloy, J. Mech. Behav. Biomed. Mater. 20 (2013) 54–60.
[21] Z. Huan, S. Leeflang, J. Zhou, W. Zhai, J. Chang, J. Duszczyk, In vitro degradation behavior and bioactivity of magnesium-Bioglass((R)) composites for orthopedic applications, J. Biomed. Mater. Res. B Appl. Biomater. 100 (2012) 437–446.
[22] S. Zhang, K. Yang, F. Cui, Y. Jiang, L. E, B. Xu, H. Liu, A novel injectable magnesium/calcium sulfate hemihydrate composite cement for bone regeneration, BioMed Res. Int. (2015) 297437 (2015).
[23] L. Canullo, G. Wiel Marin, M. Tallarico, E. Canciani, F. Musto, C. Dellavia, Histological and histomorphometrical evaluation of postextractive sites grafted with Mg-enriched nano-hydroxyapatite: a randomized controlled trial comparing 4 versus 12 Months of healing, Clin. Implant Dent. Relat. Res. 18 (2016) 973–983.
[24] K.M.R. Nuss, B. von Rechenberg, Biocompatibility issues with modern implants in bone - a review for clinical orthopedics, Open Orthop. J. 2 (2008) 66–78.
[25] T. Ozel, P.J. Bartolo, E. Ceretti, J. De Ciurana Gay, C.A. Rodriguez, J.V.L. Da Silva, Biomedical Devices: Design, Prototyping, and Manufacturing, Wiley, 2016.
[26] M.A. Lopez-Heredia, Y. Sa, P. Salmon, J.R. de Wijn, J.G. Wolke, J.A. Jansen, Bulk properties and bioactivity assessment of porous polymethylmethacrylate cement loaded with calcium phosphates under simulated physiological conditions, Acta Biomater. 8 (2012) 3120–3127.
[27] L. Wang, D.M. Yoon, P.P. Spicer, A.M. Henslee, D.W. Scott, M.E. Wong, F.K. Kasper, A.G. Mikos, Characterization of porous polymethylmethacrylate space maintainers for craniofacial reconstruction, J. Biomed. Mater. Res. B Appl. Biomater. 101 (2013) 813–825.
[28] T. Kokubo, H. Takadama, How useful is SBF in predicting in vivo bone bioactivity? Biomaterials 27 (2006) 2907–2915.
[29] P.R. Gabbai-Armelin, D.A. Cardoso, E.D. Zanotto, O. Peitl, S.C.G. Leeuwenburgh, J.A. Jansen, A.C.M. Renno, J.J.J.P. van den Beucken, Injectable composites based on biosilicate[registered sign] and alginate: handling and in vitro characterization, RSC Adv. 4 (2014) 45778–45785.
[30] R.E. Mooren, E.J. Hendriks, J.J. van den Beucken, M.A. Merkx, G.J. Meijer, J.A. Jansen, P.J. Stoelinga, The effect of platelet-rich plasma in vitro on primary cells: rat osteoblast-like cells and human endothelial cells, Tissue Eng. Part A 16 (2010) 3159–3172.
[31] H. Shin, P. Quinten Ruhe, A.G. Mikos, J.A. Jansen, In vivo bone and soft tissue response to injectable, biodegradable oligo(poly(ethylene glycol) fumarate) hydrogels, Biomaterials 24 (2003) 3201–3211.
[32] J. Oakley, J.H. Kuiper, Factors affecting the cohesion of impaction bone graft, J. Bone Joint Surg., British 88-B (2006) 828.
[33] T.H.S. Sousa, Projeto conceitual de implante bioativo com gradiente de estrutura funcional em PMMA e HA. Analises: in vitro e in vivo, Departamento de Engenharia Mecânica, Universidade de Sao Paulo, Sao Carlos, 2009, p. 152.
[34] L.C.A. Haach, Corpos compósitos de poli(metacrilato de metila) com microfibra de biovidro e poros para reparo de defeitos ósseos, Departamento de Engenharia Mecânica, Universidade de Sao Paulo, Sao Carlos, 2015, p. 204.
[35] G.M. Araújo, F.M. Vieites, A.A. Barbosa, J.G. Caramori Junior, A.L. Santos, G.H.K. Moraes, J.G. Abreu, E.S. Muller, Variação aniônica da dieta sobre características ósseas de frangos de corte: resistência à quebra, composição orgânica e mineral, Arq. Bras. Med. Vet. Zootec. 63 (2011) 954–961.
[36] S. Lopa, H. Madry, Bioinspired scaffolds for osteochondral regeneration, Tissue Eng. 20 (2014) 2052–2076.
[37] S. Rother, J. Salbach-Hirsch, S. Moeller, T. Seemann, M. Schnabelrauch, L.C. Hofbauer, V. Hintze, D. Scharnweber, Bioinspired collagen/Glycosaminoglycan-based cellular microenvironments for tuning osteoclastogenesis, ACS Appl. Mater. Interfaces 7 (2015) 23787–23797.
