Enhancing UHMWPE Antibacterial Properties Through Ion Implantation

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Abstract

We propose a new technique for the surface modification of biomedical polymers in order to strongly enhance its antimicrobial quality via ion implantation, in alternative to the well known ones that use chemical reactions or films deposition. We present the experimental results of implantation of ultra high molecular weight polyethylene (UHMWPE) samples by Ag, Cu and Ti ions. As accelerator we used "Platone", a homemade laser ion source accelerator device. The ions were extracted from the plasma generated by a KrF excimer laser operating at 248 nm. A laser beam of 12 mJ/pulse was focused on a solid target mounted in a vacuum chamber in order to obtain plasma. The laser spot was estimated to be of 0.005 cm^2. This device has an accelerating system composed by two different stages. Using pure Ag, Cu and Ti disks as laser targets and applying voltages of 40 kV to the first accelerating stage and 20 kV to the second one, we produced ion beams containing about 10^{11} ions/cm^2 per pulse. The penetration depth of ions was estimated by the Srim code and the largest depth was achieved with Ti ions. Operating 22000 laser shots we implanted our samples with doses of about 10^{15} ions/cm^2. Next we analyzed the effects of ion implantation on the bacterial adherence to UHMWPE samples. To test their antimicrobial quality, a Staphylococcus aureus strain isolated from a catheter-related bloodstream infection was used in these assays. The results demonstrate that the adherence of bacteria is reduced of 21%, 7% and 90% for Ag, Cu and Ti ions-implanted samples with respect to the control ones, confirming the effectiveness of our approach.

Introduction

In the past years, the growing demand for biomaterials of antimicrobial quality gave rise to a new and fruitful research area for material science. For example, UHMWPE is used to make components of prosthetic knee, hip and shoulder[1], while other materials are used for surgical and dental tools, sanitary equipment, parts of prosthesis type screws[2-4], etc. The essential properties of these materials must be the biocompatibility, wettability and durability. The antibacterial properties are no less essential[5]. Apart from medical applications

many other fields have demonstrated much interest in the antibacterial properties of material including the packing industry of food[6] and the mechanic industry operating in the construction of alimentary machines[7]. These properties together with surface hardness and corrosion resistance can be further improved applying the ion implantation technique[8, 9].

Ag atoms are well known as antibacterial particles while copper and its alloys are known as natural antimicrobial materials. Molecular mechanisms responsible for the antibacterial action of these atoms are today a subject of intensive research and ranges from poisoning on bacteria's respiratory enzymes and electron transport components to interfere with DNA functions and even to an induced toxicity due to the generation of reactive oxygen species. In general, the overall antibacterial effect induced by both metals seems to be the result of a combination of various causes [10-12].

Titanium dioxide is no less important as antimicrobial material, his efficiency is very high but somewhat limited by the necessity of UV light to activate its action[13]. Using ion implantation, energetic Ti ions could exploit surface impurities containing oxygen, inducing the formation of a carbon (from the substrate) doped TiO_2.

We propose to use ion implantation onto the surface of Ag, Cu and Ti metals instead of the application by chemical reactions that can potentially invalidate the biocompatibility, due to a possible formation of undesired by-products that have to be removed with complex techniques. Additionally, the application of thin film deposition could be effective only if the adherence with the bulk is guaranteed and the proprieties of the material surface are preserved. In fact, a successful film deposition needs strong chemical bounds between film and bulk. Besides, the chemical processes involve procedures that use potentially undesired solvents[14, 15]. The proposed technique, which is able to overcome the previous limitations, consists in the application of a dose of antibacterial ions inside the first surface layers of UHMWPE samples to be treated. The goal can be achieved if the ions are preventively and suitably accelerated.

Material and methods

The UHMWPE is used in many fields and particularly in the medical one due to its extreme versatility, high chemical inertness and its good biocompatibility. The samples used in this work have an average molecular weight of 4×10^6 g/mol, with a density of 0.93 g/cm^3. Their dimensions are 20x20mm^2 for a thickness of 1 mm. Instead, as target for the ion source we used thin discs with a diameter of 2 cm of silver, copper and titanium (99.99% pure).

