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Antibacterial inorganic coatings on metallic surfaces for temporary fixation devices

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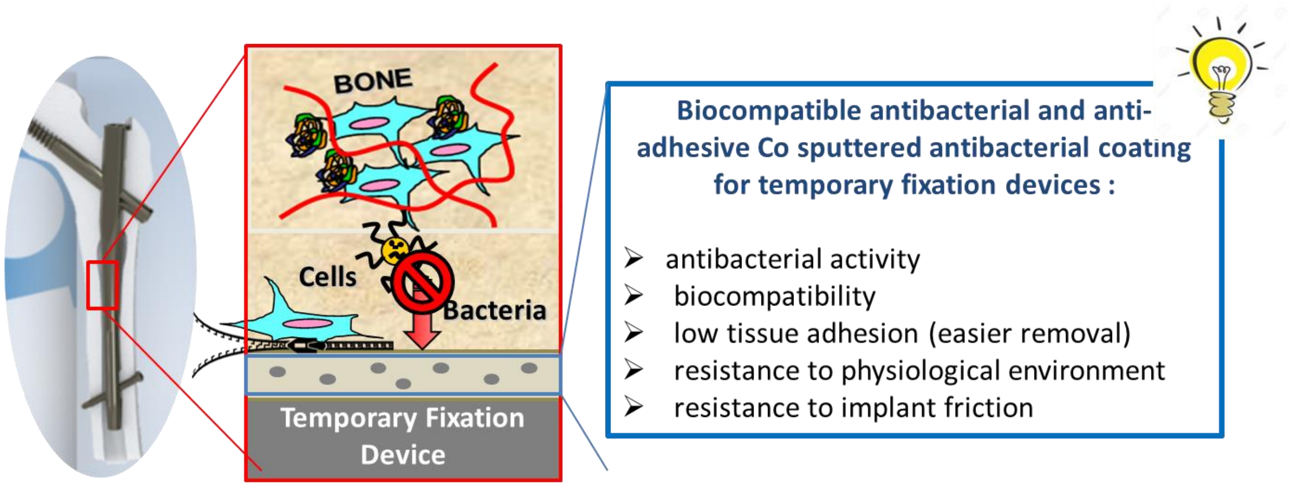
Abstract

Bacterial contamination and bone tissue overgrowth are currently the main issues for temporary fixation devices. The aim of this research is the development of innovative inorganic coatings able to reduce bacterial adhesion and bone tissue overgrowth without hampering cytocompatibility. 316L stainless-steel, Titanium grade4 and Titanium grade5 were selected as substrates, as currently used in temporary fixation devices. Starting from previous results on silica, alumina and zirconia were selected as coating matrices, according to their higher chemical stability, reduced bacterial adhesion and possible reduction of tissue overgrowth. Coatings were produced by co-sputtering. Coating adhesion was evaluated by tape test. Samples were immersed in ultrapure water up to 28 days to investigate silver release and chemical stability in fluids. Surface roughness was measured by contact profilometry. Surface wettability was determined by contact angle measurements and antibacterial activity was studied against antibiotic resistant *S aureus.* The research demonstrates the possibility to obtain coatings with roughness lower than the critical threshold for the increase of bacterial adhesion (0.2 µm) and optimal mechanical adhesion to metallic substrates. Coating chemical stability in water is strongly affected by the coating matrix composition. Silver release can be tailored to obtain antibacterial and biocompatible surfaces.

Graphical abstract



Biocompatible antibacterial and anti-adhesive Co sputtered antibacterial coating for temporary fixation devices :

- antibacterial activity
- biocompatibility
- low tissue adhesion (easier removal)
- resistance to physiological environment
- resistance to implant friction

Keywords

Coatings; Antibacterial, , temporary fixation devices, surfaces, sputtering, silver

Introduction

Temporary fixation devices are widely used in orthopedic and traumatology. They are introduced in the fractured bones in order to stabilize fractures and favor bone healing and should be removed when the bone recover its full functionality (time for fracture healing ranges from some weeks to some months depending on the body district). The main complications associated with this kind of devices are bacterial infections and tissue overgrowth.

Infection after fracture fixation is a critical problem in traumatology, which can lead to functional loss of the fractured limb, multiple surgery, prolonged antibiotic therapies and even amputation, as recently reviewed by Metsemakers et al. [1]. Bacterial contamination interests from 1% to 7% of orthopaedic implants; infection rate can reach 30% in patients with open fractures, as reported by Trampuz and Zimmerli [2].This problem is widely diffused mainly in the cases of fractures where the soft tissues surrounding the bone are limited and the lacerated skin can come in direct contact with the bone after fracture (tibia, knee, ankle, elbow and wrist fractures). Also in the case of application of an external fixator, the medical device is inserted in the bone and exits through the skin to be connected externally, thus presenting a constant “open” interface between the external and internal environment. On the other hand, bone tissue overgrowth on the implant surface can obstacle the device removal introducing the risk of secondary bone fractures associated with the extraction of the device [3, 4, 5].

Despite of a wide research on metallic surfaces, intended for permanent bone implants, able to promote a stable bone integration and/or avoid bacterial infection [6, 7], few works consider metallic surfaces for temporary implants with antibacterial and anti-adhesive characteristics (reduced bone tissue adhesion/overgrowth).

The use of smooth metallic surfaces for the reduction of bone tissue overgrowth has been reported [3,4,5], considering the crucial role of surface roughness in tissue adhesion. In the cited works a significant reduction of the force and time required for the device removal (after in vivo implantation) was observed for electropolished surfaces (roughness of at about 0.2 µm) compared to standard microrough titanium implants (roughness of at about 1 µm). Moreover, surface roughness has been evidenced as important parameter also for bacterial adhesion and a threshold for the increase in bacterial adhesion has been indicated as 0.2 µm for the average surface roughness [8, 9, 10]. In accordance to these data, the a certain reduction in bacterial adhesion was observed on the above cited electropolished titanium alloys [11].

The use of antibiotic loaded polymeric degradable coatings has also been proposed for temporary fixation devices and coated tibia nails (PROtect, DepuySyntes) already available in the market [12, 13]. This approach foresees the local release of the drug (gentamicin)allowing the therapeutic dose at the implant site but limiting its systemic toxicity [14]. However it must be noticed that antibiotics have a limited spectrum of activity (which can make difficult the treatment of polypmicrobial infections of a priori unknown pathogens) and can induce antibiotic resistance. Finally, a particular prosthesis for Tumor Applications (Mutars—Implantcast GmbH) [15] and titanium or stainless steel implants containing silver (e.g. Agluna® Technology) [16] can be cited in the framework of antibacterial fixation devices. The first one is a pure silver coating while the second one is an electrochemical treatment aimed at the creation of ionic silver reservoirs on the material surface. Both strategies replace antibiotics with silver, which possess a broader spectrum of activity and reduce bacterial resistance risk. However the dose dependent silver toxicity should be taken into account. All the above discussed solutions proposed for the obtainment of antibacterial fixation devices do not mention any effect on bone tissue overgrowth.

