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# Synthesis and characterisation of bioactive and antibacterial glass-ceramic Part 2 – plasma spray coatings on metallic substrates

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Plasma spray bioactive glass ceramic coatings on metallic substrates have been characterised and modified by a patented ion exchange process<sup>1</sup> in order to introduce silver ions onto their surface and confer antibacterial properties. Both treated and untreated materials have been analysed by means of SEM, EDS and XRD in order to verify the amount of introduced silver, and also after immersion in simulated body fluid in order to investigate bioactivity. The amount of silver released in simulated body fluid has been quantified by means of graphite furnace atomic adsorption spectrophotometry analysis. Finally cellular and microbiological test have been performed in order to verify material biocompatibility and antibacterial behaviour.

**Keywords:** Glass ceramic coatings, Antibacterial, Silver, Ion exchange

## Introduction

Metals (stainless steel, cobalt–chromium and titanium alloys) are the most common materials used in dental and orthopedic fields because of their good mechanical properties. However these materials do not promote tissue integration and often release toxic ions. Bioactive glasses and glass ceramics, on the other hand, assist tissue integration but have flexural, fracture and fatigue strength lower than bone.

Coatings of bioactive materials on metallic substrates allow combining mechanical strength of the substrate and bioactivity of the coating. In fact, one of the most important factors determining tissue implant interaction is surface behaviour, thus the presence of a bioactive coating onto a metallic substrate allows the exposition to tissues of a bioactive surface maintaining bulk mechanical characteristics.<sup>2,3</sup>

The fundamental characteristics of a coating are: good adhesion to substrate, possibility to obtain expected thickness, possibility to coat complex shapes and property maintenance after deposition. In order to obtain these results, different deposition techniques are nowadays in use in biomedical fields.<sup>4–6</sup> In this research work plasma spray technique has been chosen.

Together with tissue integration the other main problem of prosthetic implants is infection development

at the damaged site. This phenomenon is mainly due to bacterial colonisation of implanted materials through adhesion accumulation and persistence of bacteria onto material surface and to the consequent formation of a biofilm.<sup>7</sup>

This particular three-dimensional structure protects bacteria from systemic therapies and also from patient own defence, therefore it is particularly difficult to treat and often leads to the necessity of removing the implant.<sup>8</sup>

For this purpose, the development of prosthetic materials able to avoid bacterial adhesion and proliferation seems to be an interesting solution.

Silver is known since antiquity for its antibacterial properties and it has been used from centuries in the treatment of infections. In fact it presents a broad spectrum activity and a low resistance development due to its multiplicity in mode of action against bacteria.<sup>9,10</sup> Therefore silver could be a good local antimicrobial agent also in the prevention of prosthetic infections.

On the other hand, it is well known that when a material is implanted in the human body a sequence of events occurs at the surface, first water and proteins are adsorbed and then cellular colonisation starts. As far as this last passage is concerned there is a sort of ‘race for the surface’ between cells and bacteria, therefore if cellular adhesion and proliferation are improved bacteria will be obstructed.<sup>11</sup> Thus, a material which presents at the same time a bioactive and antibacterial behaviour could be an interesting proposal for prosthetic surgery.

In this work a patented ion exchange technique<sup>1</sup> has been used to introduce silver ions on the surface of glass ceramic plasma sprayed coatings on titanium substrate; this process has been yet applied to a bulk material<sup>12</sup> in

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order to optimise the technique and to a macroporous structure (scaffold)<sup>13</sup> useful to mimic the bone substituted devices. The present study aimed to confirm the possibility of extending the method to glass ceramic coated prosthetic devices.

## Materials and methods

In this work a glass ceramic (Fa-GC), previously characterised in sintered shape<sup>12</sup> has been deposited on Ti substrates, selected according to main application in dental field, by plasma spray technique to obtain glass ceramic coating.

The Fa-GC composition ( $50\text{SiO}_2-18\text{CaO}-9\text{CaF}_2-7\text{Na}_2\text{O}-7\text{K}_2\text{O}-6\text{P}_2\text{O}_5-3\text{MgO}$ ) was chosen to obtain a bioactive glass, in which the nucleation of fluoroapatite is assisted; this is very interesting for material application in dental field<sup>14,15</sup> as well as for orthopaedics devices.

As for sintered samples also in this case Fa-GC was prepared by melting the starting components in a platinum crucible at  $1550^\circ\text{C}$  for 1 h and pouring in water. The material obtained was then milled and sieved up to a grain size  $<100\ \mu\text{m}$ .

Glass ceramic powders were then deposited by plasma spray on titanium plates ( $100 \times 25 \times 2\ \text{mm}$ ) previously sand blasted with  $\text{Al}_2\text{O}_3$ , obtaining a coatings thickness between 50 and  $100\ \mu\text{m}$ . The grain size of glass ceramics powders was selected on the basis of plasma spray process parameters, used by Eurocoating S.r.l. that realised all coatings for this research work. Plates have then been cut to obtain small square samples of 0.8 cm side.

The obtained specimens were analysed by means of X-ray diffraction (XRD, X'Pert Philips diffractometer), optical microscopy (Reichert Jung MeF3 metallographic optical microscope) and scanning electron microscopy (SEM, FEI Quanta INSPECT), the last one accompanied by electron dispersion spectrometry (EDS, Philips – EDAX 9100) to verify if the plasma spray did not introduce any structural, morphological or compositional alteration.