The accelerator "Platone", installed at the LEAS laboratory at the Dipartimento di Matematica e Fisica, Università del Salento, is a homemade device[16]. It is a laser ion source (LIS) that utilizes a KrF excimer laser (Lambda Physik, Mod. COMPEX) and a stainless steel vacuum chamber as accelerating chamber (AC), Fig. 1. The laser generates a pulsed beam of 248 nm wavelength (5 eV photon energy) and a width (FWHM) variable from 23 to 30 ns. The laser beam, guided by a 15 cm focal length lens, enters the AC through a thin quartz window with an angle of 70 °C with respect to the main axis perpendicular to the target (T). The accelerating chamber has inside a second chamber called expansion chamber (EC) that enables the hydrodynamic expansion of the plasma before the ion extraction. The EC forms a hermetic contact with the support of the target in order to not allow plasma leaks. A base of the EC, together with the support 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 hole of 1.5 cm in diameter in order to extract

ions. At a distance of 3 cm from EC, it is placed a grounded electrode (GE), having the central part 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, connected to a power supply of negative polarity. This electrode was utilized either as Faraday cup (FC) or sample support. The vacuum was obtained through the use of two turbo-molecular pumps, reaching a pressure of the order of 10^{-6} mbar.

It is worth noticing that T and AC are connected through an high voltage fast capacitor (C_1) of 4 nF to ensure the stabilization of the accelerating voltage. Moreover, thanks to the presence of another high voltage capacitor (C_2) of 2 nF and a load resistor of 100 kΩ, the FC could be safely connected to an oscilloscope for diagnostic purposes.

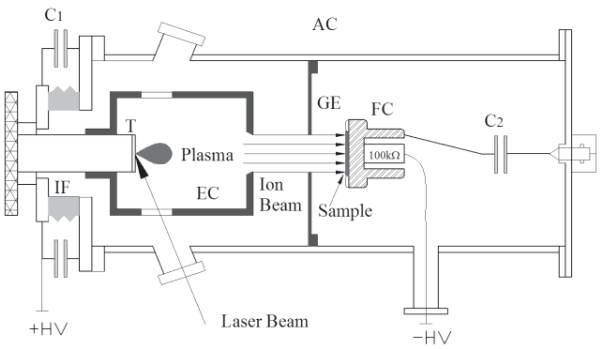


Fig. 1: Cross section of Platone accelerator. IF: insulation flange; AC: accelerating chamber; GE: ground electrode; EC: expansion chamber; FC: Faraday cup.

This device is able to accelerate the plasma ionic component up to 160 keV for charge state. Instead, to preserve the equipment from breakdown risks, during the experiments the accelerating voltages of the first and second stage were fixed at 40 and -20 kV respectively, providing a total ion beam energy of 60 keV per charge state.

Using the Srim code[17], we obtained from a simulation that the maximum implantation depth (reached in the case of +2 ions) is about 250 nm for copper and about 160 nm for silver. Fig. 2 shows the simulation results.