At the best of the author knowledge, surfaces able to reduce simultaneously bacterial contamination and bone tissue overgrowth have never been reported in this field.

The aim of the present research is the development of innovative inorganic antibacterial coatings for temporary fixation devices, able to limit bacterial adhesion (against bacteria involved in trauma infections) and tissue overgrowth, without hampering cytocompatibility. In this preliminary study an innovative inorganic coating has been designed and developed. Due to the preliminary nature of the work, the majority of the experiments were devoted to the optimization of coating deposition, composition, and of chemical and mechanical stability. Its antibacterial activity has been verified against S aureus and its biocompatibility has been indirectly analyzed verifying that the release values of the antibacterial agent are maintained below the recognized limits of cytocompatibility. The ability of coated surface to reduce tissue adhesion without hampering its healing will be investigated in future works.

The possibility to obtain mechanically and thermally resistant silver nanoclusters silica coatings with tailorable antibacterial activity on various substrates has been previously demonstrated by the authors [17-24]. However, the main drawback of silica based coatings is a poor stability in water, so in the present research, alternative coating matrices (alumina and zirconia) have been explored in order to improve coating stability and durability. Ceramic matrices and surface roughness of the coatings have been defined in order to limit also cellular adhesion and bone tissue overgrowth, however these aspect will be investigated more in depth in future works.

The main advantages of the here proposed coating, compared to the above discussed literature/market references, can be summarized as follows. The here developed surfaces present a roughness lower than 0.2 µm which can intrinsically reduce both bacterial adhesion and tissue overgrowth, as reported for polished titanium surfaces [3, 4, 5]. In addition these coatings have an active antibacterial action, associated with the presence of silver nanoclusters. The amount of silver can be finely tuned by the sputtering technique allowing the preparation of coatings with different antibacterial activities depending on the specific request. The use of silver guarantees a broad spectrum activity (crucial in case of poly-microbial infections from a priori unknown bacteria) and limited bacterial resistance development, in contrast with antibiotic loaded surfaces. Finally in the here proposed solution silver nanoclusters are stably embedded in a ceramic matrix which can increase the stability of the metal and modulate its release (compared to pure silver coatings).

The ceramic coatings containing silver nanoclusters have been deposited onto stainless steel 316L, titanium grade 4 and titanium grade 5 by means of co-sputtering, which is a technique largely affirmed on an industrial scale. Silica based coatings have been here produced and characterized as reference. Coatings have been characterized by means of contact profilometry (roughness) contact angle measurements (surface wettability), Field Emission Scanning Electron Microscopy equipped with Energy Dispersive Spectroscopy (FESEM-EDS, for morphology and chemical composition), electrokinetic measurements of zeta potential (surface charge in contact with water based media) water soaking (ion release and coating stability) and bacterial adhesion.

Materials and methods

Substrates

Stainless steel 316LVM, Titanium Grade 4 and Grade 5 (Ti6Al4V) samples (10 mm x20 mm x2 mm) were electro-cut and mirror polished. Samples were cleaned by means of one washing (5 min) in absolute ethanol in an ultrasonic bath at room temperature and two washings (10 min each) in ultrapure water, the first at room temperature and the second at 60°C in an ultrasonic bath.

Coating deposition

Metallic samples were coated with silica, alumina or zirconia coatings containing silver nanoclusters embedded inside the oxide matrices by means of co-sputtering. The sputtering system used for alumina and zirconia depositions is a Kenosistec™ equipment having a deposition chamber (Ø= 30 cm, h = 50 cm) equipped with three confocal cathodes 3 inches in diameter. Each cathode can be supplied both in DC (for conductive target) and RF (for non–conductive target) mode.

The sputtering system used for silica deposition is a MS 450 Microcoat™ equipment (as in previous works by the authors) having a deposition chamber (Ø= 50 cm, h = 50 cm) equipped with two cathodes, 6 inches and 1 inch in diameter. Each cathode can be supplied both in DC (for conductive target) and RF (for non–conductive target) mode.

The atmosphere is pure argon (99.99 %). The co – sputtering of matrix and antibacterial agent are performed simultaneously in Ar plasma, with a working pressure around 1 Pa, while the vacuum of the chamber is reach 10^{-5} Pa.

All the deposition conditions are digitally controlled and recorded by a PLC system.

Ceramic matrices (SiO_2, Al_2O_3 or ZrO_2) were deposited by applying RF power supply to one cathode, while silver (Ag) was deposited via DC (when embedded in silica matrix) or RF (when embedded in Al_2O_3 or ZrO_2) .

The sputtering parameters, summarized in Table 1, were selected according to the authors' expertize on sputtering deposition of similar coatings and the previous knowledge of their deposition rates [17-24].

Coating of at about 150 nm were obtained for all the reported conditions.

In particular for the ceramics matrices (Al_2O_3, ZrO_2) high voltage (380-450 V) in radio frequency (RF) was selected in order to achieve a reasonable deposition rate. For silver, the deposition rate is highly dependent to the power and Ag content must be fine tailored for these applications.

This issue is of particular interest in order to tailor the antibacterial activity and biocompatibility of the final coatings: 1 W in DC was used to co-sputter Ag nanoclusters and the silica matrix, while with other matrices (Al_2O_3, ZrO_2) 1 W in DC was unsuitable due to plasma instability, and it was decided to use the RF power option (10-20 W) for silver. As a consequence, the deposition rate in DC or in RF gave, as expected, a huge difference in silver content in the coatings.

This is the reason why the silver content of new coatings prepared in this work is lower compared to the previous published silver/silica based coatings. The deposition conditions for silica based coatings were selected according to previous work of the authors [17, 18] in which antibacterial activity and biocompatibility were obtained. These coatings were considered as reference and starting point for this work.

Several attempts have been done to optimize the sputtering conditions for the new matrices during the initial phase of this activity, the experimental planning and optimization, not reported for brevity.

One of the aims of this research is to obtain Al_2O_3 and ZrO_2 based coatings with a minimal amount of silver, in order to test their antibacterial activity, but also their stability towards waters and compare it to that of silica; second, their anti-adhesive feature is also of interest.

Table 1: Sputtering depositions parameters.

