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PAPER

Physicochemical, biological and drug-release properties of gallium crosslinked alginate/nanoparticulate bioactive glass composite films

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The aim of this work was to develop biodegradable and bioactive materials with sufficient structural integrity and prophylaxis effect against infection based on alginate-bioactive glass composite. The incorporation of bioactive glass nanoparticles (NBG) into Ga-crosslinked alginate films significantly improved their mechanical properties when compared with films fabricated with micron-sized bioactive glass particles. In addition, Ga-alginate films containing NBG induced a bacteriostatic effect *in vitro* towards *S. aureus* due to the presence of Ga ions (Ga^{3+}), whose release is controlled solely by crosslinking the ion with alginate. Biomineralization studies in simulated body fluid suggested the deposition of hydroxyapatite on the surface of the films indicating their bioactive nature. In addition, the films were shown to feature biocompatibility toward osteoblast-like cells. Thus, it was shown that Ga-crosslinked composite films possessed relevant physicochemical, biological and controlled bacteriostatic effects which make these materials promising candidates for bone tissue engineering applications.

Introduction

One key issue in tissue engineering is the development of suitable biodegradable materials and the subsequent production of scaffolds for seeding cells.[1] Alginates are well known biomaterials, widely used for drug delivery and tissue engineering, which are derived from brown marine algae and are composed of 1,4-linked β-D-mannuronic acid and α-L-guluronic acid residues in varying amounts.[2] Alginates have useful properties, such as the formation of gels with multivalent ions such as Ca^{2+}, Mg^{2+}, Ba^{2+}, Sr^{2+}, Ga^{3+} (ref. 2–5) which induces cross-linking of the guluronic residues of the alginate polymer. Further, bioactive glasses can be also combined with sodium alginate to improve the mechanical and physical properties of the biomaterial increasing its versatility in particular if an application in bone tissue engineering (BTE) is envisaged, *e.g.* forming a polymer–inorganic phase composite.[6] The utilisation of silicate bioactive glasses is well established particularly in the field of bone regeneration because these are surface reactive biomaterials leading to strong bonding to bone tissue and providing also antibacterial effects.[7,8] Thus the main reason for using bioactive glass particles in tissue engineering scaffolds is their high bioactivity,[7,9] particularly when used in the form of nanoparticles.[10–12] In addition, bioactive glass dissolution products have a demonstrated effect on osteoblast cell gene expression[13] and potential effect on angiogenesis,[14] providing thus a conducive environment for bone cell colonisation, proliferation, differentiation as well as enhanced vascularisation of the constructs. Nevertheless, non-desired body responses and/or related infections are to be expected when tissue engineering scaffolds are implanted.[15] In addition, many factors which are necessary or beneficial to a given tissue may be toxic for other tissues. Thus, a delivery system with local and specific delivery of therapeutic ions or drugs to the desired tissue site is highly desirable in many situations. In this context, there is a growing interest in exploring the possibility of using the scaffold itself to deliver therapeutic drugs.[15,16] Recent studies have found that gallium ion (Ga^{3+}) inhibits *P. aeruginosa*, *methicillin resistant S. aureus* and *C. difficile*.[17] The proposed mechanism of action is based on the fact that Ga^{3+} can disrupt Fe^{3+}-dependent events as many infecting bacteria are unable to differentiate between Ga^{3+} and Fe^{3+}.[18–20] Disruption of iron metabolism increases the vulnerability of these microorganisms because iron is redox active, essential in electron transport and oxidative stress, but Ga^{3+} is redox inactive.[17,21–23] Ga^{3+} (as $GaNO_3$) is a drug already approved by the FDA to treat hypercalcaemia associated with tumour metastasis to bones[24] and it is biologically active in blocking bone resorption.[25]

An interesting approach to prevent possible bacterial colonisation of a tissue engineering scaffold following implant surgery could be thus the controlled release of Ga^{3+} by the utilization of

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alginate as main ingredient of a biodegradable matrix acting as the carrier of the ion in the delivery system. The aim of the present investigation was the development of novel Ga^{3+} crosslinked alginate/nanoparticulate bioactive glass composite films (NBG/Alg/Ga) as a preliminary step for assessing the viability of using this composite in the elaboration of 3D scaffolds for bone tissue engineering, exhibiting the added value of antibacterial capability. A complete physicochemical and microstructural characterisation of the films using a range of techniques was carried out including investigating the antibacterial activity of the materials against *S. aerus*. In the context of the present investigation, it is worthwhile noting that this research topic lies in the field of therapeutic tissue engineering,[15,16] which considers the use of enhanced scaffolds incorporating drug delivery function with potential therapeutic effectiveness (bacteriostatic capability in this case).

