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45S5 Bioglass® concentrations modulate the release of vancomycin hydrochloride from gelatin–starch films: evaluation of antibacterial and cytotoxic effects

Josefina Rivadeneira[1,*], Ana Laura Di Virgilio[2], M. Carina Audisio[3], Aldo R. Boccaccini[4], and Alejandro A. Gorustovich[1]

[1] *Grupo Interdisciplinario en Materiales- Universidad Católica de Salta (IESIING-UCASAL), Instituto de Tecnologías y Ciencias de Ingeniería-Universidad Buenos Aires-Consejo Nacional de Investigaciones Científicas y Técnicas (INTECIN UBA-CONICET), Campo Castañares s/n, Salta, Argentina*

[2] *Cátedra de Bioquímica Patológica Facultad de Ciencias Exactas, Universidad Nacional de La Plata (UNLP), Calle 47 y115, La Plata, Argentina*

[3] *Instituto de Investigaciones para la Industria Química - Consejo Nacional de Investigaciones Científicas y Técnicas (INIQUI - CONICET), Universidad Nacional de Salta (UNSa), Av. Bolivia 5150, Salta, Argentina*

[4] *Institute of Biomaterials, University of Erlangen-Nuremberg, Cauerstr. 6, 91058 Erlangen, Germany*

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ABSTRACT

The aim of this work was to evaluate the release profile of vancomycin hydrochloride (VC), as well as the degradation, in vitro antistaphylococcal effect and cytotoxicity in MG-63 osteoblast-like cells of gelatin–starch (GS) films added with different concentrations of microparticles of the bioactive glass 45S5 (m-BG). The biomaterials were obtained through the gel-casting method. Four different composites were prepared at four different weight percentages of m-BG: 0, 5, 10, and 15 %. Glutaraldehyde 0.25 wt% (GA) was used as the cross-linker. The composites were characterized by scanning electron microscopy and the in vitro degradation of the films was studied by measuring the water uptake and weight loss. The drug release kinetics was quantified spectrophotometrically. The inhibition zone test and the plate count method were used to evaluate the antibacterial activity of the samples. Three staphylococcus strains were evaluated: *Staphylococcus aureus* ATCC6538, *S. aureus* ATCC29213, and *Staphylococcus epidermidis* ATCC12228. Cytotoxicity effects were evaluated through the MTT assay. The addition of m-BG to GS films showed no effects on the amount of water uptake, but led to an increase in the weight loss over time, even with m-BG content. The release rate of VC was also affected by the increasing concentration of m-BG in the composite films. However, the antibacterial effects of the composites were not improved by this modulation. All composites strongly inhibited staphylococcal cells with similar strength. On the other hand, liquid extracts from the composites resulted in cytotoxic effects on MG-63 osteoblast-like cells due to the presence of GA, but not to the concentration of VC or m-BG.

Address correspondence to E-mail: jrivadeneira@conicet.gov.ar

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Introduction

The pharmacokinetics, pharmacodynamics, non-specific toxicity, and efficacy of drugs may be controlled by various drug delivery systems [1]. The controlled and sustained delivery of a certain drug ensures that an adequate concentration will reach the target organ over a long period of time and will thus be therapeutically efficient [2]. In wound healing and tissue engineering, the modulation of the release of a drug is of particular interest because one important factor that interrupts the healing is the bacterial burden in the wound [3, 4]. *Staphylococcus aureus* and *Staphylococcus epidermidis* are important pathogens involved in wound infection [4].

Several drug delivery systems based on biopolymers have been studied to optimize drug release profiles because of their many interesting features, including good biocompatibility, biodegradability, and good mechanical properties [5, 6]. Among biopolymers, gelatin stands out strongly due to several key advantages: it is biodegradable, biocompatible, and nonimmunogenic, and has hemostatic properties [7, 8]. In addition, gelatin can be an exceptionally adaptable drug delivery carrier because drug loading and releasing kinetics from gelatin matrices can be independently tuned [8–12]. Nevertheless, due to its high brittleness and low intensity, gelatin is often used after modification by different methods such as grafting [15], crosslinking [13, 14], and blending [16–18].

Starch-based polymers and composites have also been proposed as drug delivery systems [20, 21] and have great potential to be applied in the biomedical field [19]. Biodegradable starch-based polymers present good compatibility, proper mechanical properties, and their degradation products are non-toxic [19]. Corn starch can be used together with gelatin as reinforcement to the polymeric matrix [22] and for the controlled release of drugs [23].

In the last years, some researchers have shown that the combination of bioactive glasses (BGs) with biopolymeric matrices allows modifications in the release profile of antibiotics, in particular vancomycin hydrochloride (VC) [24–26]. Unfortunately, many often, the biological response or antimicrobial effects have been overlooked. VC is used to treat infections caused by Gram-positive bacteria such as methicillin-resistant *S. aureus* (MRSA) [27].

The addition of BGs to a biopolymeric matrix can lead to several interesting biological properties [28]. BGs are being increasingly considered in bone tissue engineering, due to their ability to bind strongly to the bone and promote bone growth after in vivo implantation [29, 30]. In addition, BGs have a huge potential for applications in wound healing and soft tissue engineering [31]. In particular, 45S5 BG is a promising material for applications in soft tissue engineering. For example, it has been demonstrated that microparticles of 45S5 BG possess angiogenic effects [32–34], promote the expression of genes related to wound healing [35], and accelerate the recovery of skin wounds [36]. The aim of this work was to study the influence of increasing concentrations of 45S5 BG microparticles (m-BG) on the water uptake capacity, degradation behavior, and release profile of VC. We also investigated the in vitro antistaphylococcal effect and potential in vitro cytotoxicity on MG-63 human osteosarcoma cells of m-BG-containing composites based on gelatin–starch (GS) biopolymers.

Materials and methods

Materials

Micrometer particles of the melt-derived 45S5 BG (composition in wt%: 45 % SiO_2, 24.5 % Na_2O, 24.5 % CaO, and 6 % P_2O_5) of particle size in the 5–100 µm range were used. Edible gelatin Royal and corn starch were from Kraft Foods Argentina. VC was from Laboratorios Fabra S.A. (Buenos Aires, Argentina). Glutaraldehyde (GA) solution (50 % in water, 5.6 M) was purchased from Merck SA Argentina. Fetal bovine serum (FBS) was purchased from Natocor SA (Córdoba, Argentina). Maximum recovery diluent was prepared according to the following formula: Peptone, 1 g (Britania SA, Buenos Aires, Argentina), NaCl, 8 g (Reagents SA, Buenos Aires, Argentina), and distilled water, 1 L. Simulated wound fluid (SWF) was prepared by mixing maximum recovery diluent with FBS in equal volumes.

