Biomed. Glasses 2015; 1:128–135 DE GRUYTER OPEN

Research Article Open Access

Josefina Rivadeneira*, Gisela M. Luz, M. Carina Audisio, João F. Mano, and Alejandro A. Gorustovich

Novel antibacterial bioactive glass nanocomposite functionalized with tetracycline hydrochloride

DOI 10.1515/bglass-2015-0012
Received Aug 27, 2015; revised Aug 27, 2015; accepted Sep 02, 2015

Abstract: To prevent the high frequency of wound infections, anti-bacterial agents can be loaded onto composites. In the present study, the antibiotic tetracycline hydrochloride (TC) was incorporated, for the first time, in collagen type I membranes coated with nano-sized SiO_2-CaO-P_2O_5 bioactive glass (n-BG) obtained by a sol-gel chemical route.

Collagen membranes coated with n-BG were immersed in simulated body fluid (SBF) containing 0.25, 0.75 or 1.25 mg mL^{-1} of TC for 48 h at 37°C following a co-precipitation method. The antibiotic was released in distilled water at 37°C for up to 72 h. The antibacterial activity of the composites was evaluated *in vitro* by the inhibition zone test and plate count method. Two different *Staphylococcus aureus* strains, *S. aureus* ATCC29213 and *S. aureus* ATCC25923, were exposed to the biomaterials. The results showed that the incorporation but not the release of TC was dependent on the initial concentration of TC in SBF. The biomaterials inhibited *S. aureus* growth, although the efficacy was similar for all the concentrations. The results allow us to conclude that the new composite could have potential in the prevention of wound infections.

Keywords: Tetracycline hydrochloride; bioactive glass; collagen; Staphylococcus

1 Introduction

Antibiotic release systems such as polymeric matrices have become some of the most promising strategies for the prophylaxis and management of different kinds of wound infections (i.e. burns, pressure ulcers, post-surgical ulcers) [1–8]. However, the *in vivo* degradation process by proteases makes polymeric matrices prone to rapidly release the encapsulated drug, thus preventing long-term maintenance of therapeutic levels [9]. One alternative way to modulate antibiotic release over an extended period of time is through covalent binding of the drug so that sustained release may be maintained by the rate of biodegradation. Some authors have explored this possibility by following the co-precipitation process [10, 11].

Bioactive glasses (BGs) have started to be considered as carriers for different drugs [12] because they have been shown to be biocompatible and bioresorbable with a controllable degradation and resorption process [13]. Compared with micro-sized BGs, nano-sized BGs (n-BGs) have many advantages when polymeric-bioceramic composites are synthesized. The use of n-BGs on a polymeric matrix allows a more homogeneous distribution of particles within the polymeric matrix and many more particles for the same equivalent weight of carriers. In addition, it allows an enhanced performance of the mechanical properties of composites and enhanced [14].

In the frame of soft tissue regeneration, it has been shown that n-BGs prepared using the sol-gel method could promote the stiffness and elongation of endothelial cells and the formation of endothelial networks *in vitro* [15]. Therefore, we hypothesized that such inorganic biomaterials could facilitate the vascularization *in vivo*, which could

***Corresponding Author: Josefina Rivadeneira:** 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; Email: jrivadeneira@conicet.gov.ar; Tel.: +54 387 4268-587/85/8539
Gisela M. Luz, João F. Mano: 3B's Research Group-Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, P-4806-909 Taipas, Guimarães, Portugal; ICVS/3B's Associate Laboratory, Braga/Guimarães, Portugal
M. Carina Audisio: 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
Alejandro A. Gorustovich: 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

be relevant for the envisaged application of the devices developed in this work. Histological examination showed that n-BG can accelerate the recovery of skin wounds in both normal and diabetes-impaired healing models [16].

Tetracycline hydrochloride (TC) is a broad-spectrum antibiotic that inhibits protein synthesis [17]. TC is also considered an option to treat problems of skin and soft tissue infections (SSTIs) caused by methicillin-resistant *Staphylococcus aureus* (MRSA) [18, 19] and even considered the most efficacious and cost-effective oral treatment for MRSA [20].

This is relevant considering that the most common pathogens that cause SSTIs are streptococci and *S. aureus* [6, 21]. *In vivo* results show that TC coupled to BGs has no additional irritant effect that could prevent its usage in association with BGs [22].

On the other hand, it has been convincingly demonstrated that TC binds to synthetic crystals of apatite, especially during their formation *in vitro* [23, 24]. This implies that the antibiotic can co-precipitate with calcium on a polymeric matrix during the bioactivity process of BG incubated in simulated body fluid (SBF).

Many researchers have encapsulated TC into BGs prepared by the sol-gel method during their synthesis at room temperature [22, 25–27]. Nevertheless, biodegradable polymers plus n-BG as matrix for the release of TC have been poorly explored. Also, antibacterial effects are sometimes overlooked.

