An antibacterial coating obtained through implantation of titanium ions

D Delle Side¹, V Nassisi¹, E Giuffreda¹, L Velardi¹, P Alifano², A Talà² and S M Tredici²
¹ LEAS, Dipartimento di Matematica e Fisica “Ennio De Giorgi”, Università del Salento and INFN Section of Lecce, Lecce, Italy
² Dipartimento di Scienze e Tecnologie Biologiche ed Ambientali, Università del Salento, Lecce, Italy
E-mail: domenico.delleside@le.infn.it

Abstract. Everyday life is exposed to the risks of contracting severe diseases due to the diffusion of severe pathogens. For this reason, efficient antimicrobial surfaces becomes a need of primary importance. In this work we report the first evidences of a new technique to synthesize an antibacterial coating on Ultra High Molecular Weight Polyethylene (UHMWPE)samples, based on a non-stoichiometric, visible light responsive, titanium oxide. The coating was obtained through laser ablation of a titanium target, then the resulting ions were accelerated and implanted on the samples. The samples where tested against a *Staphylococcus aureus* strain, in order to assay their antimicrobial efficacy. Results show that this treatment strongly discourages bacterial adhesion to the treated surfaces.

1. Introduction
During the last years, the occurrence of severe threats to public health raised the attention on the conditions under which pathogens spread across the population. For example, in the cases of Avian Influenza¹ or of the Severe Acute Respiratory Syndrome², it has been shown that infections are likely in healthcare workers, although correct antiseptic strategies would ensure good safety levels. In general, several reports pointed out that infectious agents are nested in common use objects such as shopping carts handlebar³, computer keyboards⁴, clinical surfaces⁵, faucet handles⁶, door handles⁷. Although a proper treatment with antiseptic detergents would greatly reduce risks, there are situations in which such a strategy is inapplicable. Consequently, clearly emerges the necessity of permanent antimicrobial coatings.

Since 1985, the year in which Matsunaga et al.⁷ reported its antimicrobial activity, there has been a great interest in the application of titanium dioxide for sanitization purposes. The antimicrobial effect of TiO₂ arises from its photocatalytic activity. In this material, an electron could be excited by ultraviolet (UV) radiation; the corresponding excess energy promotes it to the conduction band, creating a couple electron-hole. Both the electrons and the holes generated in such processes have a strong reducing and oxidizing activity and consequently react with H₂O and/or O₂ to give reactive oxygen species (ROS). These ROS are extremely reactive when in contact with organic compounds and are supposed to perform a key role in TiO₂ antimicrobial activity. In any case, it has been found that titanium dioxide performs an efficient, long-lasting and costs-effective antimicrobial effect⁸,⁹.
Despite of the fascinating properties of TiO$_2$, the requirement of the biohazardous UV light severely limits its application. Moreover, it has to be considered that UV radiations represent only at most 4% of the solar energy spectrum reaching daily the Earth surface\cite{10}. For these reasons, a great scientific effort has been devoted to the development of methods that could activate TiO$_2$ photocatalysis in the visible range. Currently, impurity doping is the most common technique for expanding the spectral response of pure titania\cite{11}. Moreover, several reports indicate that non-stoichiometric titanium oxides develop excellent photocatalytic properties under visible light exposure\cite{10,12}. These findings suggest the possibility of a new technique for an easy synthetization of non-stoichiometric titanium oxides coatings based on ion implantation over impurities adsorbing materials.

In general, materials contain on their surfaces oxygen-rich compounds (CO or H$_2$O are clear examples). Using energetic Ti ions that impinge on the surface of such materials, it is possible to break the bonds that tie oxygen with the other elements. In such a way we consequently obtain the chance to stimulate the formation of non-stoichiometric titanium oxides on that substrate, as it is customary in ion implantation\cite{13}.

In this work we present the first proof-of-concept of this technique, using UHMMWPE as substrate. It is indeed known that in this polymer at least 11% of surface contaminants elements is represented by oxygen\cite{14}. Moreover, in order to reach our goal, it is essential that the energetic ion beams used for the bombardment process have a broad energy spread. In this way, the ions with higher energy will break the bonds, while the lower energy ones could create new bonds with the free oxygen. For this reason we choose a setup in which large energy spread ion beams obtained by laser ablation are accelerated to bombard the samples surface.

