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A NEW COMPOSITE COATING FOR 316 L STAINLESS STEEL IMPLANT

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ABSTRACT

Stainless steel (SS) is a biomaterial used widely in dental and medical fields. It is a bio-tolerant material and more susceptible to infection and corrosion. To overcome these problems SS can be coated with hydroxyapatite crystals combined with titanium. Infection resistance can be imparted by incorporating antimicrobial agent such as silver. A composite coating HA-Ti with Ag at 13wt% was prepared for SS using pulse laser technique and compared with HA and HA-Ti coatings. The coatings were characterized by X-ray diffraction and scanning electron microscopy-energy dispersive spectroscopy. The antibacterial action was investigated against *Staphylococcus aureus* and *Escherichia coli in vitro*. The corrosion behavior of the coatings was studied in Hank's balanced solution using computerized potentiated. The results revealed that the composite coating HA-Ti-Ag was uniform and thin (1.6 µm). This coating reduced the growth of *Staphylococcus aureus* and improved the corrosion resistance of SS. SS coated with (HA-Ti-Ag) coating can be used in dental and orthopedic surgery.

Key words: stainless steel, coating, corrosion, implant, silver.

INTRODUCTION

Stainless steel (SS) is a biomaterial used widely in dental and medical fields. It is used in the fabrication of surgical plates, pins and screws and certain parts of dental implant. This biomaterial has appropriate mechanical properties, relative corrosion resistance and cost effective (Sutha *et al*, 2013; Padhi, 2014). However, SS is a bio-tolerant material when used as an implant, complications such as infection, corrosion and fibrous tissue encapsulation may occur due to interaction between this material and neighboring tissues (Padhi, 2014). Infection of dental or orthopedic implant is the main reason of implant failure due to attachment of microorganisms and subsequent biofilms formation on the implant surfaces (Sutha *et al*, 2013). The bacteria and their-by products present on the implant materials impede the body immune system and prevent bone healing at the surgical site (Kocourek *et al*, 2013). Corrosion, on the other hand, occurs as a result of interaction between metallic implant and biological fluid causes the release of ions such as iron, nickel and chromium which compromise the immune system and cellular functions (Lorenzetti *et al*, 2014). Consequently, initiation of inflammation and formation of foreign body giant cells occur resulting in bone destruction and eventually implant failure (Mohammed *et al*, 2014).

In the field of biomaterials, the successful implant materials should be bioactive, biocompatible and exhibited antimicrobial action. Therefore, surfaces of stainless steel implant (SSI) necessitate modification to impart such properties and improve its quality and longevity. Coating SSI with bioactive materials such as hydroxyapatite crystals could improve bonding ability with osseous tissue (Arifin *et al*, 2014). However, delamination of HA coatings during implant insertion (Ayu *et al*, 2017) and weak mechanical properties rendering these coatings unreliable to prevent corrosion occurring. It is well known that titanium is biocompatible material and has excellent corrosion resistance in physiological condition (Kumar *et al*, 2015). Hence, developing Ti-HA composite coating could improve corrosion resistance and biocompatibility of SSI (Mansur *et al*, 2014). Further, it has been shown that bonding strength of HA-Ti composite coating to the underlying substrate is better than that of single phase HA coating (Chen *et al*, 2006).

Nevertheless, reports showed that HA coatings are more vulnerable to bacterial colonization due to rough surfaces compared to non-coating metallic implant (Norowski *et al*, 2009; Lu *et al*, 2011). Thereby, the need of antimicrobial agent is of utmost to prevent biofilm formation and subsequent implant infection. This could be enhanced by incorporating silver or silver ions into the composite coating. Silver has wide spectrum antibacterial action and non-toxic to mammalian cells at a physiological dose (Gosheger *et al*, 2004). More importantly is that there has no bacterial resistance developed against this active ion so far (Carmona *et al*, 2014).

In this work, the composite coating HA-Ti containing Ag at 13wt% was prepared for SSI. The Ag content at 13 wt% was chosen to impart biocide activity without HA-Ti coatings in terms of antimicrobial and anticorrosion behavior. All coatings were prepared using pulse laser technique. Laser coating has many advantages such as improving the properties of metallic surface without affecting its bulk, brief processing time, fast heating/cooling rate, the heating is under control and the procedure can be controlled accurately (Gaith *et al*, 2015).

