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## **Surface silver-doping of biocompatible glasses to induce antibacterial properties. Part II: plasma sprayed glass-coatings**

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**Abstract**

A glass of the composition 57%SiO<sub>2</sub>, 3%Al<sub>2</sub>O<sub>3</sub>, 34%CaO and 6%Na<sub>2</sub>O has been produced as a powder and deposited by plasma spray on titanium alloy and stainless steel substrates. The obtained coated samples have been subjected to a patented ion-exchange treatment to introduce ionic silver on material surfaces and so induce antibacterial activity. Silver surface-enriched samples have been characterized by means of X-ray diffraction, SEM observation, EDS analysis, *in vitro* bioactivity test, leaching test by GFAAS (graphite furnace atomic adsorption spectroscopy) analyses, cells adhesion and proliferation, and antibacterial tests using *Staphylococcus Aureus* strain. *In vitro* tests results demonstrate that the modified samples acquire an antimicrobial action against tested bacteria, without affecting the biocompatibility of the glass.

The ion-exchange treatment can be successfully applied to glass-coated samples without affecting the properties of the coatings. Finally, this method could be also proposed for other glass or glass-ceramic coatings, of proper composition, to produce coated devices for bone healing and/or prostheses, able to reduce bacterial colonization and reducing the infections risks.

**1. Introduction**

Medical devices for implants and prostheses are commonly realized with metallic materials, such as steel or titanium alloys, as they provide satisfactory results as regards mechanical and biological behaviour. Metals can be considered as inert materials as they do not have any interaction with bone

tissue cells, being the adhesion between the device and the host tissue based only on the mechanical bonding of surfaces. Moreover, metals do not provide any protection against bacteria adhesion, representing a good environment for the colonization of the material surfaces, especially when they are subjected to enroughness treatments (such as sand blasting, acid etching...) [1,2].

Despite the use of hygienic protocols and preventive antibiotic prophylaxes, the infections development remains one of the most serious problems connected to orthopaedic surgery, since they can cause important damages to patients [2,3], as implant failure and re-operation. Nowadays, the infections are defeated with the antibiotics use, but the increasing bacteria resistance has induced the researchers to found an alternative, such as orthopaedic implant devices realized using synthetic materials able both to be osteointegrated and to possess antibacterial properties [4-6].

Silver is well known for its antibacterial properties against most of bacteria species [7-9] and the bacterial resistance to this element is nowadays a rare phenomenon. For this reason, many researchers are studying the possibility to overwork the peculiar characteristics of silver and its compounds to realize implant materials capable to prevent bacteria colonization and consequently infection diseases [10-15]. Unfortunately silver and its compounds become toxic towards cells and tissues when their concentration is higher than threshold. This fact led to use silver to coat, for example, the surfaces of devices as urinary catheters or external fixation pins for bone fracture healing. In the first case, the real efficacy of silver is already discussed and not even completely cleared, but toxicity does not represent a limitation to the use of silver, as cell tissues does not become in direct contact with silver coated surfaces for prolonged periods. On the other side, silver coated orthopaedic pins revealed a good protection against bacteria colonization, but the amount of silver found in patients blood after implant resulted higher than tolerable limit of toxicity [16]. The silver toxicity is a still open problem, in particular the discussion concerns its potential toxic effects at local level and/or systemic one; the first one, even less dangerous than the systemic toxicity, can cause argyria phenomena and cells pain around silver containing devices.

For this reason, the possibility to control the amount of silver and to limit its presence to the external surfaces of implant materials has become the main goal to realize safe devices, both in terms of compatibility towards host tissues and in terms of reduced bacteria adhesion onto implant's surfaces.

The aim of this work was the realization of glass-coated metallic devices showing a reduced affinity towards bacteria, obtained through a silver surface-enrichment, together with the maintenance of biocompatibility towards host tissues. Several methods can be used to coat a substrate with a glassy layer. Among them, lot of literature deals with enamelling, glazing, plasma-spray, spin casting, sputtering, electrophoresis and pulsed laser ablation. Thermal spraying is a well established technology commonly used to produce coatings for a wide variety of applications [17]. Thermal spray processes can be grouped into three major categories: plasma-arc spray, flame spray, and electric wire-arc spray. The plasma spray technique made easier the development of bioactive glass coating and composites [17-25] on titanium and its alloys. By multiple scanning of the substrate, coatings of the desired thickness, usually around 70-150  $\mu\text{m}$ , can be easily developed. For these reasons, the plasma spray process was selected for the realization of the glass coatings in this work. In order to impart antibacterial properties to the glass coated substrates, the ion-exchange technique was used, following a patented and optimized experimental schedule. The glass selected for this study is a silica based glass, characterized by moderate bioactivity index, so is a good candidate for the realization of long-term stability bioactive coatings on bio-inert substrates. The glass composition was selected during previous studies, described in the first part of this work [26], aiming to reduce as much as possible the introduced silver on the glass surface, as well as to develop a glass composition which allowed the realization of coatings on metallic substrates (stainless steel and Ti6Al4V), in order to apply the ion exchange technique directly on coated devices.

## 2. Experimental

### 2.1 Materials synthesis and characterization

The glass chosen as plasma-sprayed coating on such substrates has the molar composition of 57%SiO<sub>2</sub>, 3%Al<sub>2</sub>O<sub>3</sub>, 34%CaO and 6%Na<sub>2</sub>O (named SCNA). The glass has been prepared by melting reagent-grade reactants in a platinum crucible at 1500°C for 1h and then quenching the melt in water to obtain a frit, or pouring the melt in a copper mould to obtain regular shaped bars. After drying, the frit was ball milled in a planetary miller to obtain a powder. After milling, the powder was sieved down to 100 µm (the powder size was selected on the bases of technical requirements for plasma-spray process, performed by Eurocoating s.p.a.). The SCNA glass powder has been subjected to thermal characterizations by means of Differential Thermal Analysis (DTA-7-Perkin Elmer), Differential Scanning Calorimetry (DSC-7-Perkin Elmer) in order to determine its characteristic temperatures. Thermal Dilatometry (DMA-7-Perkin Elmer) was used in order to determine the thermal linear expansion coefficient ( $\alpha$ ) on bulk samples of 8x3x3 mm<sup>3</sup> size, obtained by cutting the glass bars after a proper annealing process.