On the other hand, it has been shown that the TC release behavior changes as the BG composition changes [28].

We have previously studied the incorporation, TC release and antibacterial effects of collagen type I composites coated with 45S5 n-BG [29]. In this work, we incorporated three different initial concentrations of TC (0.25, 0.75 and 1.5 mg mL^{-1}) onto commercial type I collagen membranes coated with nano-sized $SiO_2CaOP_2O_5$ BG, quantified the incorporation and TC release, and investigated its antibacterial effects on *S. aureus* ATCC29213 and *S. aureus* ATCC25923.

2 Methods

2.1 Materials

Bioactive glass nanoparticles (n-BG, 20–30 nm) with the formulation SiO_2:CaO:P_2O_5 (mol.%) = 55:40:5 were developed by sol-gel deposition [30]. Type I collagen bovine membranes (100 μm thick) were supplied by Laboratorio Celina (Buenos Aires, Argentina). TC in powder form (Sigma-Aldrich) was used. Hank's balanced saline solution (HBSS) without Ca^{2+} and Mg^{+} was obtained from Life Technologies (Carlsbad, CA, USA).

2.2 Preparation of composites

A total of 100 mg of n-BG was used to coat type I collagen bovine membranes (9 × 9 cm^2). Each time, 10 mg of n-BG was sonicated for 5 minutes in 20 mL of isopropanol and then aliquots of 3 mL were used to coat the membranes and left to dry in air at room temperature. At the end of the process, the samples obtained were 3 × 3 cm^2. The samples were sterilized by Gamma-irradiation. Gamma-irradiation sterilization was achieved at a dose of 2.5 Mrad ^{60}Co at room temperature.

2.3 Surface analysis of the samples

The materials obtained were morphologically characterized by scanning electron microscopy (SEM). To this end, biomaterials were fixed with a 2.5% glutaraldehyde 0.1 M phosphate buffered saline (PBS) solution overnight at 4°C. The samples were then washed with distilled water and sequentially dehydrated through a graded series of ethanol solutions. After mounting on stubs and gold sputtering, the samples were examined by SEM (JSM 6480 LV, JEOL Ltd, Tokyo, Japan).

2.4 TC incorporation

First, a calibration curve was performed by measuring UV absorbance of TC solutions ranging from 0.01 to 0.1 mg mL^{-1} at 350 nm (UV-Vis, Varian 500SCAN) [24]. TC was then incorporated at 37°C in SBF, which was prepared following Kokubo's protocol [31]. The initial antibiotic concentrations were 0.25, 0.75 and 1.50 mg mL^{-1}. Each n-BG-coated membrane was incubated for 48 h in 25 mL of SBF supplemented with the above concentrations of TC. In that way, the antibiotic co-precipitated and bound to n-BG particles. The absorbance of aliquots of the TC-SBF solution was measured at different times. The initial volume of the solution was maintained by replacing the amount extracted from a solution made at the start of the experiments and maintained in similar conditions. At the end of the uptake, the samples, from now on TC-composites, were washed in distilled water and allowed to dry at 25°C. The concentrations of TC incorporated were calculated by

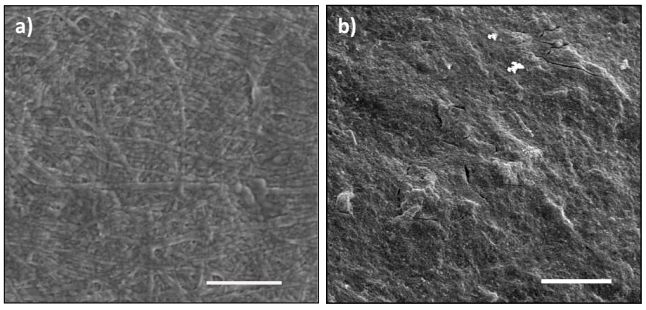

Figure 1: SEM images. Uncoated type I collagen membrane a) glass-coated collagen membrane exposed to simulated body fluid (SBF) for 48 h b). The bars represent 5 µm.

subtracting the remaining TC concentration at the different time points from the initial concentration of the immersion solutions.

The composites obtained using the above coating process were named as: CTC-025, CTC-075 and CTC-150 respectively, according to the initial concentration of TC in SBF.

2.5 Release of TC

TC release was evaluated in distilled water. TC-composites were soaked in 25 mL of distilled water at 37°C in static conditions for 72 h. TC release was calculated by measuring the UV absorbance of 4 mL extracted at regular time intervals, which was then placed back into the solution. Tests were performed in triplicate, calculating the means and standard deviations (SD).

2.6 Bacterial culture and preparation of inoculum

The following strains were used in this study: *S. aureus* ATCC29213 and *S. aureus* ATCC25923. Both strains were grown in Mueller Hinton broth (Britania S.A., Argentina) at 37°C for 24 h. For the experiments, bacterial cell suspensions were adjusted to 6–7 log cfu mL^{-1}.