To evaluate the linear expansion coefficient of Fa-GC, the glass ceramic was subjected to thermal characterisations by means of thermal dilatometry (TD-7-Perkin Elmer); the linear expansion coefficient evaluation is significant for coatings realisation, since during cooling phase, residual strains could be created inside the glass ceramic. Nevertheless the plasma spray process does not expect prolonged heating, therefore the eventual residual strains should not be evident or influence the mechanical coating adhesion.

## Mechanical tests

The covered plates were subjected to Vickers micro-indentations at the interface between the substrate and the coating to qualitatively evaluate the coating adhesion strength and to study the cracks propagation inside the coating and at the interface with the substrate.

The samples were previously incorporated in a polymeric resin and polished with diamond paste; the test was performed using a Leitz micro-Vickers indenter and applying a load of 300 g.

To quantitatively estimate the adhesion of the glass ceramic coatings to the titanium substrate, the samples were subjected to interfacial bonding tensile strength tests in accordance with ISO standard 13779-4.

## Surface modification

To introduce silver inside the glass ceramic the authors have selected, as for the previous studies, the ion exchange process.<sup>16,17</sup> This process allows the introduction of silver ions only in the outer layers of the samples, maintaining unaltered the bulk characteristics, like mechanical properties and glass bioactivity, as shown for sintered glass ceramic.

Also in the present work the ion exchange process was performed in aqueous solution of silver nitrate<sup>1</sup> and applied to finished coated samples, named Ag-Fa-GC coating from now on.

The samples were finally analysed by means of XRD, SEM and EDS to evaluate the modification induced by ion exchange process.

## In vitro bioactivity

The coatings bioactivity was tested soaking the samples in an opportune a-cellular simulated body fluid (SBF),<sup>18</sup> maintained at  $37^\circ\text{C}$  for periods up to one month, and refreshing the solution every two days to mimic the physiological turnover of body fluids.

To evaluate if and how hydroxyapatite (HAP) precipitation occurred, the samples were analysed by means of XRD and SEM EDS observations and accompanied by pH measurements of the solution after different dipping times.

## Leaching test

In order to analyse the amount of silver ions released from ion exchanged modified coatings, three Ag-Fa-GC coatings and one Fa-GC coating for the control were soaked in SBF for periods up to one month at  $37^\circ\text{C}$ . At different time of dipping, up to one month, 1 mL of the solution was spiked and analysed by means of a graphite furnace atomic adsorption spectrophotometry (GFAAS, PERKIN ELMER 4100 ZL). The amount of silver released from samples and the release rate are important parameters to asses the cytotoxicity towards bacterial cells, but not towards tissue cell.<sup>19-21</sup>

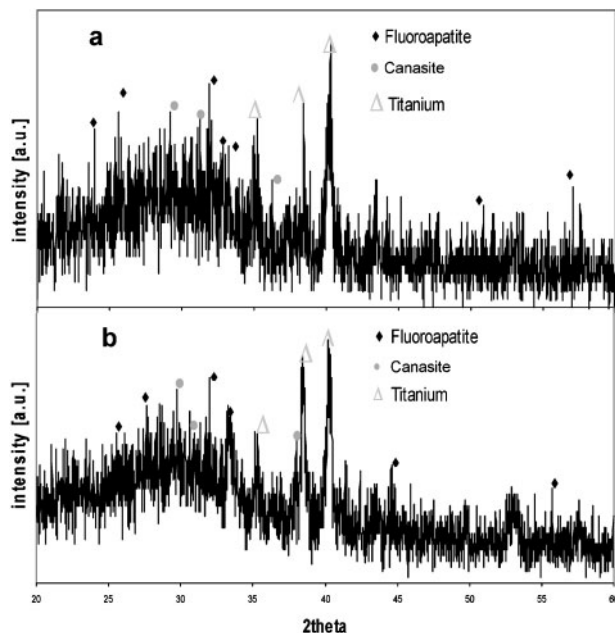
## Cytotoxicity test

The safety of silver modified coatings towards tissue cells was investigated by means of *in vitro* cytotoxicity tests; all samples were preventively dry sterilised at  $180^\circ\text{C}$  for 3 h. Tests were performed, at each time, part on cells (optical microscope observation, count, SEM observation) and part on the supernatant (pH and lactate dehydrogenase (LDH) measurements). All tests were performed in triplicate.

Osteoblasts like cells (MG-63) adhesion and proliferation were performed: samples of untreated and of ion exchanged glass ceramics were soaked in culture medium (M5650 Sigma, minimum essential medium added with 1 mM sodium pyruvate, 1% antibiotics and antimycotics solutions, 2 mM glutamine and 10% fetal bovine serum) with  $10\ 000\ \text{cells}/\text{cm}^2$  for periods of 2, 4, 8 days at  $37^\circ\text{C}$  in atmosphere with 5%  $\text{CO}_2$  and 95% air.

The cells were previously observed at optical microscope to verify their presence and morphology around the samples and subsequently at SEM to evaluate the morphology on glass ceramic surfaces.

The viable count was carried out using a Burker camera and staining the osteoblasts with trypan blue dye; this is a quantitative index of cells' viability in presence of Fa-GC coatings and Ag-Fa-GC coatings.



1 X-ray diffraction spectra of a Fa-GC and b Ag-Fa-GC

pH and LDH measurements were performed on the supernatant: the first is a measure of cellular metabolic activity, in particular medium's acidification show good cell's metabolism, the presence of LDH instead is an index of cellular death for necrosis and it's measured by spectrophotometry.