Metal substrates selected for this work have been Ti6Al4V alloy and AISI316L steel, as the major part of implant devices for bone healing are realized with those alloys. Ti6Al4V substrates were provided as disks of 25mm diameter and 5mm height, and only one surface has been plasma-spray coated with SCNA glass; this geometry was specifically selected in order to perform mechanical adhesion tests. AISI316L samples were provided as cylinders of 5mm diameter and 12mm height and their entire surface has been coated. Metal substrates have been previously sand-blasted with alumina and ultrasonically cleaned. The plasma spray process parameters have been selected by Eurocoating s.p.a., on the basis of preliminary experiences. From now on SCNA coated steel samples will be called GCS, while SCNA coated Ti-alloy samples will be called GCT. The main characteristics of coated samples are summarized in table 1. The coatings thickness has been evaluated through SEM observations of different sample sections and calculated in many section point to verify the thickness homogeneity.

## *2.2 Mechanical tests*

The uniformity and good coating adhesion are important parameters to guarantee an optimal prosthesis adherence and bond to tissue, avoiding its instability. The determination of glass coatings adhesion on titanium alloy substrates was carried out in accordance with ISO standard 13779-4 by Eurocoating s.p.a. to evaluate the interfacial bonding strength. The adhesion on stainless steel substrates was only evaluated by qualitative observations, since the geometry of the provided sample was not adequate for tensile tests.

## *2.3. Surface modification*

Sets of both GCS and GCT samples have been subjected to a silver ion-exchange process to modify glass surface by introducing a controlled amount of silver ions. The ion-exchange process is patented [27] and is based on a thermo-chemical treatment of the glassy material in an opportune solution, capable to exchange mono-valent ions, coming from the glass, with silver ions, coming from the solution itself. The parameters that regulate such process are respectively: silver ions concentration of the solution, time and temperature of the process; by opportunely tuning ion-exchange parameters it is possible to modulate both the amount of silver ions introduced in the glass and their concentration profile along the glass material section [28,29]. Such process can be applied, simply by tuning the process parameters according to the material composition and characteristics, to any material belonging to the classes of glasses, glass-ceramics and ceramics. This kind of silver-doping treatment of glasses, glass-ceramics and ceramics surfaces, allows imparting antibacterial properties to a wide range of materials, without inducing any drastic change in their peculiar properties.

GCS and GCT samples were subjected to the silver ion-exchange treatment in different aqueous solutions containing different amounts of silver ions. In this work different ion-exchange conditions have been evaluated, by varying the solution concentration between 0.5M and 0.05M, an

exchanging time and temperature process respectively between 30÷50 minutes and 80÷100°C; afterwards two silver ions concentrations were selected: one more concentrated (called I) and another one less (II). Glass-coated silver-ion-exchanged samples will be called, from now on, Ag-GC-I and Ag-GC-II; since any significant difference between GCS and GCT samples was observed, neither before nor after the ion-exchange treatment, all coated samples will be named with the same connotation.

GC and Ag-GC samples were then analyzed by means of X-Ray diffraction (XRD), using Bragg Brentano camera geometry and Cu-K $\alpha$  incident radiation, to verify if any structural change or crystallization phenomena occurred due to plasma-spray process and/or silver ion-exchange treatment. Scanning electron microscopy (SEM Philips 525M) observations and energy dispersion spectrometry (EDS) were performed to evaluate morphology and composition both on GC and Ag-GC samples.

#### 2.4. *In vitro* bioactivity test

The term “bioactivity” refers to the ability of some silica-based glasses to induce *in vitro*, by immersion in a simulated body fluid, the formation of a semi-crystalline hydroxycarbonatoapatite (HCA) rich layer. This behaviour is considered as an indication of their *in vivo* bioactivity (natural bonding ability to living tissues) through a mechanism that starts with a rapid ions exchange between the alkaline ions from the glass surface and the hydrogen ions from the solution, followed by the formation of silanols, which then undergo polycondensation to develop a silica gel layer. This layer promotes the adsorption of Ca<sup>++</sup> and PO<sub>4</sub><sup>3-</sup> ions from the solution. These ions subsequently react, forming the HCA layer. *In vitro* bioactivity tests were performed soaking GC and Ag-GC-I samples on simulated body fluid solution (SBF, as reported by Kokubo [30]) at biological temperature (37°C) for periods up to one month. Coatings and silver modified coatings reactivity was evaluated, after soaking in SBF, by means of SEM observation. The bioactivity test



was performed only on Ag-GC-I, samples subjected to the most severe ion-exchange condition, to evaluate the possible effect of silver introduction.

### *2.5. Leaching test*

The amount of released silver ions from Ag-GC surfaces was investigated on a set of three samples, having the same size and surface area (120 mm<sup>2</sup>). The procedure followed in this work was the same reported in a previous paper [26]: the samples were soaked into 30 ml of SBF solution maintained at 37°C up to 30 days. Grafite furnace atomic adsorption spectroscopy (GFAAS) analyses were performed on 1 ml of SBF spiked from each soaking solution after 3 hours, 1day, 2, 7, 14 and 30 days of dipping. For comparative purposes also the pure SBF and the solutions in contact with GC have been analysed.

### *2.6 Biocompatibility test*

Ag-GC-II biocompatible behavior has been verified using a fibroblast cell line; the cells have been cultured at 37 °C in 95% air/ 5%CO<sub>2</sub> in DMEM supplemented with 10% fetal bovine serum (FBS, Sigma), penicillin (100 U ml<sup>-1</sup>), streptomycin (100 µg ml<sup>-1</sup>) and L-glutammine 0.03%.