2.7 Antibacterial efficacy

The performance of samples was evaluated by the inhibition zone test and the plate count method. For the inhibition zone test, 100 µL of the described suspension was seeded on Mueller-Hinton agar plates on which samples were then placed with the coated and treated surface in contact with the plate. TC unloaded samples were used as controls.

After 24 h incubation, inhibition zones were observed as a halo around samples where bacteria had not grown. The area of the inhibition zones was measured in mm. All tests were performed in duplicate and the means and SD were calculated.

For the plate count method, bacterial cell suspensions were diluted to about 6 log cfu mL^{-1}.The experiments were carried out in HBSS without Ca^{2+} and Mg^{+}. The TC-composite samples were incubated for 48 h at 37°C in 25 mL of cell suspensions. Staphylococcus suspensions in the absence of biomaterial served as controls. Samples were collected after 24 and 48 h of incubation and the viability of cells at 37°C was assessed by counting in Mueller-Hinton agar plates. After incubation, composites were prepared for SEM observation. The results are expressed as log cfu mL^{-1} ± SD. All tests were performed in triplicate and the means and SD were calculated.

2.8 Statistical analysis

Statistical analysis was performed using the SPSS 15.0 statistical package software with appropriate statistical tests such as one-way analysis of variance (ANOVA) with Tukey's multiple comparison post-tests for inter-group analysis. The level of significance was set at a p value of < 0.05.

3 Results and Discussion

In this work, TC was incorporated onto collagen membranes coated with nano-sized SiO_2-CaO-P_2O_5 BG, following a co-precipitation method. Composites were incubated in SBF with different TC concentrations to promote the drug deposition considering the ability of TC to chelate calcium. This kind of methodology for antibiotic deposition has been previously reported elsewhere [10, 11, 32].

Figure 1 shows SEM micrographs of the surfaces of an uncoated type I collagen membrane (a) and a glass-coated collagen membrane exposed to SBF containing TC for 48 h (b). The collagen membrane presents randomly arranged collagen fibrils forming a dense fibrous network. The presence of n-BG plus TC introduced a continuous coating with pits and irregularities. This kind of morphology may influence the release behavior of the drug, as we will discuss later.

The TC uptake values for TC-composites are presented in Figure 2. The assay was performed for 3, 12, 24 and 48 h. The majority of TC incorporation occurred within the

Table 1: Summary of the incorporation and release behavior of tetracycline hydrochloride (TC).

Initial concentration (mg mL^{-1})	% incorporation at 48 h	Incorporation at 48 h (mg cm^2)	% release at 72 h	Release at 72 h (µg cm^2)
0.25	49.99 ± 2.96	0.38 ± 0.02	14.41 ± 2.00	56.07 ± 1.52
0.75	45.69 ± 8.00	0.94 ± 0.16	5.30 ± 0.50	50.10 ± 4.77
1.50	41.94 ± 6.40	1.83 ± 0.63	2.90 ± 0.98	53.14 ± 3.65

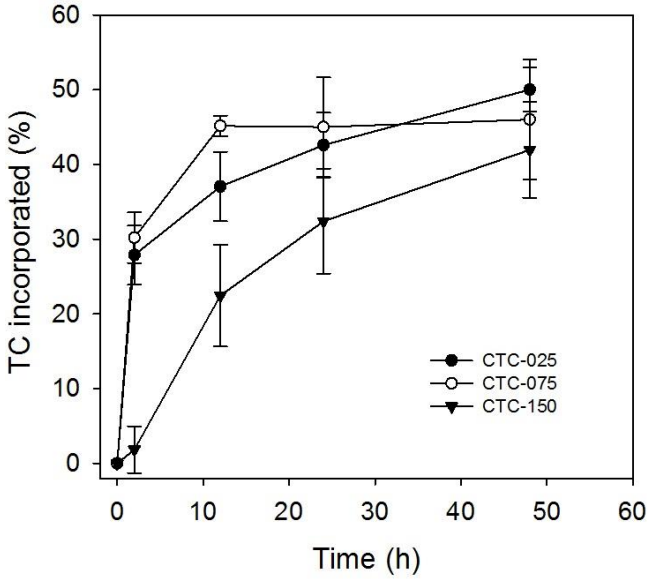

Figure 2: Drug incorporation efficiencies as a function of time of composites in TC solutions with different TC concentrations. The data are expressed as the mean ± standard deviation; n = 3.

