Title

Bactericidal activity of Gallium-doped chitosan coatings against staphylococcal infection

Authors

Arash Ghalayani Esfahani[1,3,*], Beth Lazazzera[2], Lorenza Draghi[3], Roberto Chiesa[3], Luigi De Nardo[3], Fabrizio Billi[1]

Affiliations

[1]Department of Orthopaedic Surgery, University of California, Los Angeles (UCLA), CA.

[2]Microbiology, Immunology, and Molecular Genetics Department, University of California, Los Angeles (UCLA), CA.

[3]Department of Chemistry, Materials and Chemical Engineering 'G. Natta', Politecnico di Milano, Milan, Italy.

*Email: arashghalayani@g.ucla.edu, arash.ghalayani@polimi.it

Abstract

Post-arthroplasty infections represent one the main issues after total joint replacement, challenging new solutions for their increasing incidence in both medical costs and patient quality of life. In this work, we developed a new class of Gallium (Ga)-doped Chitosan (CS) coatings fabricated by electrophoretic deposition to promise new opportunities in biofilm-associated infection therapy. The optimum conditions for uniform coating deposition and the releasing profile of Ga in different concentrations was determined. We assessed biofilm formation on CS/Ga composite coatings by *Staphylococcus epidermidis* and *Staphylococcus aureus,* which are the main strains involved in post-arthroplasty infections. The codeposition of an antibacterial agent was effective: by increasing concentrations of Ga loaded into CS matrix within the chemically bound coating reduces biofilm viability by up to 86% and 80% in *S. epidermidis* and *S. aureus* strains respectively. Finally, the in vitro influence of Pulsed Electromagnetic Field (PEMF) was investigated on modification of the bactericidal activity of the CS/Ga composite coatings. The coatings were incubated with *S. epidermidis* and *S. aureus* and exposed to the PEMF at two different frequencies and times. Biofilm viability by *S. epidermidis* decreased up to an additional 35 to 40% in the presence of low and high frequency PEMF, respectively. Biofilm viability by *S. aureus* was not further reduced in the presence of low frequency PEMF, but decreased up to an additional 38% at high frequency PEMF. The new integrated approach, of CS/Ga composite coatings and PEMF, could reduce the incidence of infection in orthopaedic implant applications.

Keywords: electrophoretic deposition (EPD) ; chitosan (CS) ; gallium (Ga); *Staphylococcus epidermidis*; *Staphylococcus aureus*; post-arthroplasty infection; PEMF; biofilm.

1 Introduction

Infections after orthopaedic surgery has increased over the recent years despite the use of antibiotics and modified surgical technique [1,2]. As the demand for orthopaedic surgery increases with the aging population, the infection cases will pass 266,000 per year in the United States by 2030 [1–4]. Bacteria (especially Staphylococci) form extracellular biofilms on implanted metallic/plastic materials, block penetration of immune cells and antibiotics, and result in bacterial survival [5–8]. The removal surgery of all the implanted materials is necessary after biofilm formation. Near 70% of these kinds of infections are caused by Staphylococcal species [9–11]. The treatment of post-arthroplasty infection is difficult due to bacteria resistant to antibiotics such as methicillin-resistant S. *aureus* (MRSA) [5–8,12].

The conventional treatment for post-arthroplasty infection usually contains two-stage procedure, first, surgical removal of all prosthetic components and placement of an antibiotic-impregnated spacer, and at the next step, revision arthroplasty after the infection has cleared [13–18]. Moreover, more medical care results in additional medical costs. All these issues lead to focus on the prevention of infection [19–21].

To promise a novel method to tackle this issue, we applied Chitosan (CS)/gallium (Ga) composite coating to surfaces, which was prepared by electrophoretic deposition (EPD). EPD is a deposition technique with different advantages such as cost-effective, versatility in materials that can be processed, reasonable control over the thickness of the coatings and a high level of homogeneity in terms of microstructure [22]. Chitosan is a cationic polysaccharide biopolymer for tissue engineering, as it is a biocompatible coating and capable of drug delivery [23]. According to the particular CS properties such as biodegradability, biocompatibility, non-toxicity, and bio-functionality, it is one of the most interesting materials for applications ranging from skin, bone, cartilage, and vascular grafts to substrates for cell culture and drug-delivery systems [24]. Previous studies have shown the feasibility of cationic EPD of chitosan [25]. Ga(III) can be potentially used as an antibacterial agent. As Ga^{3+} is similar to Fe^{3+} in radius, electronegativity, charge and coordination number [26,27], it can substitute the iron in its

process and act in a “Trojan horse” way against bacteria, such as *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* [28,29]. Ga is sequestered by the bacteria through their iron uptake systems, by the siderophores. Once inside, the metal blocks iron-dependent process where there is crucial oxidation of iron (Fe^{2+} to Fe^{3+}) because gallium III cannot be reduced to give continuity to sequential oxidation and reduction[27]. Ga(III) blocks osteoclast resorption by preventing attachment to the surface of bone without appearing to be cytotoxic to osteoclasts, nor to inhibit cellular metabolism [30,31].

In this work, after studying the morphology of the coatings and finding the optimum conditions for uniform coating, the releasing profile of Ga in different concentrations were studied, as Ga results in bactericidal activity. We assessed biofilm formation and cell growth in the presence of the composite-coated surfaces by *Staphylococcus epidermidis and Staphylococcus aureus* which are the main strains of bacteria that causes post-arthroplasty infections [5,20,32]. Cell viability was assessed using Alamar Blue™ assay and biofilm formation was investigated by counting colony-forming units (CFU) and crystal violet assay. The electrophoretic deposition of CS/Ga composite coating on orthopaedic implants show excellent bactericidal activity as well as biocompatible properties. Furthermore, the polymer-antibacterial agent (Ga) implant coating evaluated in this study was effective, suggesting the potential for this strategy as a therapeutic intervention to combat post-arthroplasty infections. Increasing concentrations of Ga loaded into CS matrix within the chemically bound coating reduces biofilm viability by up to 86% and 80% in *S. epidermidis* and *S. aureus* strains respectively. This novel coating could reduce the incidence of infection in orthopaedic implant applications.

Finally, the in vitro influence of Pulsed Electromagnetic Field (PEMF) is investigated on modification of bactericidal activity of the CS/Ga composite coatings. The coatings incubated with *S. epidermidis* strain 14990 and *S. aureus* strain 12600 were exposed to the PEMF at two different frequencies, 40,850 Hz as the high frequency and 3,846 Hz as the low frequency, for 15 minutes and 4 hours. The thera- peutic efficacy for the stimulation of bone growth with pulsating electromagnetic fields (PEMF) is already proven in controlled double-blind studies

[33–36]. The PEMF resulted in a further decrease in biofilm viability up to 40% for *S. epidermidis* and 38% for *S. aureus* compared to Ga treatment alone.