Composite films

The composite films were obtained by preparing a 5 % gelatin solution in distilled water and heating it at 80 °C for 10 min, then a 1 % starch solution in distilled water and heating it to 85 °C for 5 min, and then homogenizing the solutions. The ratio between gelatin and starch was 5:1. Glycerol was added at a concentration of 20 % in relation to the total volume of solution and used as plasticizer. Four concentrations of m-BG particles were used to obtain films with 0, 5, 10, and 15 % of m-BG respect to the polymer solution. Before the addition of m-BG, the microparticles were passivated in phosphate-buffered saline (PBS) for 12 h to avoid the sudden release of a high ion concentration from the BG. The solutions were cooled to 30 °C with constant stirring to incorporate VC at a concentration of 1 mg mL^{-1}. Alternatively, composites without VC loading were also prepared as controls. Then 20 mL of the respective mixes were placed in 8.5-cm-diameter polystyrene petri dishes and the moldings were dried at 4 °C. After drying, composites were cross-linked for 10 min in a GA solution (0.25 wt%). This concentration was chosen because it has been previously reported that 0.25 wt% of GA allows obtaining a crosslinking degree of approximately 85 % and preventing the release of gelatin into the buffer solution [37]. Finally, the composites were washed gently in distilled water and then dried at room temperature (25 °C) for 24 h.

Morphological characterization

Scanning electron microscopy (SEM) was used to characterize surface of films. According to previous work [26], the films were fixed with a 2.5 % GA 0.1 M PBS solution overnight at 4 °C. After that the materials were washed with distilled water, and then dehydrated by means of a graded series of ethanol solutions. The samples were examined using a JEOL JSM 6480 LV, Japan microscope.

In vitro degradation study

Water uptake assay

The water uptake capacity was determined as described previously in Rivadeneira et al. [26]. The films were cut and weighed. The materials were incubated in 15 mL of water at 37 °C. At regular intervals, samples were removed and the excess of water at surface was withdrawn with a filter paper. The weights of films were recorded until reaching equilibrium swelling. The water uptake capacity of the films was calculated according to the Eq. (1):

$$\text{Water uptake }(\%) = \left[(\text{Final weight} - \text{Initial weight})/\text{Initial weight}\right] \times 100. \tag{1}$$

Weight loss

The degradation pattern was studied at 37 °C in PBS through the weight loss profile of composites. Circle-shaped composites (10 mm in diameter) were immersed in PBS for up to 28 days. After each period of time, films were washed twice with distilled water to remove possible material adsorbed on the surface, and then dried at 37 °C. The PBS solution was replaced twice a week. Three samples were assessed for each group. The weight loss (WL) was calculated by means of Eq. (2), where W_0 is the initial weight and W_1 is the weight after soaking in PBS at a given time:

$$\text{WL }(\%) = [(W_0 - W_1)/W_0] \times 100 \tag{2}$$

In vitro VC release

The in vitro VC release was quantified as previously described in Rivadeneira et al. [26]. The composites were cut in 5-mm-diameter discs (area = 0.2 cm^2) with a paper punch incubated in 1 mL of distilled water at 37 °C. At predetermined intervals, the VC concentrations were determined by measuring the absorbance at a wavelength (λ) = 280 nm on a UV–Vis Thermo Spectronic Helios Beta v.460 (Thermo Electron Corporation, Massachusetts, USA). Three samples in each condition were evaluated. The data are expressed as mean ± SD.

Antimicrobial efficacy

Staphylococcus aureus ATCC6538, *S. aureus* ATCC29213, and *S. epidermidis* ATCC12228 were cultured in Mueller–Hinton broth for 24 h at 37 °C. Bacterial cell suspensions were adjusted to 6–7 log cfu mL^{-1}. Previous to antibacterial experiments, film discs were sterilized under UV light for 20 min each side. Antibacterial efficacy was evaluated through the following two methods.

Zone of inhibition assay

Film discs of 5-mm diameter were placed on Mueller–Hinton agar plates which had been previously seeded with 100 µL of described bacterial suspension. Then the plates were incubated at 37 °C for 24 h. Finally, the diameters of the inhibition zones were measured in mm. All tests were performed in triplicate and the means and SD were determined.

Viable counts

Viable counts were determined in SWF. The composite samples were incubated for 48 h at 37 °C in 2 mL of cellular suspensions. Each staphylococcus suspension in presence of SWF only was used as controls. Samples were collected at 0, 24 and 48 h and spread on agar plates containing Mueller–Hinton. The viability of cells was quantified by counting in. All tests were performed in triplicate. The results are expressed as $\log_{10}$ cfu mL^{-1} ±SD. The antibacterial activity was determined as the difference between the log number of bacteria in the control and that in the test composites. The antimicrobial activity was classified as low, moderate, or high according to the criterion of Gallant-Behn et al. [38].

MG-63 osteoblast-like cells

MG-63 osteoblast-like cells were cultured in DMEM medium supplemented with 10 % FBS, 100 µg mL^{-1} streptomycin, and 100 U mL^{-1} penicillin at 37 °C in a 5 % CO_2 atmosphere. Cells were seeded in a 75-cm^2 flask, and after reaching 70–80 % of confluence, the cells were removed from the flask by trypsinization. To determined total cell counts and the number of viable cells, a Trypan blue stain was performed using a Neubauer hemocytometer. For the experiments, cells were grown in multiwell plates. After cells reached the desired confluence, the monolayers were washed with DMEM and then incubated under the different conditions of each experiment.

Composite liquid extracts

Before cell incubation, the films (0.20 cm^2) were sterilized by UV light for 20 min each side. After, the film discs were immersed in 1 mL of DMEM for 48 h at 37 °C. Control samples were obtained by incubating under the same condition DMEM medium without the composites.

MTT assay

MTT assay was carried out as previously [39]. Briefly, 2.5×10^4 cells per well were seeded in a 96-multiwell dish and incubated overnight to allow adherence. Afterwards, the cells were exposed to liquid extracts for 48 h. After exposure, the medium was remove and cells were incubated with 0.5 mg mL^{-1} MTT under normal culture conditions for 3 h. Cells were lysed in DMSO (100 µL per well), and the absorbance was measured at 570 nm in a Microplate Reader (7530, Cambridge Technology, Inc., Karlstad, Sweden). Cell viability is shown as the percentage of the control value (assuming data obtained from untreated cells as 100 %).

Statistical analysis

Data were statistically analyzed by one-way analysis of variance, ANOVA (SPSS 15.0 statistical package software). Tukey's multiple comparison post-tests for intergroup analysis and p values of <0.05 were considered to be statistically significant.