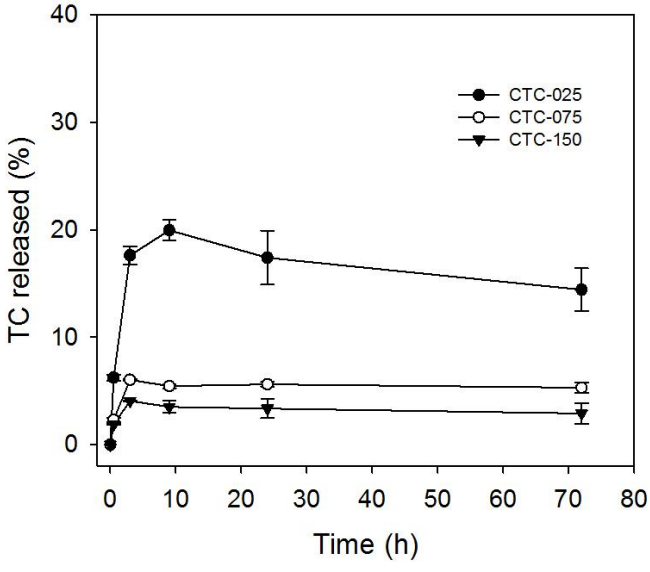

Figure 3: TC release profile from composites as a function of time, with initial TC concentrations of 0.25, 0.75, and 1.50 mg mL^{-1}. The data are expressed as the mean ± standard deviation.

first 12 h. At this time, the percentage incorporation decreased as the TC concentration in SBF increased. However, at 48 h, the percentages of incorporation were similar for the three conditions (already 50%). These results contrast with our previous research work using 45S5 bioglass nanoparticles, where the highest concentration of TC used (0.035 mg mL^{-1}) led to a higher percentage of TC deposition in collagen membranes [29]. Nevertheless, the extent of incorporation in absolute terms was dependent on the initial concentration of the drug. Therefore, while CTC-1.5 incorporated the highest absolute amount of TC, the percentage of incorporation was similar. This behavior is in accordance both with our previous findings [29] and with those of other authors [10, 24, 32].

Figure 3 shows the TC release behavior from the TC-composites as a function of time up to 72 h of incubation. The release behavior, defined by an initial rapid release rate within the first 6 h followed by a stage of zero order release (constant release rate) that lasted until the end of the experiment, was similar for all composites. The release percentage was inversely related to the initial TC concentration because a greater percentage of TC release was observed for CTC-025. However, the amount of TC released was similar for the three conditions. Table 1 summarizes the adsorption and release behavior of TC-composites.

The theoretical analysis and models of drug release used to describe the release from polymers can also be applied for bioceramics [33]. The burst effects were typical of diffusion controlled systems and observed for other related systems releasing TC [22, 25, 26, 28]. The constant release rate presumably involved degradation of the protein matrix combined with diffusion of the remaining drug that was more firmly attached to the n-BG [34]. When the percentage of cumulative release tends to decrease, like in the case of CTC-025, a phenomenon of re-adsorption takes place. This has been reported previously by other authors [11, 24] and is due to the binding of calcium ions and the TC released, which promotes the re-precipitation on collagen membranes. We have previously observed a similar phenomenon [29].

Figure 4 summarizes the model of drug incorporation proposed. Chemical incorporation occurred probably due

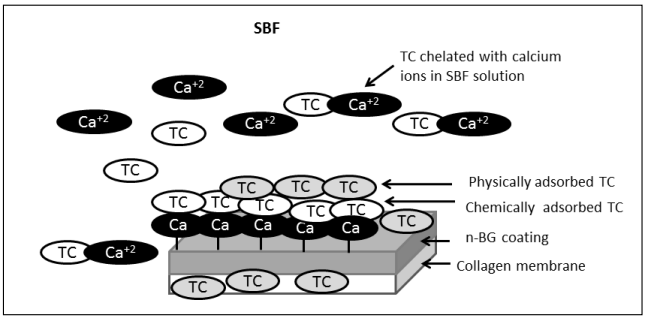

Figure 4: Proposed scheme of different types of TC incorporated on composites during incubation on SBF solution.

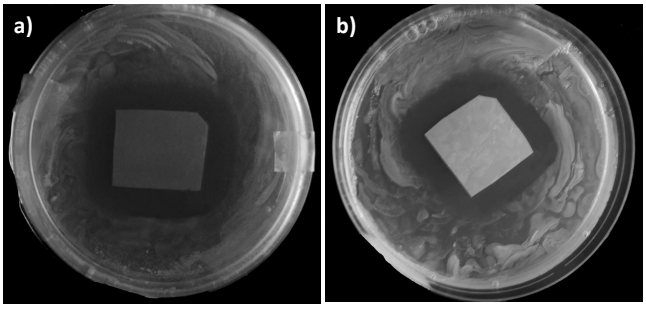

Figure 5: Inhibition zones around composites. *S. aureus* ATCC25923 (a), *S. aureus* ATCC29213 (b).

to the chelation of calcium ions [35, 36]. In line with previous findings [24], chemical incorporation is also confirmed by the change in color of membranes from yellow to brown, which may have been due to the gradual photodegradation of TC. The chelation between TC and calcium in SBF solution, observed as a precipitate, may compete with the chelation between TC and calcium in the membrane. These kinds of interactions increase with drug concentration in the loading solution and interfere with TC deposition and finally influence the release behavior [37].