Materials and methods

Materials

Sodium alginate (20–40 cps, 25 °C), calcium chloride and gallium nitrate were purchased from Sigma-Aldrich Company Ltd. (Dorset, UK). Melt-derived 45S5 Bioglass® powder (particle size ≈ 10 to 20 μm) was a gift of Dr I. Thompson (Kings College London, UK). Bioactive glass nanoparticles with the nominal composition close to 45S5 Bioglass® (46 wt% SiO_2, 23 wt% Na_2O, 27 wt% CaO, 4 wt% P_2O_5), with spherical shape and mean particle size 35–40 nm, were prepared using the flame spray synthesis process, as described by Brunner *et al.*[11] All other chemicals used were of analytical grade. Standardized cultures of *S. aureus* (ATCC29213) were used for antibacterial studies.

Methods

Preparation of gallium crosslinked alginate/bioactive glass composite films. A solution of 2 g per 100 ml alginate (ALG) was prepared by dissolving sodium alginate in distilled water. Sodium alginate solution was filtered to remove any remaining solids and impurities. Colloidal suspensions (0.5 g per 100 ml) of bioactive glass nanoparticles (NBG) were prepared with the aid of high-speed ultrasonification (BRANSON Digital Sonifier (Danbury, USA)) at pH 4.5 during 100 seconds (30% amplitude). 10 ml of the NBG solution were added to 10 ml of the homogenous ALG solution to produce colloidal suspensions with magnetic agitation. Self-supporting NBG/ALG films were obtained by casting the viscous slurry onto moulds, followed by drying in a normal atmosphere at 25 °C for 3 days. In all cases, in order to insolubilize the films, they were crosslinked in a bath containing a solution of gallium nitrate for 10 min. After crosslinking, the films were rinsed with distilled water to remove any traces of unreacted gallium nitrate and to prevent further crosslinking and were left to dry at 25 °C and 45% humidity composite. Films of 2.0 mm thickness containing NBG were produced (labelled NBG/ALG/Ga). Once the films were cast, they were stored in desiccators for further analyses, as detailed below. The average total amount of Ga^{3+} ($[Ga^{3+}]$) (730 ppm) incorporated in the films was estimated from the difference between the original Ga^{3+} concentration ($[Ga^{3+}]$) in the solution and the $[Ga^{3+}]$ remaining in the solutions after film immersion plus the $[Ga^{3+}]$ recovered from the distilled water used for rinsing the films. $[Ga^{3+}]$ was measured using inductively coupled plasma mass spectrometry (ICP-MS). The intention of these measurements was not to determine the homogeneity of Ga^{3+} incorporated in the films but to know the average total amount of Ga^{3+} in order to prove the ability of the film to sustain a constant release of the ion as a function of time at therapeutic levels. Control samples (alginate crosslinked with Ca^{2+} (labelled ALG/Ca), ALG crosslinked with Ga^{3+} (labelled ALG/Ga) and Ca^{2+}crosslinked alginate/nanoparticulate bioactive glass (labelled NBG/ALG/Ca)) were prepared by the same procedure described above. In addition, films of the same composition than NBG/ALG/Ga and NBG/ALG/Ca films but containing μm-sized Bioglass® particles (labelled m-BG/ALG/Ga and m-BG/ALG/Ca) were also prepared.

Characterisation of films

(i) Scanning electron microscopy. The morphology and microstructured features of films were characterised by scanning electron microscopy (SEM). Films were sputtered with gold to form a uniform conductive coating for SEM observation. They were then placed on a copper stub.

(ii) Mechanical testing. Mechanical properties of the films were evaluated by tensile testing using a computer-controlled universal testing machine (Instron) at a crosshead speed of 1 mm min^{-1}. Five samples of each specimen of 7 mm length, 1.4 mm width and 0.2 mm thickness were tested.

(iii) Acellular in vitro *studies by immersion in simulated body fluid.* The *in vitro* bioactivity of the films in terms of hydroxyapatite layer formation on their surfaces, as usually tested in the literature,[26] was investigated following the protocol of Kokubo *et al.*[27] Each sample of dimensions 8 × 8 × 2 mm^3 was immersed in 30 ml of simulated body fluid (SBF) and was stored in an incubator at controlled temperature of 37 °C for 7 days. After removal from the solution, films were gently washed with distilled water and dried at 37 °C for 30 min. The possible change of film microstructure after 7 days soaking in SBF was analysed using SEM. X-Ray diffraction (XRD) was used to determine the change of crystallinity of films.

(iv) Ga^{3+} release study. Gallium ion release from all films tested was investigated at 37 °C in PBS (10 ml) at pH 7.4 for 10 days. Samples were taken at 6 h, 1, 2, 3, 5, 7 and 10 days and media were replaced every other day. $[Ga^{3+}]$ was measured using inductively coupled plasma mass spectrometry (ICP-MS). The instrument was calibrated in the range 0.1–1000 μg l^{-1} by mixing Gallium standard obtained from Sigma in ultrapure water. The instrument detection limit of gallium is in the range 1–10 nm l^{-1}.