### Antibacterial tests

Antibacterial behaviour of samples has been evaluated in two ways both in accordance to NCCLS standards for antimicrobial susceptibility: inhibition zone evaluation<sup>22</sup> and broth dilution tests.<sup>23</sup>

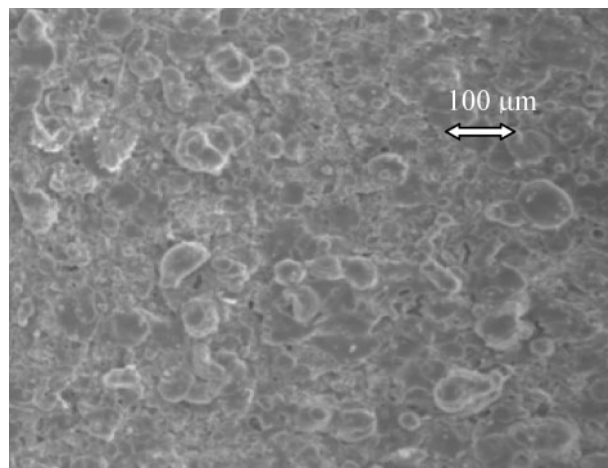
Both tests have been performed with *S. Aureus* because it is one of the main pathogen involved in prosthetic infections and each test has been carried out in triplicate. All products for this analysis have been purchased from Becton Dickinson.

In both cases a bacterial broth has been prepared dissolving a *S. Aureus* disk (ATCC 29213) in 5 mL brain heart infusion. After overnight incubation at 37°C 10 µL of the suspension have been spread on a blood agar plate and incubated 24 h. A 0.5 McFarland suspension (containing approximately  $10^8$ – $2 \times 10^8$  colony forming units (CFU) per mL) has been prepared inoculating some colonies grown on the plate in physiological solution (turbidity has been evaluated by optical instrument – Phoenix Spec BD McFarland)

For inhibition zone evaluation an aliquot of the described suspension has been spread on Mueller Hinton Agar plates on which samples have then been posed with the coated and treated surface in contact with plate.

After 24 h incubation inhibition zones have been observed as a halo around samples where bacteria did not grown up. To confirm the Ag doped samples antibacterial efficacy, inhibition zone was also evaluated using *S. Aureus* stock isolated from human prosthetic infection.

In order to perform dilution tests an aliquot of bacterial suspension has been introduced in Mueller Hinton broth tubes in order to obtain suspensions approximately containing  $5 \times 10^5$  CFU/mL.



2 Plasma spray coating morphology

A tube containing only bacteria has been prepared as growth control, in the other tubes samples and silver containing samples have been introduced.

After 24 h incubation at 35°C tubes' McFarland index has been optically evaluated (Phoenix Spec BD McFarland); the McFarland index is a measure of solution turbidity and so of bacteria proliferation.

For broth dilution tests, washing and vortexing solutions have been analysed in order to quantify CFU. Washing solution has been prepared by rapid rinsing of sample in physiological solution, while the vortexing one by 1 min 50 Hz vortex of sample in physiological solution.

Each solution has been serially diluted and spread on blood agar plates. After overnight incubation at 35°C CFU have been counted on plates.

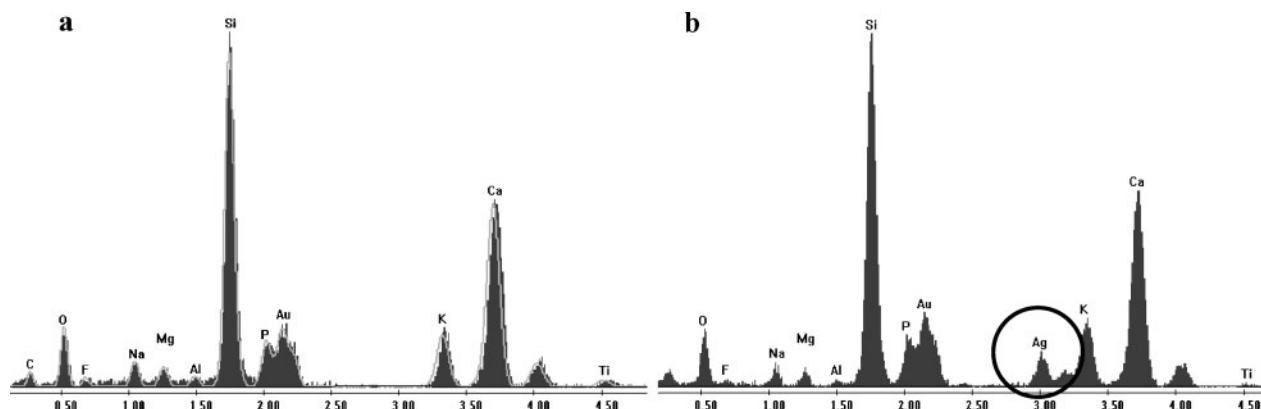
## Results and discussion

Thermal analysis measured a linear expansion coefficient of  $\alpha = 12.8 \times 10^{-6} \text{ } ^\circ\text{C}^{-1}$  between 25 and 800°C, while the linear expansion coefficient of titanium in the same temperature range is about  $\alpha_{\text{Ti}} = 8.7 \times 10^{-6} \text{ } ^\circ\text{C}^{-1}$ . The glass ceramic  $\alpha$  value is lightly different from titanium one, but even if the Fa-GC is higher than Ti one the eventual coating shrinkage did not cause adherence loss, as confirmed by mechanical test.