The greater percentage of TC released when the samples were incubated with the lowest TC concentration (CTC-025) is in agreement both with other works [38] and our previous results [29]. It has been proposed that when a scaffold is immersed in a dilute drug solution, fewer drug molecules will enter the pores, and hence, the pore channels of the scaffolds will contain fewer drug molecules [38]. A low drug entrapment will also result in the formation of thinner films at the scaffold-dissolution medium interface according to basic mass transfer phenomena. These conditions probably result in the reduced resistance to the diffusion of drug molecules, which favour faster drug release. In other words, a decrease in the concentration of the drug in the loading solution lowered the drug entrapment in the scaffold. Although we did not work with an n-BG scaffold, the coated process on collagen membranes created morphological irregularities in the composite surfaces, including pores. In that sense, a similar phenomenon could explain the results obtained in this work.

A clear difference with our previous work using 45S5 n-BG can be highlighted. Although the TC initial concentrations were not the same, the initial drug concentration of 0.25 mg mL^{-1} can be used to compare the results of both works. So we will discuss the result of this concentration in the frame of comparison with the previous research work. The concentrations of TC incorporated per cm^2 were similar but the release times were different. The rapid release of TC resulted in a shorter time in this work than that found in our previous research. A possible explanation is that sol-gel glasses tend to have an inherent nanoporosity [39] that increases their specific surface area [40]. In the view that the kinetics of antibiotic release are initially controlled to some extent by surface phenomena [41], a larger area increase the area for release and could explain the discrepancies found. If this is right, it can be concluded that adjusting the BG system, the initial release rate can be controlled.

The disc diffusion test is a good representation of the clinical situation, where the dressing material is applied to the wound surface, allowing the drug to diffuse to the wound bed [9]. The results show clear inhibition zones extending well beyond the composite margins (Fig. 5a,b). The inhibition zone around the samples was similar for the three TC-composites. This was expected because the amount of TC released was also similar for the three TC-composites. A similar strength was observed for both *S. aureus* ATCC29213 and *S. aureus* ATCC25923. This is in accordance with our previous results [29]. For the different TC-composites, the diameter of inhibition obtained was 50 ± 2 mm.

Viable bacteria counts provide valuable information on the kill rate, which is a key comparator for different formulations and physicochemical conditions [9]. The results of the viable counts are shown in Figure 6. Cell viability was significantly inhibited ($p < 0.05$) on the two *S. aureus* strains in comparison to the control after 48 h of incubation. The inhibition was not found to be dependent on TC concentration, as no statistically relevant differences were found between the samples exposed to the different concentrations of TC. In addition, no large differences in inhibition strength were observed between the *S. aureus* strains after 24 or 48 h of incubation. In accordance with the zone inhibition test, the reduction in cell growth was neither time- nor dose-dependent.

Drug delivery systems follow two distinct strategies, according to the two different targets: treatment or prophy-

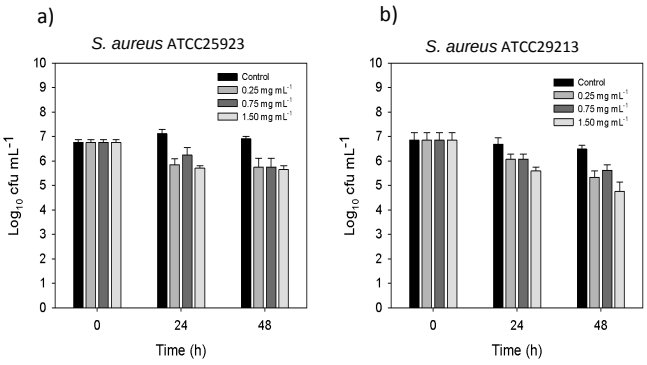

Figure 6: Viable counts of *S. aureus* ATCC25923 (a) and *S. aureus* ATCC29213 (b) in the presence of TC-composites.

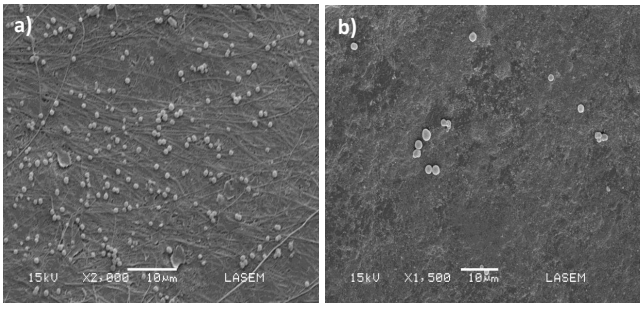

Figure 7: Interaction between *Staphylococcus* cells and the different surfaces of the composites: uncoated collagen face (a), n-BG-coated face (b).

laxis [42]. Many authors have defined that an infection is established when the concentration of micro-organisms is close to or exceeds 10^5 cells per gram of tissue or due to the presence of beta-hemolytic streptococci [43, 44]. The results found in this work show that the TC-composites are able to inhibit cell concentration up to 10^5 cfu mL^{-1} but failed to establish a cell concentration lower that 10^5 cfu mL^{-1} that would promote wound healing.

