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Injectable composites based on biosilicate® and alginate: handling and *in vitro* characterization

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The objective of the study was to prepare an injectable composite for bone regeneration based on the combination of a highly bioactive glass-ceramic (Biosilicate®) and alginate by optimizing the ratio of Biosilicate®/alginate. These formulations were evaluated in terms of injectability, visco-elastic properties, degradation (*i.e.* mass loss, pH, calcium deposition) and cytotoxycity. The results showed that by mixing Biosilicate® and alginate, it is possible to obtain an injectable biocomposite material that exhibits interesting elastic properties. Furthermore, the formulations with higher alginate (up to 20 wt%) content showed higher mechanical stability compared to pure Biosilicate®. All formulations mineralized in Simulated Body Fluid (SBF) during the initial 4 days of testing. The cytotoxicity of conditioned media obtained *via* incubation of the formulations showed negative effects on cell viability but this effect was nullified with increasing the number of washing post-treatments (especially in the case of the formulation containing Biosilicate®/alginate of 42.5/7.5 wt%). Based on the results of the present study, it can be concluded that the material properties of injectable Biosilicate®/alginate formulations seem suitable for bone regenerative applications, for which future studies should aim at biological evaluation in animal experimental models.

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Introduction

The incidence of bone fractures is rising significantly, mainly due to the ageing of the population and the increase in accidental traumas.[1] Among those fractures, 5–10% either fail completely or demonstrate a delay in healing.[1] For these indications, surgical procedures are required that use bone grafts to treat such non-union fractures.[2]

Facing increasing demands for bone grafts, research efforts have explored many possible directions for alternatives, including the development of synthetic biomaterials,[3] such as calcium phosphate (CaP) ceramics[4] and polymer-based materials.[5] Furthermore, bioactive glasses and glass ceramics have been widely used as bone graft materials, especially due to their high bioactivity.[6,7] These materials belong to a group of silica-based melt-derived glasses, that possess an unique ability to rapidly bond to living tissues and stimulate the attachment of bone progenitor cells, hence increasing organic matrix deposition.[6–8]

Despite the osteogenic potential of the available bioactive glasses, their use has been limited because of their crystallization tendency that follows heat treatment.[9] In order to overcome this limitation, bioactive glass-ceramics have been obtained by controlled crystallization of certain glasses.[10] In this context, glass-ceramics based on the P_2O_5–Na_2O–CaO–SiO_2 system, including Biosilicate®, have been developed.[10] The osteogenic effects of Biosilicate® have been demonstrated using *in vitro* and *in vivo* studies. The *in vitro* studies showed formation of a bone-like matrix deposition in Simulated Body Fluid (SBF) and significantly larger areas of calcified matrix at day 17 of osteogenic cell culture.[11] Additionally, *in vivo* studies conducted in osteoporotic and healthy rats showed significantly higher bone formation and increased mechanical properties of tibia bone defects treated with Biosilicate®.[12,13]

The current availability of glass ceramics is still mainly in the form of powders, blocks or scaffolds. One of the main disadvantages of those forms is that optimal filling of bone defects with irregular and complex shapes and sizes is hardly possible.[14] Consequently, injectability is a desirable characteristic, which offers several clinical advantages as compared to solid prefabricated material forms that do not allow for minimally invasive surgery.

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In that respect, alginate is an interesting material with appealing properties for biomedical applications in terms of biocompatibility, biodegradability and injectability.[14] Alginate is a natural polysaccharide that efficiently cross-links by interaction with divalent cations (*e.g.* calcium).[15–17] Furthermore, alginate hydrogels have previously been used for cell encapsulation[18] as well as for vascular[19] and cartilage regeneration.[20] Bioactive glass has been previously mixed application of a bioactive glass ceramic together with alginate for the obtainment of scaffold blocks for cell seeding.[21] Alginate has been combined previously with nano-sized CaP particles, generating an injectable and moldable bone substitute material, while its implantation into femoral condyle defects of rabbits showed that the composite disintegrated over time, leaving behind osteoconductive CaP particles.[22]