X-ray diffraction analysis show, as for sintered material, that the main crystalline phases are fluoroapatite and canasite. However in this case amorphous phase is predominant because the glass ceramic, poured in water, did not undergo further thermal treatment in the crystallisation range, as for sintered samples (Fig. 1a). In addition, plasma spray process involves high temperatures and rapid cooling that favour glassy structure development. Titanium peaks at 35.2, 38.4 and 40.3 are related to the metallic substrate.

X-ray diffraction on Ag-Fa-GC coatings shows that any new crystalline phases grows up during the treatment, as for sintered samples (Fig. 1b).

Observations using SEM shows a morphology typical of plasma spray coatings (Fig. 2). Analysis of EDS confirms Fa-GC composition and this further proves that plasma spray process does not influence glass characteristics. Spectrum of EDS evidences the presence of titanium, due to the substrate (Fig. 3a).



3 Spectra of EDS for *a* as done coating and *b* ion exchanged coating

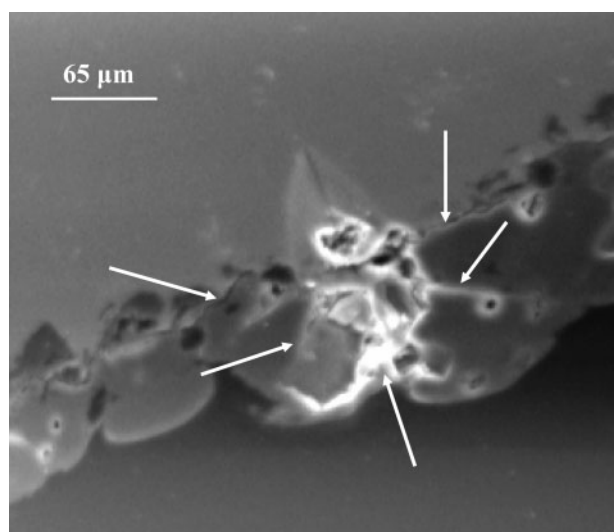
Scanning electron microscopy observation on Ag–Fa–GC samples did not show significant difference from those of Fa–GC. Analysis of EDS shows the presence of Ag peak (Fig. 3*b*). These results confirm that ion exchange process is effective to introduce silver on material surface without altering its characteristics (no dissolution phenomena).

Silver amount measured on coating samples surface is higher than for sintered ones. This is due to the bigger surface available for ion exchange process and to the higher amount of amorphous phase in the plasma sprayed glass ceramic.

In order to evaluate the coating mechanical adhesion on substrate, Vickers micro-indentations were performed at the interface with different load. Figure 4 shows a Vickers indentation with a load of 200 g; fracture cracks propagate both inside the Fa–GC coating and at the interface, thus it can be underlined that the cohesion strengths into the coating are comparable to the coating adhesion ones.

The coating adhesion strength evaluated on Fa–GC coating samples shows an adhesion value of  $41.6 \pm 9.6$  MPa; this value is very promising, since it is higher than hydroxyapatite, glasses or glass matrix composites adhesion strengths reported.<sup>24–26</sup>

Samples' weight has been measured before and after ion exchange process. Results are identical before and after the treatment, thus it did not induce any corrosion



4 Vickers micro-indentation on Fa–GC coating

phenomena. Results are in accordance to SEM observation previously reported.

### *In vitro* bioactivity

In order to evaluate material's bioactivity before and after ion exchange process, samples soaked in SBF for periods up to one month, were analysed by XRD and observed at SEM. As for sintered samples these analyses confirm the formation of HAP layer on both Fa–GC and Ag–Fa–GC coating sample's surface already after 14 days dipping in SBF.

Images of SEM show the presence of uniform layer of a substance with the typical morphology of HAP and EDS analyses underline the enrichment in Ca and P for both samples (Fig. 5). X-ray diffraction spectra confirm the presence of hydroxyapatite on materials surface, after 28 days dipping in SBF (see Fig. 6, where the XRD pattern registered on the silver doped glass ceramic coating is reported). This result indicates that Fa–GC, in the form of plasma spray coatings on titanium substrates, is still bioactive<sup>18,27</sup> and that silver introduction by ion exchange technique does not affect its bioactive behaviour.