Biocompatibility tests consisted in fibroblasts adhesion and proliferation studies, performed using a starting cell density of 10<sup>4</sup> cells/cm<sup>2</sup>; incubated cells have been counted in a Burkler camera after being removed with SDS solution from samples surface, respectively after 6 hours culture, to evaluate their adhesion, and 24 hours culture to estimate the proliferation.

Ag-GC-II behaviour has been compared with GC sample and with a control one; both adhesion and proliferation have been investigated on a set of three samples.

### *2.7 Antibacterial test*

The zone of inhibition test [31] was performed on Ag-GC samples and on GC as control. A *Staphylococcus Aureus* standard stock (ATCC 29213) was used to characterize Ag-GC-I and Ag-

GC-II samples; furthermore the inhibition halo of Ag-GC-I was observed employing one stock isolated from human prosthetic infection: a bacterial broth has been prepared dissolving a *S. Aureus* disk in 5ml of brain heart infusion; after overnight incubation at 37°C, 10µl of the suspension have been spread on a Blood-Agar plate and incubated 24 hours in order to allow the bacterial colonies growth.

A standard 0.5 Mc Farland (containing approximately  $1 - 2 \cdot 10^8$  CFU/ml) solution was prepared dissolving some bacterial colonies in a physiological solution (turbidity has been evaluated by optical instrument – Phoenix Spec BD McFarland), an aliquot of this suspension has been spread on Mueller Hinton agar plates. Silver containing samples surfaces were drawn close to agar plate and incubated 24 hours at 35°C.

Broth dilution tests have been also performed on Ag-GC-I and Ag-GC-II samples: a 0.5 Mc Farland suspension has been prepared in the same way described above, an aliquot of bacterial suspension has been introduced in Mueller Hinton broth tubes in order to obtain suspensions approximately containing  $5 \cdot 10^5$  CFU/ml. After 24h incubation at 35°C tubes' turbidity has been optically evaluated.

As for samples washing and vortexing solutions have been analyzed in order to quantify colony forming units (CFU). Washing solution has been prepared by rapid rinsing of sample in physiological solution, while the vortexing one by one minute 50Hz vortex of sample in physiological solution. Mc Farland index of each solution has been measured, then they have been serially diluted and spread on Blood-Agar plates, after overnight incubation at 35°C CFU have been counted on plates.

The percentage reduction in bacteria count was calculated by the formula:

$$\frac{CFU_0 - CFU_{Ag}}{CFU_0} * 100$$

Where  $CFU_0$  is the number of colonies forming units counted on plate containing the solution of untreated samples and  $CFU_{Ag}$  is the number of bacteria counted on doped silver samples; this formula has been used as for adhered bacteria as for proliferated ones.

All samples were previously dry sterilized at 170°C for 1 hour and all products for antibacterial analysis have been purchased from BD-Becton Dickinson.

### **3. Results and discussion**

#### *3.3 Thermo mechanical properties of the coatings*

Thermal analyses on SCNA powders measures  $T_g$  at 685°C. This temperature was detected in order to set the annealing procedure for the glass bars (for ten hours at 600°C), to be cut in small specimens for the determination of the thermal expansion coefficient ( $\alpha$ ). The value of  $\alpha$  varies between  $6,8 \cdot 10^{-6} \text{ }^\circ\text{C}^{-1}$  and  $8,6 \cdot 10^{-6} \text{ }^\circ\text{C}^{-1}$  in the range from 100°C to 600°C. In this temperature interval,  $\alpha$  for Ti6Al4V ranges between  $8,7 \cdot 10^{-6} \text{ }^\circ\text{C}^{-1}$  and  $10 \cdot 10^{-6} \text{ }^\circ\text{C}^{-1}$ , while for AISI316L it ranges between  $16 \cdot 10^{-6} \text{ }^\circ\text{C}^{-1}$  and  $17,5 \cdot 10^{-6} \text{ }^\circ\text{C}^{-1}$  (being  $\alpha$  for Ti-alloy nearer to the glass than the steel one). Taking into account the theories that describe how to control the interface between a glass coating and a metal substrate, the glass should have a slightly lower thermal expansion than the metal. This feature could induce only small compressive stresses in the glass coating, avoiding the generation of tensile thermal stresses during cooling from the processing temperature to room temperature, which can induce coating cracking or delaminating during processing. Bioactive glasses are typically silica-based glasses, with silica content below 60 wt.% (glasses with silica contents greater than 60 wt.% are no longer bioactive). Most of these glasses have thermal expansion coefficients much higher than those of Ti alloys. Thermal expansion of the glass can be reduced by increasing the  $\text{SiO}_2$  content, or by adding  $\text{Al}_2\text{O}_3$  in the composition, but this reduces bioactivity as well [32,33], so these compositional modifications should be done in a very limited amount, as in the present work. Despite the differences in the thermal expansion coefficient between the glass and the two metal substrates, no significant differences between the two metals were observed after plasma spray

coating, verifying that, for the plasma spray technique, the different thermal properties of the coating, respect to the substrate one, do not affect in great amount the main characteristics of the resulting coated sample, as could happen, on the contrary, during enamelling processes.

Moreover, any significant difference can be observed between GCS and GCT samples, being the structure, the morphology and the mechanical adhesion of the glass coating the same on both metals' substrates. Table 1 summarizes some dimensional features of the coated GCS and GCT samples. The coating adhesion, estimated on GCT samples showed a value up to  $41.2 \pm 9.6$  MPa. This value is higher than those detected for various plasma spray coatings of similar thickness made of hydroxyapatite, glasses or glass-matrix composites on Ti6Al4V substrates, as reported in literature [24,34,35].

### *3.2 Phase analysis (XRD) and morphology.*

The plasma spray process did not provoke any crystallization phenomena on the SCNA glass, as clearly evident in figure 1.a and figure 1.b, where the XRD patterns of the as done glass and a titanium alloy coated sample have been reported: the only difference is represented by the signal of Ti substrate at  $40.3^\circ$ . Figure 1.c reports the XRD pattern of a coated GCT sample after ion exchange with silver solution, where no evidence of structural modifications after ion exchange on the glass coating appears.