	Cathode	Power [W]	Voltage at cathode [V]	Mode	Instrument
SiO2-Ag coatings	SiO_2	200	250	RF	Microcoat™
	Ag	1	270	DC	Microcoat™
Al2O3-Ag coatings	Al_2O_3	200	450	RF	Kenosistec™
	Ag	10	37	RF	Kenosistec™
ZrO2-Ag coatings	ZrO_2	200	380	RF	Kenosistec™
	Ag	20	42	RF	Kenosistec™

Surface characterization

Surface roughness was measured by means of a contact profiler equipped with a diamond stylus tip (Taylor-Hobson, Form Talysurf 120L) in two perpendicular directions on all the samples before and after coating. Three measurement were done for each direction. Sampling lengths of 1.5 mm were taken using a cut-off of 0.25 mm (ISO 4288).

Surface wettability by water was determined through contact angle measurements (sessile drop method, DSA-100, KRÜSS GmbH, Hamburg, Germany) on bare and coated substrates. Briefly, a drop (5 µl) of ultrapure water was deposited on the material surface and the angle formed with the surface measured by the instrument software. Test were performed in triplicate on both coated samples and reference materials.

Surface charge was investigated in contact with a water based electrolyte (0.001m KCl) by means of electrokinetic measurements (SurPASS, Anton Paar). The pH of the electrolyte solution was varied in the acid and basic regions through the addition of 0.05M HCl and 0.05M NaOH with the instrument automatic titration unit. Two separate measurements were performed (one in the acid and one in the basic region) on the same set of samples, separated by extensive washing of samples and instrument.

Coating mechanical adhesion to the substrate was estimated by means of tape adhesion tests, according to the Standard Test Methods for Rating Adhesion by Tape Test (ASTM D 3359) [25]: a grid of cuts was prepared on the coated surface using a specific cutter, after surface cleaning with a brush, a pressure sensitive tape was applied and rapidly removed (parallel to the surface). The adhesion was estimated observing the portion of coating eventually removed by the tape.

Surface morphology and chemical composition were investigated by means of Field Emission Scanning electron Microscopy equipped with energy dispersive Spectroscopy (FESEM-EDS, SUPRATM 40, Zeiss^{TM} and Merlin Gemini Zeiss^{TM}).

For the most promising samples, surface chemical composition, as well as the chemical state of elements were analyzed by means of X-ray Photoelectron Spectroscopy (XPS, ESCA-5000, Physical Electronics, Versa Probe system) with beam size diameter: 200 µm; beam power: 50W; voltage: 15kV and 2.10^{-6} Pa pressure in the analysis chamber. The XPS data were collected using monochromatic Al K-alpha radiation at 1486.6 eV. Both survey spectra and high resolution spectra of Zr and Ag were acquired. Photoelectrons were collected at take-off angle of 45° to the surface normal. The elements were analyzed with a pass energy of 23.5eV (high resolution spectra). Atomic compositions were derived from peak areas using photoionisation cross-sections calculated by Scofield, corrected for the dependence of the escape depth on the kinetic energy of the electrons and corrected for the analyzer transmission function of the spectrometer. Atomic compositions were derived from peak areas after a Shirley background subtraction. All the XPS spectra were corrected for charge composition with C1s at a binding energy of 285eV.

In order to investigate silver release and coating chemical stability in water based media, samples (three samples per type) were soaked in ultrapure water at 37°C up to 28 days. The amount of silver in solution was analyzed by means of Inductively Coupled Plasma (ICP-MS; iCAP™ Q spectrometer, Thermo Fisher Scientific, Waltham, MA, USA), as well as the release of elements characteristic of the coating matrices (Si, Al and Zr). Coating stability was assessed also by visual inspection of the samples and EDS analyses after soaking.

Antibacterial properties

The antibacterial activity of the treated specimens was evaluated according to the International Standardization Organization (ISO) test 22196 (Measurement of antibacterial activity on plastics and other non-porous surfaces). Accordingly, 0.1 ml of *Staphylococcus aureus* suspension (1x10^5 cells per ml, multi-drug resistant (MDR) *S. aureus*, ATCC 43300, purchased from the American Type Culture Collection, Manassas, USA) were directly dropped onto specimens' surface and then covered with a sterile polyethylene film. The inoculated specimens were placed in an incubator at 35°C for 24 hours. After incubation, each specimen was washed with a soybean casein digest broth containing lecithin and polyoxyethylene sorbitan monooleate (SCDLP broth, 5 ml) to recover the bacteria. Then, colonies forming unit (CFU) were counted as follow. Briefly, 100 µl of supernatant were collected from each specimen and used to perform six-serial ten-fold dilutions, mixing 20 µl of bacterial suspension with 180 µl of sterile saline (0.9% NaCl). 20 µl were then collected from each dilution, spotted onto plates containing LB agar medium, and incubated for 24 hours at 37°C. CFU ml^{-1} were counted as follows [26]:

CFU = [(number of colonies x dilution factor)^(serial dilution)]

where:

- number of colonies = countable single round colonies;
- dilution factor = dilution made from the initial 1 mL suspension;
- serial dilution = 1–6 ten-fold dilution areas where colonies were counted.

Results and discussion

The visual appearance of bare and coated samples is shown in Figure 1.

Uncoated samples have the typical metal grey shiny appearance (Figure 1, first row). No evident variation in the color can be observed after Al_2O_3-Ag coating deposition, except of a moderate darkening (Figure 1, third row). Differently, a brownish color characterize the SiO_2-Ag coated samples (Figure 1, second row), analogously to what previously observed by the authors on different substrates [17-24]. Finally ZrO_2-Ag coatings have a characteristic light blue color (Figure 1, last row).