Related to the growing interest in the development of bone graft materials with optimal handling properties, it was hypothesized that the combination of alginate and Biosilicate® would be appealing in terms of injectability and bioactivity, by exploiting the bioactivity of the glass ceramic with the moldability and cross-linking ability that arises from the interaction between alginate and soluble calcium ions derived from the Ca-releasing Biosilicate®. Consequently, the aim of the current study was to optimize the ratio of Biosilicate® and alginate *via* different formulations and evaluate the biocomposite's physicochemical features, mechanical properties and morphological characteristics. To this end, different Biosilicate®/alginate formulations were evaluated in terms of injectability, viscoelastic properties and degradation [*i.e.* mass loss quantification (cohesion), pH, calcium deposition]. All these properties should be carefully considered while designing a ceramic bone substitute.[23,24] Additionally, a preliminary study was performed to investigate the cytotoxicity of the new compositions.

Materials and methods

Materials

Biosilicate® parent glass – particle size: 2.5 μm[25,26] was provided by Vitreous Materials Laboratory (LaMaV; Department of Materials Engineering, Federal University of São Carlos, São Carlos, São Paulo, Brazil). Biosilicate® is the designation of a particular composition of a group of fully crystallized glass-ceramics of the Na_2O–CaO–SiO_2–P_2O_5 system, with additions of Li_2O and K_2O. Detailed compositions and thermal treatment schedules to obtain the Biosilicate glass-ceramic have been reported previously.[27,28] Ultrapure, high-molecular-weight alginate[22] (molecular weight of 237 kDa, 65% guluronic content and a particle size less than 100 μm) was provided by EMCM (Nijmegen, Netherlands), obtained according to the ASTM F 2064-00 (re-approved 2006) standard. The purification procedure was performed by EMCM according to a previously disclosed method.[29]

Preparation of pre-set composites

Different formulations of Biosilicate® and sodium alginate (with different wt% of Biosilicate® and alginate) were evaluated. These formulations were defined based on cohesion, handling and the presentation of a putty-like appearance. Table 1 presents an overview of the different formulations used. The formulations were prepared by manual mixing of Biosilicate® and alginate powder with the liquid component consisting of phosphate-buffered saline solution (PBS), and subsequently put inside a 2 ml closed tip syringe (BD Plastipakt, Becton Dickinson S.A., Madrid, Spain) for injection (Fig. 1).

For the analysis of mass loss quantification, calcium deposition and pH, formulations were injected into Polytetrafluoroethylene (PTFE) molds (6 mm diameter × 12 mm height); for the cytotoxicity assay, pre-set disks (5 mm diameter × 3 mm height) were obtained using PTFE molds The disks were sterilized by soaking in 70% ethanol followed by immersion in PBS in order to remove all the ethanol residues from the samples.[30] Scanning electron microscopy (SEM, JEOL 6310) was performed to analyze the morphology of the composites.

Injectability

Injectability of the formulations ($n = 3$) was measured by using a tensile bench (Model 858, Mini-Bionix II, MTS Systems Corp., Eden Prairie, MN, USA). After mixing and putting the composites inside a 2 ml syringe (orifice diameter of 1.7 mm), the syringe was fitted vertically in a fixture and adapted under the

Table 1 Different formulations of the Biosilicate®/alginate composites

Formulation	Biosilicate (wt%)	Alginate (wt%)	PBS (wt%)
A	40	10	50
B	42.5	7.5	50
C	45	5	50
D	47.5	2.5	50
E	50	0	50

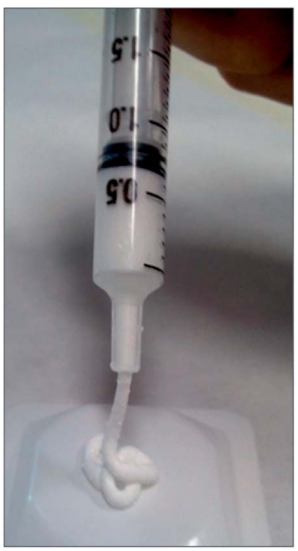

Fig. 1 Photo of a representative formulation immediately after injection (formulation D).

plates of a tensile bench set in compression mode. Compression force was applied to the syringe at a constant velocity of 20 mm min^{-1} up to a final force of 100 N. The injectability (defined as the period of time necessary to reach the final force of 100 N) was recorded as a function of the plunger travel time. All tests were performed in triplicate.