MATERIALS AND METHODS

Samples preparation: The chemical composition of SS 316 L used in present study is shown in Table 1. Rectangular samples (3x2x1) cm^3 were ground and polished with silica carbide (sic) emery papers (140-1100 mesh size) and diamond paste. The mirror polished samples were washed with ethanol for 15 minutes to remove any debris and left in air to dry before coating procedure.

Table 1: Chemical composition of 316L stainless steel in weight percent

Fe	Cr	Ni	Mo	Cu	Co	Si	Al	Mn	V	P	S	C	N
Bal*	16.48	10.30	2.11	0.32	0.19	0.53	0.005	1.38	0.058	0.028	0.0006	0.021	0.023

*Bal: balance

Coating process: Three discs of 12 mm in diameter and 2mm in thickness were prepared from commercially available powder form of HA (Seelze Hannover, Germany), HA and titanium (TC Titanium, Germany) and HA, Ti and silver (Chem, China). The disc of HA-Ti were mixed in a weight ratio of 75:25 and the third disc is a composite of HA-Ti-Ag in a weight ratio of 62:25:13. The powder form of HA, HA-Ti and the composite of HA-Ti-Ag was pressed with 40 MPa mold to prepare the discs. These prepared discs were used as a target to coat SS substrate using CO_2 laser technique with pulse energy at 1000 J. The substrate-target distance was maintained at 45 cm. The laser pulse of 1000 P was applied on target for each coating. The substrate was maintained at a fixed temperature of 300 °C with ramping rate of 10 °C/min for the three depositions.

Microstructure characterization: The prepared HA, HA-Ti and HA-Ti-Ag coatings for SS substrate were characterized by X-ray diffraction (XRD) ADX-2700 (USA) and scanning electron microscopy-energy dispersive spectroscopy (SEM-EDS) S50.fei (Netherland).

Antimicrobial test:

Microbial isolates: The diagnosis of clinical isolates *Staphylococcus aureus* and *Escherichia coli* was carried out using conventional biochemical tests as described by (Forbes *et al*, 2007).

Sensitivity assay: The sensitivity assay was performed against *S. aureus* (Gram +ve) and *E. coli* (Gram -ve) *in vitro*. The isolates were obtained from dental clinic and inoculated in brain heart broth. The inoculated media was incubated at 37°C for 24 hours in an incubator. Then, the broth media were centrifuged at 2000 rpm for 15 minutes. The supernatant was removed and the turbidity was adjusted to 0.5 McFarland tube. A swab of each inoculum was taken and streaked in a prepared Muller-Hinton agar plate. Sterilized coated and uncoated samples were placed in such a way that the coated surface in contact with the culture media. Then after, the petri-dishes incubated at 37 °C for 24 hours in an incubator. The zone of clearance was measured using a transparent ruler and uncoated sample was used as a control. The experiment was repeated thrice for each inoculum.

Turbidity assay: About 250 ml of brain heart broth (BHB) was prepared and autoclaved at 121 °C for 20 minutes. The prepared (BHB) was poured in two sterilized flasks so that each one contains 125 ml. A swab was taken from stored broth media contaminated with *S. aureus* and *E. coli* and activated with fresh one and incubated at 37 °C overnight in an incubator. Then after, 1 ml of the inoculated broth media was added into each flask contains 125 ml (BHB). Sterilized coated and uncoated samples were placed in 100 ml sterilized plastic cups. About 30 ml of bacterial suspension were poured in the plastic cups to submerge the coated and uncoated samples and incubated at 37 °C for 24 hours. Bacterial optical density was measured at 590 nm by taking about 2 ml of the culture media from each plastic cup containing sample using UV/Vis spectrophotometer (Biotech.CO.UK). The measurement was carried out at zero time point (before incubation) and after 24 hours incubation. A sample of broth media was used as a blank and the reading of bacterial optical density was repeated three times for each sample.

Corrosion test: The corrosion conduct of the uncoated and coated SS substrate with (HA, HA-Ti and HA-Ti-Ag) coatings was evaluated by potentiodynamic polarization using computerized potentiostat (Wenking, China) with software analysis. The samples were connected as a working electrode, a saturated silver electrode was utilized as a reference and a platinum electrode was the auxiliary electrode. Hank's balanced salt solution (Hbss) was prepared using de-ionized water and the commercially available chemical ingredients shown in Table 2. The solution was kept at 37 °C and a pH ranged between 7.4-7.8 to simulate the physiological condition of body fluid. The samples were wrapped with nitrocellulose substance which was used as an insulator during immersion in Hank's balanced salt solution.