Microbiological studies. The effect of the incorporation of Ga^{3+} in the films on the viable counts of *S. aureus* was conducted on NBG/ALG/Ga, m-BG/ALG/Ga and ALG/Ga films with NBG/ALG/Ca, m-BG/ALG/Ca and ALG/Ca films as controls. Prior to the study, films were sterilized using UV treatment for 30 min. The samples (of circular cross-section of 1 cm diameter) were immersed at 37 °C in PBS (10 ml) at pH 7.4 for 10 days. At predetermined time intervals (1, 2, 3, 5, 7 and 10 days) three films of every series were removed, gently washed by distilled water and dried at 37 °C

for 30 min. The collected samples were used to investigate their antibacterial properties, using *S. aureus* (ATCC 29213), through the antimicrobial disk susceptibility test (NCCLS M2-A9 Performance Standards for Antimicrobial Disk Susceptibility Tests, Approved Standard, Ninth Edition).[27,28] Typically, aliquots of standard 0.5 McFarland suspension (approximately (1–2) $\times$ 10^8) colony forming units per ml (CFU per ml) were spread onto Mueller Hinton agar plates where the samples were placed. After 24 h of incubation, the zones of inhibition were measured.

In vitro cell culture studies

(i) Cell culture methodology. In vitro cell culture assays to study the effect of NBG and Ga^{3+} on the viability and differentiation of MG-63 was conducted using osteoblast like human osteosarcoma cells line (MG-63) from ATCC (The American Type Culture Collection). The cell lines were cultured in growth medium (Dulbecco's modified Eagle's medium (DMEM), PAA, Coelbe, Germany) with 10%, v/v foetal calf serum (FCS) and 100 U ml^{-1} *penicillin–streptomycin* solution and incubated at 37 °C in a humidified atmosphere (5% CO_2 and 85% humidity). The medium in the flasks was changed every 2 days. Cultures of 80–85% confluence were used for seeding on the films for investigating cell proliferation. Prior to cell seeding, films were sterilized using UV treatment for 30 min and pre-incubated with culture medium for 12 h at 37 °C in a humidified incubator with 5% CO_2 and 85% humidity. Cells were seeded drop wise onto the top of the films at an initial density of 2.5 $\times$ 10^6 cells cm^{-2}. Two measures of cell viability/number were used: Live/Dead cell counting and AlamarBlue assay. All measurements were performed on three independent samples; there were 2 sample series for each time point (NBG/ALG/Ga and NBG/ALG/Ca). Tissue culture polystyrene (TCP) substrates were used as control.

(ii) Live/dead staining. Films were incubated at 37 °C for 30 min in PBS containing 2 μm calcein AM (Live) and 2 μm ethidium homodimer-1 (Dead) (Invitrogen). Cells stained green (live) and red (dead) were imaged using an inverted epifluorescence microscope (Nikon Eclipse TE 300). The ratio of the number of live cells divided by the sum of the number of live and dead cells was defined as the percent of live cells.

(iii) AlamarBlue assay. AlamarBlue (BioSource International, CA, USA) is a metabolic assay, which is intimately linked and directly proportional to the number of cells present at a given time point. Thus, the fluorescent and colorimetric signals generated from the assay are proportional to the number of living cells in the sample. The films were removed from the culture plates on days 1, 3 and 7. They were washed with PBS after aspirating the medium and then, 1.8 ml of media and 0.2 ml of the AlamarBlue dye were added to allow for a 4 h incubation period at 37 °C (with 5% CO_2). Aliquots of 200 μl from each sample well were transferred into a black 96-well plate and the fluorescence of the samples was measured at room temperature using a fluorescence plate reader (Fluorescence Microplate Reader, Biotek, VT, USA) using excitation and emission wavelengths of 560 nm and590 nm, respectively.

Data analysis. Unless otherwise stated, the experiments were carried out in triplicate per series and all data were presented as mean $\pm$ SD. Statistical analyses were performed using the one-way analysis of variance (ANOVA). The difference was regarded statistically significant when $p < 0.05$.

Results

Film characterization

(i) Scanning electron microscopy. The morphology and microstructure of ALG/Ga and NBG/ALG/Ga films examined using SEM are shown in Fig. 1. It can be seen from micrographs that the ALG/Ga film exhibits a smooth top surface (the cracks observed are artefacts of the coated technique for SEM analysis) (Fig. 1a). With the incorporation of NBG, the surface morphology and topography become rougher (Fig. 1b). The increase of surface roughness plays an important role in cell attachment. The particle distribution seems to be homogeneous however some agglomeration of NBG particles is clearly visible by SEM observation. The cross-section morphology of NBG/ALG/Ga films is shown in Fig. 1c and d at different magnifications. EDX plots confirmed that Ga^{3+} was successfully incorporated within the films (*e.g.* inset in Fig. 1b).