Viscoelastic properties

The viscoelastic properties of the composite gels ($n = 4$) were analyzed using an AR2000ex rheometer (TA Instrument, New Castle, NJ, USA) equipped with a flat steel-plate geometry (20 mm diameter) at 37 °C. Storage moduli (G') were determined in oscillatory time sweep tests for 10 min at a gap distance of 500 mm by subjecting the samples directly after injection to a stress of 0.1 Pa and a frequency of 1 Hz.

In vitro degradation

To determine the cohesion of the formulations in liquids, an *in vitro* mass loss study was carried out. After preparation of the pre-set samples ($n = 3$), these samples were placed in 5 ml of PBS (pH 7.4) and incubated at 37 °C in a water bath on a shaker table (70 Hz) for 1, 3, 7 and 14 days. At each time point, the samples were removed from the solution and the absorbed liquid removed using tissue paper. The calculated mass loss was obtained using the formula:

$$\text{Mass loss } \% = ((W_t - W_0)/W_0) \times 100\%$$

where W_t is the weight of the samples after immersion in PBS and W_0 is the weight of the samples before immersion in this same solution.

Calcium uptake

For the calcium deposition study, pre-set samples ($n = 3$) were placed in 5 ml of conventional Simulated Body Fluid (ionic concentrations: 142.0 mm Na^+, 5.0 mm K^+, 1.5 mm Mg^{2+}, 2.5 mm Ca^{2+}, 103, 0 mm Cl^-, 4.2 mm $HCO3^{2-}$, 1.0 mm $HPO4^{2-}$ and 0.5 mm $SO4^{2-}$) according to the protocol from Kokubo.[23] The solution was changed at days 4, 8, 12 and 16, and saved for analysis of the calcium content using the orthocresolphtalein complexone (OCPC) assay.[31] These supernatants were incubated overnight in 1 ml 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-wells 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-Tech Instruments, Winooski, VT, USA). The standards (range: 0–100 μg ml^{-1}) 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 SBF solution in the presence of Biosilicate®/alginate formulations.

pH measurements

Directly after removal of the supernatant, the pH of the SBF medium was measured using a pH electrode (Meterlab PHM210 calibrated with IUPAC buffers, S11M002, S11M004, S11M007 from Radiometer Analytical, Villeurbanne, France).

Cytotoxicity

An indirect assay was used to determine the cytotoxycity of the Biosilicate®/alginate formulations. After preparation of the pre-set formulations ($n = 4$), these were put in contact with 2 ml of cell medium (alpha Minimal Essential Medium without ascorbic acid; α-MEM; Gibco BRL, Life Technologies, Breda, The Netherlands) supplemented with 10% fetal bovine serum (FBS; Gibco) for two periods of three days. After the first three days, the medium was collected and new medium was put in contact with the formulations for another three days, after which the medium was collected again. As a control, four empty wells were filled with the same amount of medium and left for two periods of three days.

MC3T3-E1 subclone cells (ATCC CRL-2593) from passage 20 were cultured in proliferation medium containing αMEM (Gibco) supplemented with 10% FCS (Gibco) in a humidified incubator set at 37 °C and 5% CO_2. Upon 80% confluency, cells were detached using trypsin/EDTA. Cells were seeded at a density of 4×10^4 cells per cm^2 and placed in a 96-well plate containing 200 μl new medium. After overnight incubation the medium was changed for the conditioned medium that was previously collected and the cells were incubated for 24 hours. Afterwards, the alamarBlue™ assay (Thermo Fisher Scientific; Landsmeer, The Netherlands) was used on all the samples to determine cell viability.

Statistical analysis

Data are presented as mean ± standard deviation. Statistical analyses were performed using GraphPad InStat® 3.05 (GraphPad Software, San Diego, CA, USA). Significant differences were determined using a one-way analysis of variance (ANOVA) with a Tukey multiple comparison post-test. Differences were considered significant at p-values < 0.05.

Results

Formulations morphological structure and injectability

After injection, all materials displayed a putty-like appearance (Fig. 1). The different composites displayed a well distributed microstructure, confirming that the mixing procedure was efficient without causing extensive agglomeration of the Biosilicate® particles (Fig. 2).