Results

Composite films

Figure 1 shows the surface morphologies of VC-loaded composites studied by SEM. GS films showed a dense, smooth, and uniform surface (Fig. 1a). The presence of m-BG particles led to the formation of irregular protrusions on the surfaces of the films (see arrows). An increase of the roughness of the surface with increasing concentration of m-BGs in the films can be qualitative assessed by inspection of the SEM micrographs (Fig. 1b–d).

The addition of VC to the films did not affect the morphology of the films in comparison to non-releasing composites (not shown). The coating was approximately 65.08 ± 3.75 µm for GS-BG5 %, 82.1 ± 2.55 µm for GS-BG10 %, and 106.67 ± 5.77 µm for GS-BG15 %.

In vitro degradation

The values of water uptake capacity for each composite (in %) are shown in Fig. 2a. We found no statistically significant differences in the water

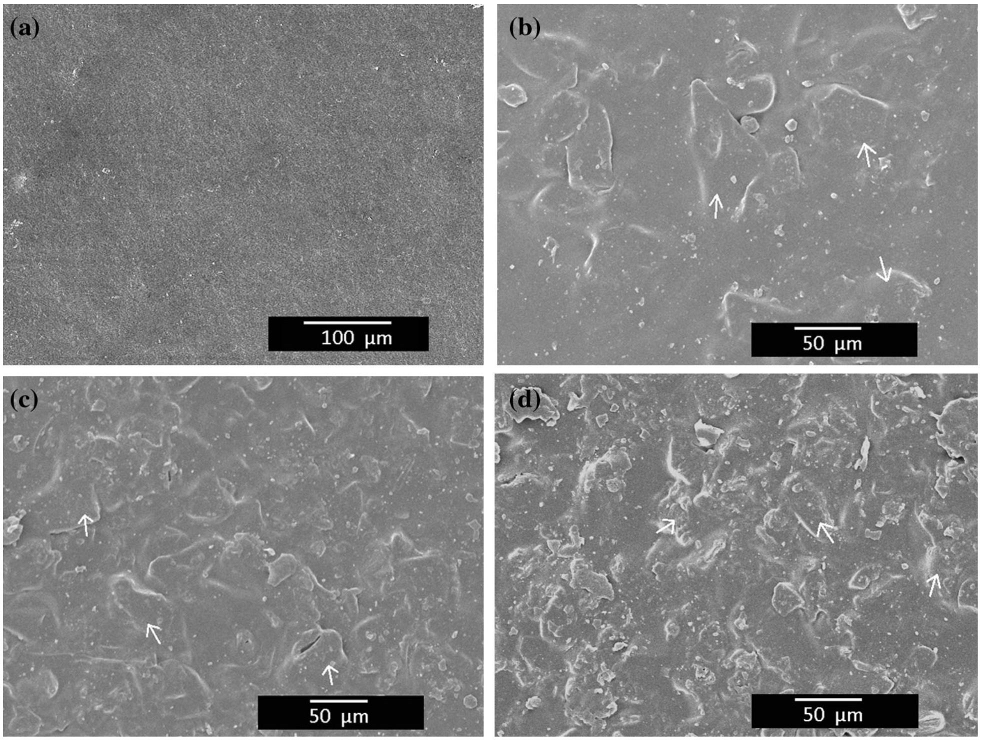


Figure 1 SEM images of films. GS films (**a**), GS-BG5 % (**b**), GS-BG10 % (**c**), GS-BG15 % (**d**).

absorption capacity of the composites. The water uptake capacity of the films was of the order of 250–300 %. The swelling reached equilibrium after 15 min.

Figure 2b shows the weight loss of films containing VC grown in PBS for 28 days. The results show that the mass loss increased gradually over time. After a week, the weight loss of the composites was similar. Nevertheless, after this time, the GS-BG films showed faster degradation than the GS films. GS-10 % BG and GS-15 % BG were completely degraded by day 20. On the other hand, after 28 days of degradation, GS had a maximum mass loss of 43 % and GS-5 % BG a maximum mass loss of 57 %. However, the difference was not statistically significant.

Effects of 45S5 m-BG on VC release

Figure 3 shows the release behavior of VC from the films. A burst release was expected during the first hours due to the hydrophilic nature of VC. In general, the release profiles followed typical three phases: a rapid release phase during the first hours; a decrease in the release rate and a change in the slope of the curves; and a zero order release (constant release rate), which lasted until the end of the experiment. The release rate of VC from the composites was modified by the presence of 45S5 m-BG. The amount of VC released to the medium increased as the m-BG concentration increased. Most of the VC was released during the first 24 h. At this time, the amount of VC released was 0.147 ± 0.080 mg mL^{-1} for GS, 0.2078 ± 0.087 mg mL^{-1} for GSBG-5%, 0.2526 ± 0.060 mg mL^{-1} for GSBG-10%, and 0.2867 ± 0.020 mg mL^{-1} for GSBG-10%.

In the first stage, GS-BG films showed an abrupt initial burst release, while GS films showed a much lower initial release of VC. In the second stage, GS films exhibited a longer release period than GS-BG composites and showed a controlled release of VC.

Antibacterial effects

Inhibition zone

The values of the inhibition zone (in mm) around the composites are shown in Table 1. The presence of the composites inhibited the growth of the three staphylococcus strains tested. *S. aureus* ATCC6538 showed the largest inhibition zone, whereas *S. aureus* ATCC29213 and *S. epidermidis* ATCC12228 showed inhibition zones of similar size. The addition of 45S5