The coated and uncoated area of the SS substrate that subjected to electrochemical test had dimensions of 2 x 2 cm^2. The samples were immersed in Hank's balanced salt solution for 14 minutes during the electrochemical investigation. The electrochemical test was run by measuring the open circuit potential. For each sample the experiment was repeated twice. The corrosion rate (CR) was calculated according to the following equation:

$$CR=0.13\ (i_{corr.}).(Ew)/A.p$$

Where 0.13 is a constant, $i_{corr.}$ is the electrical current (μA), Ew is the equivalent weight of the elements of stainless steel substrate (g/mol), A is the sample surface area subjected to electroche-

mical test (cm^2) and *p* is the density of stainless steel substrate which is (7.8 g/cm^3).

Table 2: composition of Hank's solution

Ingredient	Concentration g/L
NaCl	0.4
KCl	0.4
KH_2PO_4	0.06
Na_2HPO_4	0.048
$NaHCO_3$	0.35
$MgSO_4$	0.098
$CaCl_2$	0.14
Glucose	1.0
pH	7.4-7.8

RESULTS AND DISCUSSION

Coating characterization: The Figure 1 showed XRD plots of the HA, HA-Ti and HA-Ti-Ag composite coating, as can be seen that the diffraction peaks present in the plots are not associated with HA, Ti and Ag. However, the high intensity peaks at 2Θ at 44.3° and 50.8° present in these plots are mostly associated with SS substrate (Starbova *et al*, 2008). The inability to detect HA, Ti and Ag peaks could be attributed to the thickness of the coating which is about 1.6 µm as shown by cross-sectional micrograph of SEM examination of the composite coating. Such thin films might be beyond the detection level of X-ray diffraction. It is worth mentioning that the concentration of Ag in the composite coating HA-Ti-Ag was 13wt% which is difficult to be detected by XRD.

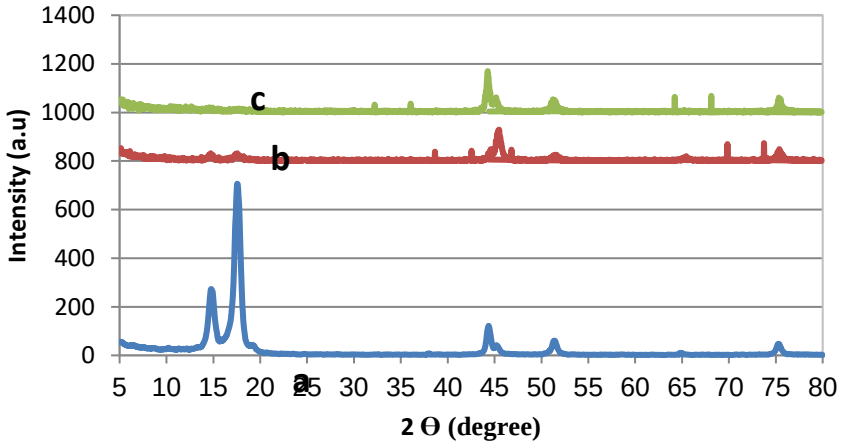
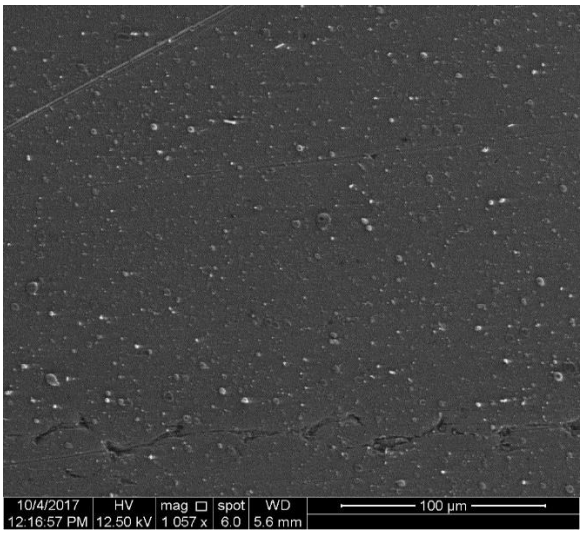