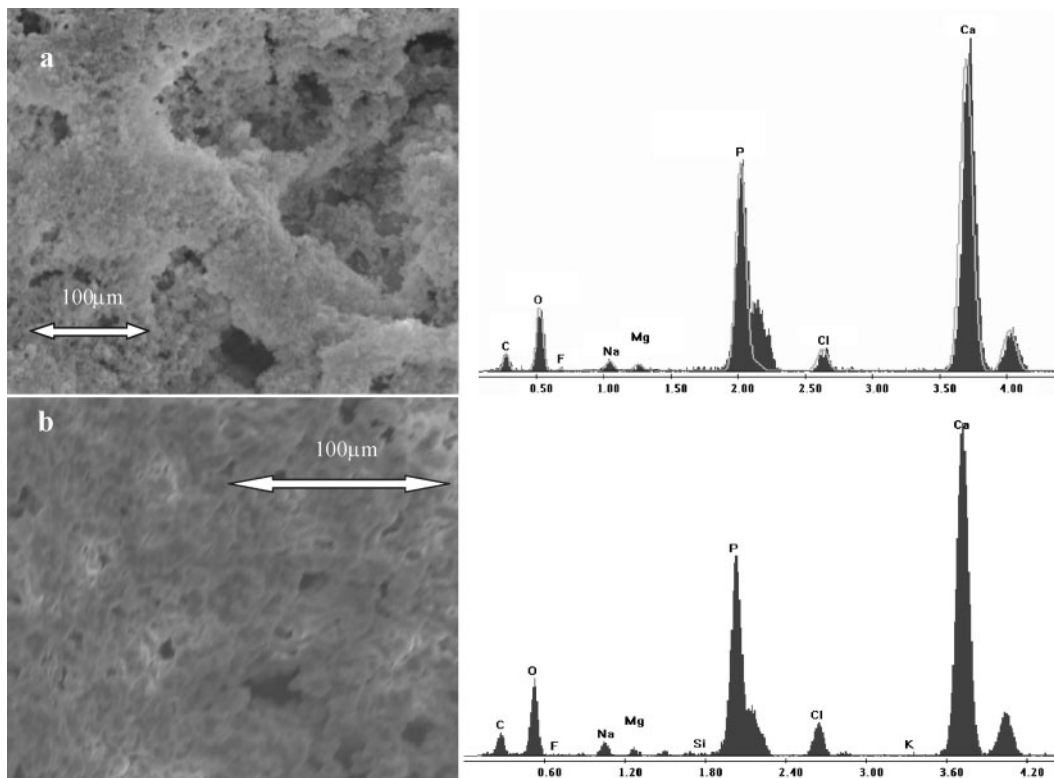
The pH trend during dipping shows, also in this case, little variations, in accordance to physiological values (Fig. 7). Any difference in pH trend was observed between Fa–GC coatings and Ag–Fa–GC coatings.

Scanning electron microscopy observations and EDS analysis on Ag–Fa–GC coatings after 24 h dipping in SBF show the formation of small AgCl particles on samples' surface. They are still present after longer dipping times and gradually covered by a hydroxyapatite layer. However their quantity is small respect to surface area. In fact if a significant amount of AgCl is present, it is detected by XRD analysis also under a thick hydroxyapatite layer.<sup>17</sup>

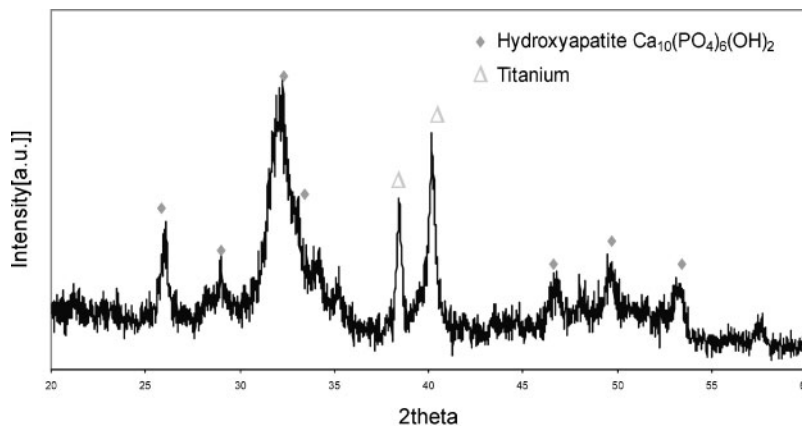
### Leaching tests

In order to quantify the amount of silver released in solution from Ag–Fa–GC coatings, aliquots of SBF after Ag–Fa–GC coatings dipping have been analysed at different soaking times by GFAAS technique. Figure 8 represents the amount of silver in solution, expressed in  $\mu\text{g mm}^{-2}$  and compared with sintered samples, as well as the leaching speed, expressed in  $\mu\text{g h}^{-1} \text{mm}^2$ .

Figure 8 demonstrate that silver is gradually released in SBF and particularly in the first period which is the most critical for infections' development. According to expectances the amount of silver released from coatings



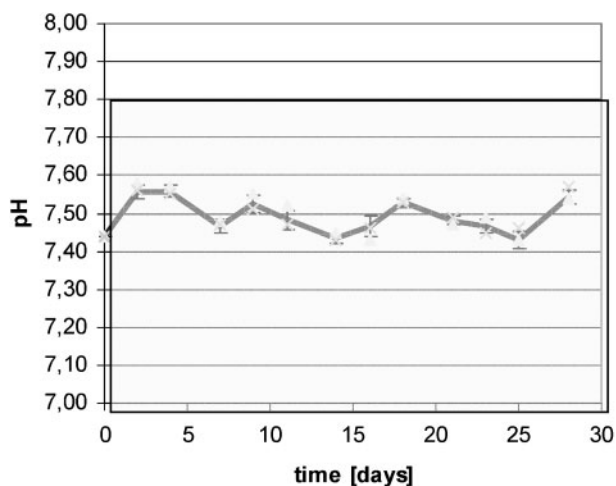
5 Morphology and EDS spectra of hydroxyapatite on a Fa-GC coating and b Ag-Fa-GC coating after 28 days in SBF