As regards the effect on the substrates of silver ion-exchange process, both metallic substrates did not undergo any significant change in their structure or morphology, thus verifying that the process did not provoke metal corrosion or structure modifications (Figure 2). Morphological observations by SEM on coated samples did not evidence any dissolution phenomena or variation in the morphology occurring on the glass after silver ion-exchange process. Figure 3 shows a detail of the interface between the Ti substrate and the coating after ion exchange (performed after the sample sectioning) where no sign of corrosion are evident. The material composition, by means of EDS measurements, evidenced the presence of silver on Ag-GC surfaces (Figure 4). This is a further

confirm that the ion-exchange process allow only the modification of the outer layer of glass surfaces.

### *3.3. Leaching tests*

Since the specific surface area of GC samples is reasonably higher than that of SCNA massive samples, we can expect that all processes linked to surface mechanisms would be influenced and could affect the final characteristics of the device. So, though silver ion-exchange process was performed in the same conditions used for massive SCNA glass samples (as reported in the first part of the present study [26], data collected by GFAAS measurements showed higher amounts of released silver ions, on equal time both for Ag-GC-I and Ag-GC-II. Figure 5a show the release trend comparison between silver containing coatings, ion-exchanged in both  $\text{Ag}^+$  concentration selected for this work, and glass massive samples. Figure 5b reports the comparison of silver released rate from different samples. The greater part of silver is released during the first days of contact with SBF solution.

Leaching test show an amount of released silver in agreement with previous works and with the chosen ion-exchange parameters: coatings, having a higher specific surface area, release a higher amount of silver, proportional to the silver concentration selected. The release trend of Ag-GC-I samples is lightly different from Ag-GC-II ones, probably the more critical conditions of ion-exchange lead to a deeper diffusion of Ag ions and to a more gradual release.

Nevertheless, in both condition, silver is fast released during the first day of immersion in the simulated body fluid; this aspect is very important because the first period after a surgical treatment is particularly dangerous for the infections incidence, so it is very helpful to inhibit the bacteria adhesion immediately after a device implant, when a fight to colonize its surfaces occurs between host cells and bacteria, and promote the regeneration of a healthy tissue.

### *3.4. Bioactivity*

The *in vitro* tests, performed only on Ag-GC-I, confirm the low bioactivity of the glass, as verified in a previous work [14]. Since the glass maintains the same degree of bioactivity both after the ion-exchange and after the plasma spray process, the introduced silver, as well as the plasma spray process, are completely irrelevant on the glass reactivity. This result is very interesting in expectation to apply the ion-exchange technique to a medical devices, because this technique does not influence the substrate characteristics, both the bulk and surface properties, useful to promote the osteo-integration with the surrounding tissues.

### *3.5. Biocompatibility*

Biocompatibility tests confirmed the safety of materials also in the case of Ag-treated samples. Figure 6 shows the amount of alive cells counted on untreated and treated samples and on control ones, after 6 hours for the adhesion and after 24 hours for the proliferation evaluation. The fibroblasts amount adhered and proliferated on GC and on Ag-GC samples is lightly lower than cells grown on control, but the cells grown on silver doped samples are comparable with those on untreated glass. These cytotoxicity tests confirm the safety of silver doped coatings toward fibroblast cells.

### *3.6 Antibacterial properties*

The zone of inhibition tests disclose the antibacterial behaviour of both Ag-GC-I and Ag-GC-II doped samples: as expected the inhibition halo due to Ag-GC-I (3-4 mm) is larger than Ag-GC-II halo (2 mm), indeed Ag-GC-I was obtained using higher concentration silver solution than Ag-GC-II. Figure 7.a and figure 7.b show two Mueller Hinton Agar plate including everyone three silver doped samples and one untreated as control.

A further confirmation of the antibacterial behaviour of Ag-GC comes from the zone of inhibition test on the stock isolated from human prosthetic infection: Ag-GC-I show a halo of about 5 mm, larger than halo obtained with a standard stock (Figure 8).

Broth dilution tests (Figure 9) show an important antibacterial effect of Ag-GC-I and a more limited one for Ag-GC-II, nevertheless Ag-GC-I show a reduction of 99.8% for adhered bacteria and of 99.5% for the bacteria proliferated closely the material. Ag-GC-II provide an adhered bacteria reduction of 94.8% and of 78.7 % for proliferated colonies.

Mc Farland index measurement confirm this trend (Figure 10), there is a gap of 0.53 Mc Farland between GC and Ag-GC-II, and a gap of 2.02 Mc Farland between GC and Ag-GC-I.

In summary, antibacterial test showed a marked bacteriostatic behaviour of Ag-treated samples, proportional to the introduced and consequently released silver amount. The antibacterial effect is proved by different tests: broth dilution test, Mc Farland index evaluation and zone of inhibition test; the last one, performed on a stock isolated from human prosthetic infection, is an important confirmation of the antibacterial behaviour of silver doped samples.

As seen in the previous work, the introduced silver has a bacteriostatic effect, it inhibits the proliferation and most of all the adhesion of bacteria on coatings surfaces, but does not kill bacterial cells; nevertheless this is actually the aim of this research: the realization of a biocompatible and antibacterial glass-coated metallic devices. Future works will evaluate the possible bactericidal effect of silver ions released from Ag-GC samples.

#### **4. Conclusions**

A silica based glass with a low degree of bioactivity was successfully used to coat Stainless steel and Ti6Al4V samples By Plasma Spray in air (APS). The coated samples have been treated by a patented surface treatment to enrich their surface with small amounts of silver ions, in order to induce antibacterial activity. The parameters of the treatment have been carefully selected in order to obtain a surface with bacteriostatic properties but no danger for cytotoxicity; in particular the total amount of the released silver is under the toxic threshold, and on the glass surface any silver compound potentially responsible of toxic effect, precipitated during soaking.