Figure 1: XRD plots of (a) HA, (b) HA-Ti and (c) HA-Ti-Ag coatings

The Figure 2 showed SEM micrograph of the composite coating HA-Ti-Ag, as can be seen that the coating was homogenous and uniform. However, fine cracks could be observed which might be associated with thermomechanical stresses created during cooling after laser treatment. This might be related to the difference in thermal expansion coefficient of HA and Ti ($17.3x10^{-6}$ °C and $8.4x10^{-6}$ °C, respectively) (Basu and Gosh, 2017). A fine agglomeration was noted in the SEM micrograph of the composite coating HA-Ti-Ag at higher magnification which could be associated with Ag particles, as this metal tends to agglomerate during coating process (Lue *et al*, 2011).

Table 3 depicted EDS analysis and the chemical composition of the composite coating HA-Ti-Ag. The Ca/P ratio observed in the EDS analysis was about 2 which are close to that found in bone (1.67). This proves that the SS surface was bioactive and HA did not undergo decomposition during laser coating.

SEM micrograph of the composite coating-metal interface revealed that the visible thickness of the composite coating about 1.6 µm. The coating-metal interface cannot be seen as there was no space between the two phases and the coating seemed to be melting and fused with the SS substrate during laser treatment, as shown in Figure 3.



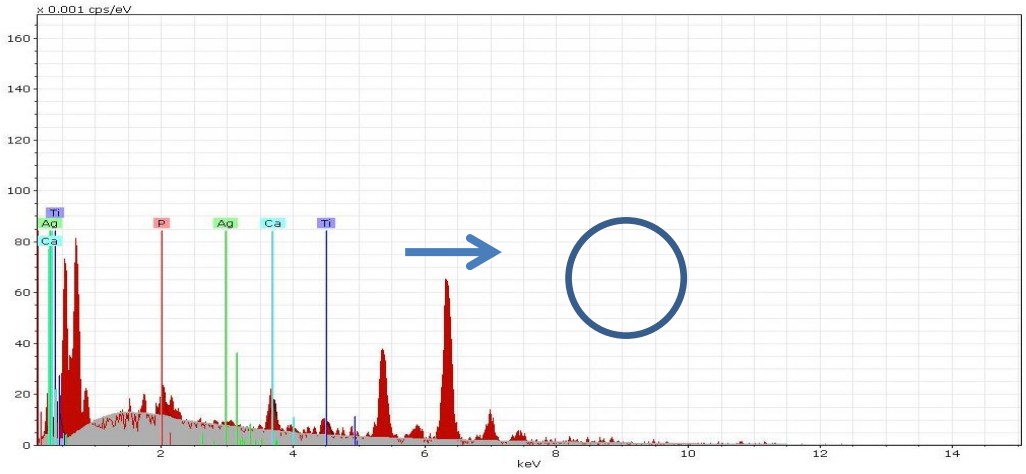


Figure 2: SEM micrograph of the HA-Ti-Ag coating surface and (below) EDS-spectrum of the coating surface. The arrow showing fine cracks and the circle demonstrating fine Ag agglomeration .

Table 3: EDS analysis of the coating surface HA-Ti-Ag

Element	Weight%
Phosphorus	0.14
Calcium	0.28
Titanium	0.34
Silver	0.18

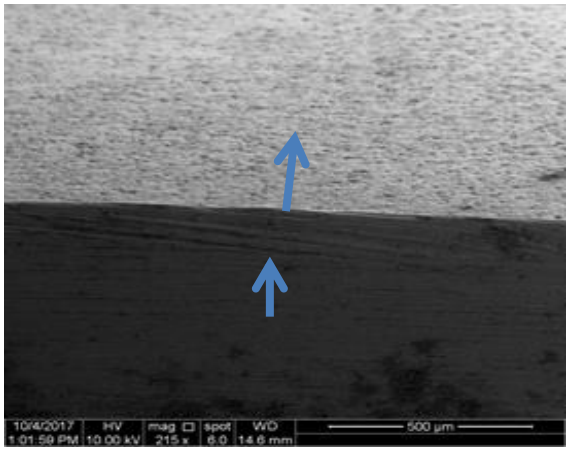


Figure 3: SEM micrograph of the HA-Ti-Ag coating-metal interface. Blue arrows showing coating thickness.