The injectability test showed that all Biosilicate®/alginate formulations were injectable at the force applied and also extruded as homogeneous pastes. Moreover, the injectability time for the groups containing 40/10%, 42.5/7.5% and 45/5% (wt%) in Biosilicate®/alginate (198 ± 10 s, 144 ± 12 s, 119 ± 9 s, respectively) was significantly higher ($p < 0.05$) compared to groups containing 47.5/2.5% and 50/0% (wt%) in Biosilicate®/alginate (50 ± 6 s, 50 ± 12 s) (Fig. 3).

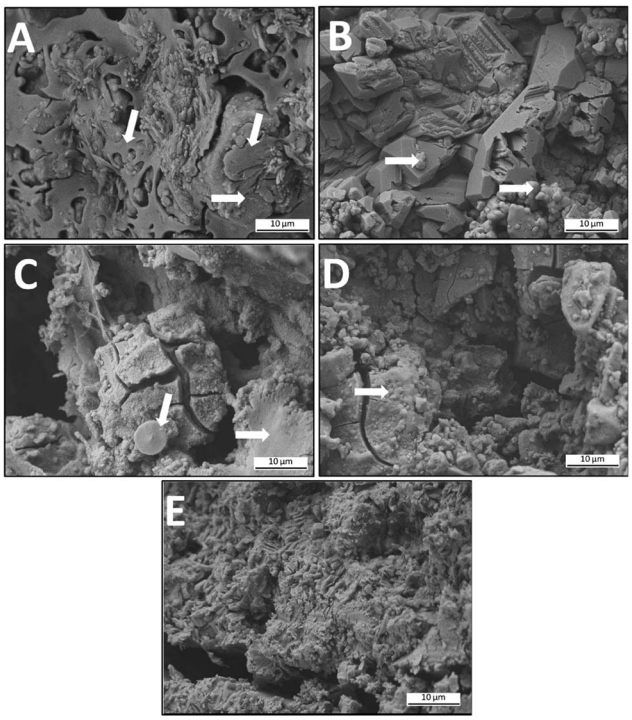

Fig. 2 SEM micrographs of formulations A, B, C, D and E (white arrows indicate alginate polymer).

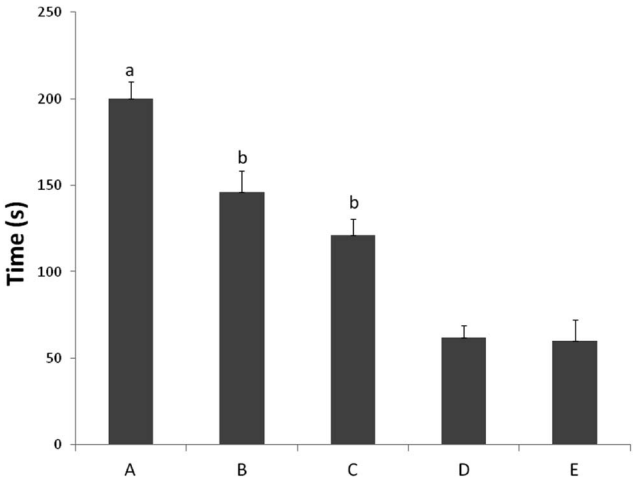

Fig. 3 Injectability of Biosilicate®/alginate formulations ((a) compared to B, C, D and E $p < 0.001$; (b) compared to D and E ($p < 0.001$)).

Viscoelastic properties

The viscoelastic properties of the different formulations are shown in Fig. 4, which reveals that the storage modulus gradually increased with increasing amount of alginate. All the formulations presented a tan(δ) lower than one (not shown in figure) which indicated an elastic behavior, including the formulation containing only Biosilicate®. The highest storage moduli were observed for the formulations containing 40/10%, 42.5/7.5% (wt%) in Biosilicate®/alginate with no statistical difference ($p > 0.05$) between these groups. All formulations

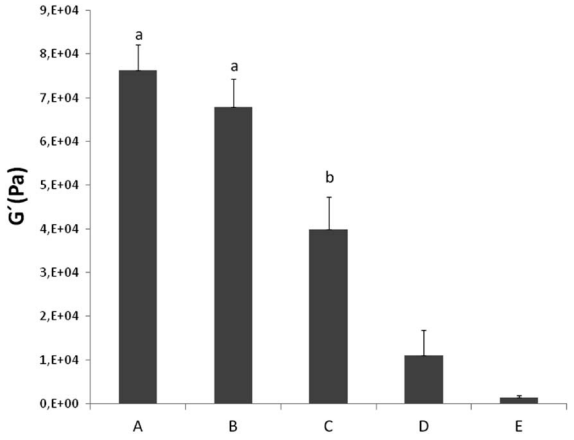

Fig. 4 Storage modulus of Biosilicate®/alginate formulations ((a) compared to C, D and E ($p < 0.001$); (b) compared to D and E ($p < 0.001$)).

containing alginate presented higher storage modulus values (ranging between ~10 to ~77 kPa) compared to the material containing only Biosilicate® (~1 kPa) ($p < 0.001$).