2. Materials and Methods

The main apparatus used to reach our scope is the “PLATONE” accelerator\cite{15}, which is a laser ion source (LIS) coupled to a double stage electrostatic accelerator. The LIS uses a KrF excimer laser (Lambda Physik, Mod. COMPEX 205) and a stainless steel vacuum chamber as accelerating chamber (AC), Fig. 1. The accelerating chamber has inside an expansion one (EC) that enables the hydrodynamic expansion of the plasma before the ion extraction. The EC forms a hermetic contact with the target support T. A base of the EC, together with T, is fixed to the AC by an insulating flange (IF) that allows the application of a positive high voltage to the EC. At the opposite EC side there is a 1.5 cm diameter hole in order to extract ions. The distance T-EC is fixed at 18 cm. A ground electrode (GE) is placed at a distance of 3 cm from EC, having the center drilled by a hole of the same diameter of the EC one. After the GE, at a distance of 2 cm, it is placed a further third electrode. This electrode was utilized either as Faraday cup (FC) or sample support. The vacuum was operated through the use of two turbo-molecular pumps, reaching a pressure of the order of $10^{-6}$ mbar. During the experiments

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{Figure1.png}
\caption{Cross section of the \textit{PLATONE} accelerator. IF: insulating flange; AC: acceleration chamber; GE: ground electrode; EC: expansion chamber; FC: Faraday cup.}
\end{figure}

the accelerating voltages of the first (T+EC-GE) and second (GE-FC) stage were fixed at +40 and −20 kV respectively, providing a maximum ion beam energy of 60 keV per charge state. We
used such an apparatus because the resulting ion beams are known to have the broad energetic spectrum that we require\cite{16}.

The target used was a commercial thick Ti disk with a diameter of 2 cm, 99.99% pure. The UHMWPE samples used as substrates, instead, have a density of 0.93 g/cm\(^3\), while their dimensions are 20 × 20 mm\(^2\) for a thickness of 1 mm. These samples have been fixed on the FC and implanted with 22000 laser shots, for a total dose of about 8.8 × 10\(^{15}\) ions/cm\(^2\).

After the treatment, the samples were analyzed through scanning electron microscopy (SEM), energy dispersive spectroscopy (EDS), atomic force microscopy (AFM) and UV-Visible (UV-Vis) spectroscopy.

Moreover, in order to test the efficacy of the treatment, we challenged the samples with 10\(^4\) CFU/ml of Staphylococcus aureus SA-1 in micro-wells filled with 4 ml of Nutrient Broth at 37°C, under moderate shaking. The S. aureus strain was isolated from a catheter-related bloodstream infection\cite{17}. Before biological testing, samples were UV sterilized for 1 h. Treated and untreated UHMWPE samples were placed on direct day/artificial light exposure in the same tank, sealed to prevent any contaminations. During incubation, the samples have been washed 3 times with saline solution (NaCl0.9%) in order to remove from samples the bacteria that were firmly adherent to the surfaces. After a week of incubation, the biofilm matured on the samples surface was stained using green-fluorescent nucleic acid stain (SYTO9; Molecular Probes, USA). After 15 minutes of dark incubation, the biofilm development was viewed with a Nikon Optiphot-2 microscope with an episcopic-fluorescence attachment (EFD-3, Nikon). This technique enabled us to have a direct evidence of the bacteria effectively adhered to the surfaces. In particular, the survival shares have been computed by means of the formula

\[
\text{survival } \% = \frac{\text{microbe count on treated sample}}{\text{microbe count on blank sample}} \times 100.
\]

3. Results
In order to obtain insights on the effect of the technique under development, we performed some measurements to understand the status of the UHMWPE surfaces before and after treatment. In Fig. 2, the SEM images of a blank sample and of a treated one are shown. These images show qualitatively that surface morphology undergoes drastic changes. In order to estimate the morphology changes illustrated before, we performed also an AFM analysis. Such measurements (Fig. 3) showed that the root mean square surface roughness increased from 23.2 to 94.6 nm, the average height from 172 to 637 nm and the average roughness from 18.4 to 73.7 nm. Moreover,
Together with the measurements focused at the surface changes, we performed also a qualitative investigation of the visible-light response of the treated material with respect to the blank one. For this reason we carried out an UV-Vis spectra, measuring the absorbance of the samples, as shown in Fig. 5. Finally, we performed a bacterial surface adhesion test on a blank and 3 different treated samples, which differed for the exposure time on the normal air atmosphere after treatment (respectively: 2, 4 and 7 days). The resulting fluorescence microscope images are shown in Fig. 6. Quantitative analysis, performed by counting the bacterial cells observed in 50 microscopic fields randomly selected, revealed that the mean values of percentages of adherence to the substrate with respect to the blank were $50 \pm 14\%$ (2 days), $32 \pm 11\%$ (4 days) and $10 \pm 5\%$ (7 days). Fig. 7 shows the histogram of the results with the relative standard deviation.