3.2Antimicrobial action: The results of sensitivity assay revealed that the composite coating (HA-Ti-Ag) had no effect on the studied bacterial strains, likewise other coatings prepared in this study. Silver is an inert metal and can be reduced into an active form (Ag^+) in the presence of biological fluid, water and inflammatory exudate (Lansdown, 2010). Therefore, the findings of the sensitivity assay could be ascribed to the insufficient moisture present in the culture media that required changing silver metal into its biologically active form silver ions. The other possible reason is that silver could be ionized but the solid media restricted its movement and hence there was no antimicrobial action. (Results not shown for brevity).

Turbidity assay was performed to assess the effect of Ag in the composite coating on the growth of the studied bacteria. Optical density was measured at zero time point and after 24 hours to assess the growth of viable bacteria. In this test the composite coating should be in contact with liquid broth media which supposed to provide sufficient moisture to convert silver into Ag^+. Figure 4A showed that the composite coating containing Ag reduced the growth of *S. aureus* after 24 hours in compareson to other coatings and the control sample. However, this effect was little, if any, against *E. coli*, as depicted in Figure 4B. The results of turbidity assay of this study disagreed with the elaboration of Pradhaban *et al* who found that silver in silver-zirconia composite coating produced by laser technique exhibited similar biocide action against *S. aureus* and *E. coli in vitro*.

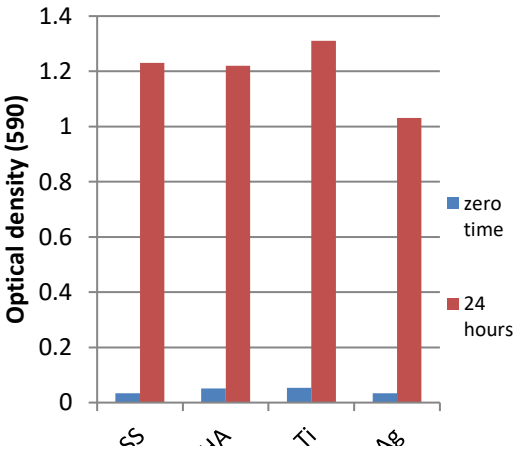


Figure 4: A growth of *S. aureus* in broth media containing different coatings and SS (stainless steel) defined by measuring optical density.

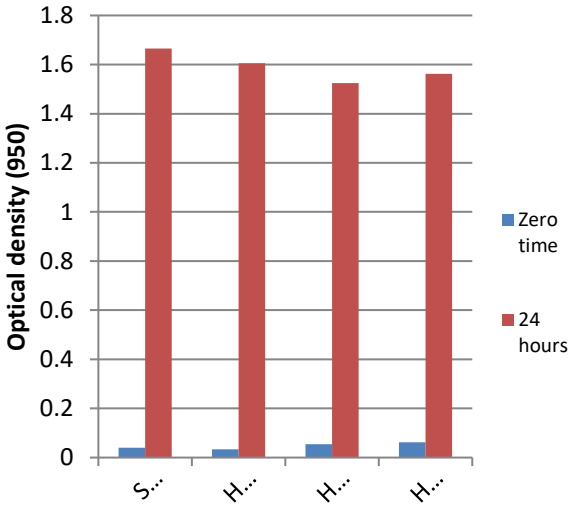


Figure 4B: Growth of *E.coli* in broth media containing different coatings and SS(stainless steel) defined by measuring optical density.

The results of turbidity assay could be ascribed to the differences in the structure of *S. aureus* (Gram +ve) and *E. coli* (Gram -ve). The cell wall of gram positive bacteria is composed of thick peptidoglycan layer and thin plasma membrane; whereas, in gram negative bacteria the cell wall contains thin peptidoglycans layer and the plasma membrane covered by outer membrane (Nathanael *et al*, 2011). The permeability of the outer membrane in gram negative bacteria may influence the passage of certain antimicrobial agent. Therefore, in certain situations gram negative bacteria can develop more resistance to chemical agent compared to gram positive bacteria (Tortora *et al*., 2001). Feng *et al*., 2000 suggested that silver ion can exert antimicrobial effect only when released from implanted materials, as released silver ions penetrate bacterial cell wall and bind with DNA phosphate group, resulting in DNA condensation and further reaction with protein causing bacterial damage.