(ii) Mechanical testing. The mechanical properties of NBG/ALG/Ga composite films were evaluated through static tensile tests and the effect of NBG on the tensile modulus of NBG/ALG/Ga films is shown in Fig. 2. The addition of 0.25 wt% m-BG or NBG particles to the films resulted in a significant increase in the tensile modulus ($p < 0.05$). Moreover, it was observed that at the same contents of NBG and m-BG in ALG/Ga (0.25 wt%), there was a significant increase in the tensile modulus for NBG/ALG/Ga films ($p < 0.05$).

(iii) Acellular *in vitro* study in SBF. SEM images of films immersed in SBF are shown in Fig. 3.The *in vitro* bioactivity of NBG/ALG/Ga was confirmed by the deposition of hydroxyapatite on the surface of the films after 3 and 7 days of immersion in SBF, as suggested by SEM observations (Fig. 3a and b respectively). ALG/Ga films displayed no apatite particles deposition on their surfaces (Fig. 3c). The XRD spectrum of a sample immersed in SBF for 7 days did not conclusively confirm the crystalline form of apatite[29,30] due to the development of calcite,[31] as shown by the sharp peaks in the XRD spectrum (Fig. 3d). No other phases were detected by XRD.

(iv) Ion release. The Ga^{3+} release profile in PBS is shown in Fig. 4. NBG/ALG/Ga films exhibited controlled Ga^{3+} release, and this was compared to unfilled ALG/Ga films and m-BG/ALG/Ga films. No significant difference was found among the samples suggesting that, at the concentration used, the inorganic particles incorporated (either in NBG/ALG/Ga or mBG/ALG/Ga films) have no influence on the ion release. NBG/ALG/Ga films released almost 20% of Ga^{3+} within the first 10 days following a zero order reaction which is desirable when delivering therapeutic drugs because it is possible to deliver a constant [Ga^{3+}] to prevent possible bacterial colonisation of the biomaterial following implant surgery.

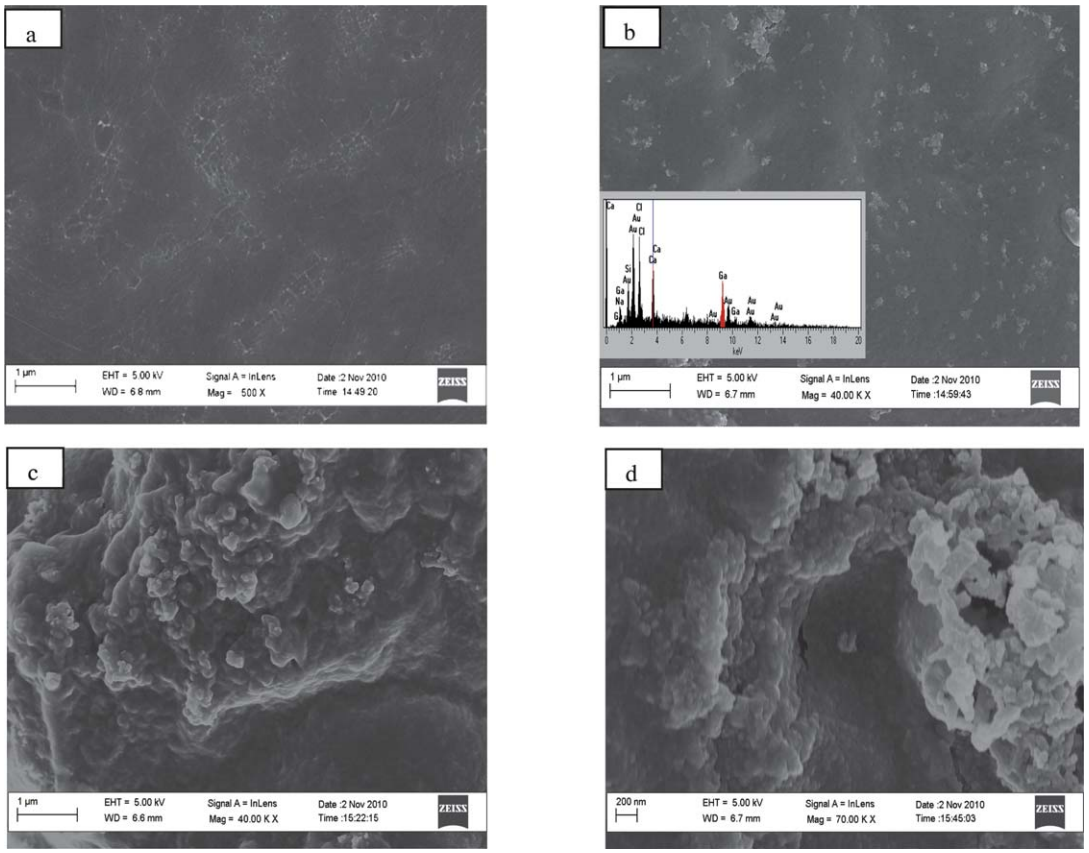

Fig. 1 SEM images showing the typical microstructure of the top surfaces of (a) alginate cross-linked with Ga^{3+} film (ALG/Ga) and (b) nano-bioactive glass–sodium alginate cross-linked with Ga^{3+} film (NBG/ALG/Ga). SEM images showing the cross-section of NBG/ALG/Ga films at different magnifications (c and d). Inset in (b) is an EDX plot showing the presence of Ga^{3+} within the film.