The surface silver enrichment did not affect the morphology and the structure of the coatings. The bacteriostatic effect of the as treated coatings has been proved by three different antibacterial tests; at the same time the cytotoxicity test confirm the safety of materials and most of all of silver-doped samples toward fibroblast cells.

The plasma spray process did not induce any changing in glass composition and reactivity and the realized coatings have a good mechanical strength. The SCNA glass powders can be used to coat several metallic devices, on which the ion-exchange technique can be applied to impart antibacterial properties.

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**Table legend**

Table 1: Dimensional parameters of GCS and GCT

Name	Substrate	Diameter [mm]	Height [mm]	Coating material	Coating thickness [μm]	Coated surface area [mm2]
GCS	AISI316L	25	3	SCNA glass	80	491
GCT	Ti6Al4V	5	12	SCNA glass	80	228

**Figure legends**

Figure 1: XRD spectra of: a) SCNA glass, b) GCT and c) GCT after ion-exchange.

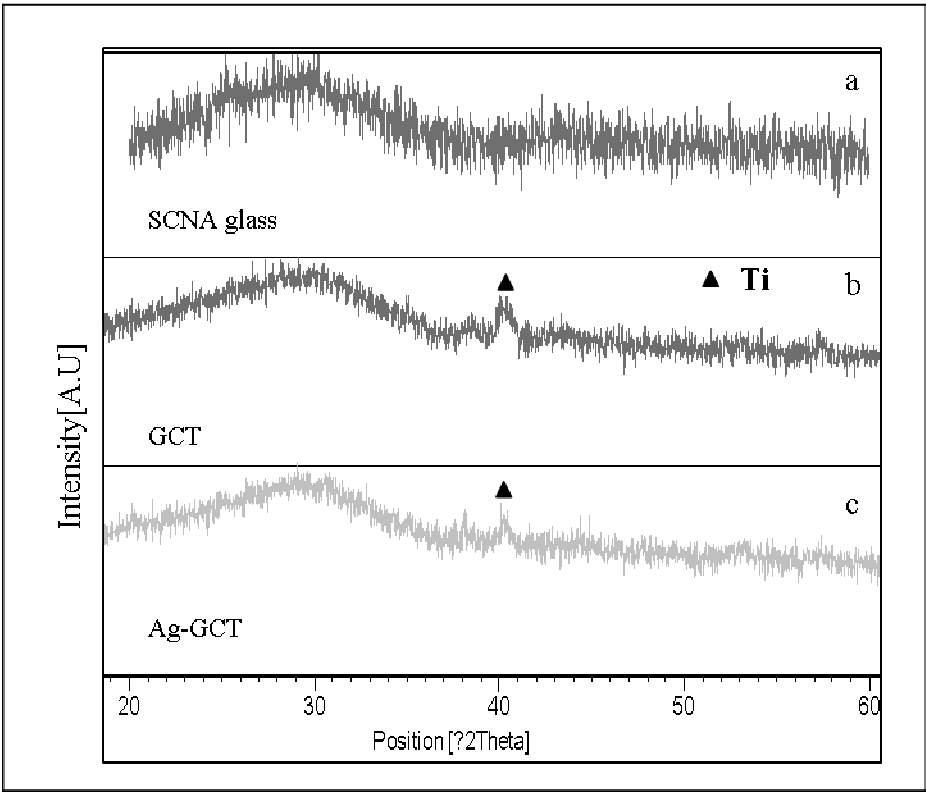


Figure 2: Influence of ion-exchange on Ti6Al4V substrate.

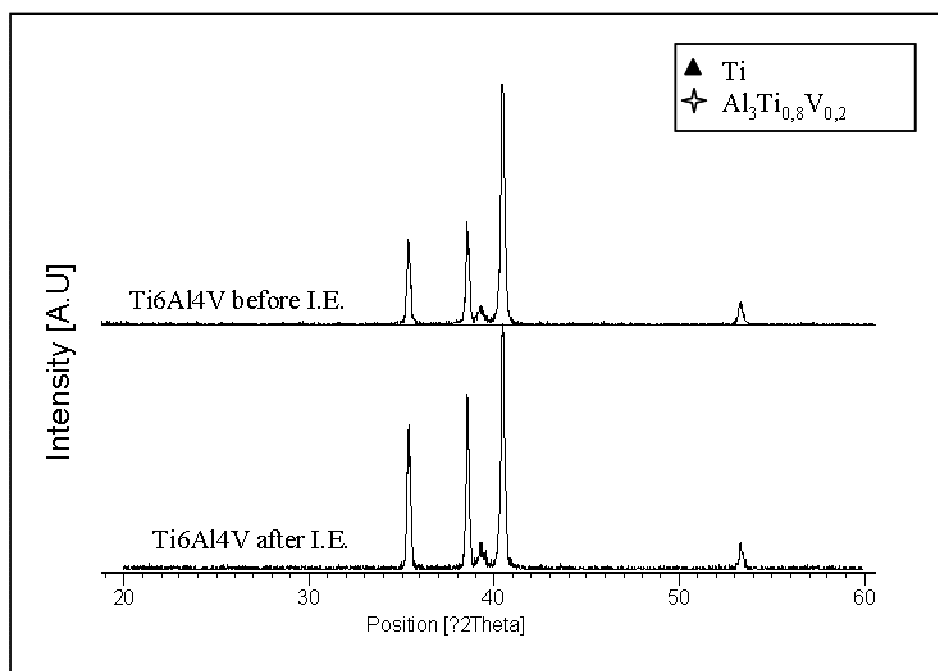


Figure 3: The interface between the Ti substrate and the coating after ion exchange