Mass loss quantification

In order to study the effect of the different ratios of Biosilicate® and alginate on the cohesion of the formulations, the mass change upon soaking in PBS was determined (Fig. 5). Formulations containing 40/10%, 42.5/7.5% (wt%) in Biosilicate®/alginate maintained their cohesion (preserving 95 and 94% of the initial mass respectively), while the formulations containing 45/5% and 47.5/2.5% in Biosilicate®/alginate lost their mass (initial mass decreasing until 0) and cohesion after day 3.

The formulations containing only Biosilicate® showed immediate loss of cohesion after 1 day of immersion, which impeded mass loss quantification. In contrast, formulations containing 45/5% and 47.5/2.5% in Biosilicate®/alginate

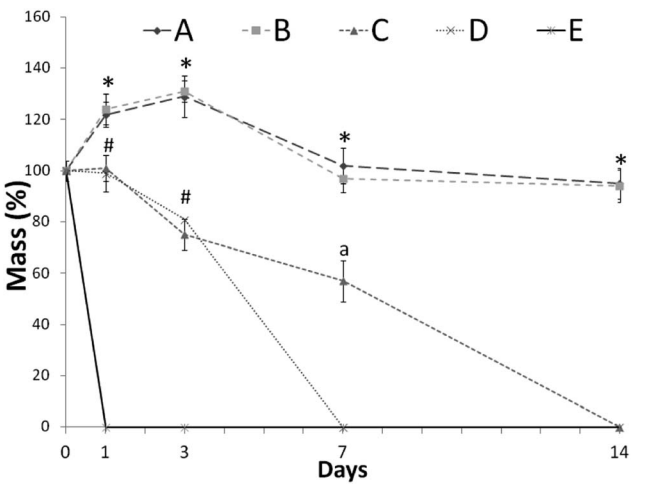

Fig. 5 Mass loss of Biosilicate®/alginate samples in PBS for up to 14 days ((*) groups A and B compared to group C, D and E ($p < 0.05$); (#) groups A and B compared to group C, D and E ($p < 0.05$); (a) groups A and B compared to group C, D and E ($p < 0.05$)).

showed higher stability after 1 day in solution, but lost mass and cohesion during the following time point resulting into complete disintegration at day 14 and 7 respectively. On the other hand, formulations with the highest amount of alginate (*i.e.* formulations containing 40/10%, 42.5/7.5% (wt%) in Biosilicate®/alginate) maintained their cohesion during the entire experimental period, with no significant difference between both groups. For formulations containing 40/10%, 42.5/7.5% (wt%) in Biosilicate®/alginate, swelling was observed during the initial 3 days, leading to an increase in the original mass.

Calcium uptake

The calcium uptake by the formulations was measured quantitatively by assessing the calcium concentration as a function of soaking time (Fig. 6). For samples containing both Biosilicate® and alginate, the amount of calcium uptake increased with time until day 12, where after a plateau was reached. Afterwards, calcium release was observed for all formulations. At none of the experimental time points, significant differences between the formulations were observed ($p > 0.05$). For the formulation containing only Biosilicate®, however, a continuous release of calcium into the medium was noticed, even after starting with similar values of Ca uptake at day 4 (73.5 ± 8.2 μg) compared to other formulations (values between 71.6 ± 5.4 μg and 73.5 ± 5.1 μg).

pH measurements

The results of the pH measurements during degradation are presented in Fig. 7. Compared to the control (*i.e.* SBF solution), all formulations revealed pH increase to a value of approximately pH 10 within 24 hours of incubation.