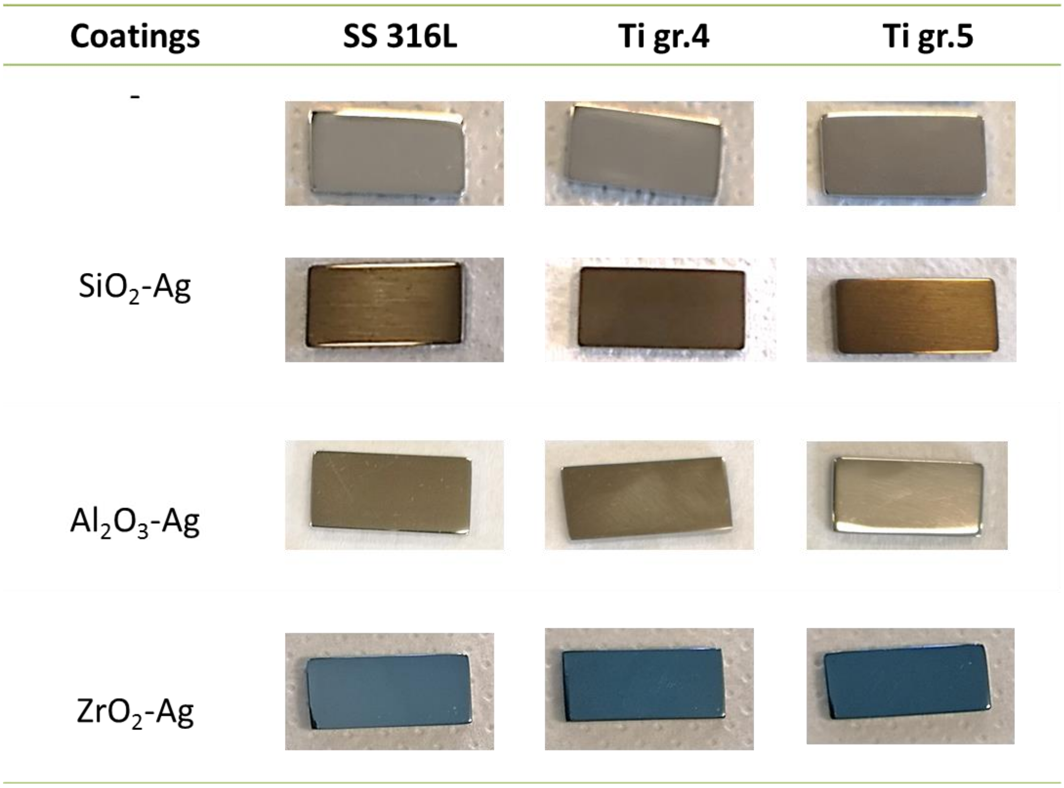


Figure 1: Visual appearance of bare and treated samples.

Results of roughness measurements are reported in Figure 2.

The average roughness is reported as measured in the same direction of the polishing tracks and in the perpendicular one, where, as expected, the values are slightly higher. All the values are lower than the threshold reported in literature for increasing of bacterial adhesion (0.2 µm) [8, 9, 10]. This is a first goal of the work, in fact it allows to limit bacterial adhesion, in a passive way by using surface topography. Moreover, smooth surfaces can also reduce adhesion strength of cells, without hampering cytocompatibility [3,4,5].

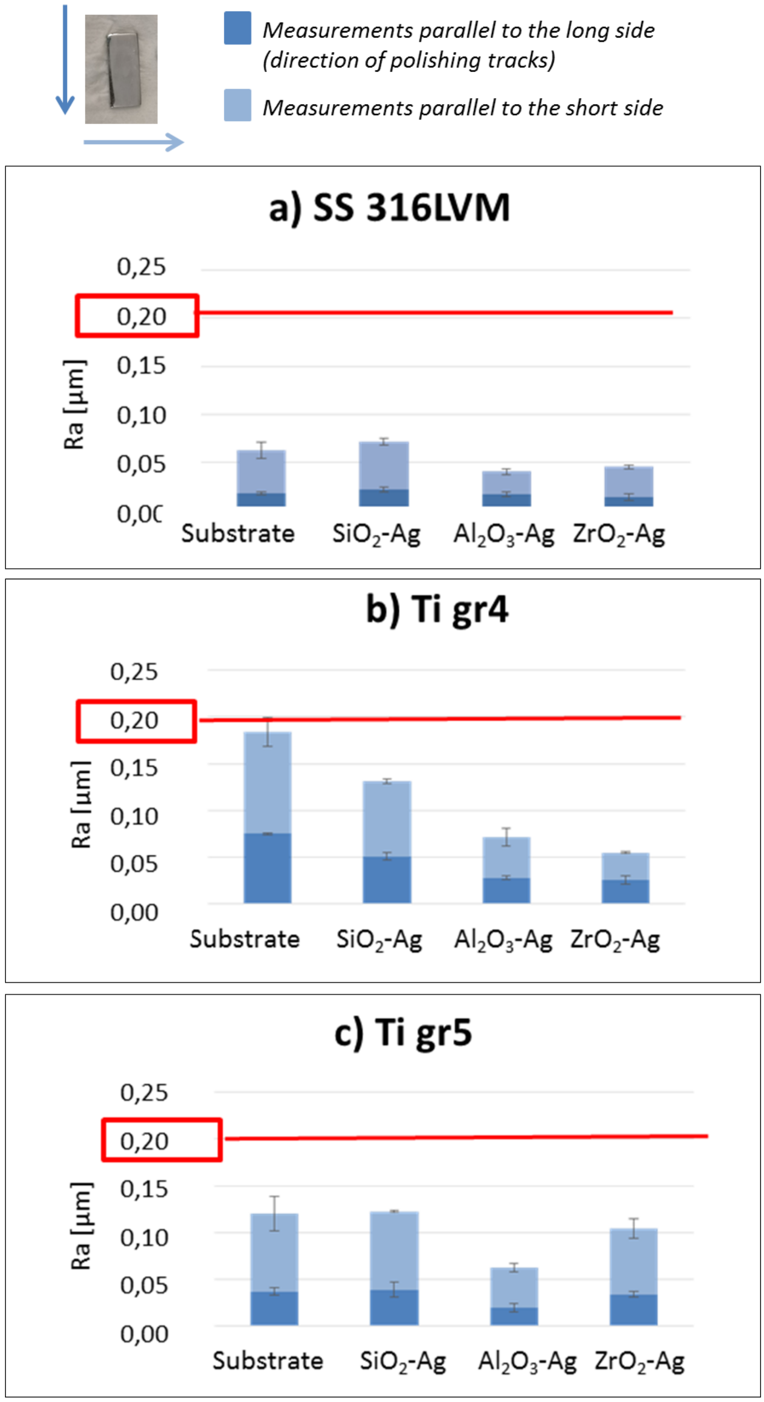


Figure 2: Roughness measurements before and after coatings deposition on the different substrates.

The results of contact angle measurements are reported in figure 3.

All the tested coatings increase water wettability of the metallic substrates. The phenomenon is particularly evident for silica-based coatings, in accordance with the high affinity of silica for water and to its significant exposition of surface hydroxyl groups [27].