4. Discussion and Conclusions
As shown by surface analysis, the treated polymer underwent significant morphology changes. Important is the fact that EDS revealed that Ti and O are often together on the treated samples,
giving an indication of the oxide formation. The UV-Vis spectra showed that the modified polymer has a slight, although sensible, increase of the absorbance in the visible range, indicating that the treatment went into the right direction. Antimicrobial tests are really interesting, since gave good results on the efficacy of the resulting coatings. Moreover, it has been clearly shown that the effectiveness increases with the time of the exposure to air. This is probably due to titanium natural tendency to develop an oxide layer on its surface. This circumstance is important for applications, since it ensures that the efficacy of the treatment is not compromised while the surfaces are in the atmosphere.

Concluding, we showed that bombarding an oxygen rich surfaces with a broad energy spread dose of Ti ions the formation of a (non-stoichiometric) titanium oxide film with high antimicrobial activity in the visible range is induced. This technique seems to be promising and could simplify the commonly used treatment modality. Despite of this, these results are just preliminary and deserve more attention. In particular more insights on the nature of the titanium oxide and on the experimental parameters affecting the results are needed.

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References
[1] The Writing Committee of World Health Organization 2005 N. Eng. J. Med. 353 1374–1385 pMID: 16192482 URL. http://www.nejm.org/doi/full/10.1056/NEJMra052211
[2] Peiris J S, Yuen K Y, Osterhaus A D and Stöhr K 2003 N. Eng. J. Med. 349 2431–2441 pMID: 14681510 URL. http://www.nejm.org/doi/full/10.1056/NEJMra032489
[3] Gerba C P and Maxwell S 2012 Food Protection Trends 32 747–749 URL http://www.foodprotection.org/publications/food-protection-trends/article-archive/2012-12bacterial-contamination-of-shopping-carts-and-approaches-to-control/
[4] Bures S, Fishbain J T, Uyehara C F, Parker J M and Berg B W 2000 Am. J. Infect. Control 28 465–471 URL. http://www.ajicjournal.org/article/S0196-6553(00)900655-2/abstract
[5] Chopra I and Hacker K 1992 J. Antimicrob. Chemother. 29 19–25 URL. http://jac.oxfordjournals.org/content/29/1/19.abstract
[6] Wojgani H, Khalsa C, Cloutman-Green E, Gray C, Gant V and Klein N 2012 PLoS ONE 7 e40171 URL. http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0040171
[7] Matsunaga T, Tomoda R, Nakajima T and Wake H 1985 PEMS Microbiol. Lett. 29 211–214 ISSN 1574-6968 URL. http://dx.doi.org/10.1111/j.1574-6968.1985.tb00864.x
[8] Liu J W and Chang H H 2012 Arch. Immunol. Ther. Exp. 60 267–275 ISSN 0004-060X URL. http://dx.doi.org/10.1007/s00005-012-0178-x
[9] Visai L, De Nardo L, Punta C, Melone L, Cigada A, Imbriani M and Arciola C R 2011 Int. J. Artif. Organs 34 929–946 URL. http://www.artificial-organs.com/article/titanium-oxide-antibacterial-surfaces-in-biomedical-devices-ijao-d-11-00132
[10] Dholam R, Patel N, Adami M and Miotello A 2008 Int. J. Hydrogen Energ. 33 6896 – 6903 ISSN 0360-3199 URL. http://www.sciencedirect.com/science/article/pii/S0360319908012108
[11] Pelaez M, Nolan N T, Pillai S C, Seery M K, Kontos A G, Dunlop P S M, Hamilton J W, Byrne J, O’Shea K, Entezari M H and Dionysiou D D 2012 Appl. Catal. B: Environ. 125 331 – 349 URL. http://dx.doi.org/10.1016/j.apcatb.2012.05.013
[12] Kitano M, Matsuoka M, Ueshima M and Anpo M 2007 Appl. Catal. A: Gen. 325 1 – 14 ISSN 0926-860X URL. http://www.sciencedirect.com/science/article/pii/S0926860X06001937
[13] Chen J, Wan G, Leng Y, Yang P, Sun H, Wang J and Huang N 2004 Surf. Coat. Tech. 186 270 – 276 URL. http://www.sciencedirect.com/science/article/pii/S0257899X04002828
[14] Mittal K (ed) 2004 Polymer Surface Modification: Relevance to Adhesion vol 3 (Taylor & Francis Group)
[15] V. Nassisi, D. Delle Side and L. Velardi 2013 App. Surf. Sci. 272 114 – 118 URL. http://dx.doi.org/10.1016/j.apsusc.2012.03.128
[16] D. Delle Side and L. Velardi 2013 Rev. Sci. Instrum. 83 02B717 URL. http://dx.doi.org/10.1063/1.3672476
[17] Paladini F, Pollini M, Tala A, Alfano P and Sannino A 2012 J. Mater. Sci. - Mater. Med. 23 1983–1990 ISSN 0957-4530 URL. http://dx.doi.org/10.1007/s10856-012-4674-7