2 Materials and methods

2.1 Materials

Chitosan (Deacetylated chitin, Poly(D-glucosamine), medium molecular weight, Lot#STBG1894V), Gallium(III) nitrate hydrate (crystalline, 99.9% trace metals basis, Lot#MKBQ1999V), acetic acid (99.7%) and water (CHROMASOLV® Plus, for HPLC) were all supplied by Sigma-Aldrich and used without further purification. To evaluate the in vitro biological response, cell culture experiments were performed on pure chitosan and Ga-doped coatings using human primary osteogenic sarcoma cell line SAOS-2 (ECACC 89050205) as a model. Cell culture medium was prepared using McCoy's 5a medium, with 15% fetal bovine serum, 1% [v/v] L-glutamine 2 mM, 1% [v/v] sodium pyruvate 1 mM and 1% [v/v] penicillin/streptomycin.

Trypticase soy broth (TSB) (30 g L^{-1}in purified water, autoclave at 121 ^{o}C for 15 minutes, Becton, Dickinson and Company) was used for routine growth of bacterial cells, crystal violet stain was used to quantify biofilm viability (0.41% W/V in ethanol and DI water, Fisher scientific company), and Wash Buffer was used to change the medium of the bacterial cells [37].

Staphylococcus epidermidis strain 14990 and *Staphylococcus aureus* strain 12600 both from the ATCC were used in the study.

2.2 Preparation and chemico-physical characterization of CS/Ga composite coating

2.2.1 EPD of CS/Ga composite coating

Titanium sheets (Ti, grade 2) were used as cathode in an electrophoretic deposition cell: electrodes were positioned at distance of 10 mm [38] in a lab made EPD cell. Processing conditions have been optimized in order to achieve a uniform, homogeneous and consistant deposition of coatings; Square waves (75-100 V, duty cycle = 0.17) have been used in water-based bath (pH=3.67, [CS] = 1g L^{-1}, [Gallium(III) nitrate hydrate] = 10 mg L^{-1} (LGa) and [Gallium(III) nitrate hydrate] = 100 mg L^{-1} (HGa)).

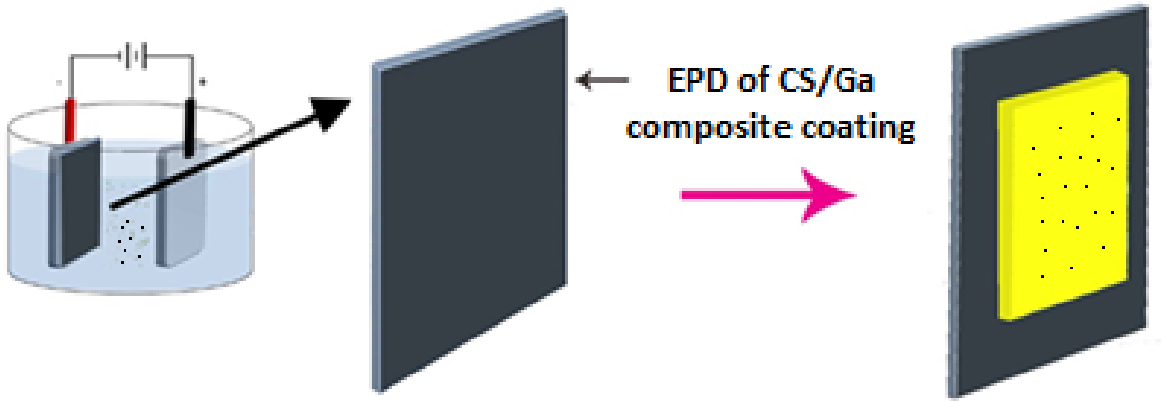

Figure 1. Schematic of Chitosan (CS)-based coatings that were prepared using EPD.

2.2.2 Microstructural characterization

In the first phase, the feasibility of coating with Inductively Coupled Plasma - Optical Emission Spectrometry (ICP-OES, Perkin Elmer Optima 2000DV OES, Wellesley, USA) technique was evaluated. In order to study the morphology of CS/Ga composite coating, which was prepared by EPD, scanning electron microscope (SEM) (Zeiss EVO 50EP) was used. The SEM was fitted with an Oxford Instruments INCA energy-dispersive X-ray spectrometer (EDS) which was used for qualitative elemental analysis of the coatings. To measure the conductivity of deposition bath, conductivity meter (Crison, CM 35) has been used.

2.2.3 Antibacterial agent (Ga) release study

The in vitro release of Ga from the EPD chitosan matrix was studied by incubating composite coating (20 mm × 20 mm × 0.2 mm) in 7.5 mL of phosphate buffered saline (PBS, Sigma-Aldrich P4417-50TB) at 37 °C. Inductively Coupled Plasma - Optical Emission Spectrometry (ICP-OES, Perkin Elmer Optima 2000DV OES, Wellesley, USA) analysis was used to investigate the release of antibacterial agent. To determine release of Ga from the CS matrix, 3 specimens for each treatment at each time point (1, 2, 4, 8, 14, 24 hours and 3, 7 days) were incubated in 7.5 ml of PBS. They were fixed vertically in 15 mL falcon tubes with conical end, to allow release of the antibacterial agent from both sides of the specimen. The tubes were

maintained at 37 °C in a thermostatic oven under constant gentle shaking (75 rpm) (VDRL DIGITAL MOD. 711/D). After 1, 2, 4, 8, 14, 24 hours and 3, 7 days, aliquots of PBS from three specimens was analyzed by ICP-OES to determine the concentration of Ga released. The PBS solution was also analyzed by ICP-EOS to standardize the data.

2.3 Biological and microbiological characterization

2.3.1 Cytotoxicity tests on extract

For cytotoxicity assessment, samples eluates were obtained, according to UNI EN ISO 10993-5, by incubating the samples in culture medium for 24 h. The extraction ratio (sample surface area/eluates volume) was 3 cm^2 mL^{-1}. SAOS-2 cells were seeded at a density of 10^4 cells cm^{-2} in 96-well microtiter plate and cultured with fresh complete medium until 70% confluent. The medium was then replaced with eluates or control and cells were returned to incubator. After 24 h, Alamar Blue™ assay (BioReagent, Sigma-Aidrich R7017) was performed to evaluate cell viability. Plates fluorescence was spectrophotometrically read (Tecan, Genios Plus plate reader) to evaluate the possible cytotoxic effects of the tested material.