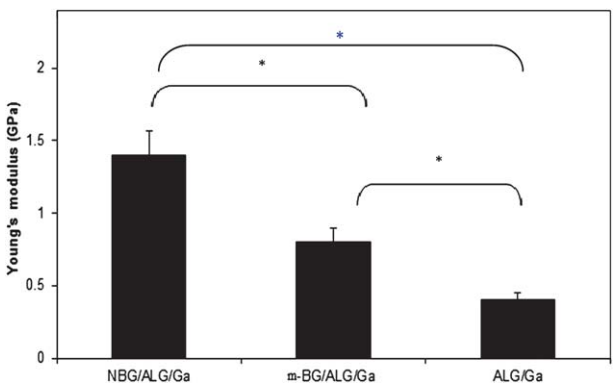

Fig. 2 Values of Young's modulus of NBG/ALG/Ga, mBG/ALG/Ga and ALG/Ga. The addition of 0.25 wt% m-BG or NBG particles to the films resulted in a significant increase in the tensile modulus ($p < 0.05$). At the same contents of NBG and m-BG in ALG/Ga (0.25 wt%), there was a significant increase in the tensile modulus for NBG/ALG/Ga films ($p < 0.05$). Average values ± SD; significantly different *$p < 0.05$; $N = 5$.

Microbiological test

The antimicrobial disk susceptibility test showed that NBG/ALG/Ga films submitted to 10 day immersion in PBS developed zone of inhibitions between 14 and 17 mm during the mentioned period, thus confirming the effectiveness of the NBG/ALG/Ga films to induce a bacteriostatic effect *in vitro* towards *S. aureus*. Fig. 5 shows the relative length of zone of inhibitions for different films tested after different periods of immersion in PBS. No significant differences were observed between NBG/ALG/Ga and m-BG/ALG/Ga films for each day. However, significant differences were observed between NBG/ALG/Ga and NBG/ALG/Ca films; similar results were obtained when compared with m-BG/ALG/Ga and m-BG/ALG/Ca films. The zones of inhibition corresponding to NBG/ALG/Ca and m-BG/ALG/Ca films could be due to the change in pH during the bioactive glass degradation.[10,32]

Cell culture studies

Two complementary bioassays were used to measure osteoblast viability on films: (1) Live/Dead stain measures membrane integrity and cellular esterase activity; and (2) AlamarBlue assay measures cellular metabolic activity. NBG/ALG/Ga films and controls (NBG/ALG/Ca films and tissue culture plastic (TCP)) supported osteoblast like human osteosarcoma cells line (MG-63) proliferation over the 7 day period of study and the results are shown in Fig. 6. The percentage of live cells after 1 d of cell culture is shown in Fig. 6a. NBG/ALG/Ga and NBG/ALG/Ca films have an adequate percentage of live cells but significantly lower than control ($p < 0.05$). No significance difference was found between NBG/ALG/Ga and NBG/ALG/Ca films. The growth of MG-63 cells on all films increased over the

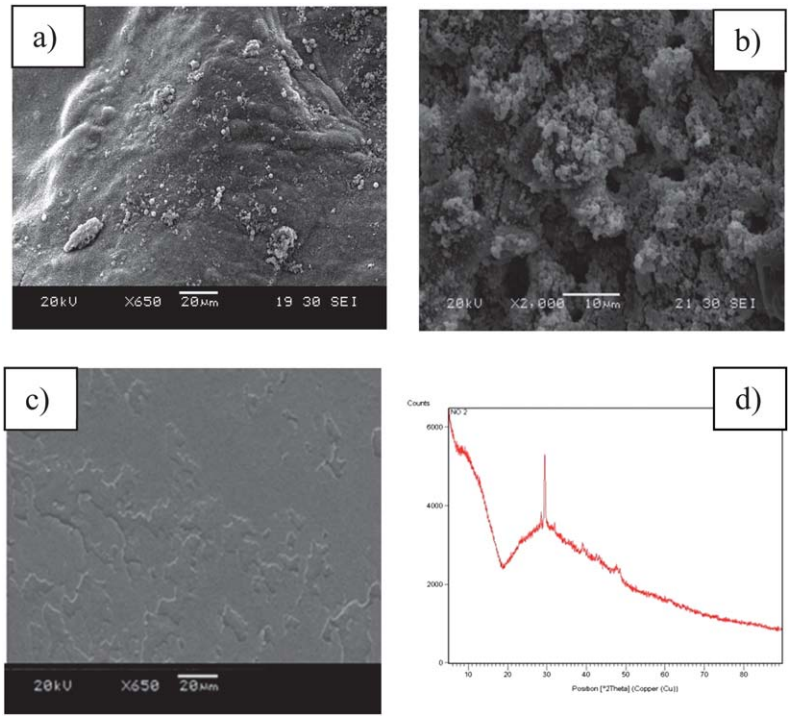

Fig. 3 SEM micrographs showing (a) hydroxyapatite formed on the surface of NBG/ALG/Ga after 3 and (b) 7 days of immersion in SBF. No hydroxyapatite was formed on the surface of ALG/Ga (c). The main crystalline phase identified on the surface of NBG/ALG/Ga films after 7 days in SBF by XRD analysis is calcite (d).