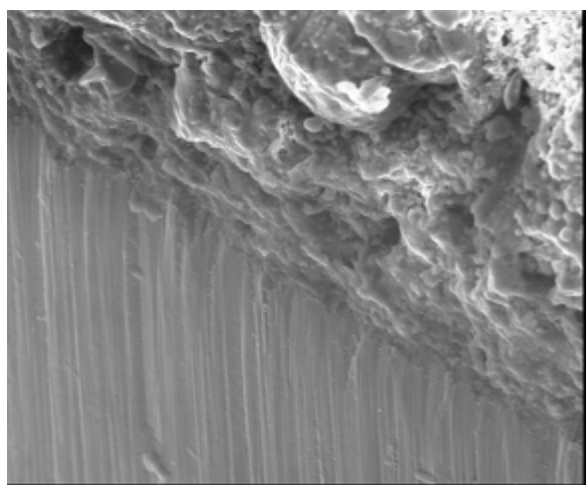


Figure 4: EDS analyses on a) GC, b) Ag-GC-I and c) Ag-GC-II

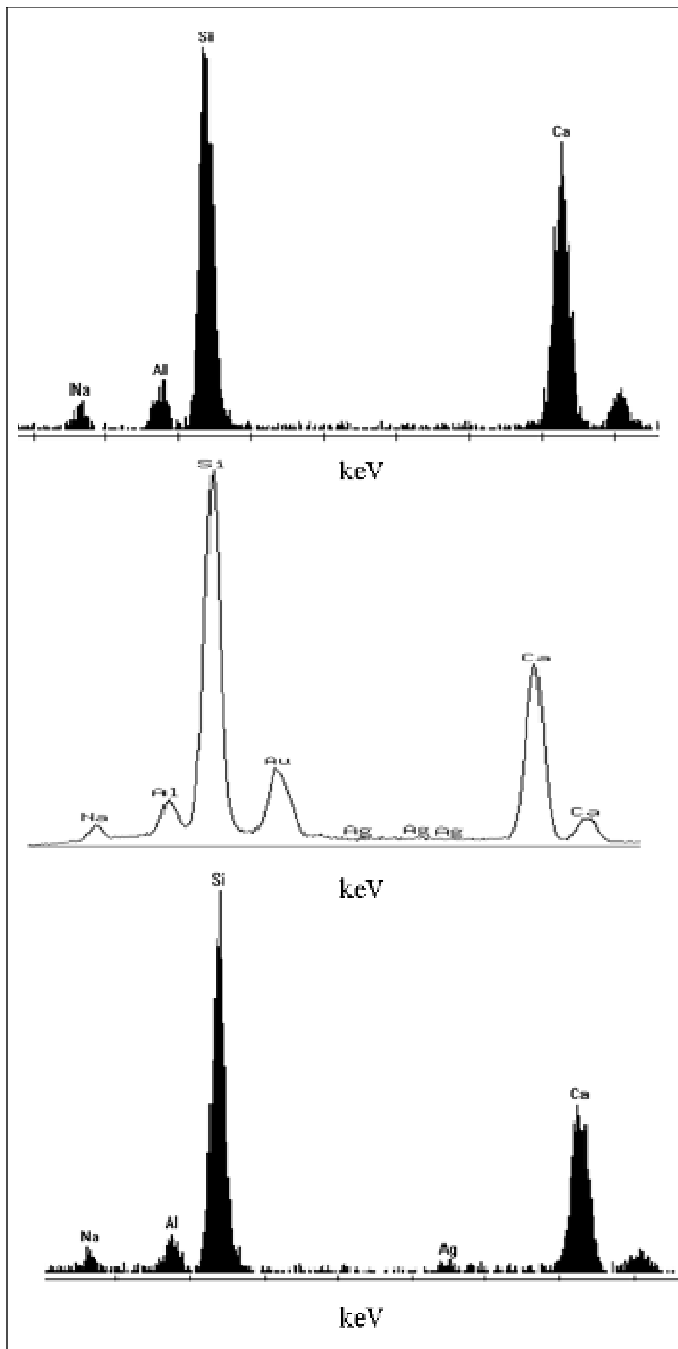


Figure 5: Comparison of silver release trend from massive glass and coatings

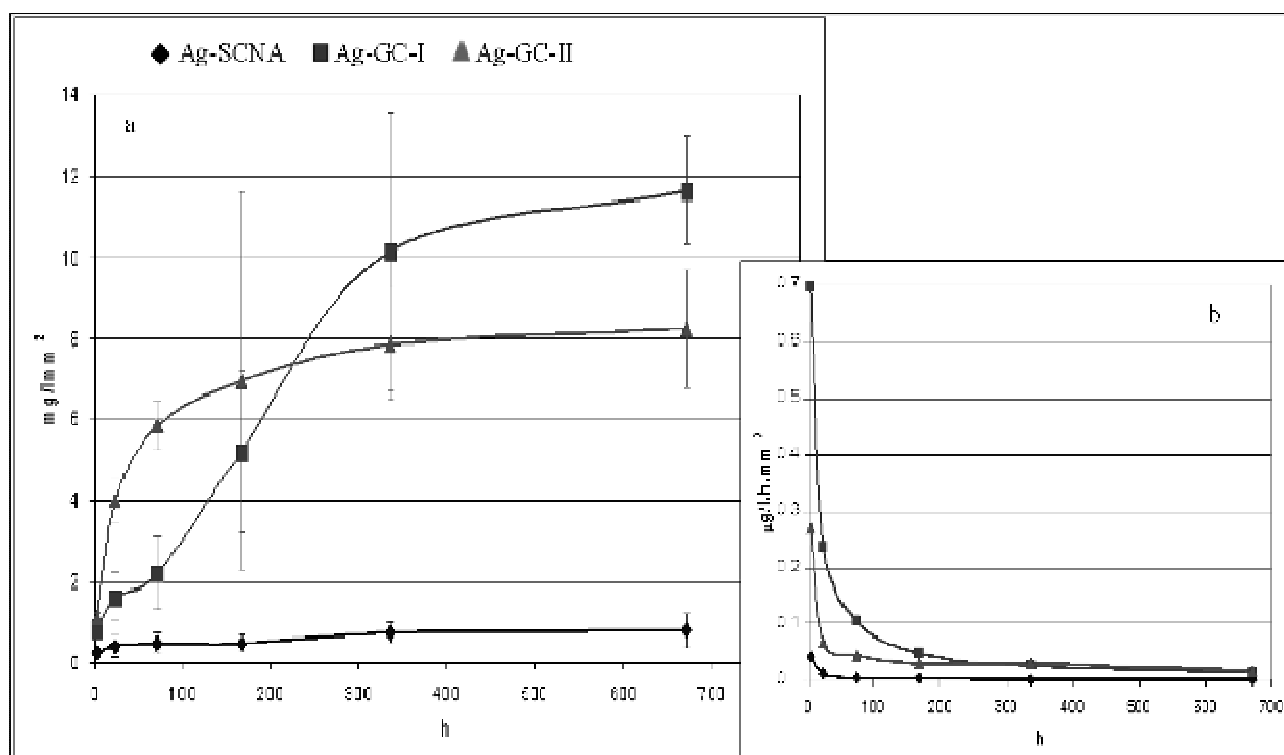


Figure 6: Number of alive cells adhered (6h) and proliferated (24h) on control (PE), glass samples (GC) and Ag-treated samples (AgGC)