2.3.2 Crystal violet assay and colony-forming units counting

To evaluate biofilm viability by *S. epidermidis* and *S. aureus*, crystal violet assay was performed. A 1 ml of diluted (1:100) overnight culture of *S. epidermidis* in TSB was added to each sample in each well of 24 well, flat bottom microtitre plate. All the coatings autoclaved (Tuttnauer cat2007) for 1 hour in 150 °C to be sterilized. The plate was incubated under static conditions at 37 °C for 24 hours. The growth media removed, and the wells washed 3 times with 1 ml Wash Buffer. At the next step, 1 ml crystal violet was added to each well and incubated at room temperature for 15 minutes. Crystal violet was removed, and the wells were washed twice with 1 ml of distilled water and 1 ml of an 80% ethanol, 20% acetone solution was added. The liquid was transferred to a fresh 96 well PVC round bottom microtiter plate to

measure the absorbance at 570 nm (A_{570}) by plate reader (BMG FLUOstar Omega). Control is CS coating without any Ga.

Biofilm viability (%) = $\frac{Composite\ coating Ab_S}{Control\ Ab_S}$ x 100 (Equation 1)

To allow the biofilm to detach from the coating surface, coating containing biofilms were resuspended in 1 mL of TSB, vortexed, and sonicated at 60 Hz (Aquasonic 250HT, VWR International) for 30 s; this was repeated five times. The suspension was used to prepare six, ten-fold dilutions. A 100 µL volume of each dilution were spotted onto Lysogeny broth (LB) plates and incubated for 24 h at 37 °C. The following day, the number of CFUs per ml was counted, working blind, and using the following formula [39–41]:

CFU/ml = (no. of colonies x dilution factor) / volume of culture plate.

2.3.3 Biofilm morphology

To study the morphology of biofilm which was formed by *S. epidermidis* on the CS/Ga coatings, Variable-Pressure (VP) Scanning Electron Microscopy (SEM) (Zeiss supra 40VP) was used.

2.4 Pulsed electromagnetic field (PEMF)

To investigate the in vitro effect of a pulsed electromagnetic field (PEMF) on the efficacy of antibacterial agent (Ga) in the treatment of coated orthopaedic implants infection, two different frequencies, 40,850 Hz as the high frequency and 3,846 Hz as the low frequency, were used to expose the specimens to the PEMF. PEMF was applied for 15 minutes and 4 hours to the coatings which were incubated into *S. epidermidis* strain 14990 and *S. aureus* strain 12600 in 24 well microtiter rack. The rack was located in the incubator (figure 2).

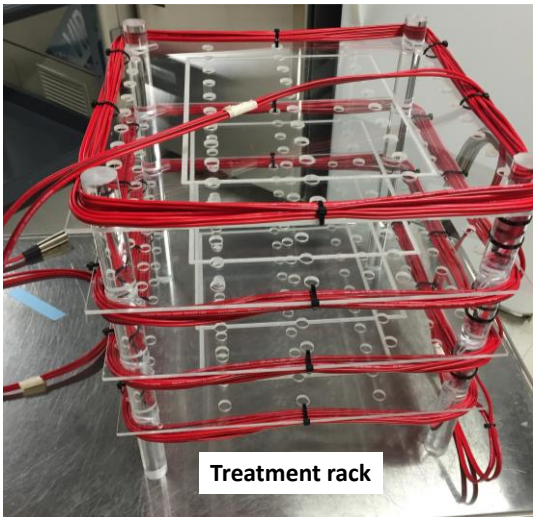

Figure 2. Pulsed ElectroMagnetic Field (PEMF) setup.

2.5 Statistical data analysis

All results are reported as mean ± standard deviation. Significant differences between two sets of data were determined by one-way ANOVA followed by Tukey post-hoc test for pairwise comparisons and $p < 0.05$ was considered statistically significant. The Statistical Package for Social Science was used for the calculations (Minitab Express™ Version 1.4.0)

3 Results

3.1 Feasibility of EPD CS/Ga composite coating

Figure 3 shows the SEM images of (a) pure CS and (b,c) CS/Ga composite coating in different Ga concentrations ([Gallium(III) nitrate hydrate] = 10 and 100 mg L^{-1}): a porous structure of pure CS coating is evident, due to hydrolysis of water during EPD process [42]. Ga-doped coatings show a different morphology and an homogeneous presence of bright spots. EDX analysis allow the identification of such clusters, mainly deposited on the pore borders (Figure 3 d,e). The EDX spectrum (Figure 3(f,g)) contains peaks associated with Ga atoms.

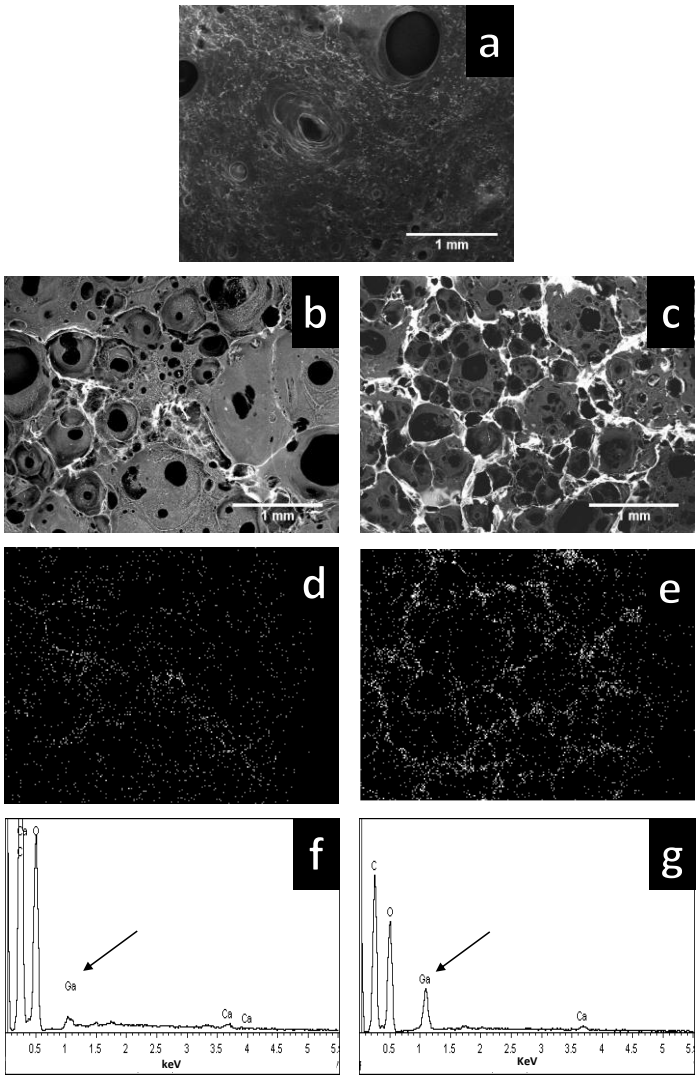

Figure 3. (a) SEM image of EPD of CS, (b,c) SEM images of EPD of CS/Ga composite coating ((b) [Gallium(III) nitrate hydrate] = 10 mg L^{-1} and (c) [Gallium(III) nitrate hydrate] = 100 mg L^{-1}), (d,e) corresponding X-ray map; (f,g) corresponding EDX spectrum.