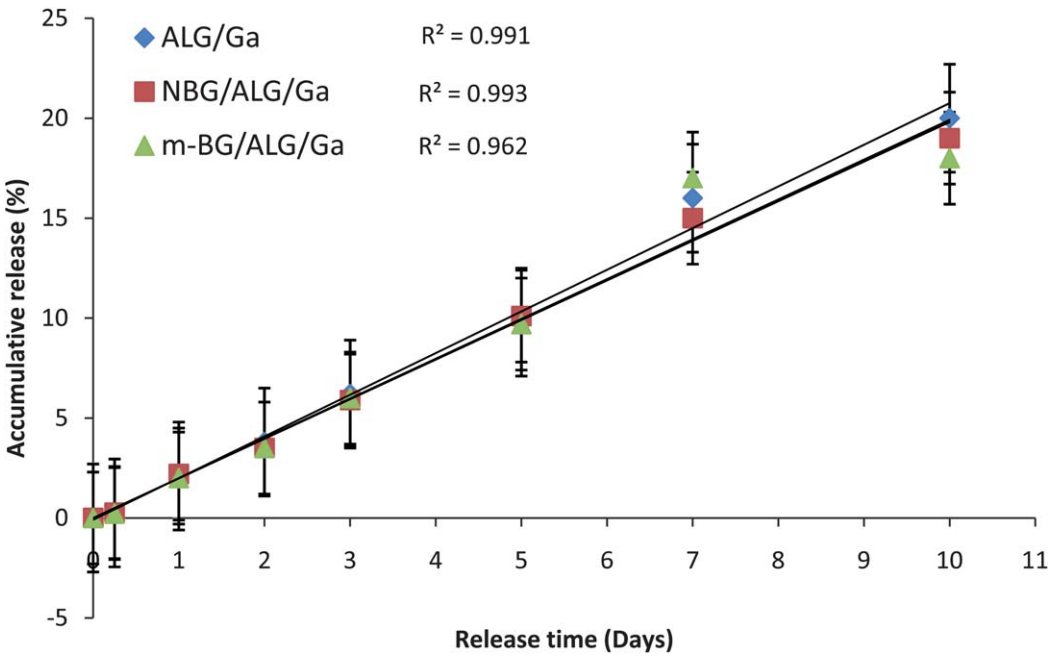

Fig. 4 Ga^{3+} release profile from NBG/ALG/Ga, m-BG/ALG/Ga, ALG/Ga films. NBG/ALG/Ga released almost 20% of Ga^{3+} within the first 10 days following a zero order reaction. No significant difference was found among the samples suggesting that, at the concentration used, the glass particles incorporated (either in NBG/ALG/Ga or mBG/ALG/Ga) have no influence on the Ga^{3+} ion release.

observed 7-day period indicating the suitability of the samples to allow for cell proliferation (Fig. 6b). Even though there was a significant difference in relative cell proliferation between the control (TCP) and the NBG/ALG/Ga samples during the study period, there was no significant difference between the NBG/ALG/Ga and NBG/ALG/Ca samples at any point during the study period. These findings suggest the suitability of the incorporation of Ga^{3+}, at the concentration used, for cell proliferation.