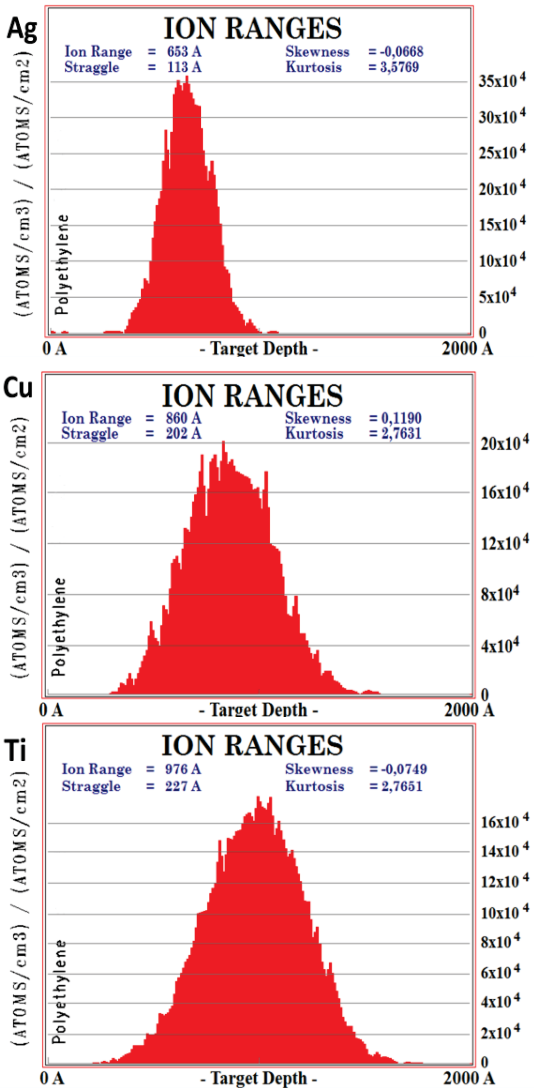


Fig. 2: Simulation results of the penetration depth for Ag, Cu and Ti 1+ ions ; abscissas and ordinates are in linear scale.

Samples implantation

The samples were fixed on the FC collector and implanted with 22000 laser shots, both in the case of silver and copper. Fig. 3 shows a photo of a sample mounted on the FC support. The total dose obtained is roughly the same[18] corresponding to 10^{15} ions/cm^2.

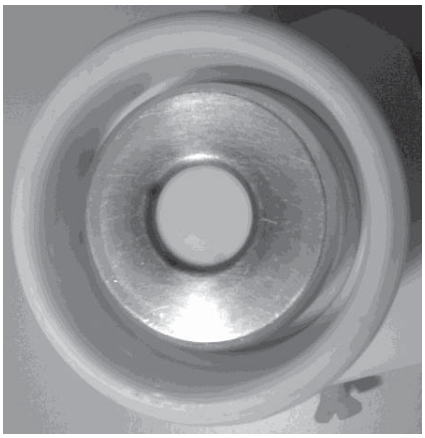


Fig. 3: Photo of the UHMWPE sample mounted on the FC support; the support consists of an inner body of stainless steel to which is applied a negative high voltage and an external insulating cover made of PVC.

Antibacterial experiments

After this treatment the UHMWPE samples were challenged with 10^4 CFU of *Staphylococcus aureus* in micro-wells filled with 4 ml of Nutrient Broth at 37 °C, under moderate shaking; this high concentration was chosen in order to assay the samples under extreme conditions. The *S. aureus* strain was isolated from a catheter-related bloodstream infection. We chose these bacteria since they are among the first causes of infections in humans and in food related pathologies.

Treated and untreated UHMWPE samples were placed in the same tank. After a week of incubation on daylight exposure, when the concentration of *S. aureus* should have reached a value of about 10^{10} CFU, the biofilm maturated on the samples surfaces was stained using green-fluorescent nucleic acid stain (SYTO9; Molecular Probes, USA). After 15 min of dark incubation, the biofilm development was viewed with a Nikon Optiphot-2 microscope with an episcopic-fluorescence attachment (EFD-3, Nikon). The images are shown in Figs 4 and 5.

Results

In order to test the antimicrobial effectiveness of our technique, we conducted a fluorescence microscopy analysis on all the samples. This revealed a sensible reduction of bacterial adhesion on Cu- and Ag-treated UHMWPE samples (Fig. 5a) and an even stronger one on Ti-treated ones (Fig. 5b), with respect to the control. 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 were 93%, 79% and 10% with Cu, Ag and Ti implanted samples respectively. Fig. 6 shows the histogram of results with the relative standard deviation.