Ga appears distributed mainly on pore bordes, probably due to the higher current density in such area (Figure 3 d,e) [22,43,44]. From EDX analysis a difference is evident in the relative peak of Ga according to the different bath concentrations. Before studying the Ga release rate, the total amount of Ga loaded in the chitosan matrix during EPD was determined as a function of Ga in the suspension by ICP-OES analysis(Figure 4a). As seen, the efficiency during EPD was approximately 66-55% which is quite acceptable (Figure 4a) (supporting information).

a	[Ga]/[CS] in EPD bath (%)	[Ga]/[CS] in the coating (%)	Ga in the coating (mg)
LGa	0.27	0.18 ± 0.05	0.054
HGa	2.7	1.5 ± 0.08	0.34

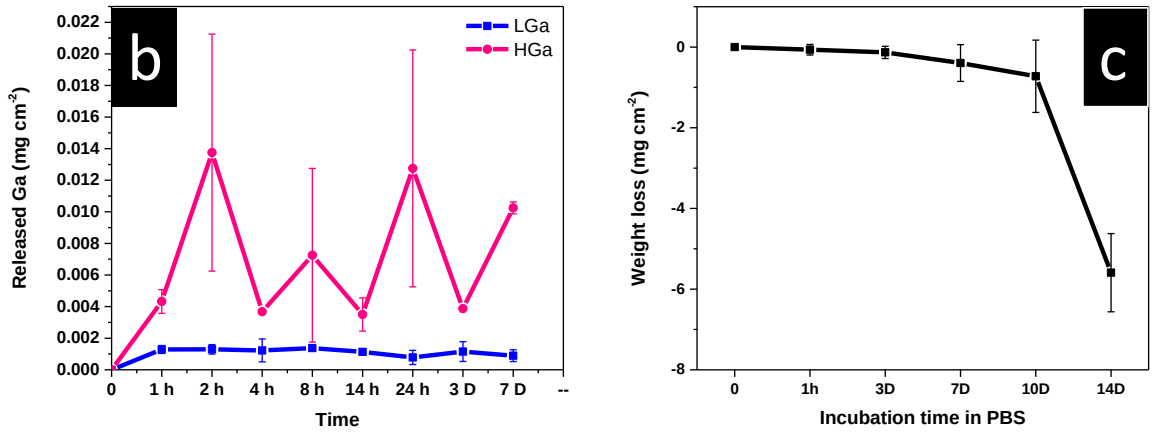

Figure 4. (a) Effect of the Ga concentration in the suspension on the loading efficiency of the EPD process, (b) Release of Ga from the composite coatings, (c) Chitosan degradation test in PBS.

The release of Ga from CS matrix, measured by ICP-EOS, occurred during the first 7 days. The released Ga was detected in 7.5mL of PBS with a maximum of 0.0137 and 0.0013 mg/cm^2 from high Ga concentration ([Gallium(III) nitrate hydrate] = 100 mg L^{-1}) and low Ga concentration ([Gallium(III) nitrate hydrate] = 10 mg L^{-1}) deposition baths, respectively (Figure 4b). The critical period to inhibit biofilm formation after the implantation surgery is 6 h [45].

However, at extended periods, certain species of adhered bacteria are capable of forming a biofilm at the implant–tissue interface [19,45,46]. Therefore, we performed the drug release studies for an extended time. Two release phases can be highlighted. In the early phase, a burst of release was observed (after 2h). The second phase occurs after that in which was very little additional release was detected. To evaluate whether the release rate is controlled by degradation of the chitosan matrix in the PBS solution over long incubation, the cumulative weight loss of the CS coating in PBS was determined (Figure 4c). As seen, the amount of chitosan weight loss in PBS was noticeable after a long period of incubation (90 h). [47] suggesting that water molecules destroy the hydrogen bond among chitosan fibers, disordering the macromolecule alignments that may lead to dissolution/degradation.

3.2 Cytotoxicity assessment and bactericidal activity

3.2.1 Cytotoxicity - test on extracts

Figure (5) shows the results of cell viability for the control plate, chitosan coating without gallium, and the CS/Ga composite coatings with different Ga concentrations. The first set of bar graph data demonstrates the cell viability in extracted culture, where the next two sets of bar graph data show the results of cultures diluted, 50% and 90% (w%), into fresh SAOS-2 media. No cytotoxicity was observed in all examined samples. This observation suggests that the prepared composite coatings are not toxic to human osteoblast-like cells in good agreement with previous studies [30].

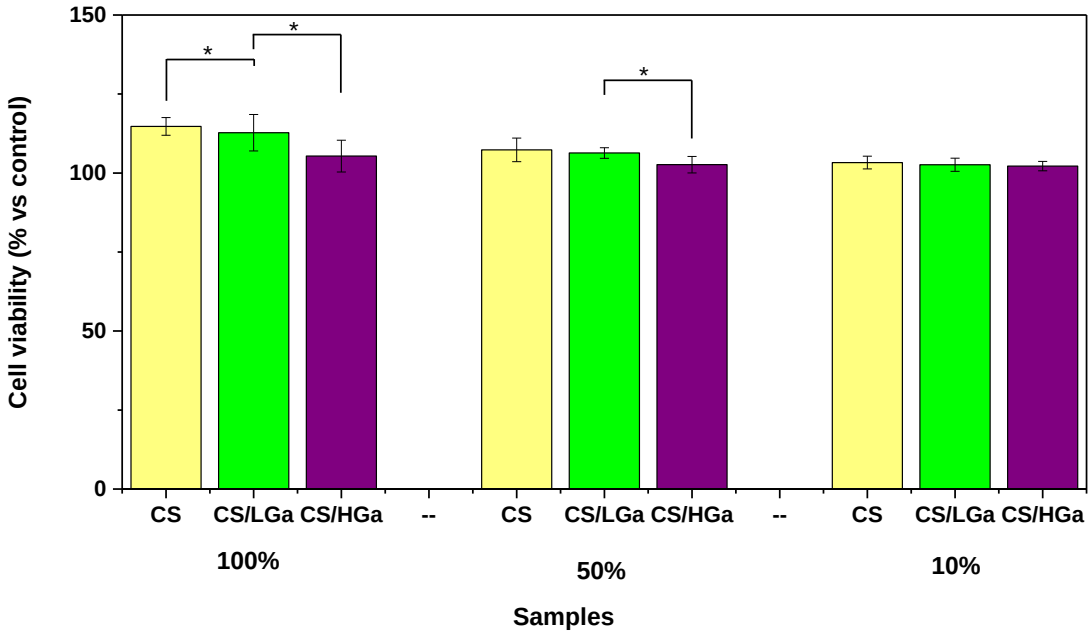

Figure 5. Cell viability (% vs. control) of SAOS-2 cells cultured with coatings extracts as determined by a Alamar Blue ™ assay ($p < 0.05$, indicated by *). For 50% and 10%: cultures diluted, 50% and 90% (w%), into fresh SAOS-2 media.