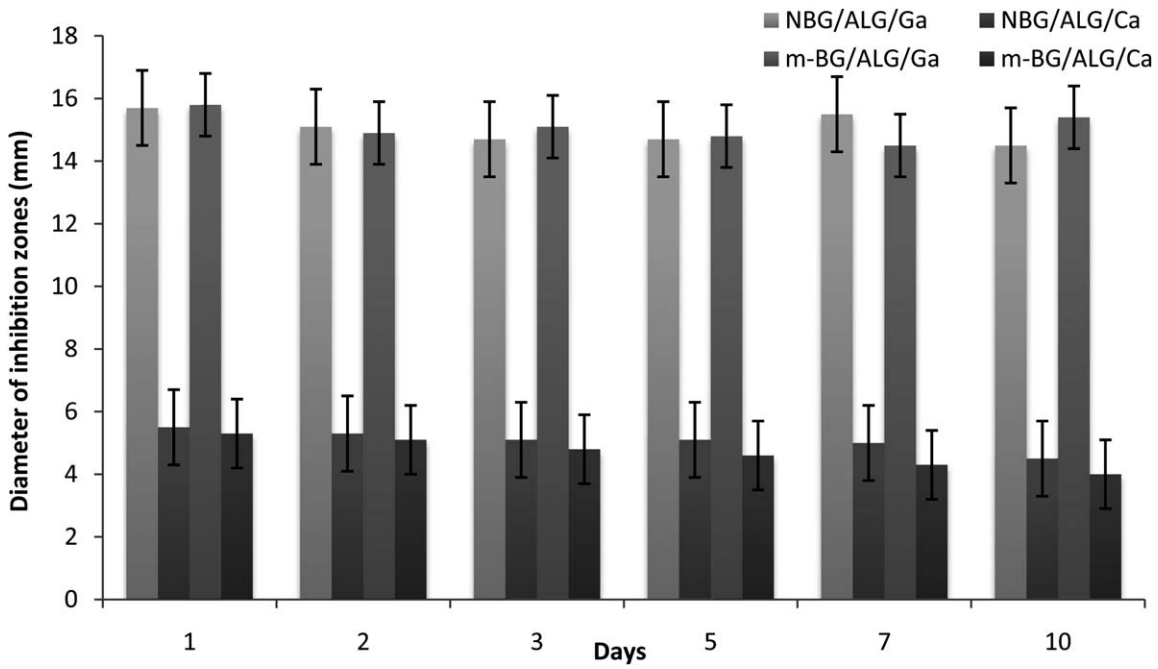

Fig. 5 Microbiological test results showing the relative diameter of zone of inhibitions after different periods of immersion in PBS for NBG/ALG/Ga and m-BG/ALG/Ga films. Data were obtained from three independent experiments and are shown as means ± SD.

Discussion

This investigation has shown that the incorporation of Ga^{3+} as the crosslinker ion resulted in the controlled release of the ion itself as bacteriostatic agent in agreement with previous results.[3] Moreover, this investigation has shown that the incorporation of bioactive glass nanoparticles (NBG) into Ga^{3+}-crosslinked alginate (NBG/ALG/Ga) films significantly improved the mechanical properties when compared with films of the same composition but with μm-sized Bioglass® (m-BG). Further, NBG/ALG/Ga composite films were confirmed to possess attractive biological properties considering cell proliferation results that showed the biocompatibility of the composites towards MG-63 osteoblasts. The results of the cytotoxicity test showed that gallium ions are not measurably toxic under the conditions of the test. In this study, Ga^{3+} was crosslinked with alginate in order to enable its immobilization within the films and to control its release in concordance with our previous work.[3]

The achievement of controlled drug-release capability (the ability of a drug delivery system to release a drug over an extended period of time at a controlled rate) is one of the important features characterising new generation of scaffolds for regenerative medicine.[15,16] The present experiments showed that the release rate of Ga^{3+} with bacteriostatic capability can be controlled by crosslinking the ion with alginate and a model of how this process could be done is shown in Fig. 7.

Additionally, the strategy to bound ions by a matrix to improve ion immobilization is very often utilised. The release rate of Ga^{3+} was found to be constant during assay time and it was also observed that the Ga^{3+} release rate was independent of the presence of either nano- or micro-sized bioactive glass in the ALG matrix. This can be explained assuming that (a) the erosion process of the crosslinked ALG/Ga matrix occurs also at a constant rate, and (b) the diffusion rate of Ga^{3+} is very low against the erosion rate of the matrix. Immobilization of Ga^{3+} within the alginate film is shown thus to improve the release kinetics of the antibacterial ion and therefore to enhance its effectiveness; the release of the ion will be controlled solely by the erosion of the film. In suggested *in vivo* applications, alginate biodegradation in the body will be likely the result of exchange of the multivalent ion cross-linker (Ga^{3+} in this particular case) with monovalent cations present in the biological environment surrounding the material.[3,33–35] Most polymer biomaterials for bone tissue engineering applications have low stiffness, and therefore the development of composites, *e.g.* incorporating inorganic fillers, has been proposed.[6,32] Our study has shown that NBG/ALG/Ga films possess significantly improved elastic constant compared to films of the same composition but with μm-sized Bioglass® inclusions (m-BG/ALG/Ga). Interestingly, there seems to be a limitation in the amount of either nanosized or μm-sized Bioglass® that can be incorporated in the polymer soft matrix. A potential explanation may be that higher contents of either nano-scale or μm-sized particles will disrupt the structure of alginate leading to a detrimental effect on the mechanical properties of the composite film, possibly due to an excessive agglomeration of the bioactive glass particles. This effect was more evident with μm-sized Bioglass® possibly due to its irregular shape and bigger size (compared with nanosized bioactive glass). These observations are in agreement with the ones reported by Misra *et al.* in their work on P(3HB)/bioactive glass composites.[36] In the present study, the investigated amount of NBG or m-BG was sufficient to improve the stiffness and the bioactivity without interfering with the release of Ga^{3+}.