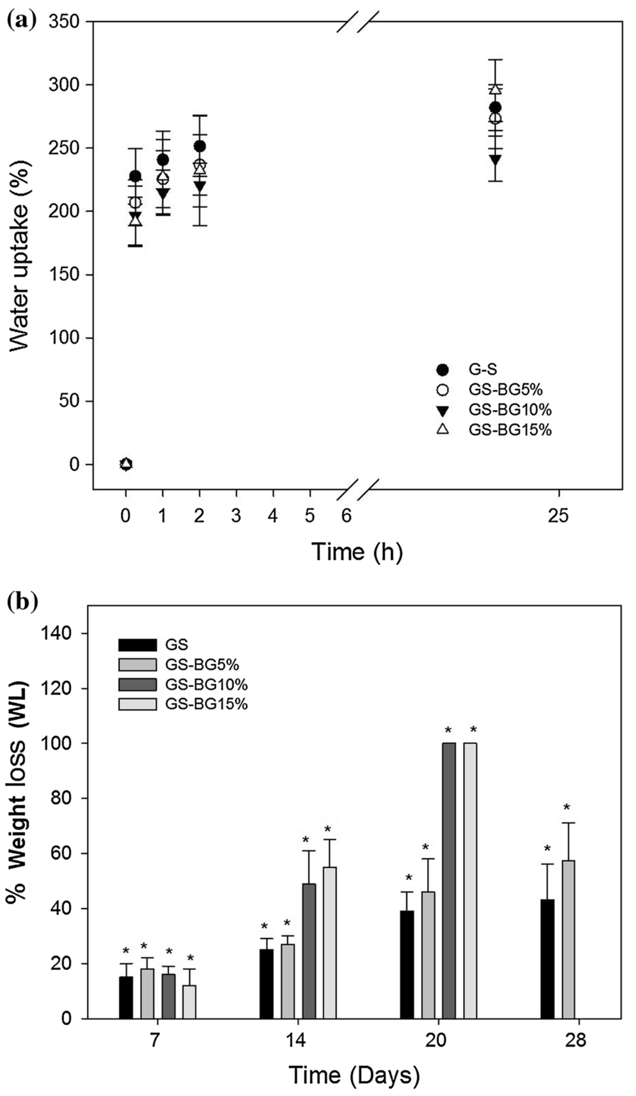


Figure 2 In vitro degradation study. Water uptake capacity of films along the incubation time (**a**); weight loss of films incubated in PBS at 37 °C for the time period indicated (**b**). Results are reported as mean ± SD. *Asterisk* indicates $p < 0.05$.

m-BG to the composites showed no effects on the efficacy of bacterial inhibition. Films not releasing VC developed no inhibition zones.

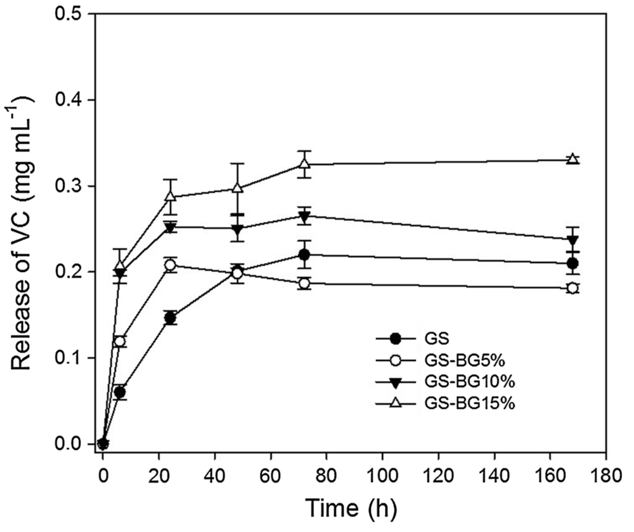


Figure 3 Vancomycin hydrochloride release profile from films as a function of time. The data are expressed as the mean ± SD.

Table 1 Inhibition zone around composites $N = 3$ (mm)

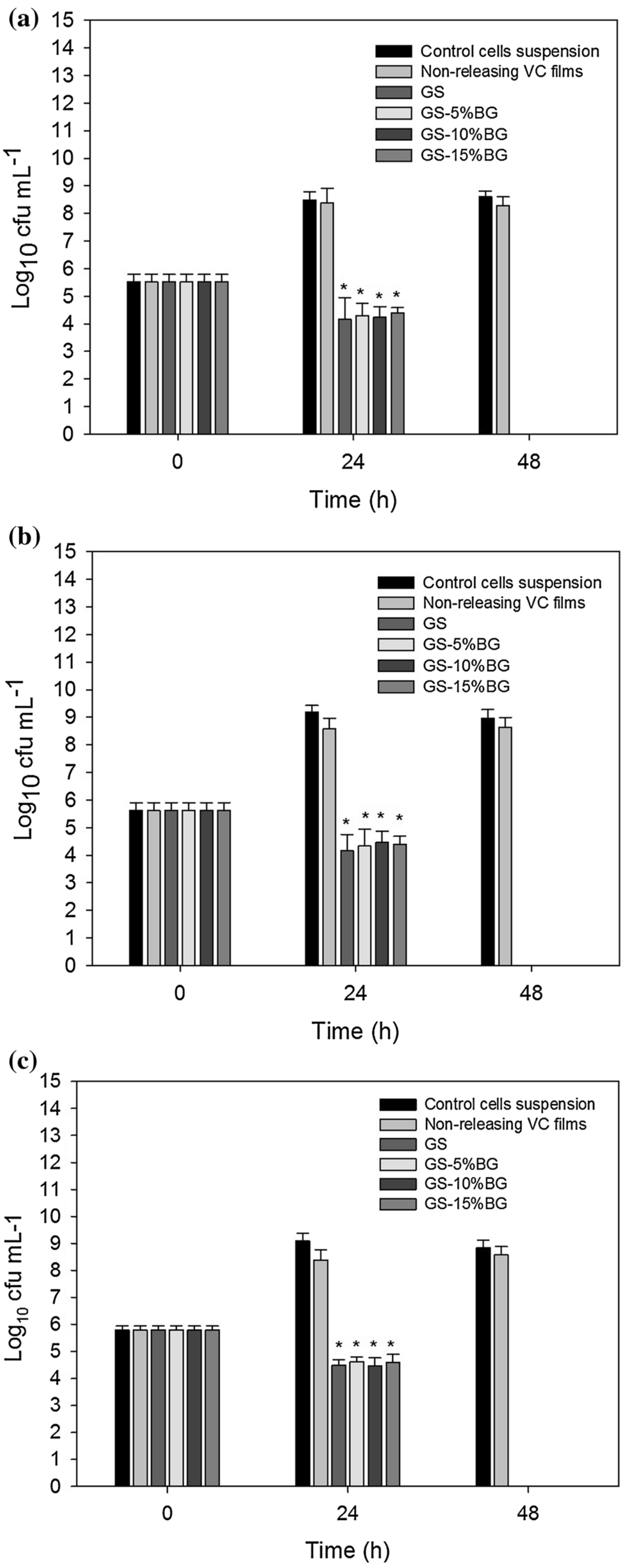
Staphylococcus strain			
Composites	ATCC6538	ATCC29213	ATCC12228
GS	21 ± 2.83	17.5 ± 1.75	18 ± 1.41
GS-BG5%	17.5 ± 0.71	17 ± 2.12	15.25 ± 2.47
GS-BG10%	18 ± 1.41	15 ± 0.71	17 ± 2.43
GS-BG15%	19.75 ± 1.77	14 ± 1.04	15.5 ± 2.12

Viable counts

The initial cell concentrations (expressed as $\log_{10}$ cfu mL^{-1}) were 5.52 ± 0.27 for *S. aureus* ATCC6538, 5.66 ± 0.28 for *S. aureus* ATCC29213, and 5.79 ± 0.14 for *S. epidermidis* ATCC1228 (Fig. 4). No statistical differences were observed in the initial inoculum size between the strains. The composites strongly inhibited the cell viability of the Staphylococcus spp. tested, with similar strength. After 24 h, cell viability was around 4.30 $\log_{10}$ cfu mL^{-1} for *S. aureus* ATCC6538, 4.33 $\log_{10}$ cfu mL^{-1} for *S. aureus* ATCC29213, and 4.50 $\log_{10}$ cfu mL^{-1} for *S. epidermidis*. After 48 h, the cell viability of the three strains was below the detection limits. In terms of antimicrobial activity, the composites presented high antimicrobial activity at 48 h, since growth inhibitions were higher than 3-log reduction.