During incubation stage the composite coating containing Ag exposed to the inoculated broth media and some bacteria have a chance to adhere to the coating surface. The released silver ions from the composite coating exert biocide action against the attached and non-attached bacteria (Pradhapan *et al*, 2014) and hence reducing the bacterial mass in the culture media.

Corrosion test: The results of the electrochemical test of the coated and uncoated SS substrate in Hank's balanced salt solution for 14 minutes are illustrated in Table 4. Hank's balanced salt solution was used in the present study as most in *vitro* corrosion research on biomedical implants was conducted in this solution due to its chemical composition which is similar to body fluid (Manivasagam *et al*, 2010).

Table 4 Electrochemical analysis of coated and uncoated SS substrate

Sample	E_{corr}(mV)	$i_{corr.}$ (µA)	CR (mpy)
Uncoated SS	-221.1	2.29	0.214
SS coated with HA	-21.5	2.11	0.197
SS coated with HA-Ti	-22.2	1.26	0.118
SS coated with HA-Ti-Ag	-21.3	1.30	0.121

***SS: stainless steel.**

It can be seen that the SS substrate coated with HA-Ti coating had the lowest corrosion rate compared with other coated samples. This could be assigned to titanium as this metal has excellent corrosion resistance in physiological environment (Kumar *et al*, 2015). In addition, this metal may improve the mechanical properties of HA particles (micro-hardness and bond strength) (Zaho *et al*, 2012) and consequently reduce the released ions from the metallic substrate by the protective action of HA coating. It has been reported that the cohesion strength of HA coating increased by adding Ti powders, as this metal eliminates the crater like defect in the coating (Zheng *et al*, 2000). Further, Anawati *et al*, (2013) stated that by combining Ti and HA powders the corrosion resistance increased and stabilized the surface as a result of passive layer formation.

The results of this work revealed that the incorporation of Ag at 13wt% into HA-Ti coating had no effect on the corrosion behavior of SS substrate in Hank's solution, as the corrosion rate of the composite coating containing Ag was very close to that of HA-Ti coating. It is found that silver is more stable than stainless steel and has a reasonable corrosion resistance (Feng *et al*, 2011). The results of the composite coating (HA-Ti-Ag) could be assigned to the low concentration of Ag in the coating. In addition, silver particles agglomerated in the composite coating as observed in the SEM micrograph and had no role to influence the corrosion conduct of SS substrate. Lu et al., (2011) stated that the most challenge factor that compromised the coating application is the agglomeration of Ag particles in the coating materials. Therefore, there was no difference in the corrosion rate of the composite coating and the HA-Ti coating.

The sample coated with HA exhibited very little difference from that of uncoated SS substrate. This reveals that HA coating may have little, if any, impact on the corrosion resistance of the metallic substrate. This result disagrees with findings of other research where they found that HA coatings decrease the corrosion rate at short immersion time (Kayali *et al*, 2016). The weak corrosion resistance of SS sample coated with HA could be associated with porosity of HA coatings (Riahi *et al.*, 2015). These porosities permit penetration of the corrosive solution into the substrate and lead to leak of metal ions from the bulk metal into the solution. The results of corrosion rate of different coatings are in agreement with i_{corr} and E_{corr} values.

Conclusion

The composite coating (HA-Ti-Ag) for 316 L stainless steel produced by laser technique was thin film which was about 1.6 µm. The coating was homogenous and uniform and appeared to be fused to the underlying stainless-steel substrate. A slight agglomeration of Ag was noted in the composite coating but had no effect on the coating structure. Incorporation of Ag at 13wt% into the HA-Ti coating reduced the growth of *S. aureus* but had no effect on *E. coli in vitro*. The composite coating improved the corrosion resistance of stainless steel. However, Ag at 13wt% had no influence on the anticorrosion conduct of the coating.

Since the HA-Ti-Ag composite coating enhanced the antimicrobial and anticorrosion conduct of stainless steel, stainless steel coated with HA-Ti-Ag coating can be used in dental and orthopedic field as in case of dental implant and pins and screws for bone fixation.

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