[38] H.H. Lu, S.F. El-Amin, K.D. Scott, C.T. Laurencin, Three-dimensional, bioactive, biodegradable, polymer-bioactive glass composite scaffolds with improved mechanical properties support collagen synthesis and mineralization of human osteoblast-like cells in vitro, J. Biomed. Mater. Res. 64A (2003) 465–474.
[39] P. Habibovic, T.M. Sees, M.A. van den Doel, C.A. van Blitterswijk, K. de Groot, Osteoinduction by biomaterials - physicochemical and structural influences, J. Biomed. Mater. Res. 77A (2006) 747–762.
[40] K.C. Laurenti, L.C.d.A. Haach, A.R.d. Santos Jr., J.M.D.d.A. Rollo, R.B.d.M. Reiff, A.M.M. Gaspar, B.d.M. Purquerio, C.A. Fortulan, Cartilage reconstruction using self-anchoring implant with functional gradient, Mater. Res. 17 (2014) 638–649.
[41] W.M. Haynes, CRC Handbook of Chemistry and Physics, 93rd Edition, Taylor & Francis, 2012.
[42] N. Sezer, Z. Evis, S.M. Kayhan, A. Tahmasebifar, M. Koç, Review of magnesium-based biomaterials and their applications, J. Magnes. Alloy. 6 (2018) 23–43.
[43] M.P. Staiger, A.M. Pietak, J. Huadmai, G. Dias, Magnesium and its alloys as orthopedic biomaterials: a review, Biomaterials 27 (2006) 1728–1734.
[44] M.P.H. Khandaker, Y.L. Li, S. Tarantini, Interfacial Fracture Strength Measurement of Tissue-Biomaterial Systems, Amer Soc Mechanical Engineers, New York, 2012 Asme.
[45] L. Xu, E. Zhang, D. Yin, S. Zeng, K. Yang, In vitro corrosion behaviour of Mg alloys in a phosphate buffered solution for bone implant application, J. Mater. Sci. Mater. Med. 19 (2008) 1017–1025.
[46] F. Witte, V. Kaese, H. Haferkamp, E. Switzer, A. Meyer-Lindenberg, C.J. Wirth, H. Windhagen, In vivo corrosion of four magnesium alloys and the associated bone response, Biomaterials 26 (2005) 3557–3563.
[47] M.N. Rahaman, D.E. Day, B. Sonny Bal, Q. Fu, S.B. Jung, L.F. Bonewald, A.P. Tomsia, Bioactive glass in tissue engineering, Acta Biomater. 7 (2011) 2355–2373.
[48] X. Li, C.L. Chu, L. Liu, X.K. Liu, J. Bai, C. Guo, F. Xue, P.H. Lin, P.K. Chu, Biodegradable poly-lactic acid based-composite reinforced unidirectionally with high-strength magnesium alloy wires, Biomaterials 49 (2015) 135–144.
[49] W. Wen, Z. Zou, B. Luo, C. Zhou, In vitro degradation and cytocompatibility of g-MgO whiskers/PLLA composites, J. Mater. Sci. 52 (2017) 2329–2344.
[50] A.M.P. Magri, K.R. Fernandes, F.R. Ueno, H.W. Kido, A.C. da Silva, F.J.C. Braga, R.N. Granito, P.R. Gabbai-Armelin, A.C.M. Rennó, Osteoconductive properties of two different bioactive glass forms (powder and fiber) combined with collagen, Appl. Surf. Sci. 423 (2017) 557–565.
[51] Z. Lei, P. Jia, H.D. Wang, Y.J. Shi, J.L. Niu, Y. Feng, H. Hua, Z. Hua, G.Y. Yuan,

Facile preparation of poly(lactic acid)/brushite bilayer coating on biodegradable magnesium alloys with multiple functionalities for orthopedic application, ACS Appl. Mater. Interfaces 9 (2017) 9437–9448.
[52] L. Fiocco, S. Li, M.M. Stevens, E. Bernardo, J.R. Jones, Biocompatibility and bioactivity of porous polymer-derived Ca-Mg silicate ceramics, Acta Biomater. 50 (2017) 56–67.
[53] P.R. Gabbai-Armelin, M.T. Souza, H.W. Kido, C.R. Tim, P.S. Bossini, A.M. Magri, K.R. Fernandes, F.A. Pastor, E.D. Zanotto, N.A. Parizotto, O. Peitl, A.C. Renno, Effect of a new bioactive fibrous glassy scaffold on bone repair, J. Mater. Sci. Mater. Med. 26 (2015) 177.
[54] P.R. Gabbai-Armelin, M.T. Souza, H.W. Kido, C.R. Tim, P.S. Bossini, K.R. Fernandes, A.M. Magri, N.A. Parizotto, K.P. Fernandes, R.A. Mesquita-Ferrari, D.A. Ribeiro, E.D. Zanotto, O. Peitl, A.C. Renno, Characterization and biocompatibility of a fibrous glassy scaffold, J. Tissue Eng. Regenerat. Med. 11 (2017) 1141–1151.