3.2.2 Biofilm viability

Figure 6 a and 7 a summarizes the Biofilm viability results assayed by the crystal violet method on *S. epidermidis* strain 14990 and S. *aureus* strain 12600, cultured in TSB medium. After 24h, all Ga-doped specimens differed significantly from untreated pure CS coating as a control ($p < 0.05$, figure 5 and 6, indicated by *) resulting in a significant bacterial inhibition. For *S. epidermidis*, both CS/LGa and CS/HGa coatings caused a reduction in biofilm viability of about 15% and 60%, respectively, after 24 hrs, and a reduction of 82% and 86%, respectively, after 3 days (Fig. 6a). For *S. aureus*, both CS/LGa and CS/HGa coatings caused a reduction in biofilm viability of about 10% and 55%, respectively, after 24 hrs, and a reduction of 40% and 80%, respectively, after 3 days (Fig. 7a).

This assay was repeated with TSB media adjusted to different pHs; regarless of the medium pH the CS/Ga coated reduced in the number of viable bacterial cells (Figure 6b and 7b). Generally, the pH drops in presence of bacteria [48]. CS matrix was confirmed to release Ga

both passively and actively, in response to lowering pH. At lower pH, the amide groups on the chitosan can become protonated, forming the hydrophilic NH_3^+ group. The resulting electrostatic repulsion between the protonated amino groups weakened the intermolecular and intramolecular hydrogen bonding interaction of chitosan molecules, as a result, the buffer solution can diffuse into the network easily which would facilitate the equilibrium swelling ratios to increase. According to the swelling, the diffusion rate increases from matrix to the exterior. As a consequence the embeded agents in the matrix will be released easier and faster [49].

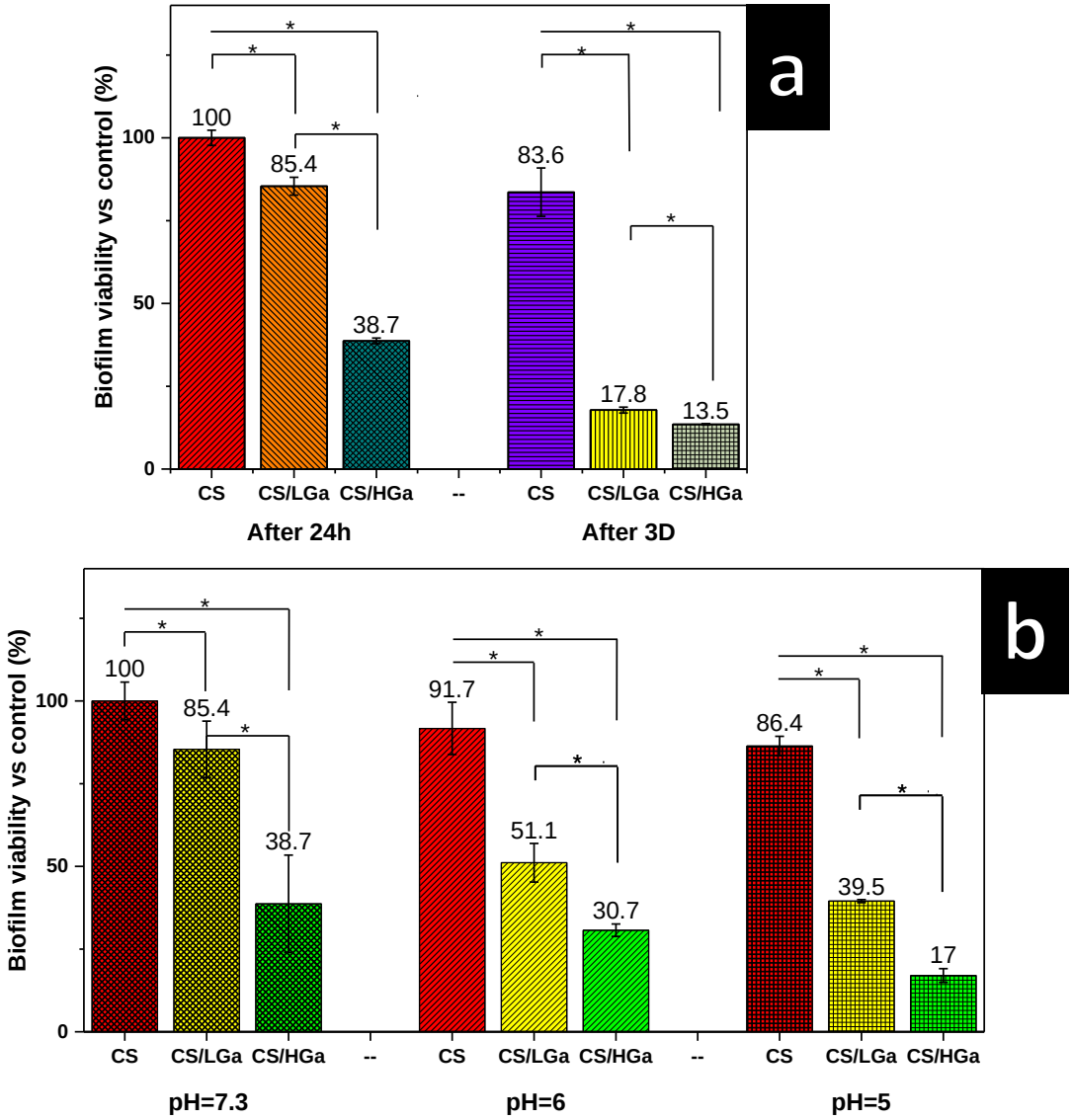

Figure 6. Crystal violet assay indicates in vitro biofilm viability for *S. epidermidis*, (a) after 24h and 3 days (3D) in comparison with control (CS) ($p < 0.05$, indicated by *) , (b) after 24h in different pH (all the absorbance values normalized with CS value at pH 7.3).