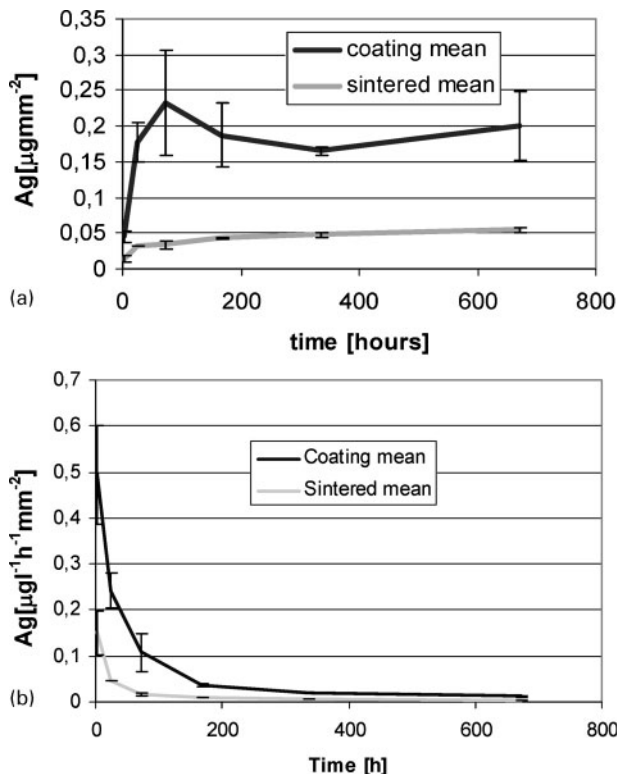
6 X-ray diffraction pattern of Ag-Fa-GC coating after 28 days in SBF

is higher than that analysed from sintered. Coatings, as said before, received a higher amount of silver because of their bigger specific area and their more amorphous nature, thus the release in solution is higher.

Figure 8 presents also a particular behaviour of coatings in solution. In fact, despite the study is relative to a cumulative release (no refresh is made in dipping period), a decrease of silver amount in solution is registered after about three days dipping. This is due to AgCl precipitation on samples' surface, as confirmed from SEM observation, which is affected by the surrounding medium (SBF). The composition of SBF is complex and rich of different ionic species, in particular chlorides and phosphates. Weak aqueous complexes and precipitates of silver and phosphate ( $\text{Ag}(\text{H}_2\text{PO}_4)^{2-}$ ,  $\text{AgHPO}_4^-$ ,  $\text{Ag}(\text{HPO}_4\text{H}_2\text{PO}_4)^{2-}$ ,  $\text{AgH}_2\text{PO}_4$ , and  $\text{Ag}_3\text{PO}_4(\text{s})$ ) have been reported in the literature.<sup>28</sup> The presence of these complexes in solution could compete with the formation of other ion pairs



7 pH trend during soaking in SBF (▲: Bioriv; ×: Ag-Bioriv; —: mean)

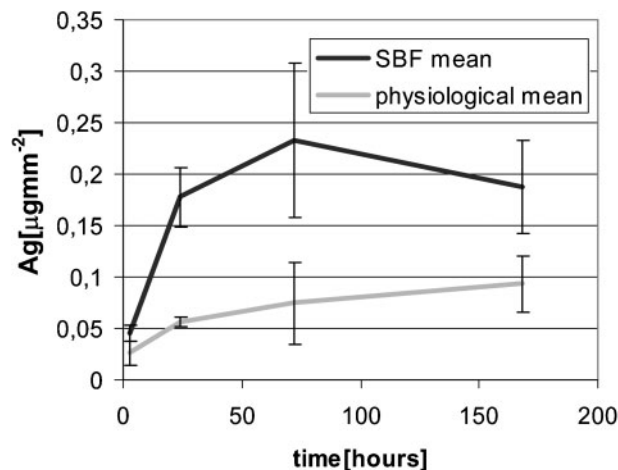


a amount; b rate

8 Silver release in SBF of sintered and coatings samples

such as  $\text{AgCl}_2^-$ ,  $\text{AgCl}_3^{2-}$ ,  $\text{AgCl}_4^{3-}$ . These complexes can affect the AgCl solubility, since only the  $\text{Ag}^+\text{Cl}^-$  ion pair is responsible of its precipitation. This can explain the high release of silver ions detected during the first three days of soaking. However SBF is also rich of  $\text{Ca}^{2+}$  ions, which have higher affinity with phosphates than silver, and causes precipitation of hydroxyapatite on samples' surface. This process subtract phosphates from solution, promotes AgCl precipitation and consequently silver decrease in solution. These effect is less evident after longer times, due to the progressive stabilisation of the diffusion phenomena through the hydroxyapatite layer grown on the glass ceramic surface.

For comparative purposes the amount of silver release in physiological solution (0.9 wt-%NaCl) was also evaluated up to one week. Testing procedure is the same of SBF leaching test. Figure 9 shows that silver release is higher in SBF than in simple physiological solution and the decrease of silver release after three days is not observed. This could be due to the absence of phosphates in physiological solution, since their presence could promote silver release avoiding the precipitation of AgCl, as discussed before. Scanning electron microscopy observation on the samples soaked



9 silver release in physiological solution

in physiological solution (here not reported) showed, as expected, the presence of AgCl, some signs of glass corrosion and no sign of hydroxyapatite precipitation.