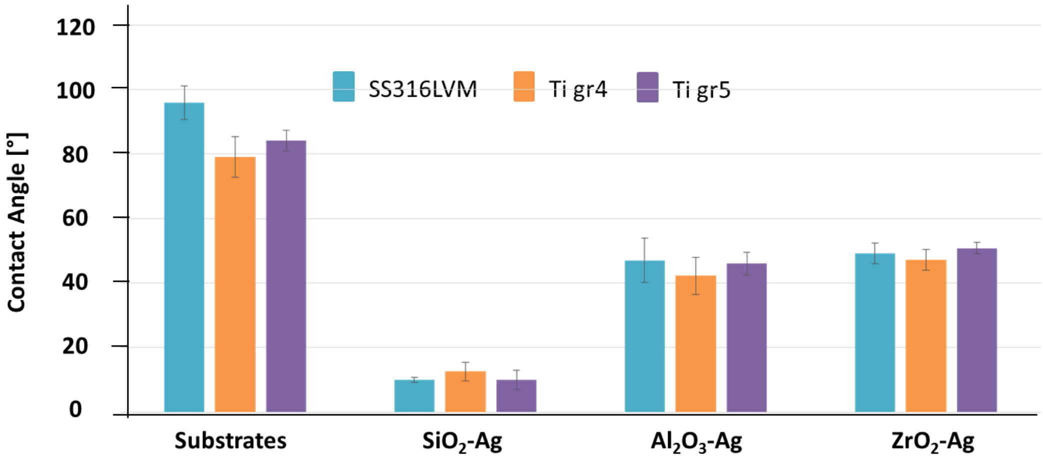
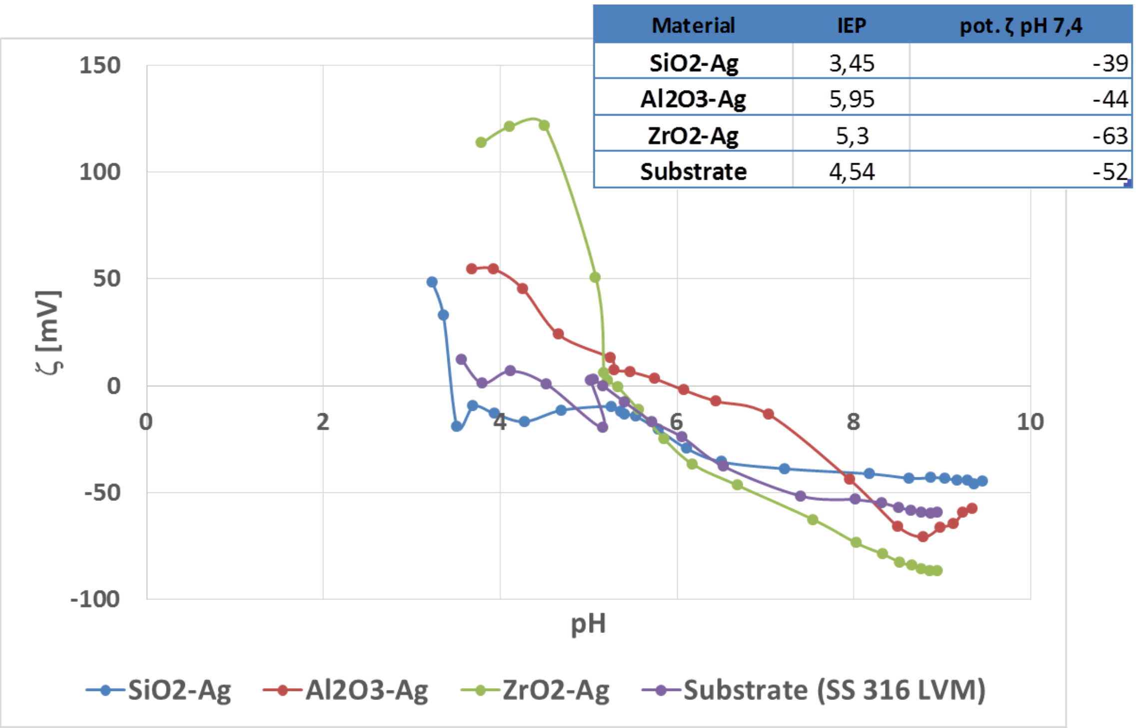


Figure 3: Water wettability before and after coating deposition on the different substrates.

Zeta potential titration curves are reported in Figure 4.



Material	IEP	pot. ζ pH 7,4
SiO2-Ag	3,45	-39
Al2O3-Ag	5,95	-44
ZrO2-Ag	5,3	-63
Substrate	4,54	-52

Figure 4: Zeta potential titration curves before and after coating deposition in the case of SS316LVM as substrate.

Zeta potential measurements were performed on stainless steel as a model substrate. Besides of the data obtained on the bare substrate, the results of coated ones can be extended also to coated Ti and Ti - alloy substrates, because the measurement interests only the outermost surface layer of the sample.

All the samples exhibited an isoelectric point lower than 7.4 and consequently are negatively charged at physiological pH. Silica based coatings show a plateau in the basic region which can be ascribed to the presence of acidic functional groups (OH groups) [27, 28], thus confirming the results of wettability tests.

The different coatings have an increasing value of zeta potential at pH 4-5, that is the value occurring during inflammation events [29], moving from silica (slightly negative) to alumina (slightly positive) and finally to zirconia (positive). This aspect could be of interest considering that electrostatic effects have a role in adhesion of bacteria and cells; anti-adhesive materials, able to inhibit growth of bacteria, with a consistent positive surface charge are reported in literature [30]. This point is worthy of investigation and will be examined more in detail in future works.

Coating adhesion to the substrates was investigated by means of tape test. After the preparation of the grid on the surface, the application and removal of the pressure sensitive tape the amount of coating eventually detached from the sample is observed and estimated, looking at the sample surface, but also to the tape surface, as schematized in Figure 5a. If the color of the coating is significantly different from the substrate one (as in the case of silica and zirconia based coatings) the operation can be easily performed by visual inspection (eventually with the help of a magnification lens). On the other hand, when the coating is colorless, further analyses (e.g. EDS post tape removal) are required to determine the presence/absence of the coating after the test.

As shown in figure 5b and 5d, all the coatings are completely unaltered (no portion of coating removed by the tape) after the tape test at visual inspection (Figure 5c) , but considering that visual inspection is not sufficient, it was supported by FESEM-EDS analyses (not reported) of the samples after the test. In conclusion, all the coatings can be classified as 5B, optimal adhesion, according to ASTM D 3359 standard.