The effect of the CS/Ga composite coating on cell viability of *S. epidermidis* was measured as the total CFU present in the plantonic phase of the cultures incubated with the coatings (Figure 8). The composite coating with a high Ga concentration (HGa) gave the lowest number of planktonic CFU, yeilding. 8.3% and 4.5% of the population on untreated CS after 24 hours and 3 days, respectively. The effect of the CS/Ga compositie coating of the viability of cells in the biofilm phase of the culture was measured as the total CFU presence in the medium after detachment of the cells from the surface. The coatings with highest Ga concentration resulted in the lowest CFU, yielding 27% and 37% of the population on untreated CS for *S. epidermidis* strain 14990 and *S. aureus* strain 12600, respectively (Figure 9).

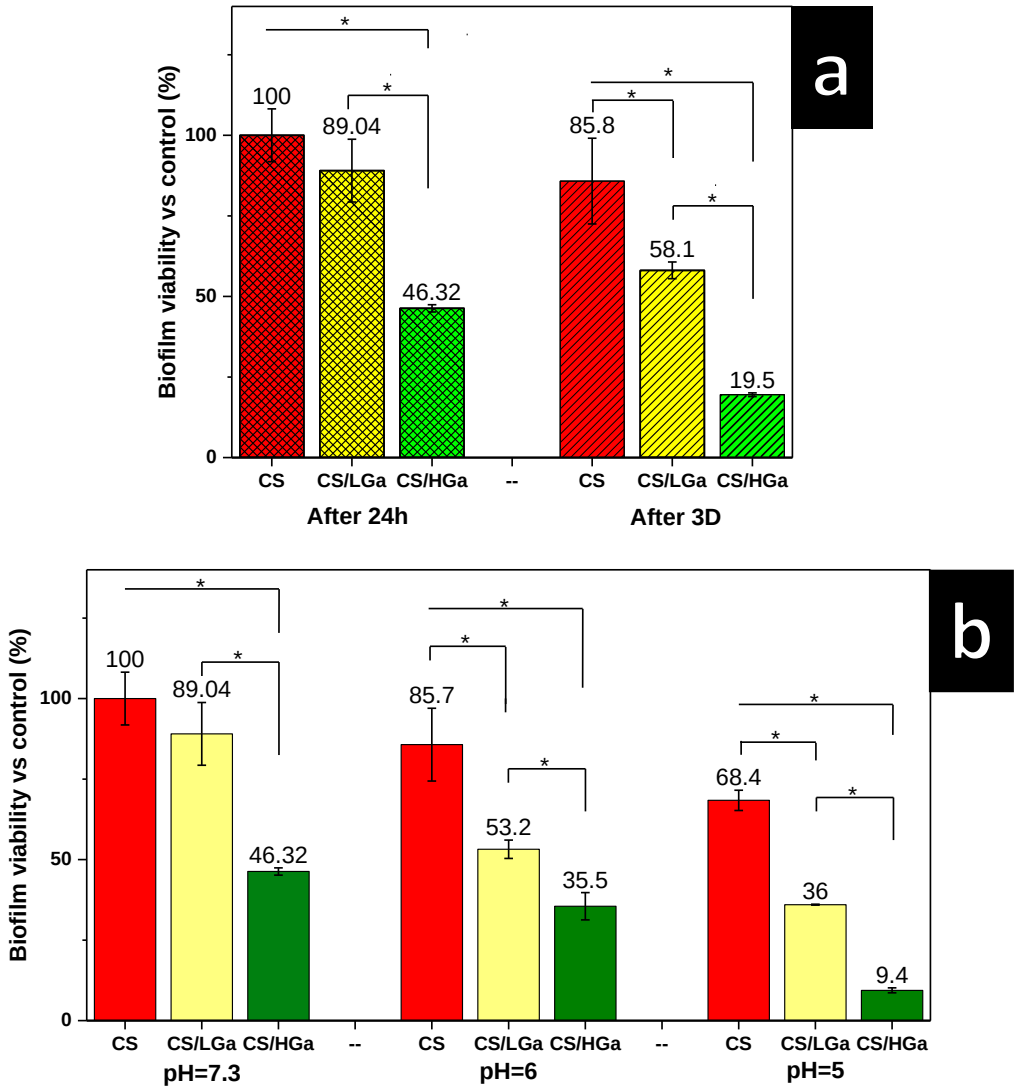

Figure 7. Crystal violet assay indicates in vitro biofilm viability for *S. aureus*, (a) after 24h and 3 days (3D) in comparison with control (CS) ($p < 0.05$, indicated by *) , (b) after 24h in different pH (all the absorbance values normalized with CS value at pH=7.3).

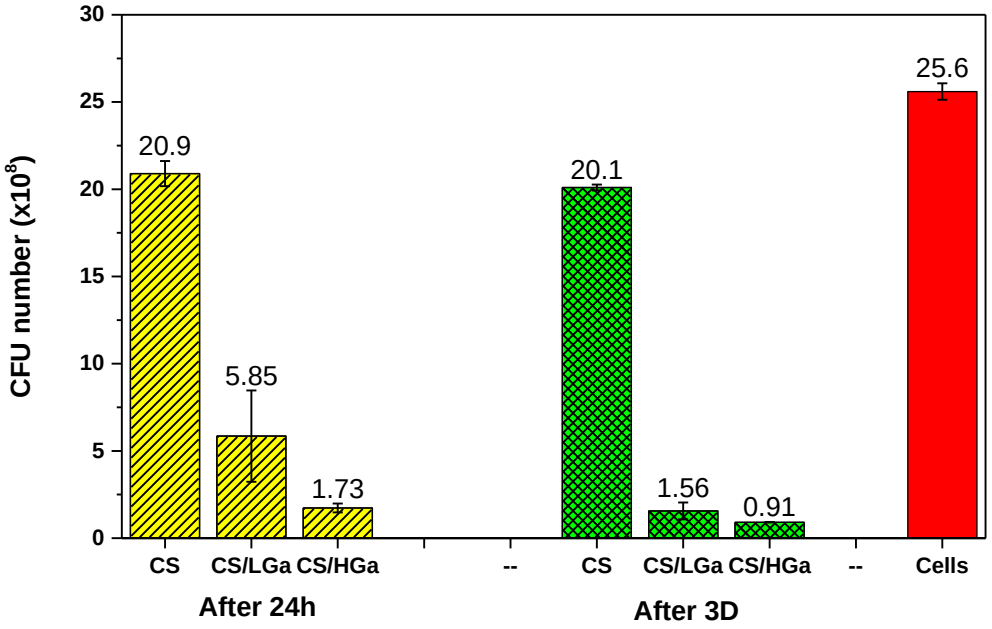

Figure 8. Total CFU of *S. epidermidis* incubated in LB medium, (planktonic cells).