Similar antibacterial performance was found in our previous work [29]. In that way, it seems that changing the BG system or the TC concentration does not reflect any improvement in terms of antibacterial effects.

The design of a drug delivery system for prophylaxis includes, among other characteristics, a broad spectrum antibiotic and concentration of antibiotic well above MICs that last for at least a few days/a week [42]. The clinical MIC breakpoint for *Staphylococcus* spp. is ≤ 1 mg L^{-1} (www.eucast.org). Despite the low percentage of TC released, the drug levels achieved in this study exceeded the MIC values by several fold.

Besides materials eluting antimicrobials, the development of anti-adhesive materials against bacteria is another strong strategy to prevent infections [45]. With less bacterial adhesion, the risk of biofilm formation could be reduced or delayed. Figure 7 shows the SEM images of the presence of bacteria on the two surfaces of composites (coated and uncoated). Much fewer bacterial cells were present on the surface coated with n-BG, which was also the surface exposed to the TC solutions (Fig. 7). These results also corroborate the antibacterial properties of TC-composites. Reduced presence of bacteria has also been observed on agar-gelatin films coated with 45S5 BG [46] and on other polymers containing BG [47, 48]. Possible explanations have been discussed in previous research work [49] and include the repulsion that can occur between the bacterial cells and the BG surfaces and surface phenomena.

4 Conclusions

This research work confirms the possibility to effectively incorporate TC on type I collagen membranes coated with nanoparticles of a ternary system of BG ($SiO_2CaOP_2O_5$). However, the technique also showed some limitations since we found that a higher concentration not necessarily leads to greater drug release or better antibacterial performance. In future works, a further optimization of the initial drug concentration should be foreseen. TC-composites showed a sustained release of TC over time and the kinetics of the release achieved the requirements of a biomaterial eluting antibiotic for prophylaxis of wound infections, i.e. a first fast releasing step to prevent the risk of infection followed by a sustained one. The amount of TC released by TC-composite samples was several-fold higher than the MICs required for TC for the *S. aureus* strains evaluated. This was confirmed by the inhibition zone test and plate count method. Because of these reasons, the TC-composites have potential for the prophylaxis of wound infection.

Acknowledgement: This work was supported by the Consejo Nacional de Investigaciones Científicas y Técnicas, CONICET (PIP0184 to A.A.G). The authors declare no conflict of interest related to this work.

References

[1] Diefenbeck M., Mückley T., Hofmann G.O., Prophylaxis and treatment of implant-related infections by local application of antibiotics, Injury 2006, 37 Suppl 2, S95–104.