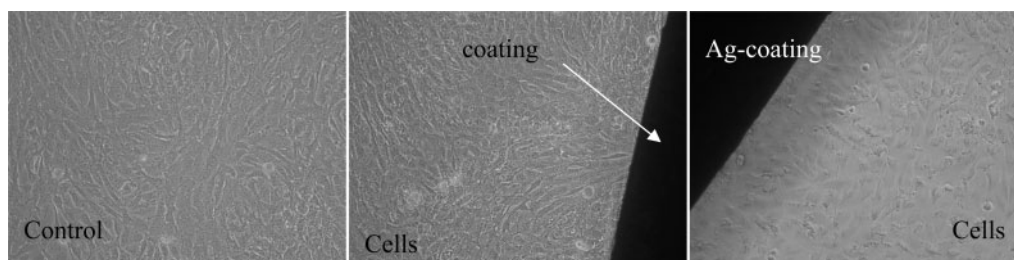
Cytotoxicity tests

Figure 10 collects optical observations of cells after four days culture and represents a comparison between control (cells cultured without coating), Fa-GC coatings and Ag-Fa-GC coatings. While images are similar for both morphology and number of cells for control and Fa-GC coatings, Ag-Fa-GC coatings present a lower number of cells and a significant amount of suffering cells (globular ones). This shows good biocompatibility for Fa-GC coatings but a starting sign of toxicity for Ag-Fa-GC coatings.

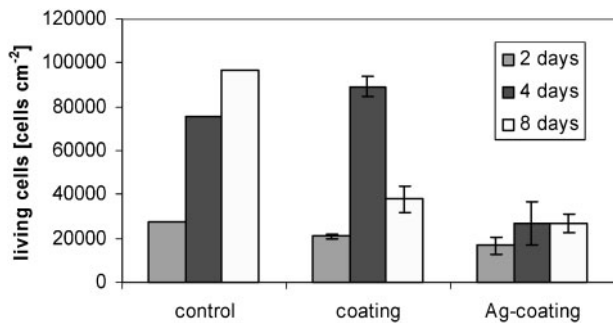
Fa-GC samples, in the coating form, receive and release a higher amount of silver respect to sintered form, that affect cellular tolerability. The amount of silver introduced onto coatings is probably at bound of safety silver gap and the count of viable cells confirms this behaviour, as shown in Fig. 11.

Nevertheless it is important to highlight that culture medium was not substituted during cellular test, so  $\text{Ag}^+$  ions are accumulated on medium, otherwise in the human body, fluids clear the wound bed and remove the products rising from an implant device (clearance process). Therefore a starting cytotoxic behaviour during *in vitro* test could have no effect *in vivo*.

pH value is 8.37 for basal medium. Table 1 reports pH mean values for control, Fa-GC coatings and Ag-Fa-GC coatings. An acidification after cell culture indicate active cellular metabolism. Ag-Fa-GC coatings samples present a lower medium's acidification in the first four days culture, in accordance to optical observation and counts results. At eight days culture medium acidification is the same for both coatings.



10 Optical observations after four days culture



11 Viable cells counts

Table 1 pH and LDH values

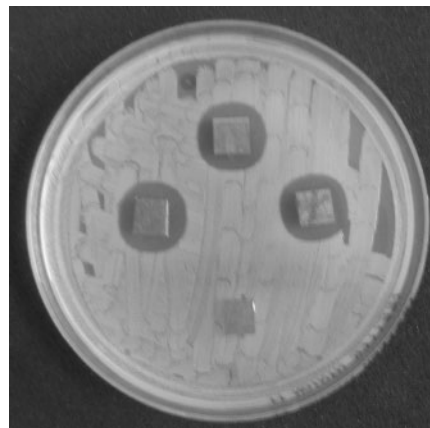
	pH		LDH			
	2 days	4 days	4 days	2 days	4 days	8 days
Control	8.12	7.98	8.23	5.71	9.49	67
Coating	8.06	8.02	7.92	3.12	0.77	89.38
Ag coating	8.10	8.16	7.92	0.00	0.00	14.32

Lactate dehydrogenase in culture medium is sign of necrosis. At two and four days culture there is not LDH neither for control nor for Fa-GC coatings and Ag-Fa-GC coatings. At eight days culture LDH measurement reveals a significant presence of the enzyme for control and Fa-GC coatings but not for Ag-Fa-GC coatings. At eight days culture optical observation and counts underline confluences reaching for control and Fa-GC but not for Ag-Fa-GC, which have determined a slowing down in culture growth, inhibiting confluence reaching. This explains cellular death in control and Fa-GC and a static situation for Ag-Fa-GC after eight days culture. At this time for the first two samples cellular death is not due to the presence of an external material in culture medium, nor to silver release, but to natural development of the culture.

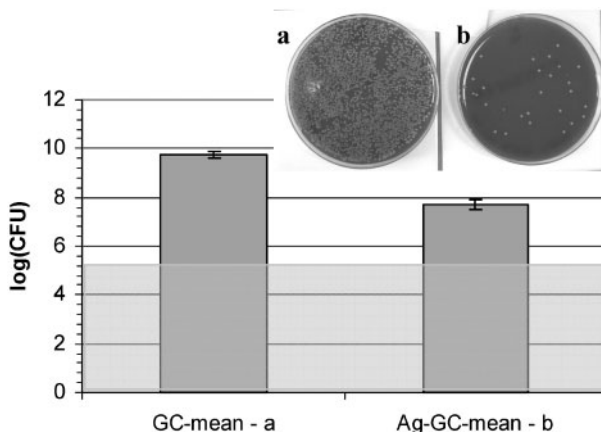
**Antibacterial tests**

Figures 12 and 13 show that silver containing samples are able to produce a significant inhibition zone both with standard bacterial strains and with clinical ones. The antibacterial behaviour also on bacteria stock isolated from human prosthetic infection is a significant confirmation, since it is very probable that clinical stocks are more resistant to antibacterial agents and/or antibiotics than standard one.

Broth dilution tests give quantitative information about antibacterial behaviour of silver doped samples.