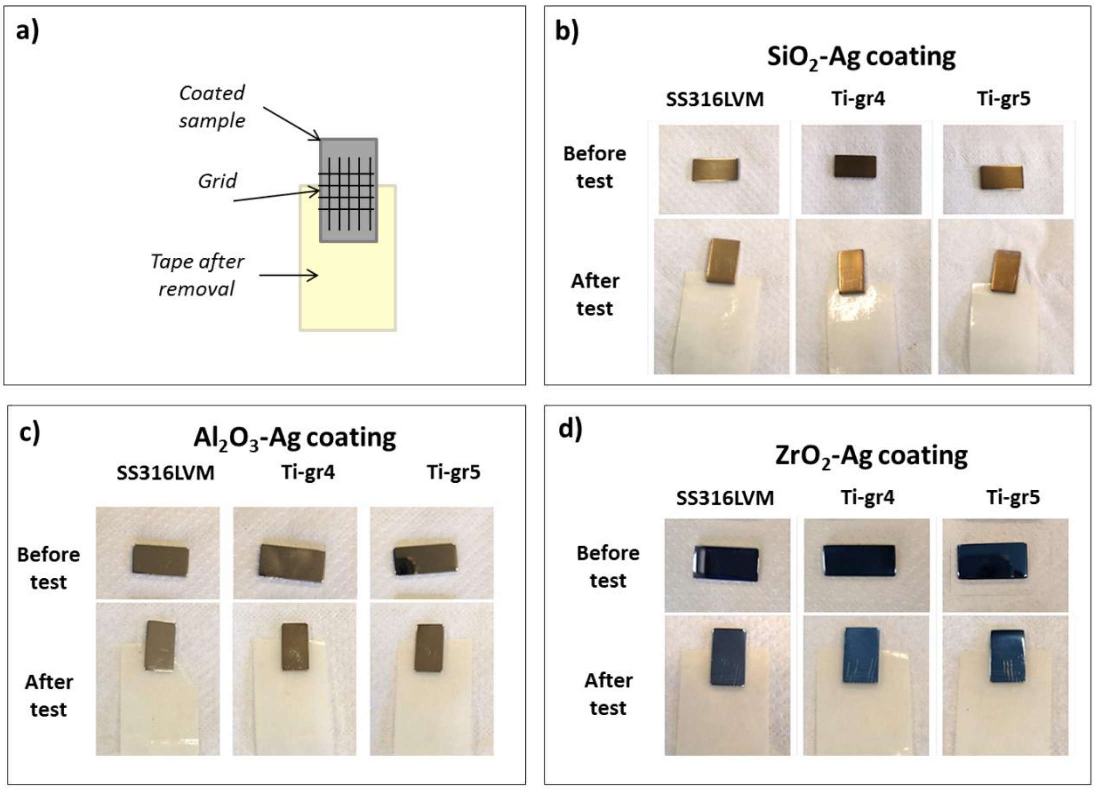


Figure 5: Coating adhesion by tape test of the different coatings on the different substrates.

The morphology of the coatings, observed at FESEM is reported in Figure 6. The appearance is the typical one observed for sputtered coatings, with "island-like" texture of the matrix in which silver nanoclusters are homogeneously dispersed. This morphology of silica-based coatings (Figure 6a) is analogous to the one previously reported by the authors [17-24]. Alumina and zirconia based coatings (Figure 6 b and c, respectively), evidence a smother texture with islands on a smaller scale and a reduced amount of silver nanoclusters. The observation of a lower concentration of silver nanoclusters on alumina and zirconia based coatings can be associated with the lower silver content also detected with EDS analyses on these samples. The concentration of silver measured on the surface is, in fact, comprised between 0.3 and 0.7 at % for silica based coatings while it is below 0.1 for alumina and zirconia ones.

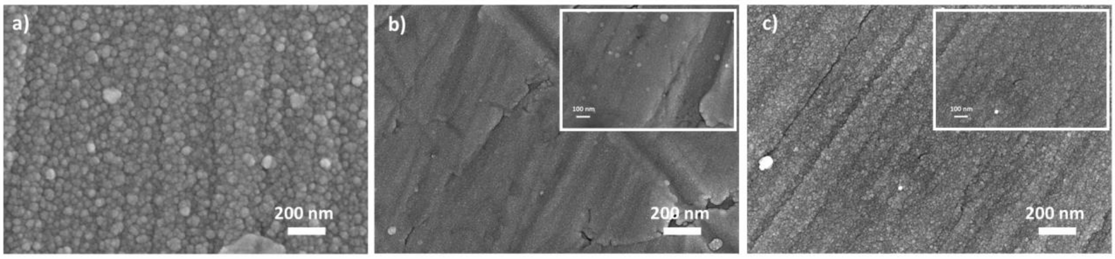


Figure 6: FESEM images (150000x) of SiO_2-Ag (a), Al_2O_3-Ag (b) and ZrO_2-Ag (c) coatings on Ti-gr5 substrates. Higher magnification insets (300000x are present for Al_2O_3-Ag and ZrO_2-Ag)

In order to evaluate silver release and coating chemical stability in water based media, some samples were soaked in ultrapure water at 37°C up to 28 days.

The visual appearance of the coatings before and after 28 days of soaking is reported in Figure 7a.

Silica coated samples turn to grey metallic appearance after soaking, while zirconia coated ones maintain their light blue color. It is more difficult to discriminate color changes for alumina coatings (which are almost colorless, as discussed for tape test) however a more pronounced metallic aspect can be evidenced after soaking also for these samples.

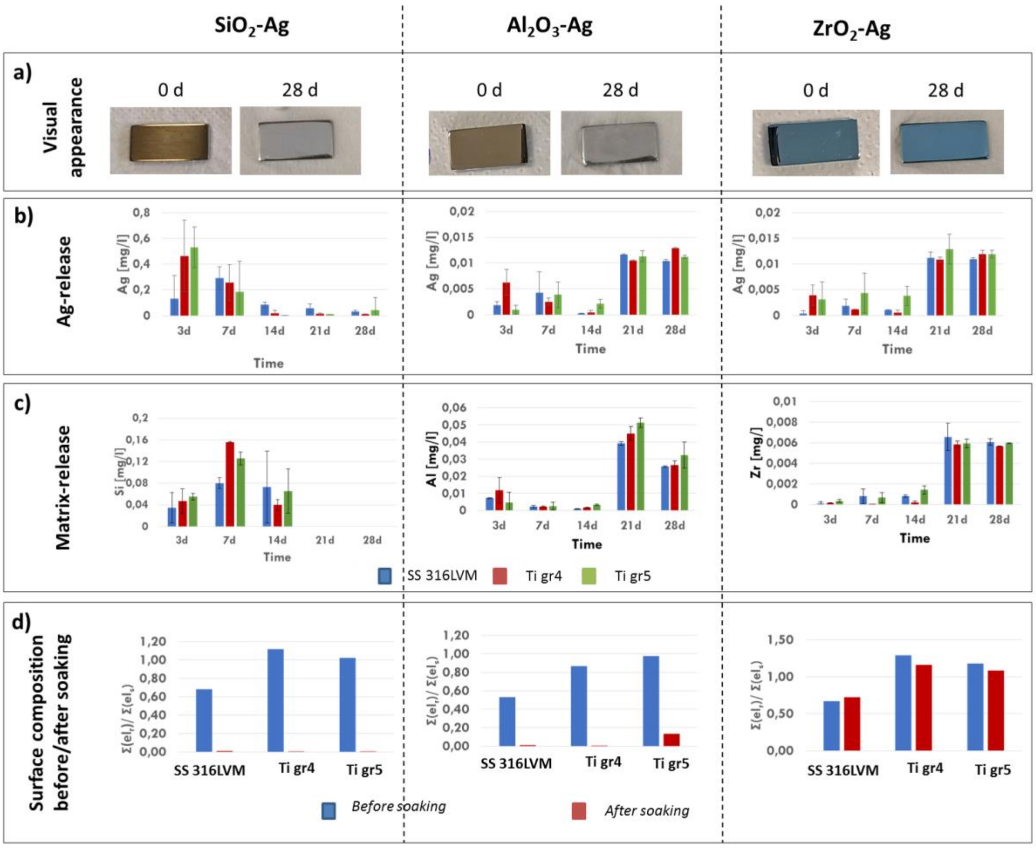


Figure 7: Water soaking of coated samples: a) visual appearance before and after soaking, b) Ag release in water, c) Release of the ions characteristic of the coating matrix (Si, Al, Zr) in water, d) EDS analyses before and after soaking (ratio between coating and substrate elements).