The development of this novel film based on bioactive glass particles embedded in alginate cross-linked with Ga^{3+} (NBG/Alg/Ga) represents a first step for assessing the viability of using this

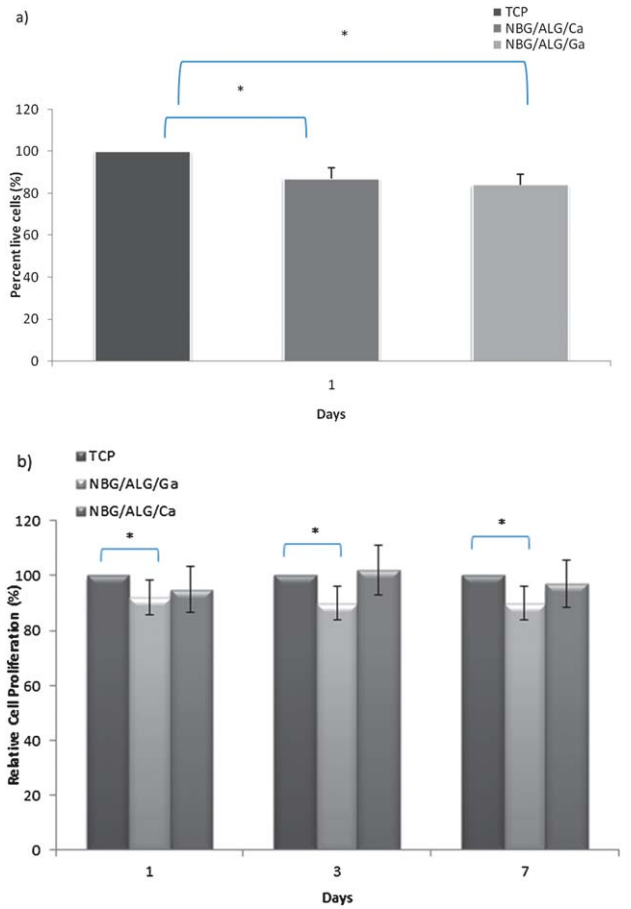

Fig. 6 Osteoblast like human osteosarcoma cells line (MG-63) response to NBG/ALG/Ga and NBG/ALG/Ca films as a function of time: (a) fractional viability determined by Live/Dead staining, and (b) metabolic activity from Wst-1 assay. Error bars represent means ± SD for $n = 3$.

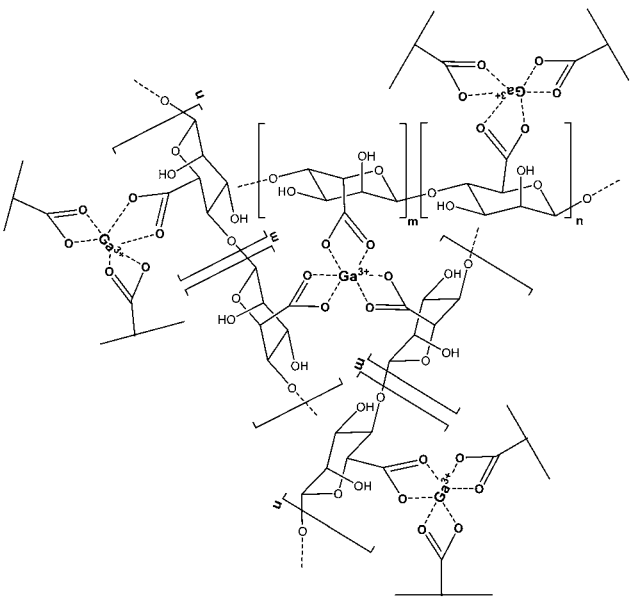

Fig. 7 Schematic diagram showing possible alginate gel network formation by trivalent ions.

composite in the elaboration of 3D scaffolds for bone tissue engineering with the added value of exhibiting antibacterial capability. The novel film developed here enables to adjust its composition in order to achieve optimal performance *in vivo* (*e.g.* increase or decrease of the concentration of sodium alginate, increase or decrease of the concentration of gallium accordingly and also increase or decrease of NBG concentration within certain limits). These adjustments will be done in the framework of *in vivo* experiments and they will be discussed in future publications.

Conclusions

NBG/ALG/Ga multifunctional composite films were prepared as novel biodegradable and bioactive materials with prophylaxis effect against infections and potential use in bone tissue engineering. The incorporation of bioactive glass nanoparticles into Ga crosslinked alginate films significantly improved the elastic constant and acellular bioactivity. Biomineralization studies (in SBF) indicated the deposition of apatite on the surface of the films suggesting their bioactive behaviour which is a consequence of the high bioreactivity of NBG, however the crystallinity of the formed layer was not confirmed by XRD. In addition, NBG/ALG/Ga films supported osteoblast like cell proliferation and it was shown that Ga^{3+} release is controlled by crosslinking the ion with alginate. We conclude that NBG/ALG/Ga composites have potential application in bone tissue engineering and the material, as coating or as porous matrix, will be useful to design 3D scaffolds with enhanced performance.

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