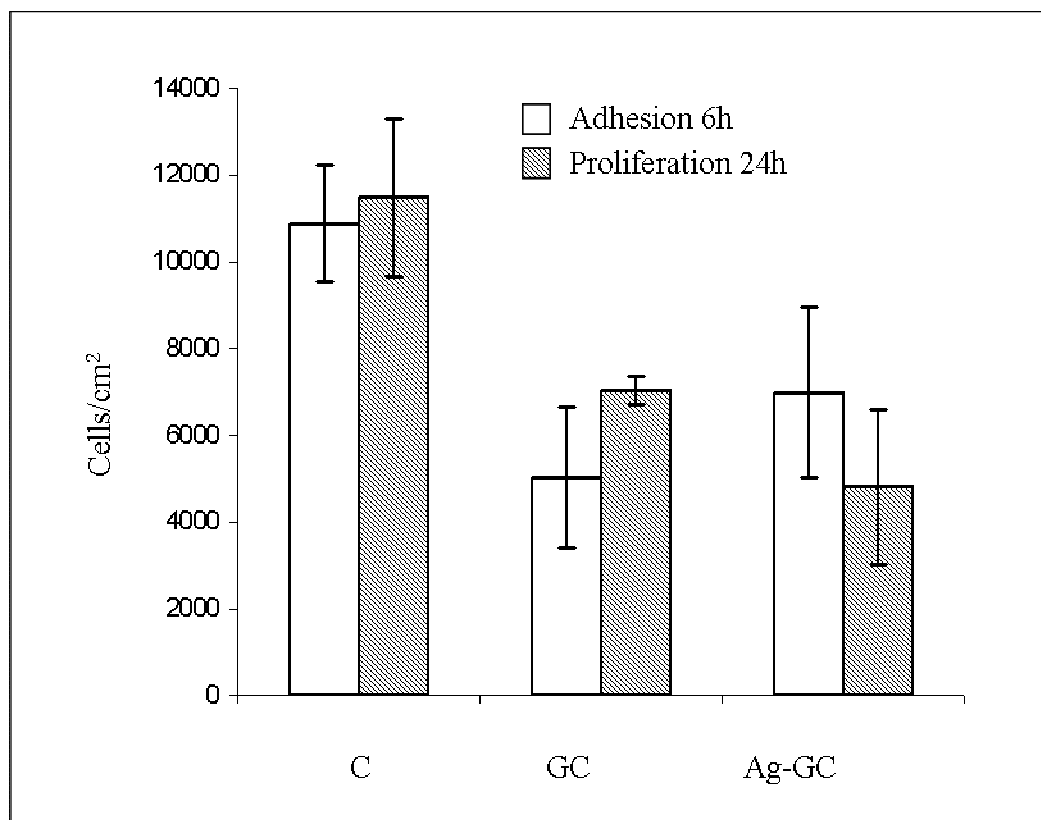


Figure 7: Zone of inhibition test: inhibition halo of a) three Ag-GC-I and one GC as control, b) three Ag-GC-II and one GC as control

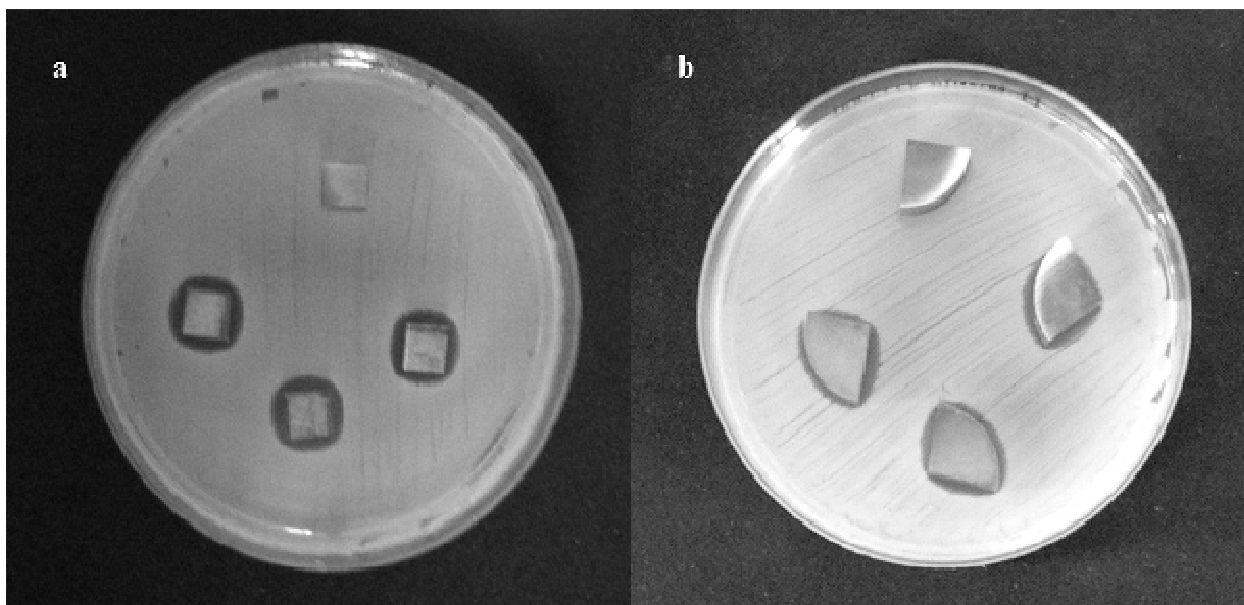


Figure 8: Zone of inhibition test on the stock isolated from human prosthetic infection of four Ag-GC-I and one GC as control