The amount of silver released in water is reported in Figure 7b. Ag release from SiO_2 based coatings is the highest one and it is concentrated in the first days, sometimes exceeding the cytotoxicity limit reported in literature (0.38 mg/l) [31], but with high statistical variability. Ag release from Al_2O_3 and ZrO_2 based coatings is two order of magnitude lower than from the SiO_2 based ones (significantly lower than cytotoxicity limits reported in literature for silver ions) and it is delayed in time (the highest values are reported after 21 and 28 days). The absolute amount of the released silver can be correlated with the initial low silver content of the coatings, while the slower release kinetic depends on the coating matrix type, as discussed below.

The release of ions characteristic of the coating matrices can be an indication of coating chemical degradation in solution. The values obtained are reported in Figure 7c. It can be observed that The release of Si^{4+} is the highest and it is concentrated in the first days, while the release of Al^{3+} is one order of magnitude lower and it is delayed in time (maximum at 21 days). Finally, the release of Zr^{4+} is almost negligible and it is also delayed in time (the highest values are detected at 21 and 28 days).

The comparison between EDS analyses before and after soaking can be a further indication of coating chemical stability in water. Figure 7d reports the ratio between the coating elements and substrate elements (%at). When no differences in EDS data before/after the release test is evidence, coating permanence can be highlighted. It must be noticed that results are not completely reliable when the same element is present both in the coating and in the substrate (Si in SS31LVM coated with SiO_2 based coating, Al in Tigr5 for Al_2O_3 based coating), but a good trend can be in any case, extrapolated. Results evidenced an almost complete dissolution of the coating in the cases of silica and alumina based layers (absence of the coating elements after soaking), while surface composition is almost unaltered for zirconia based coatings suggesting their better stability.

By summarizing all the results (Figure 7), it can be concluded that silica based coatings are the most sensitive to wet environments and completely dissolve within 1 week (Ag and Si rapid release), alumina based coating are slightly more stable (from Ag and Al release a dissolution within two weeks can be supposed). Zirconia based coatings are the most stable (no degradation up to 1 month from Ag and Zr release, EDS analyses and visual appearance).

The quantity of silver release can be correlated to the amount of Ag initially introduced in the coating while the time of its release depends also on the chemical stability in solution of the coating matrix.

Stability in wet environments is a crucial requisite for non-biodegradable coatings intended for implantable applications. Since zirconia based coatings showed the best stability in water among the tests ones, XPS analyses and preliminary antibacterial tests were performed only on these coatings. Ti-gr5 was selected as substrate. Despite of the result of the analysis on the bare metal, the effect of the coating can be considered the same for the other substrates.

The chemical composition of the outermost surface layer of a control Ti-gr5 sample and of a ZrO_2-Ag coated one is reported in table 2.

Table 2: Chemical composition (at%) of bare and ZrO_2-Ag coated Ti-gr 5 samples detected by XPS.

	Tigr5 [at%]	**Tigr5 ZrO2-Ag coated [at%]**
C	28.02	28.97
O	50.27	49.53
Ti	18.09	n.d.
Al	3.04	n.d.
V	0.58	n.d.
Zr	n.d.	19.42
Ag	n.d.	0.38

n.d. not detected

The presence of a significant amount of carbon on both samples can be associated with unavoidable hydrocarbon contaminations from the atmosphere [32].

Besides contaminations, the control sample shows the characteristic elements of the alloy together with a significant amount of oxygen, attributable to the native oxide layer, typical of titanium surfaces. On the coated samples, substrate characteristic elements are no longer visible (the penetration depth of XPS, few nanometers, is lower than the coating thickness, at about 150 nm) while Zr and Ag appear, confirming the presence of the coating. According to the other reported analyses, the amount of silver is very low (<1%at).

The high-resolution spectra of Zr and Ag (of the coated sample) are reported in Figure 8.