[2] Boateng J.S., Matthews K.H., Stevens H.N., Eccleston G.M., Wound healing dressings and drug delivery systems: a review, J. Pharm. Sci. 2008, 97, 2892–2923.
[3] Gao P., Nie X., Zou M., Shi Y., Cheng G., Recent advances in materials for extended-release antibiotic delivery system, J. Antibiot (Tokyo) 2011, 64, 625–634.
[4] Kittinger C., Marth E., Windhager R., Weinberg A.M., Zarfel G., Baumert R., Felisch S., Kuehn K.D., Antimicrobial activity of gentamicin palmitate against high concentrations of *Staphylococcus aureus*, J. Mater. Sci. Mater. Med. 2011, 22, 1447–1453.
[5] Chang W.K., Srinivasa S, MacCormick A.D., Hill A.G., Gentamicin-collagen implants to reduce surgical site infection: systematic review and meta-analysis of randomized trials, Ann. Surg. 2013, 258, 59–65.
[6] Bertesteanu S., Triaridis S., Stankovic M., Lazar V., Chifiriuc M.C., Vlad M., Grigore R., Polymicrobial wound infections: pathophysiology and current therapeutic approaches, Int. J. Pharm. 2014, 463, 119–126.
[7] Perumal S., Ramadass S.K., Madhan B., Sol-gel processed mupirocin silica microspheres loaded collagen scaffold: a synergistic bio-composite for wound healing, Eur. J. Pharm. Sci. 2014, 52, 26–33.
[8] Perchyonok V.T., Reher V., Zhang S., Basson N., Grobler S., Evaluation of nystatin containing chitosan hydrogels as potential dual action bio-active restorative materials: in vitro approach, J. Funct. Biomater. 2014, 5, 259–272.
[9] Elsner J.J., Berdicevsky I., Zilberman M., In vitro microbial inhibition and cellular response to novel biodegradable composite wound dressings with controlled release of antibiotics, Acta Biomater. 2011, 7, 325–336.
[10] Stigter M., Bezemer J., De Groot K., Layrolle P., Incorporation of different antibiotics into carbonated hydroxyapatite coatings on titanium implants, release and antibiotic efficacy, J. Control Release 2004, 99, 127–137.
[11] Miola M., Vitale-Brovarone C., Mattu C., Verné E., Antibiotic loading on bioactive glasses and glassceramics: an approach to surface modification, J. Biomater. Appl. 2012, 28, 308–319.
[12] Hum J., Boccaccini A.R., Bioactive glasses as carriers for bioactive molecules and therapeutic drugs: a review, J. Mater Sci. Mater. Med. 2012, 23, 2317–2333.
[13] Arcos D., Vallet-Regí M., Bioceramics for drug delivery, Acta Mater. 2013, 46, 890–911.
[14] Caridade S.G., Merino E.G., Alves N.M., Bermudez Vde Z., Boccaccini A.R., Mano J.F., Chitosan membranes containing micro or nano-size bioactive glass particles: evolution of biomineralization followed by in situ dynamic mechanical analysis, J. Mech. Behav. Biomed. Mater. 2013, 20,173–183.
[15] Hong Z., Luz G.M., Hampel P.J., Jin M., Liu A., Chen X., Mano J.F., Mono-dispersed bioactive glass nanospheres: preparation and effects on biomechanics of mammalian cells, J. Biomed. Mater. Res. A 2010, 95A, 747–754.
[16] Lin C., Mao C., Zhang J., Li Y., Chen X., Healing effect of bioactive glass ointment on full-thickness skin wounds, Biomed. Mater. 2012, 7, 045017.
[17] Sapadin A.N., Fleischmajer R., Tetracyclines: nonantibiotic properties and their clinical implications, J. Am. Acad. Dermatol. 2006, 54, 258–265.
[18] Ruhe J.J., Menon A., Tetracyclines as an oral treatment option for patients with community onset skin and soft tissue infections caused by methicillin-resistant Staphylococcus aureus, Antimicrob. Agents Chemother. 2007, 51, 3298–32303.
[19] Harless K., Borlaug G., Monson T.A., Stemper M.E., Davis J.P., Abing A.E., Shelerud J.F., An investigation of antibiotic susceptibility to empiric therapy for community-associated methicillin-resistant Staphylococcus aureus, W.M.J. 2014, 113, 59–63.
[20] Enoch D.A., Karas J.A., Aliyu S.H., Oral antimicrobial options for the treatment of skin and soft-tissue infections caused by methicillin-resistant Staphylococcus aureus (MRSA) in the UK, Int. J. Antimicrob. Agents 2009, 33, 497–502.
[21] Amin A.N., Cerceo E.A., Deitelzweig S.B., Pile J.C., Rosenberg D.J., Sherman B.M., Hospitalist perspective on the treatment of skin and soft tissue infections, Mayo Clin. Proc. 2014, 89, 1436–1451.
[22] Domingues Z.R., Cortés M.E., Gomes T.A., Diniz H.F., Freitas C.S., Gomes J.B., Faria A.M., Sinisterra R.D., Bioactive glass as a drug delivery system of tetracycline and tetracycline associated with beta-cyclodextrin, Biomaterials 2004, 25, 327–333.
[23] Kaitila I., The mechanism by which tetracycline hydrochloride inhibits mineralization in vitro, Biochim. Biophys. Acta 1971, 244, 584–594.
[24] Dashti A., Ready D., Salih V., Knowles J.C., Barralet J.E., Wilson M., Donos N., Nazhat S.N., In vitro antibacterial efficacy of tetracycline hydrochloride adsorbed onto Bio-Oss bone graft, J. Biomed. Mater. Res. B Appl. Biomater. 2010, 93, 394–400.
[25] Andrade A.L., Manzi D., Domingues R.Z., Tetracycline and propolis incorporation and release by bioactive glassy compounds, J. Non-Cryst Solids 2006, 352, 3502–3507.
[26] Andrade A.L., Souza D.M., Vasconcellos W.A., Ferreira R.V., Domingues RZ., Tetracycline and/or hydrocortisone incorporation and release by bioactive glasses compounds, J. Non-Cryst Solids 2009, 355, 811–816.
[27] Cavalu S., Banica F., Gruian C., Vanea E., Goller G., Simon V., Microscopic and spectroscopic investigation of bioactive glasses for antibiotic controlled release, J. Mol. Struct. 2013, 1040, 47–52.
[28] Zhao L.Z., Yan X.X., Zhou X.F., Zhou L., Wang H.N., Tang H.W., Chengzhong Y., Mesoporous bioactive glasses for controlled drug release, Micropor Mesopor Mat 2008, 109, 210–215.
[29] Rivadeneira J., Di Virgilio A.L., Audisio C., Boccaccini A.R., Gorustovich A., Evaluation of antibacterial and cytotoxic effects of nano-sized bioactive glass/collagen composites releasing tetracycline hydrochloride, J. Appl. Microbiol. 2014, 116, 1438–1446.
[30] Luz G.M., Mano J.F., Preparation and characterization of bioactive glass nanoparticles prepared by sol-gel for biomedical applications, Nanotechnology 2011, 22, 494014.
[31] Kokubo T., Takadama H., How useful is SBF in predicting in vivo bone bioactivity? Biomaterials 2006, 27, 2907–2915.
[32] Stigter M., de Groot K., Layrolle P., Incorporation of tobramycin into biomimetic hydroxyapatite coating on titanium, Biomaterials 2002, 23, 4143–4153.
[33] Melville A., Rodríguez-Lorenzo L., Forsythe J., Effects of the calcination temperature on the drug delivery behaviour of ibuprofen from hydroxyapatie powders, J. Mater. Sci. Mater. Med. 2008, 19, 1187–1195.
[34] Peles Z., Zilberman M., Novel soy protein wound dressings with controlled antibiotic release: mechanical and physical properties, Acta Biomater. 2012, 8, 209–217.
[35] Wang D., Miller S.C., Kopeckova P., Kopecek J., Bone targeting macromolecular therapeutics, Adv Drug Deliv Rev 2005, 57,