13 Inhibition zone evaluation: efficacy of silver doped coating against clinical strains

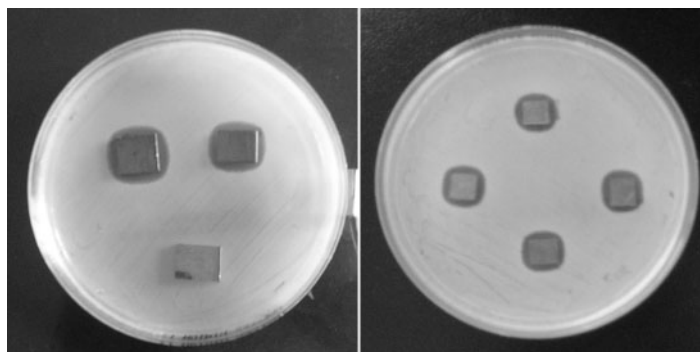


14 Colony forming units count for broth

Solution analysis describes bacterial proliferation in the whole medium were both bacteria and samples have been incubated. Counts show two order magnitude reduction of CFU number for silver containing samples (one way ANOVA,  $p < 0.05$ ), as reported in Fig. 14 (the box indicates the amount of initial inoculum).

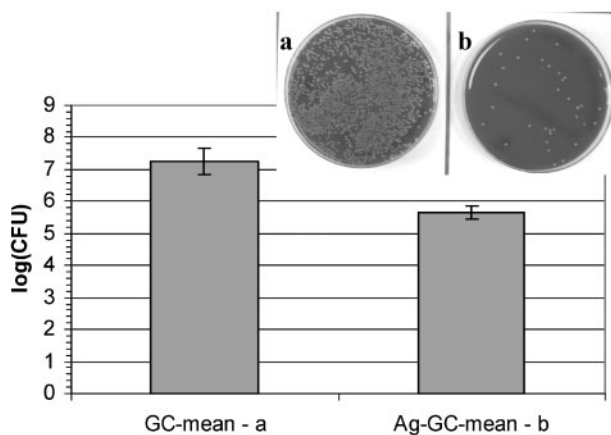
Washing solution quantifies bacterial proliferation in the liquid film just around the sample. Also in this case there is a two order magnitude reduction in CFU number for Ag-GC (Fig. 15).

Vortexing solution determines the amount of bacteria adhered on samples' surface and detached by vortex treatment. Silver containing samples show two order magnitude reduction in CFU number (Fig. 16; one way ANOVA,  $p < 0.05$ ).

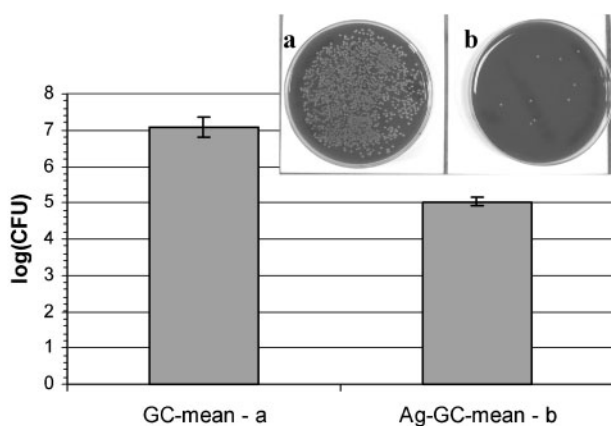


12 Inhibition zone evaluation

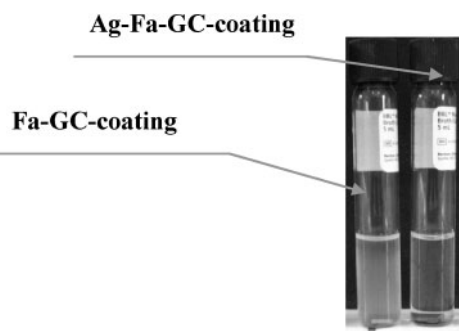




15 Colony forming units count for washing solution



16 Colony forming units count for vortexing solution

17 McFarland index and turbidity observations: mean Fa-GC coating,  $2.58 \pm 0.17$ ; mean Ag-Fa-Gc coating,  $0.08 \pm 0.01$ 

Finally, the turbidity evaluation is reported in Fig. 17: the solution containing untreated samples shows a McFarland index clearly higher than Ag-Fa-GC coating containing solution, as confirmed by tubes pictures; therefore the obtained results from all antibacterial tests are concordant.

## Conclusions

Bioactive glass ceramic coatings onto titanium substrates are an interesting solution in order to promote tissue integration of implants maintaining mechanical bulk properties of metallic structures. The possibility to confer antibacterial properties to these coatings represents a challenge. In this research work ion exchange

technique, previously optimised on bulk glass ceramic samples,<sup>12</sup> has been successfully applied to glass ceramic coatings. Results show that this kind of modification is effective also on coating samples. Owing to the higher specific surface exposed to treatment, plasma spray coatings are able to acquire a larger amount of silver compared to bulk samples, if submitted to the same process conditions, so a higher amount of ions is released from these materials. Treated samples present a significant antibacterial behaviour against *S. Aureus*, reductions up to two order magnitude in CFU number have been observed for silver containing material compared to untreated ones. Cytotoxicity tests on modified glass ceramic coatings in static conditions globally showed a good cellular response. In view of future applications, an optimisation of the biological behaviour of silver doped glass ceramic plasma spray coatings on prosthetic device is possible, case by case, by tailoring the processing conditions both in terms of coating production and ion exchange modifications.

## Acknowledgement

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