Cell cytotoxicity

Figure 5 exhibits the viability of MG-63 cells which had been cultured for 24 h in the presence of the composites. The control group consisted of MG-63 cells cultured without addition of composites. Cells incubated in the presence of the composites with or without VC were significantly inhibited. The cytotoxicity was related to the presence of GA and not to



◀**Figure 4** Viable counts of *Staphylococcus aureus* ATCC6538 (**a**), *S. aureus* ATCC29213 (**b**), and *S. epidermidis* ATCC1228 (**c**) in the presence of films. *Asterisk* indicates a statistically significant difference at $p < 0.05$ between VC-releasing films and control cell suspensions at time 0.

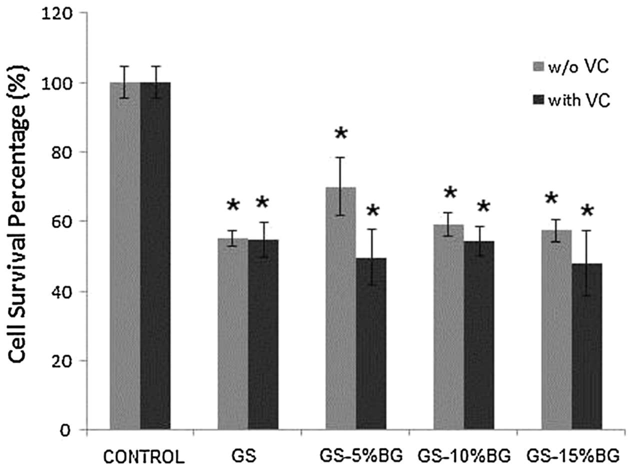


Figure 5 Cell survival percentage of the human osteosarcoma cell line MG63 in the presence of VC-releasing and non-releasing films. *Asterisk* indicates a statistically significant difference at $p < 0.05$ between VC-releasing films and control groups.

the concentration of m-BG or the presence of VC. This result follows from the fact that there were no significant differences between GS films and GS plus m-BG films. In addition, composites with VC and without VC exhibited similar cytotoxicity.

Discussion

Several researchers agree that BG systems including 45S5 composition can modify the release profile of a drug [24–26]. Unfortunately, the biological consequences of this effect have been often overlooked. Here, for the first time, we studied the release profile of a drug (VC), and the water uptake capacity, degradation, in vitro antistaphylococcal effect, and cytotoxicity (on MG-63 osteoblast-like cells) of GS films with increasing concentration of 45S5 m-BG.

When chemical cross-linked biopolymer films are developed for drug delivery applications, a very important parameter should be considered is the water uptake capacity. This parameter will affect not only the film morphology and structure [40] but also the drug release rate in diffusion-controlled systems [41].

In the present study, the water uptake capacity of the polymer was not affected by the presence of m-BG in the polymeric matrix. Although this is in accordance with our previous results [26] and with results of other authors [42], other studies have reported opposite findings [43–45]. These discrepancies can be explained by the nature of the biopolymeric matrix or the BG particle size. More specifically, when 45S5 m-BG nanoparticles are included in hydrophobic polymers, the water uptake capacity is improved [43, 44]. On the other hand, 45S5 BG nanoparticles seem to enhance the water uptake capacity, probably due to the higher time of exposure and larger surface area [45].

Another parameter that should be considered is the degradation behavior of biomaterials in a physiological environment, since this parameter plays an important role in the regeneration of new cells [46]. After implantation, biomaterials interact with the fluids of the tissue and a degradation process starts. The in vitro degradation rate of composite materials is determined as a function of the hydrolysis time in PBS in normal physiological conditions (pH 7.4 at 37 °C). The weight loss increases over the incubation period and with the increasing concentrations of 45S5 m-BG [44, 47, 48]. Nevertheless, some studies have shown that the addition of BGs reduces the degradation rate of biomaterials [49–52]. The proposed explanations for the slower degradation lies on that the BG particles induce a rapid exchange of protons in the water for the alkali in the BG, providing a pH buffering effect that neutralizes the acidic degradation of the by-products produced during hydrolysis, thus preventing the autocatalytic effect [43, 44]. Another possible effect is that the degradation rate decreases because the incorporation of inorganic filler into the polymer matrix decreases the porosity of composites [51]. A decrease in porosity leads to a decrease in the surface area exposed to the medium [52]. In this study, the presence of BG particles could have increased the surface area of the composites and could have accelerated the degradation of GS-BG samples. Indeed, as mentioned in the materials and methods section, 45S5 m-BG samples were previously incubated in PBS before being added to the polymeric matrix to eliminate the increase in pH that could induce cell damage or that could degrade the antibiotic [53]. In that way, the aforementioned pH buffering effect is likely not taking place in the present composites. Finally, it has been proposed that the increased weight loss in the presence of BG could be related to the dissolution of the BG particles, as previously reported for other composites [47, 54, 55].

Contextualizing these results for a potential application of the composites in wound dressing engineering, some considerations can be made. In wound dressing engineering, the choice of a polymer or polymer combination depends on the fact that degradation rate matches the typical wound healing time frame for proper tissue influx. Many factors affect wound healing but, in normal conditions for skin wounds, the expected healing time frame is about 4 weeks [56]. Here, we found that, in general, films containing 45S5 m-BG degraded too fast in relation with the wound healing time frame. Nevertheless, the degradation of biomaterials can be modulated and improved by different techniques like blending [57], incorporation of enzymatic inhibitors [58], or crosslinking [59].

The drug release rate of VC was found to be affected by the presence of m-BG. This modulation depended, in general, on the m-BG concentration. This result is in agreement with previous research data [52, 60]. A possible explanation for this behavior arises from chemical and physical considerations. Chemically, the drug and the biopolymeric matrix may have a greater bounding affinity [52]. Physically, BG particles in the polymeric matrix leave no free space for the antibiotic, promoting the fast release of the drug [52, 61]. This can also be explained by the increase in the surface area of the composite by the addition of m-BG, as previously discussed [62].