1049–1076.
[36] Huang W.C., Zeng H., Weng L.L., Synthesis of tetracycline analogs, Chinese Chem. Lett. 2008, 19, 19–22.
[37] Oyane A., Yokoyama Y., Uchida M., Ito A., The formation of an antibacterial agent-apatite composite coating on a polymer surface using a metastable calcium phosphate solution, Biomaterials 2006, 27, 3295–3303.
[38] Soundrapandian C., Mahato A., Kundu B., Datta S., Sa B., Basu D., Development and effect of different bioactive silicate glass scaffolds: in vitro evaluation for use as a bone drug delivery system, J. Mech. Behav. Biomed. Mater. 2014, 40, 1–12.
[39] Sepulveda P., Jones J.R., Hench L.L., Characterization of melt-derived 45S5 and sol–gel-derived 58S bioactive glasses, J. Biomed. Mater. Res. 2001, 58, 734–740.
[40] Lei B., Chen X.F., Wang Y.J., Zhao N.R., Du C, Fang L.M., Surface nanoscale patterning of bioactive glass to support cellular growth and differentiation, J. Biomed. Mater. Res. Part A 2010, 94A, 1091–1099.
[41] van de Belt H., Neut D., Uges D.R., Schenk W., van Horn J.R., van der Mei H.C., Busscher H.J., Surface roughness, porosity and wettability of gentamicin-loaded bone cements, and their antibiotic release, Biomaterials 2000, 21, 1981–1987.
[42] Campoccia D., Montanaro L., Speziale P., Arciola C.R., Antibiotic-loaded biomaterials and the risks for the spread of antibiotic resistance following their prophylactic and therapeutic clinical use, Biomaterials 2010, 31, 6363–6377.
[43] Edwards R., Harding K.G., Bacteria and wound healing, Curr. Opin. Infect. Dis. 2004, 17, 91–96.
[44] Zilberman M., Elsner J.J., Antibiotic-eluting medical devices for various applications, J. Control Release 2008, 130, 202–215.
[45] Desrousseaux C., Sautou V., Descamps S., Traoré O.J., Modification of the surfaces of medical devices to prevent microbial adhesion and biofilm formation, Hosp Infect 2013, 85, 87–93.
[46] Rivadeneira J., Carina Audisio M., Boccaccini A.R., Gorustovich A.A., In vitro antistaphylococcal effects of a novel 45S5 bioglass/agar–gelatin biocomposite films, J. Appl. Microbiol. 2013, 115, 604–612.
[47] Pratten J., Nazhat S.N., Blaker J.J., Boccaccini A.R., In vitro attachment of S. epidermidis to surgical sutures with and without Ag-containing bioactive glass coating, J Biomater Appl 2004, 19, 47–57.
[48] Misra S.K., Ansari T.I., Valappil S.P., Mohn D., Philip S.E., Stark W.J., Roy I., Knowles J.C., Salih V., Boccaccini A.R., Poly(3-hydroxybutyrate) multifunctional composite scaffolds for tissue engineering applications, Biomaterials 2010, 31, 2806–2815.
[49] Rivadeneira J., Di Virgilio A.L., Audisio M.C., Boccaccini A.R., Gorustovich A.A., Evaluation of the antibacterial effects of vancomycin hydrochloride released from agar-gelatin-bioactive glass composites, Biomed Mater 2015, 10, 015011