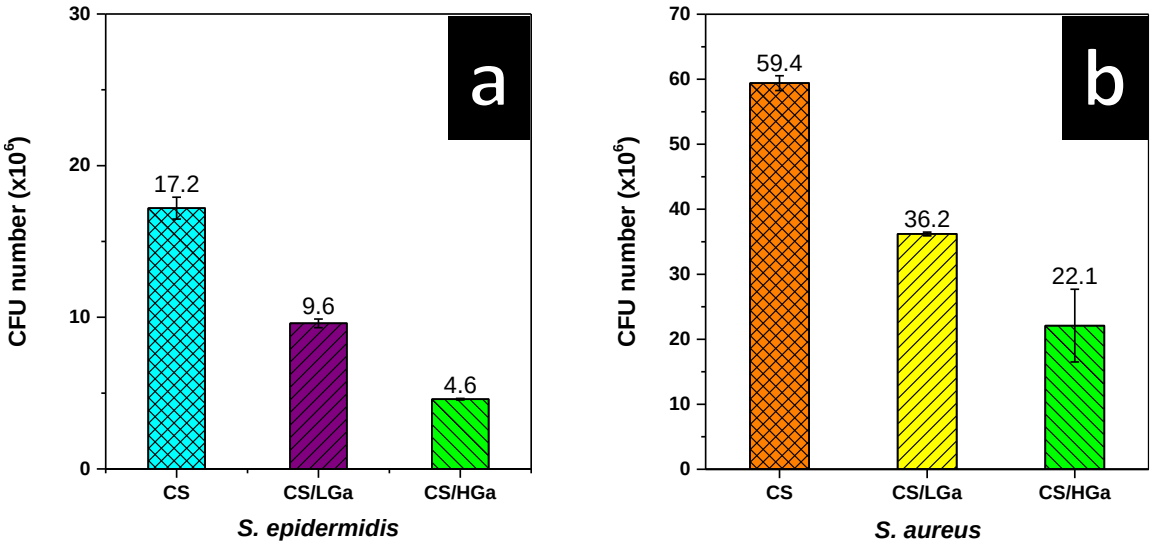

Figure 9. Total CFU of bacteria cells incubated in LB medium for 24 h and then biofilm cells were detached from the coatings; (a) *S. epidermidis*, (b) *S. aureus*.

Besides affecting planktonic bacteria, Ga seems to be efficient to reduce biofilm cells (Figure 9). Consequently, Ga, can be effective against either planktonic or biofilm cells. Gallium is metabolically very similar to Fe^{3+}, acting as an iron substitute in several biological pathways. Respect to its chemical similarity to Fe^{3+} in terms of charge, ionic radius, electronic configuration, and coordination number, Ga can substitute iron in siderophore dependent biological systems; this capability underlies its antibacterial action. Since Ga^{3+} cannot be reduced under the same conditions as Fe^{3+}, sequential redox reactions critical for the biological functions by Fe^{3+} are impaired when iron is replaced: Ga thus inhibits Fe^{3+} by a "Trojan horse" strategy [29,50,51].

3.2.3 Biofilm Morphology

At different Ga concentration, colonies were attached on the surface of the coating specimen over 24h incubation. These results were substantiated by the VP-SEM images (Figure 10 and 11). The SEM micrographs revealed reduced biofilm formation by *S. epidermidis* and *S. aureus* grown on CS/Ga composite coatings compared with the pure CS control (Figure 10a and 11a). The biofilm formed on Ga were poorly structured, very thin, arrested at the microcolony stage, and had reduced surface area coverage. It is also evident that the biofilm structure on the CS/HGa composite coating (Figure 10c and 11c) is only a single-layer of cells compare to CS/LGa composite structure biofilm (Figure 10b and 11b).

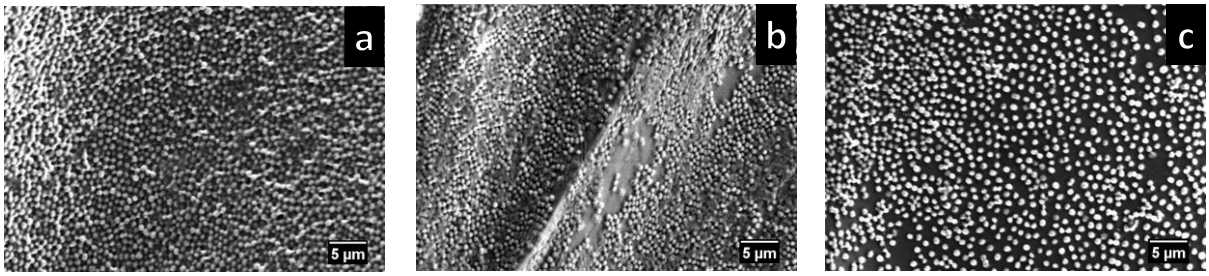

Figure 10. Representative VP-SEM images of the EPD CS/Ga coatings incubated with *S. epidermidis* for 24h, (a) pure CS, (b) CS/LGa composite coating ([Gallium(III) nitrate hydrate]= 10 mg L^{-1}) and (c) CS/HGa composite coating ([Gallium(III) nitrate hydrate]= 100 mg L^{-1}).

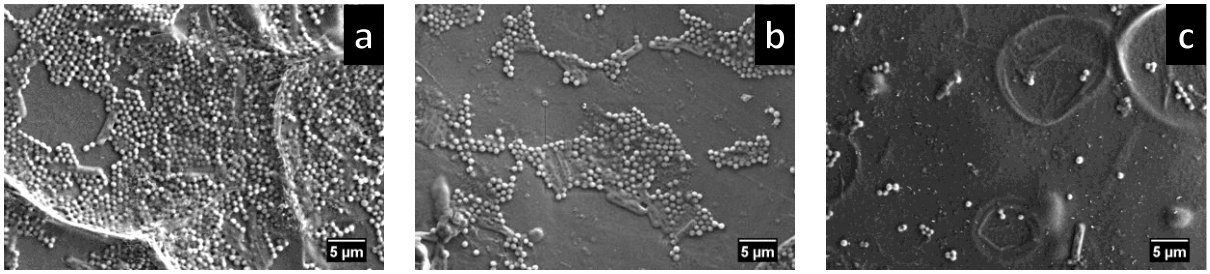

Figure 11. Representative VP-SEM images of the EPD CS/Ga coatings incubated with *S. aureus* for 24h, (a) pure CS, (b) CS/LGa composite coating ([Gallium(III) nitrate hydrate]= 10 mg L^{-1}) and (c) CS/HGa composite coating ([Gallium(III) nitrate hydrate]= 100 mg L^{-1}).