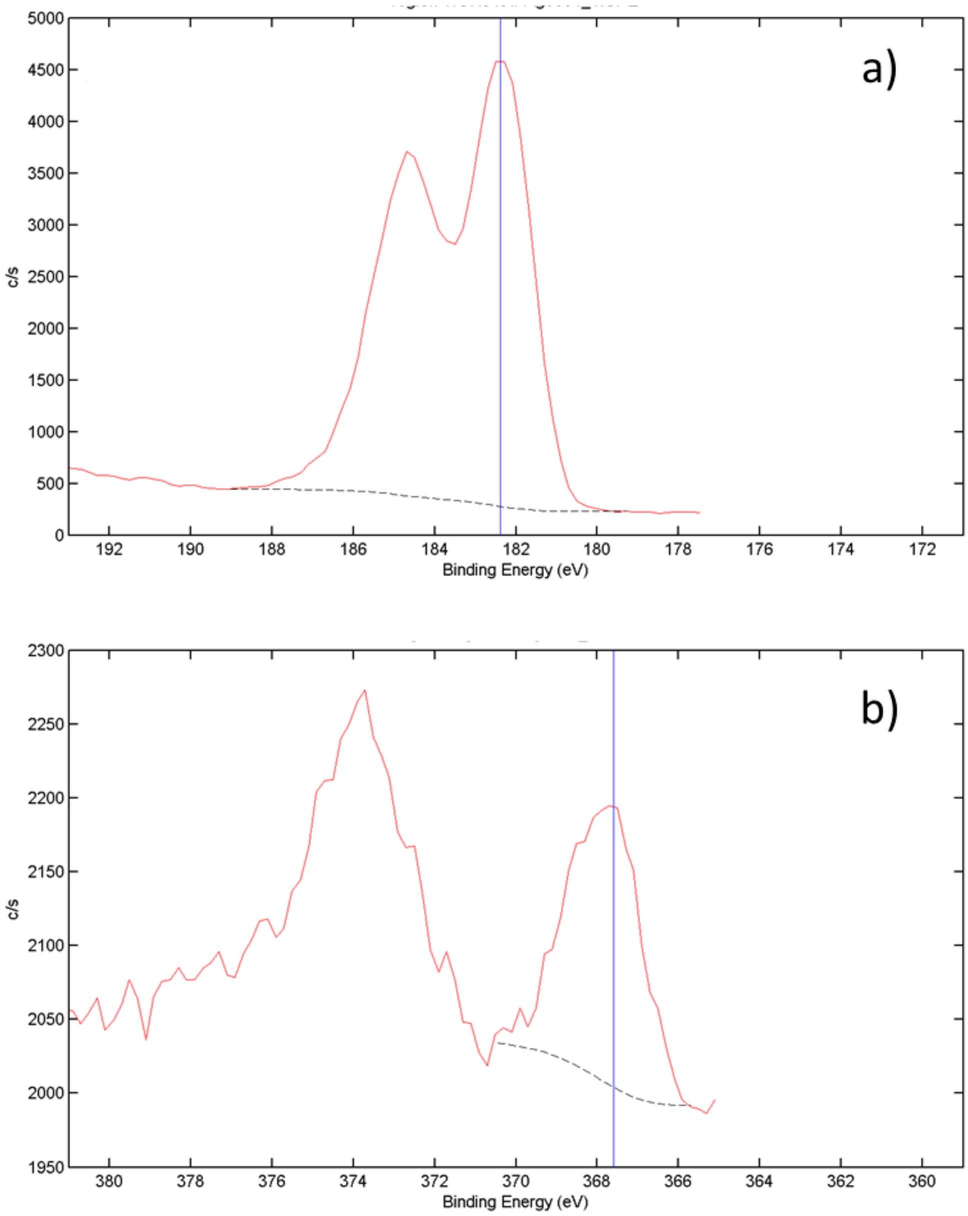


Figure 8: XPS high resolution spectra of Zr (a) and Ag (b) regions for a Zirconia based coating on a Ti-gr5 substrate.

The examination of the Zr3d peak shows a binding energy of ~182.3 eV typical of a ZrO_2 [33] and the examination of the Ag3d peak shows a binding energy of ~368eV typical of an Ag in a metallic form [23, 34]. As expected, silver is present as metal clusters embedded in the ceramic coating matrix.

The results of preliminary antibacterial tests are reported in Figure 9.

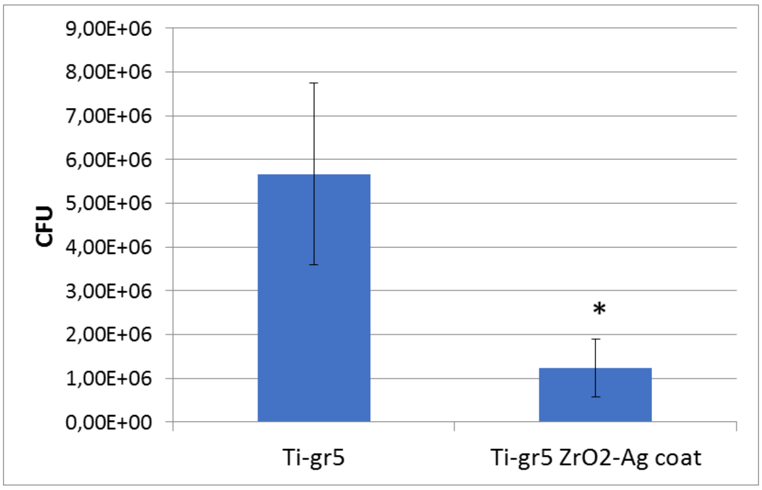


Figure 9: Antibacterial tests on bare/ZrO2-Ag coated Tigr5 samples (*=*p<0.05*)

Even if the amount of silver introduced in zirconia based coatings, in the here reported setup, is considerable low (<0.1%at), ZrO_2-Ag coated samples are able to significantly reduce the colony forming units of one order of magnitude, compared with uncoated ones (p<0.05, ONE-way ANOVA and Tukey's test, indicated by *). This result is extremely promising. In fact, the first objective of this coating is to reduce bacterial adhesion/proliferation on the surface of the fixation device. Usually, antibiotic-free anti-adhesive surfaces against bacteria are suitable only considering implantation in non-infected sites: they have the advantage of avoiding the introduction of cytotoxicity effects, which are often associated with an active antibacterial action. Moreover, a very low silver release may not require the classification of the final coated device as a therapeutic device, which significantly simplify the certification procedures. The observed effect can be explained by the evidence that silver antibacterial activity does not necessarily require release into the medium and bacterial internalization; it can be affective only by contact. In fact, while internalization process is based on DNA interference and replication stop, the contact inhibition is based on membrane accumulation and permeabilization [35]. Hence, when silver get in touch with the membrane, it is fast accumulated within the membrane thus causing the formation of permanent pores; therefore, bacteria metabolism is irreversibly unpaired due to the loss of homeostasis.

Based on these preliminary results, these Ag/ZrO_2 co-sputtered coatings seem a good starting point: if necessary, the amount of silver can be increased for specific situations (e.g. for potential application in conditions with high risk of infection).

Conclusion

Silver nanoclusters doped SiO_2, Al_2O_3 and ZrO_2 coatings were obtained by sputtering onto SS316LVM, Ti-gr4 and Ti-gr5 substrates, following the rationale of surfaces antibacterial and anti-adhesive against cells for temporary fixation devices. All the coatings have a mean roughness value lower than 0,2 µm (threshold for not increasing bacterial adhesion). Coated samples (especially SiO_2 ones) have surface wettability by water higher than the uncoated metals and negative charge at physiological pH. All the coatings have high mechanical adhesion to the substrates (5B classification according to ASTM D 3359). The Ag content and its release in water is higher in SiO_2 based coatings which are the starting point of this research; in this case, it is close to the literature cytotoxicity limit and the coating matrix is not chemically stable in water . On the other hand, the Ag content and release is significantly lower for Al_2O_3 and ZrO_2 based coatings due to the rationale of the work aimed at coatings with minimal silver addition in a chemically stable coating matrix; its release in water is significantly lower than the cytotoxicity limit reported in literature. ZrO_2 – Ag coatings have the best stability in water, stable up to one month of soaking, and they are able to reduce S. aureus adhesion/growth of one order of magnitude.

At the moment, we can assess that the sputtering technique allows to tailor the silver content of coatings in order to have the required antibacterial activity. Moreover, it can be easily scaled up making possible the industrial production of real fixation devices with reasonable costs. Biocompatibility and adhesion strength of cells on ZrO_2-Ag coated surfaces are actually under investigation in order to verify the anti-adhesive potential of the proposed coatings against cells.

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