At day 16 after incubation, a pH decrease was observed, dropping to values of about 8.5 for formulations 40/10%, 42.5/7.5% and 45/5% (wt%) in Biosilicate®/alginate without significant differences between these groups ($p > 0.05$). Regarding the formulations with higher amounts of Biosilicate® (47.5/2.5% and 50/0% (wt%) in Biosilicate®/alginate), significantly higher values of pH were observed at day 8 compared to the other formulations ($p < 0.05$). At the end of the experimental period, formulation containing only Biosilicate® and PBS induced a higher pH ~9 compared to the other experimental groups ($p < 0.05$).

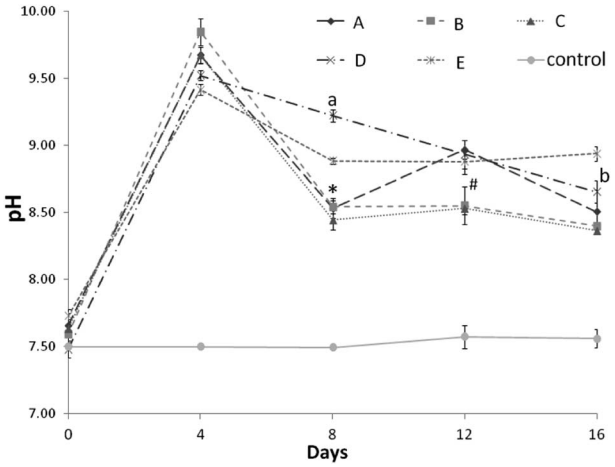

Fig. 7 pH of SBF solution in contact with Biosilicate®/alginate formulations for up to 16 days ((a) of group D compared to group E ($p < 0.05$); (*) groups A, B and C compared to group E ($p < 0.05$); (#) groups C and B compared to group E ($p < 0.05$); (b) groups A, B, C and D compared to group E ($p < 0.05$)).

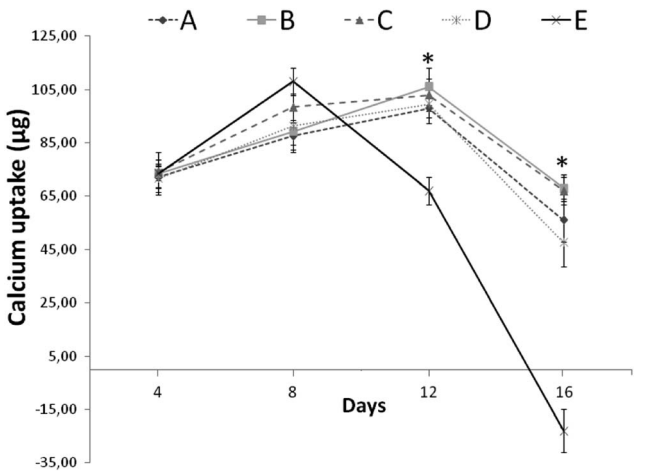

Fig. 6 Cumulative calcium uptake by Biosilicate®/alginate formulations immersed in SBF for up to 16 days ((*) groups A, B, C and D compared to group E ($p < 0.05$)).

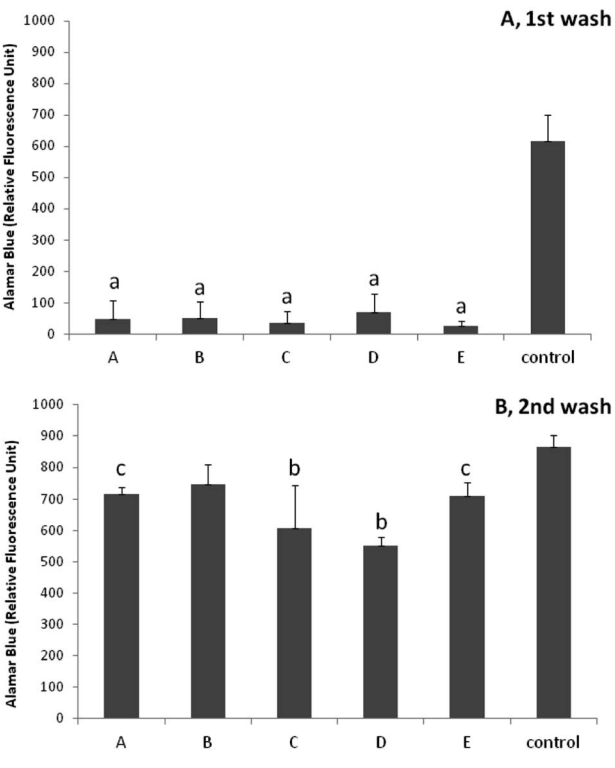

Fig. 8 Cytotoxicity (alamar blue assay) of Biosilicate®/alginate formulations. (A) first wash. (B) second wash ((a) compared to control ($p < 0.001$); (b) compared to control ($p < 0.01$); (c) compared to control ($p < 0.05$)).

