Prosthetic infections interest 1% of total joint replacement and represent one of the major complications of orthopedic implants [Miron S. 2010, Papageorgiou P-I 2009, Campoccia D. 2000]. They can lead to prolonged antibiotic therapies and even to the need of implant removal. The results are patient health concerns and discomfort, together with increased hospitalization times and costs. In this context, the development of implant surfaces able to reduce infection risk and enhance reintegration rate constitutes a challenge. After implantation, a sort of “face for the surface” between tissue cells and bacteria has been described in the scientific literature [Simoni A.G. 1997, Ferrari S. G. 2006]. An ideal surface for implants should improve cellular adhesion and reduce bacterial one. A proper stimulation of the cell activity is the last request to the new biomaterials, intended for bone substitution and ossification. In this regard, the scientific literature suggests that the surface modification on a nanoscale is a major source of innovation. The nano features and multiple topographies can stimulate cell differentiation and activity. Moreover, the presence of specific biological molecules is grafted onto the biofaces can properly stimulate cells to tissue regeneration [Simoni S. 2015]. Furthermore, numerous solutions have been proposed to face the problem of the bacterial contamination and to prevent the development of infectious processes. The considered solutions are mainly based on inorganic antibacterial agents (such as metal ions: silver, copper or zinc), which have been introduced in order to overcome the growing problem of bacterial resistance to antibiotics [Ferrari S. 2016]. Nevertheless, despite of a wide research in the field of antibacterial surfaces, the optimal solution is still far from the clinical application. The aim of the present research work is the development of innovative antibacterial and bioactive titanium alloy (Ti6Al4V) surfaces, able to promote fast and physiological bone integration and to avoid bacterial contamination.

**Methods**

**Antibacterial testing**

Titanium discs of Ti6Al4V (4 mm diameter and 2 mm thick, ASTM 6-8) were surface-modified by means of a patented process [Sparano S, Fiatchi PCT/IT2013000010, Ferrari S. 2015] that foresaw a first treatment (electrochemical) and a subsequent control solution in hydroxyacetic acid, added with silver.

**Microbiological analysis**

The morphology of the modified surfaces was characterized by means of field-emission scanning electron microscopy (FESEM) [Tatari E, Agresti R., Chiesa S, Spectroscopy 2014]. The bacterial cultures were performed on agar plates and incubated for 5 to 7 days at 37°C. After the incubation, the samples were observed using a light microscope. All experiments were performed in triplicate.

**Antibacterial testing**

The microbial models were tested against five strains: ATCC 29213 (E. coli ATCC 29213), ATCC 35218 (S. aureus ATCC 35218), ATCC 49739 (B. subtilis ATCC 49739), ATCC 9542 (S. pneumoniae ATCC 9542) and ATCC 11228 (K. pneumoniae ATCC 11228). After incubation, the colonies were counted on agar plates stained with crystal violet. The plates were observed under a light microscope.

**Antibacterial testing**

**Results**

Modified Ti6Al4V samples presented a titanium oxide layer with a peculiar nanotexture (Figure 2) that can be described as a nanometric sponge with Ra of 10 nm [Sparano S, Patent PCT/IT2013000010, Ferrari S. 2015]. Silver nanoparticles are embedded in this surface layer (Figure 3b) by the addition of silver precurs in the hydrogen peroxide bath [Ferrari S. 2014]. XPS analyses indicate that silver is in the metallic from (Figure 3b). Silver nanoparticles (50-200 nm) are responsible of silver ion release in water. The released silver amount (Figure 3c) is higher than what required to have an antibacterial action and lower that the cytotoxic limit reported in the literature [Ferrari S. 2013].

**Conclusions**

An innovative and patented surface treatment has been applied to Ti6Al4V alloy. A nanotextured titanium oxide layer rich in hydroxyapatite and embedded with silver nanoparticles has been obtained. Modified surfaces are bioactive by inducing hydroxyapatite precipitation in SBF, release silver ions and present an antibacterial action against S. aureus. Considering the reported results, the obtained Ti6Al4V surfaces are promising for orthopaedic applications to induce fast and physiological bone integration associated to a reduced incidence of prosthetic infections.