Both the water uptake capacity and the degradation rate also play important roles in the release kinetics of a drug [60]. If water uptake is higher, the matrix would be more openly spaced and thus easier for drugs to diffuse out. Material degradation also offers relatively large open channels, thus accelerating drug diffusion [60]. Since the addition of m-BG accelerates the degradation of composite films, this effect could explain the results found here.

The treatment of the agar plates with drug-loaded composite films led to obvious inhibition zones. The inhibition zones were not affected by the m-BG concentration. On the other hand, there were no inhibition zones in films not releasing VC, which indicates the lack of bactericidal activity in the absence of the antibiotic. This result is relevant since previous research has reported antibacterial effects of 45S5 BG per se [63–66]. Here, the antibacterial effects depended on the release of VC. The plate count method showed that the composites strongly inhibited the growth of staphylococcal cells in SWF. The exposure of the three staphylococcal strains tested to composites after 48 h resulted in a reduction of the total viable count. Interestingly, the composite exhibited similar antibacterial strength. This means that the greater concentration of VC released by the GS-BG composites (Fig. 3) does not indicate an improvement in their antibacterial effects. This phenomenon, where the drug concentration is not related to the antibacterial strength, is in agreement with our previous results [26, 39] and with those of other authors with related biomaterials [67].

In the context of wound regeneration, the release of a drug to a wound depends on the condition of the wound [68]. Often, a burst release is considered as a negative consequence of long-term controlled release systems [69]. Nevertheless, high initial delivery rates or rapid release may sometimes be desirable [69] and should be considered in the immediate performance of dressings [68]. A high burst release followed by a decreased release over several days may allow a robust response and eliminate large numbers of bacteria, and can therefore be suitable in cases of developing infection. In a wound, it is commonly accepted that a concentration of bacteria above 10^5 cfu mL^{-1} determines an infection [70, 71]. In this context, the presence of m-BG on films led to an abrupt initial burst of VC release, which is suitable to control the large number of bacteria present in infected wounds.

To complete the results of bacterial inhibition, the composites should be non-toxic to mammalian cells. Biomaterials may be toxic to cells due to the dressing material itself, the material processing or the incorporation of antimicrobials [68]. Here, the results obtained by the MTT assay (Fig. 5) showed that the composites exerted some cytotoxicity. Nevertheless, this cytotoxicity was related to the presence of GA but not to the concentration of m-BG or to the amount of VC released to the medium. This finding supports the idea of BGs as promising materials for soft tissue engineering and wound healing [31], since the presence of BGs can impart superior functionalities to a biopolymeric film than the biopolymeric matrix by itself.

As mentioned above, some of the limitations of biomaterials, such as the lack of adequate mechanical properties, can be overcome by crosslinking [59]. According to other authors, the cytotoxicity of GA depends on the concentration used, and up to 8 % GA has been found to be non-cytotoxic [72]. In this work, 0.25 % GA resulted in cytotoxicity to human osteoblast-like cells. Nevertheless, this kind of limitation can be overcome using other crosslinking agents like genipin, which has been demonstrated to be much less cytotoxic [71, 73].

Conclusions

The present research demonstrated that the presence of 45S5 m-BG in gelatin–starch films can modulate a number of physicochemical parameters of relevance in drug delivery system engineering. In particular, the degradation and VC release rates can be affected by increasing concentrations of 45S5 m-BG, but the water uptake capacity of GS films does not depend on the BG content. These modifications did not lead to changes in the antibacterial effects of the composites or to cytotoxicity effects. Future research should focus on finding a proper cross-linker agent for gelatin and investigating the biological response of composites in relation to wound healing.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

References

[1] Park K (2014) Controlled drug delivery systems: past forward and future back. J Control Release 190:3–8

[2] Huang CL, Steele TWJ, Widjaja E, Boey FYC, Venkatraman SS, Loo JSC (2013) The influence of additives in modulating drug delivery and degradation of PLGA thin films. NPG Asia Mater 5:e54

[3] Bowling FL, Jude EB, Boulton AJ (2009) MRSA and diabetic foot wounds: contaminating or infecting organisms? Curr Diabetes Rep 9:440–444

[4] Bertesteanu S, Triaridis S, Stankovic M, Lazar V, Chifiriuc MC, Vlad M, Grigore R (2014) Polymicrobial wound infections: pathophysiology and current therapeutic approaches. Int J Pharm 463:119–126

[5] Zafar N, Fessi H, Elaissari A (2014) Cyclodextrin containing biodegradable particles: from preparation to drug delivery applications. Int J Pharm 461:351–366

[6] Ivanova P, Bazaka L, Crawford RJ (eds) (2014) New functional biomaterials for medicine and healthcare, Chapter 2. Woodhead Publishing, Oxford. ISBN 9781782422655

[7] Chiono V, Pulieri E, Vozzi G, Ciardelli G, Ahluwalia A, Giusti P (2008) Genipin-crosslinked chitosan, gelatin blends for biomedical applications. J Mater Sci Mater Med 19:889–898

[8] Santoro M, Tatara AM, Mikos AG (2014) Gelatin carriers for drug and cell delivery in tissue engineering. J Control Release 190:210–218

[9] Gaowa A, Horibe T, Kohno M, Sato K, Harada H, Hiraoka M, Tabata Y, Kawakami K (2014) Combination of hybrid peptide with biodegradable gelatin hydrogel for controlled release and enhancement of anti-tumor activity in vivo. J Control Release 176:1–7

[10] Foox M, Zilberman M (2015) Drug delivery from gelatin-based system. Expert Opin Drug Deliv 5:1–17

[11] Patel ZS, Ueda H, Yamamoto M, Tabata Y, Mikos AG (2008) In vitro and in vivo release of vascular endothelial growth factor from gelatin microparticles and biodegradable composite scaffolds. Pharm Res 25:2370–2378

[12] Young S, Wong M, Tabata Y, Mikos AG (2005) Gelatin as a delivery vehicle for the controlled release of bioactive molecules. J Control Release 109:256–274

[13] Su Y, Mo X (2011) Genipin crosslinked gelatin nanofibers for tissue engineering. J Control Release 152(Suppl1):e230–e232

[14] Amadori S, Torricelli P, Rubini K, Fini M, Panzavolta S, Bigi A (2015) Effect of sterilization and crosslinking on gelatin films. J Mater Sci Mater Med 26:69