Cytotoxicity

The cytotoxicity results showed that all the conditioned media obtained from incubating formulations for a first period of 3 days had a deleterious effect on cell viability (Fig. 8A, 1st wash). However, the conditioned media obtained from incubating formulations for a second period of 3 days showed high cell viability (Fig. 8B, 2nd wash).

Of all the materials tested, only the medium in contact with the formulation containing 42.5/7.5% (wt%) in Biosilicate®/alginate showed similar cell viability compared to the control (~800 RFU). All the other Biosilicate®/alginate formulations showed lower cell viability in comparison to the control with significant differences between the experimental groups ($p < 0.05$ and $p < 0.01$).

Discussion

The present study evaluated the properties of injectable formulations containing Biosilicate® and a polymeric phase of alginate, *via* quantitative determination of injectability, viscoelastic properties, degradation behavior, calcium uptake and cytotoxicity. It was hypothesized that the combination of Biosilicate® and alginate would be appealing in terms of injectability and bioactivity, exploiting the bioactivity of the glass ceramic with the moldability and cross-linking ability between calcium ions and alginate. The results showed that Biosilicate® and alginate can be mixed successfully to obtain a bioactive paste, injectable using standard syringes, with appropriate cohesion and accelerated mineralization compared to pure Biosilicate®. Furthermore, cell culture experiments showed that after one washing, the formulations induced only limited lower cellular viability.

The formulations were maximized with respect to Biosilicate® content to obtain the highest possible amount of bioactive filler without compromising the injectability and moldability. Beside Biosilicate® content, also the variation of alginate and PBS were investigated. In attempting to increase amounts of alginate, greater PBS content could have been added, but this provoked the loss of putty-like appearance of the materials. To avoid this, the established amount of PBS was 50 wt%, so all the combinations could be mixed homogeneously (decreased contents of PBS led to a lack of cohesion between materials). On the other hand, the highest amount of alginate to be introduced into Biosilicate® was found to be 10 wt%, since the addition of higher amounts of alginate gave rise to non-homogenous formulations that could not be loaded into syringes. All the selected formulations were extruded from syringes with a 1.7 mm diameter orifice,[32] resulting in homogenous microstructures as evidenced by SEM. Furthermore, the introduction of alginate significantly increased the injectability time and storage modulus to a maximum of respectively >3 minutes and >75 kPa (*i.e.* for 40/10 Biosilicate®/alginate wt%). This increase in storage modulus with higher alginate content corroborates earlier reports[33–35] and indicates that the polymer influences the rheological properties of the materials due to its gel forming ability, generating a stiffer and cross-linked material. Glass ceramics in solution release several types of ions, including Ca,[6,7] which will interact with the alginate polymer in the initial formation of cross-linking polymer chains. Furthermore when alginate is mixed with materials with different rates of Ca release in solution, when there is not sufficient Ca in solution the polymer does not cross-link leading to the obtainment of a non-elastic material.[22] Even though the formulation containing only Biosilicate® also showed a gel like behavior ($\tan(\delta)$ lower than one) we attribute this to the Biosilicate® particles in solution.

The evaluation of the degradability showed that pure Biosilicate® is not cohesive upon immersion in PBS. On the other hand, formulations containing the highest amounts of alginate (*i.e.* 10 and 7.5 wt%) remained stable for up to 14 days despite a slight swelling in the first 7 days. Previous tests on degradation of glass ceramic/polymer composites also showed an increase in the stability of the materials with the addition of a polymer component to the bioactive glass.[36] Both the mechanical stability and the swelling were not observed for the other formulations, which disintegrated directly upon immersion in PBS and leading to complete loss of cohesion in a period ranging from 7 (*i.e.* 2.5 wt% alginate) to 14 days (*i.e.* 5 wt% alginate). Apparently, above a threshold alginate content (*i.e.* 7.5 wt% or higher), water absorption into the polymer resulted in an increase in mass from swelling.