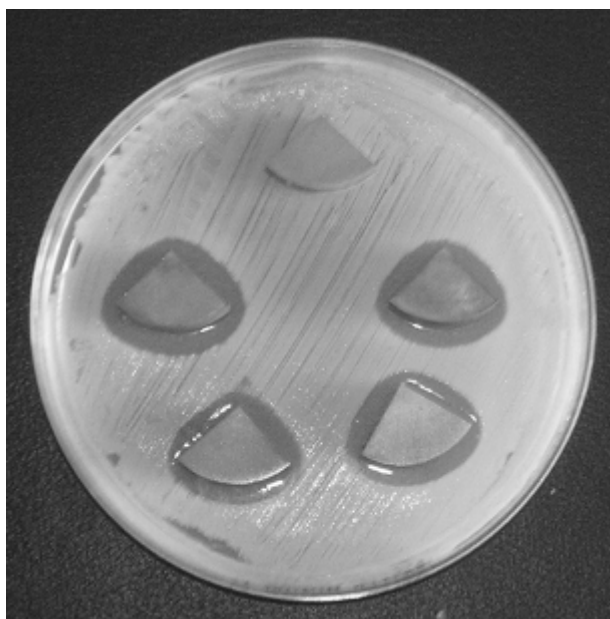


Figure 9:

CFU proliferated (a) and adhered (b) on GC □ , Ag-GC-I ■ and Ag-GC-II ▒

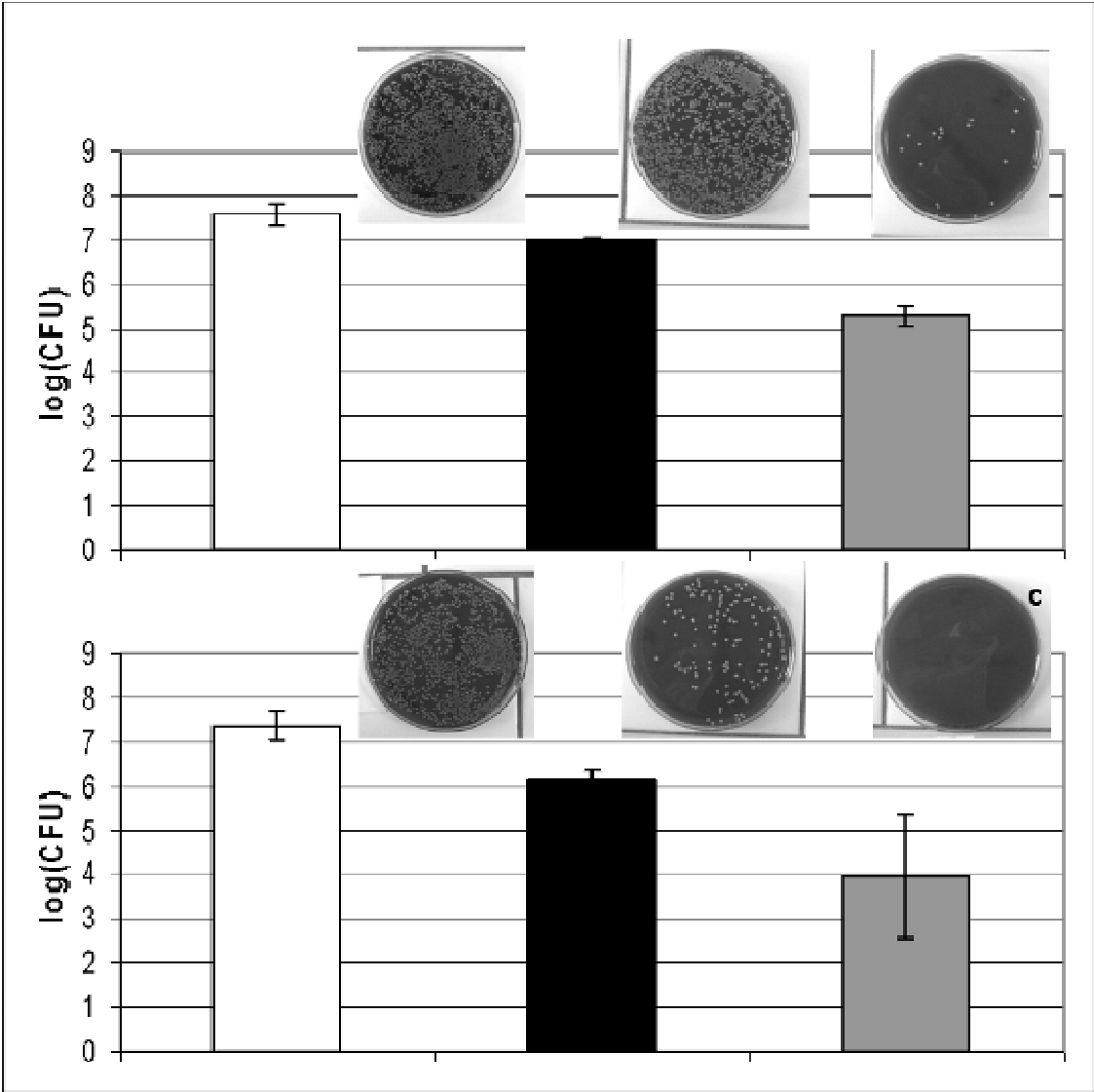




Figure 10:

Mc Farland index of GC , Ag-GC-I  and Ag-GC-II