[15] Leng YG, Huang YQ, Dong CL, Huang MZ (2002) Research of grafting acrylamide on gelatin. Polym Mater Sci Eng 18:93–97

[16] Shalumon KT, Deepthi S, Anupama MS, Nair SV, Jayakumar R, Chennazhi KP (2015) Fabrication of poly (L-lactic acid)/gelatin composite tubular scaffolds for vascular tissue engineering. Int J Biol Macromol 72:1048–1055

[17] Xue J, Zhong Q (2014) Blending lecithin and gelatin improves the formation of thymol nanodispersions. J Agric Food Chem 62:2956–2962

[18] Kowalczyk D, Kordowska-Wiater M, Nowak J, Baraniak B (2015) Characterization of films based on chitosan lactate and its blends with oxidized starch and gelatin. Int J Biol Macromol 77:350–359

[19] Lu DR, Xiao CM, Xu SJ (2009) Starch-based completely biodegradable polymer materials. Express Polym Lett 6:366–375

[20] Vieira AP, Ferreira P, Coelho JF, Gil MH (2008) Photocrosslinkable starch-based polymers for ophthalmologic drug delivery. Int J Biol Macromol 43:325–332

[21] Chen L, Li X, Li L, Guo S (2007) Acetylated starch-based biodegradable materials with potential biomedical potential applications as drug delivery systems. Curr Appl Phys 7S1:e90–c93

[22] Fakhouri FM, Costa D, Yamashita F, Martelli SM, Jesus RC, Alganer K, Collares-Queiroz FP, Innocentini-Mei LH (2013) Comparative study of processing methods for starch/gelatin films. Carbohydr Polym 95:681–689

[23] Phromsopha T, Baimark Y (2014) Preparation of starch/gelatin blend microparticles by a water-in-oil emulsion method for controlled release. Drug Deliv Int J Biomater 2014:829490

[24] Li W, Ding Y, Rai R, Roether JA, Schubert DW, Boccaccini AR (2014) Preparation and characterization of PHBV microsphere/45S5 bioactive glass composite scaffolds with vancomycin releasing function. Mater Sci Eng C Mater Biol Appl 41:320–328

[25] Olalde B, Garmendia N, Sáez-Martínez V, Argarate N, Nooeaid P, Morin F, Boccaccini AR (2013) Multifunctional bioactive glass scaffolds coated with layers of poly(D, L-lactide-*co*-glycolide) and poly(*n*-isopropylacrylamide-*co*-acrylic acid) microgels loaded with vancomycin. Mater Sci Eng C Mater Biol Appl 33:3760–3767

[26] Rivadeneira J, Di Virgilio AL, Audisio MC, Boccaccini AR, Gorustovich AA (2015) Evaluation of the antibacterial effects of vancomycin hydrochloride released from agar–gelatin–bioactive glass composites. Biomed Mater 10:015011

[27] Cabanas MV, Pena J, Roman J, Vallet-Regi M (2009) Tailoring vancomycin release from beta-TCP/agarose scaffolds. Eur J Pharm Sci 37:249–256

[28] Rezwan K, Chen QZ, Blaker JJ, Boccaccini AR (2006) Biodegradable and bioactive porous polymer/inorganic composite scaffolds for bone tissue engineering. Biomaterials 27:3413–3431

[29] Hench LL (2009) Genetic design of bioactive glass. J Eur Ceram Soc 7:1257–1265

[30] Rahman MN, Day DE, Bal BS, Fu Q, Jung SB, Bonewald LF, Tomsia AP (2011) Bioactive glass in tissue engineering. Acta Biomater 7:2355–2373

[31] Miguez-Pacheco V, Hench LL, Boccaccini AR (2015) Bioactive glasses beyond bone and teeth: emerging applications in contact with soft tissues. Acta Biomater 13:1–15

[32] Gorustovich AA, Roether JA, Boccaccini AR (2010) Effect of bioactive glasses on angiogenesis: a review of in vitro and in vivo evidences. Tissue Eng B 16:199–207

[33] Day RM (2005) Bioactive glass stimulates the secretion of angiogenic growth factors and angiogenesis in vitro. Tissue Eng 11:768–777

[34] Leach JK, Kaigler D, Wang Z, Krebsbach PH, Mooney DJ (2006) Coating of VEGF-releasing scaffolds with bioactive

glass for angiogenesis and bone regeneration. Biomaterials 27:3249–3255

[35] Yu H, Peng J, Xu Y, Chang J, Li H (2016) Bioglass activated skin tissue engineering constructs for wound healing. ACS Appl Mater Interfaces 8:703–715

[36] Lin C, Mao C, Zhang J, Li Y, Chen X (2012) Healing effect of bioactive glass ointment on full-thickness skin wounds. Biomed Mater 7:045017

[37] Bigi A, Cojazzi G, Panzavolta S, Rubini K, Roveri N (2001) Mechanical and thermal properties of gelatin films at different degrees of glutaraldehyde crosslinking. Biomaterials 2001(22):763–768

[38] Gallant-Behm CL, Yin HQ, Liu S, Heggers JP, Langford RE, Olson ME, Hart DA, Burrell RE (2005) Comparison of in vitro disc diffusion and time kill-kinetic assays for the evaluation of antimicrobial wound dressing efficacy. Wound Repair Regen 13:412–421

[39] Rivadeneira J, Di Virgilio AL, Audisio MC, Boccaccini AR, Gorustovich AA (2014) Evaluation of antibacterial and cytotoxic effects of nano-sized bioactive glass/collagen composites releasing tetracycline hydrochloride. J Appl Microbiol 116:1438–1446

[40] Thakur G, Mitra A, Basak A, Sheet D (2012) Characterization and scanning electron microscopic investigation of crosslinked freeze dried gelatin matrices for study of drug diffusivity and release kinetics. Micron 43:311–320

[41] Coimbra P, Gil MH, Figueiredo M (2014) Tailoring the properties of gelatin films for drug delivery applications: influence of the chemical cross-linking method. Int J Biol Macromol 70:10–19

[42] Mota J, Yu N, Caridade SG, Luz GM, Gomes ME, Reis RL, Jansen JA, Walboomers XF, Mano JF (2012) Chitosan/bioactive glass nanoparticle composite membranes for periodontal regeneration. Acta Biomater 8:4173–4180

[43] Maquet V, Boccaccini AR, Pravata L, Notingher I, Jérôme R (2003) Preparation, characterization, and in vitro degradation of bioresorbable and bioactive composites based on Bioglass filled polylactide foams. J Biomed Mater Res A 66A:335–346