The pH measurements confirmed that incorporation of Biosilicate® results in alkalinization of the immersion medium (up to pH 10 in the first day). Reactions taking place at the composite interface are likely responsible for these observations, releasing cations (Si, Na, Ca and PO_4^{3-}) immediately after the immersion of Biosilicate® into fluids, thereby inducing an increase in pH.[37,38] Nevertheless, it has been shown in previous *in vivo* studies that the alkaline pH as observed *in vitro* for Biosilicate® does not impede bone healing, since these investigations indicated higher amounts of newly formed bone and increased biomechanics of bone defects treated with Biosilicate®.[10,12,13] In contrast, the mixture of Biosilicate® with alginate partially neutralized the pH increase. This phenomenon was observed previously when bioactive glasses were mixed with poly(L-lactic acid).[39]

Calcium-containing SBF was selected as incubation medium, allowing for monitoring of calcium concentrations in the supernatant as a measure for mineralizing capacity.[29] It was assumed that the differences in calcium concentration result from calcium phosphate precipitation on the composite surface.[40] It should be realized, however, that alginate chains that were not cross-linked by the calcium released from Biosilicate® can also interact with calcium ions present in SBF. Therefore, only a very small amount of calcium present in the SBF solution was available for interaction with the alginate network, whilst the majority would be available for precipitation on the gel surface. The formulation containing only Biosilicate® showed deposition of Ca during the first days, which should be related to the initial formation of a HA-like layer on the surface of Biosilicate®.[41,42] Afterwards, there was a continuous release of calcium ions in the solution due to material degradation. Similar behavior was observed for the

formulations containing alginate, although these released lower amounts of calcium to the environment in comparison with formulations containing only Biosilicate®. This difference in release pattern could arise because of higher surface area for particle dissolution (formulations containing only Biosilicate®). It is suggested that this increased time for calcium release from Biosilicate® may indicate a controlled release of these ions, which could have a positive impact on the *in vivo* behavior of this material.[40,43]

In order to study the possible cytotoxycity of the different formulations, an indirect method was used by culturing MC3T3 cells in conditioned medium. The results showed that all the composite formulations and pure Biosilicate® had a deleterious effect on cell viability using conditioned media obtained *via* incubation of formulations for day 1–3. Materials that induce an alkalinization (such as bioactive glasses) or acidification (calcium phosphate cements) of the *in vitro* environment are known to affect cell viability.[40,44–46] and this phenomenon was also observed in the present study, specially due to the fine Biosilicate® particles used which have a high reactivity in solution. In contrast, it has been shown that the *in vivo* performance of several bioactive glasses and calcium phosphate cements led to bone tissue repair.[12,13,47–49] This fact should be attributed to the buffering capacity of an organism due to the constant fluid exchanges *in vivo*, allowing cell recruitment and a normal progression of the bone healing.[40] Taking this in consideration, this buffering effect was mimicked by incubating the cells with conditioned media obtained after incubating the formulations for another 3 day period after the first wash. With these conditioned media, cells were capable of surviving, indicating a substantial decrease in cytotoxicity after removal of the deleterious content by the first wash. As such, this finding justifies further *in vivo* investigations on Biosilicate®/alginate formulations to evaluate histocompatibility and long-term efficacy.

Conclusions

Injectable composites for bone regeneration have been developed based on the combination of the glass-ceramic Biosilicate® and alginate. The introduction of alginate made it possible to obtain injectable, cohesive and elastic composite materials that are injectable in less than 4 minutes even at high concentrations using appropriate force. All composites mineralized in SBF which indicates that the Biosilicate® maintains its bioactive characteristics after mixing with the polymeric alginate phase. The cytotoxycity of conditioned media obtained *via* incubation of the formulations showed negative effects on cell viability, but this effect diminished with increasing the number of washing post-treatments. Based on the results of the present study, it can be concluded that the material properties of injectable Biosilicate®/alginate formulations (combination of bioactive glass-ceramic and moldable/injectable polymer) seem suitable for bone regenerative applications and that the preliminary cytotoxity data justify further biological experimentation.

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