3.3 PEMF effect on bactericidal activity of the coatings

Four separate experimental setups were used to expose coatings incubated in bacterial cultures of *S. epidermidis* and *S. aureus* in TSB media, to (1) a low-frequency PEMF, 3,846 Hz for 15 minutes and 4 h and (2) a hi-frequency PEMF, 40,850 Hz for 15 minutes and 4 h as well. In each of the four applied fields showed a biofilm viability reduction of *S. epidermidis and S. aureus* in the presence of Ga within 24 h of the experiment (Figure 12 and 13). The best results were obtained by low frequency for 4 h in both strains. Exposure to a PEMF increased the effectiveness of Ga against the one-day biofilms of *S. epidermidis* and *S. aureus*.

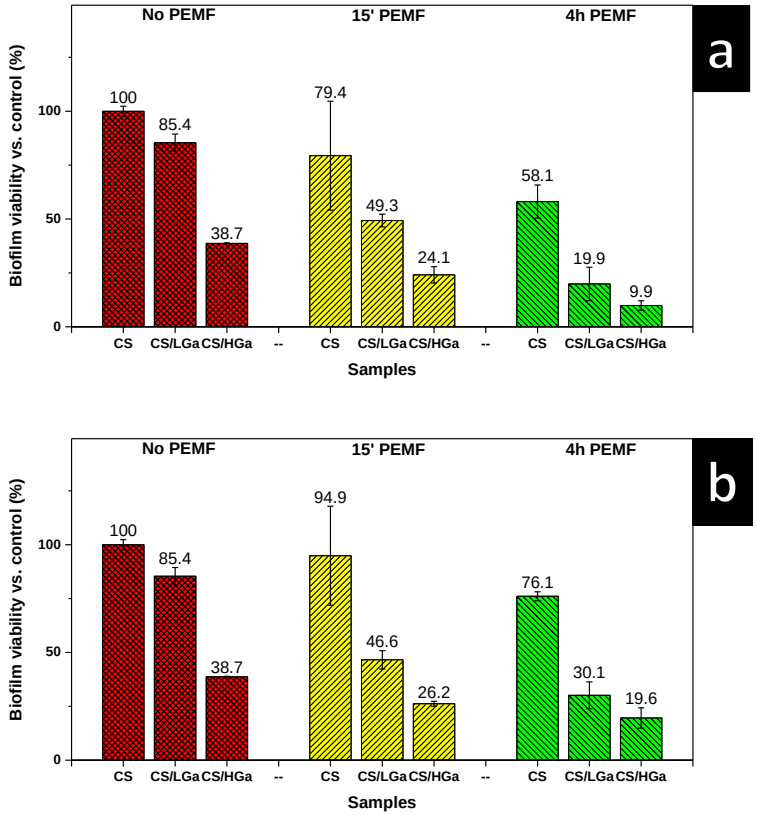

Figure 12. PEMF impact on biofilm viability of *S. epidermidis* strain 14990 in EPD CS/Ga composite orthopaedic coating; after 24h; (a) at low frequency, (b) at high frequency (all the absorbance values are normalized to no PEMF CS value).

Hydroxyl and oxygen radicals are known to destroy cell membranes of bacteria and may be present with the application of an electromagnetic field. This is the so-called bioelectric effect [52–55]. This may facilitate the penetration of antibacterial agents into the biofilm and subsequently in the cells, and could be an explanation for the detected modification of Ga efficacy.

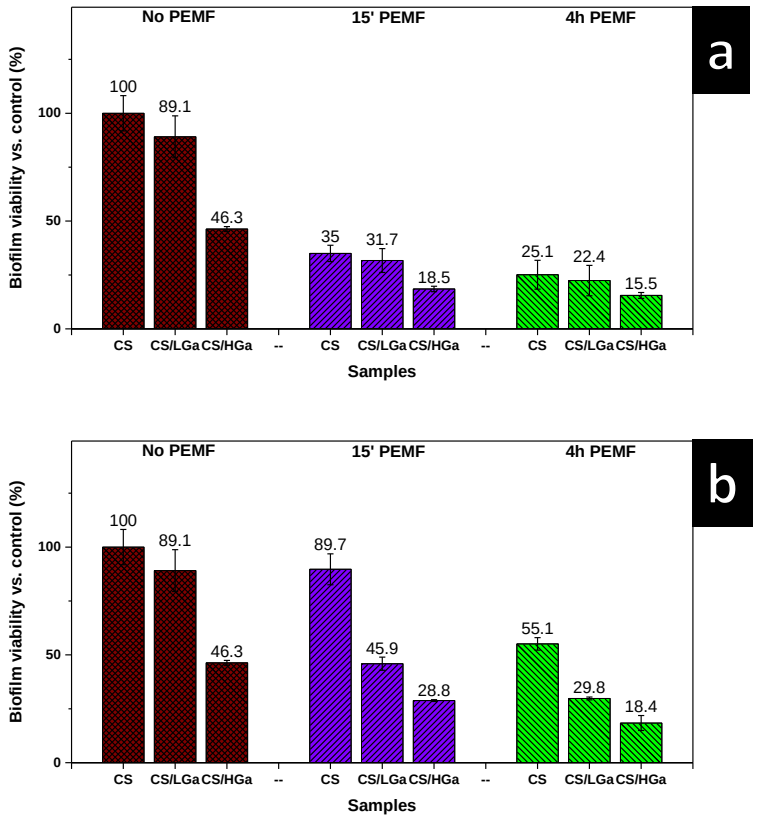

Figure 13. PEMF impact on biofilm viability of *S. aureus* strain 12600 in EPD CS/Ga composite orthopaedic coating; after 24h; (a) at low frequency, (b) at high frequency (all the absorbance values are normalized to no PEMF CS value).

4 Conclusions

In this study, we have demonstrated that a combination of pulsed electromagnetic fields with the antibacterial agent, improves bactericidal activity of Ga against *S. epidermidis* strain 14990 and *S. aureus* strain 12600. We conclude that the combination of Ga treatment with low-frequency PEMF could promise new opportunities in biofilm-associated infection therapy due to the improved Ga efficiency. Furthermore, the therapeutic efficacy of the Ga under the used fields, should be proven in well-designed, evidence-based, randomized clinical studies in the future. This novel modification could result in the lower use of antibacterial agents, which has the potential to decrease antibacterial resistance.

Acknowlegement

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Supporting information:

S.1 Electrophoretic deposition efficiency during Gallium-doped chitosan coatings fabrication

In the case of HGa (high gallium concentration):

We know that: $[Ga(NO_3)_3xH_2O]/[CS]=1/10$ and according to atomic weight:

$(Ga)/Ga(NO_3)_3xH_2O = 70/255.74 = 0.27$

Then: $[Ga]/[CS]= 0.027$ (2.7%) (Weight % of Ga in the deposition bath)

ICP result : 1.5 % (Weight % of deposited Ga in the scaffold)

Then: 1.5/2.7 = 55% (EPD Efficiency).