[44] Maquet V, Boccaccini AR, Pravata L, Notingher I, Jérôme R (2004) Porous poly(alphahydroxyacid)/bioglass composite scaffolds for bone tissue engineering: I. Preparation and in vitro characterization. Biomaterials 25:4185–4194

[45] Misra SK, Mohn D, Brunner TJ, Stark WJ, Philip SE, Roy I, Salih V, Knowles JC, Boccaccini AR (2008) Comparison of nanoscale and microscale bioactive glass on the properties of P(3HB)/bioglass composites. Biomaterials 29:1750–1761

[46] Grover CN, Cameron RE, Best SM (2012) Investigating the morphological, mechanical and degradation properties of scaffolds comprising collagen, gelatin and elastin for use in soft tissue engineering. J Mech Behav Biomed Mater 10:62–74

[47] Misra SK, Ansari T, Mohn D, Valappil SP, Brunner TJ, Stark WJ, Roy I, Knowles JC, Sibbons PD, Jones EV, Boccaccini AR, Salih V (2010) Effect of nanoparticulate bioactive glass particles on bioactivity and cytocompatibility of poly(3-hydroxybutyrate) composites. J R Soc Interface 7:453–465

[48] Larrañaga A, Aldazabal P, Martin FJ, Sarasua JR (2014) Hydrolytic degradation and bioactivity of lactide and caprolactone based sponge-like scaffolds loaded with bioactive glass particles. Polym Degrad Stab 110:121–128

[49] Boccaccini AR, Maquet V (2003) Bioresorbable and bioactive polymer/bioglass composites with tailored pore structure for tissue engineering applications. Compos Sci Technol 63:2417e29

[50] Blaker J, Nazhat SN, Maquet V, Boccaccini AR (2011) Long-term in vitro degradation of PDLLA/bioglass bone scaffolds in a cellular simulated body fluid. Acta Bio-mater 7:829e40

[51] Yao Q, Nooeaid P, Roether JA, Dong Y, Zhang Q, Boccaccini AR (2013) Bioglass -based scaffolds incorporating polycaprolactone and chitosan coatings for controlled vancomycin delivery. Ceram Int 39:7517–7522

[52] Mabrouk M, Mostafa AA, Oudadesse H, Mahmoud AA, El-Gohary MI (2014) Effect of ciprofloxacin incorporation in PVA and PVA bioactive glass composite scaffolds. Ceram Int 40:4833–4845

[53] Das Gupta V, Stewart KR, Nohria S (1986) Stability of vancomycin hydrochloride in 5 % dextrose and 0.9 % sodium chloride, injections. Am J Hosp Pharm 43:1729–1731

[54] Li H, Chang J (2005) In vitro degradation of porous degradable and bioactive PHBV/wollastonite composite scaffolds. Polym Degrad Stabil 87:301–307

[55] Loher S, Reboul V, Brunner TJ, Simonet M, Dora C, Neuenschwander P, Stark WJ (2006) Improved degradation and bioactivity of amorphous aerosol derived tricalcium phosphate nanoparticles in poly(lactide-co-glycolide). Nanotechnology 17:2054–2061

[56] Guo S, DiPietro LA (2010) Factors affecting wound healing. J Dent Res 89:219–229

[57] Roh D-H, Kang S-Y, Kim J-Y, Kwon Y-B, Kweon HY, Lee K-G et al (2006) Wound healing effect of silk fibroin/alginate-blended sponge in full thickness skin defect of rat. J Mater Sci Mater Med 17:547–552

[58] Vasconcelos A, Pêgo AP, Henriques L, Lamghari M, Cavaco-Paulo A (2010) Protein matrices for improved wound healing: elastase inhibition by a synthetic peptide model. Biomacromolecules 11:2213–2220

[59] Reddy N, Reddy R, Jiang Q (2015) Crosslinking biopolymers for biomedical applications. Trends Biotechnol 33:362–369

[60] Kouhi M, Morshed M, Varshosaz J, Fathi MH (2013) Poly(e-caprolactone) incorporated bioactive glass nanoparticles and simvastatin nanocomposite nanofibers: preparation, characterization and in vitro drug release for bone regeneration applications. Chem Eng J 228:1057–1065

[61] Wang Q, Du YZ, Kennedy J (2007) Controlled release of ciprofloxacin hydrochloride from chitosan/polyethylene glycol blend films. Carbohydr Polym 69:336–343

[62] Li W, Ding Y, Rai R, Roether JA, Schubert DW, Boccaccini AR (2014) Preparation and characterization of PHBV microsphere/45S5 bioactive glass composite scaffolds with vancomycin releasing function. Mater Sci Eng C 41:320–328

[63] Allan I, Newman H, Wilson M (2001) Antibacterial activity of particulate bioglass against supra- and subgingival bacteria. Biomaterials 22:1683–1687

[64] Waltimo T, Brunner TJ, Vollenweider M, Stark WJ, Zehnder M (2007) Antimicrobial effect of nanometric bioactive glass 45S5. J Dent Res 86:754–757

[65] Hu S, Chang J, Liu M, Ning C (2009) Study on antibacterial effect of 45S5 bioglass. J Mater Sci Mater Med 20:81–86

[66] Rivadeneira J, Audisio MC, Boccaccini AR, Gorustovich AA (2013) In vitro antistaphylococcal effects of a novel 45S5 bioglass/agar–gelatin biocomposite films. J Appl Microbiol 115:604–612

[67] Dashti A, Ready D, Salih V, Knowles JC, Barralet JE, Wilson M, Donos N, Nazhat SN (2010) In vitro antibacterial efficacy of tetracycline hydrochloride adsorbed onto Bio-Oss bone graft. J Biomed Mater Res B Appl Biomater 93:394–400

[68] Elsner JJ, Berdicevsky I, Zilberman M (2011) In vitro microbial inhibition and cellular response to novel biodegradable composite wound dressings with controlled release of antibiotics. Acta Biomater 7:325–336

[69] Huang X, Brazel CS (2001) On the importance and mechanisms of burst release in matrix-controlled drug delivery systems. J Control Release 73:121–136

[70] Robson MC, Mannari RJ, Smith PD, Payne WG (1999) Maintenance of wound bacterial balance. Am J Surg 178:399–402

[71] Edwards R, Harding KG (2004) Bacteria and wound healing. Curr Opin Infect Dis 17:91–96

[72] Umashankar PR, Mohanan PV, Kumari TV (2012) Glutaraldehyde treatment elicits toxic response compared to decellularization in bovine pericardium. Toxicol Int 1:51–58

[73] Bigi A, Cojazzi G, Panzavolta S, Roveri N, Rubini K (2002) Stabilization of gelatin films by crosslinking with genipin. Biomaterials 23:4827–4832