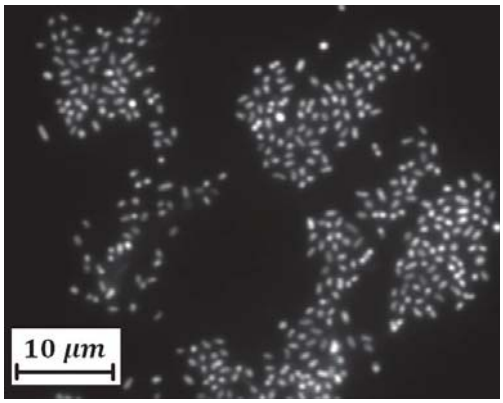
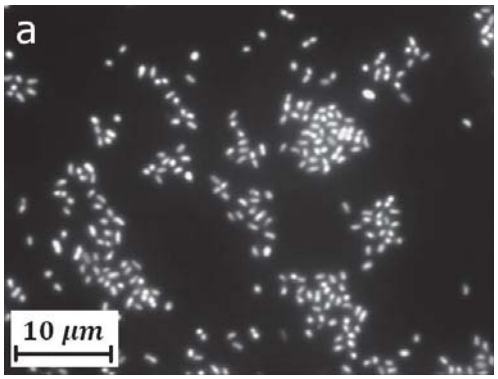


Fig. 4: Representative images showing adherence of Staphylococcus aureus to UHMWPE untreated (control) samples.



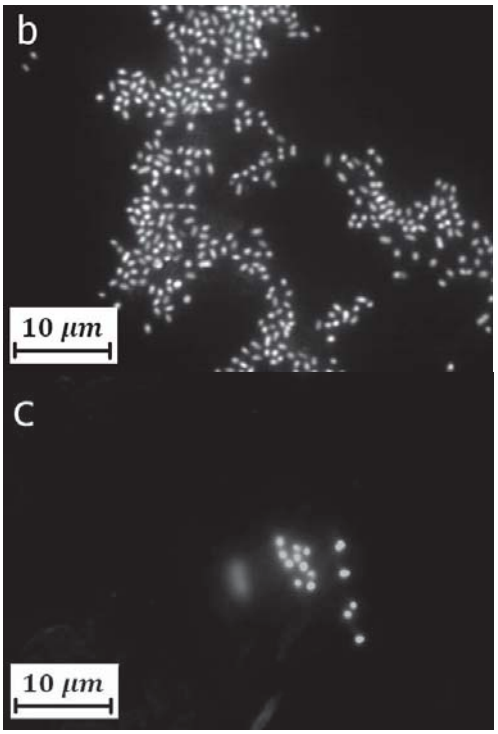


Fig. 5: Representative images showing adherence of Staphylococcus aureus to Cu- (a), Ag- (b) and Ti-implanted (c) UHMWPE samples.

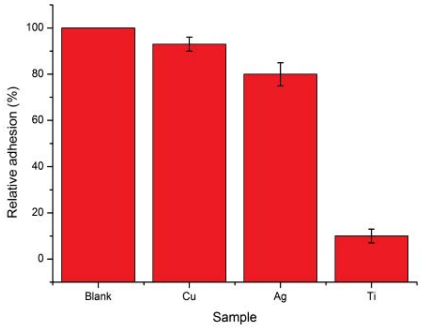


Fig. 6: Relative adhesion (%) of Staphylococcus aureus on the sample surfaces. The graph shows the reduction of bacteria on treated samples with respect to the untreated one.

The higher efficiency of the Ti-treated samples in preventing *Staphylococcus aureus* adhesion is very interesting. This behaviour should be ascribed to the fact that the titanium dioxide is formed directly on the surface of the sample, in direct contact with the site that hospited bacteria. Therefore next experiments will be devoted to clarify these aspects.

Moreover, we performed some morphological analysis to understand if the implantation process altered the roughness and wettability of the samples surface.

Using a commercial PCE-RT1200 tester, we measured the arithmetic roughness R_A, given by the arithmetic mean of the absolute value of the vertical distance y_i from the mean line to the i_{th} data point

$$R_A = \frac{1}{N}\sum_{i=1}^{N}|y_i|;$$

the values obtained are shown in table I.

	Blank	**Ag**	**Cu**	**Ti**
R_A (μm)	1.176	1.435	1.474	1.211

Table I: *arithmetic roughness of the samples*

As could be seen from the values, the implantation process increases the roughness of the surface.

The wettability of the surfaces has been estimated through the method of the contact angle.

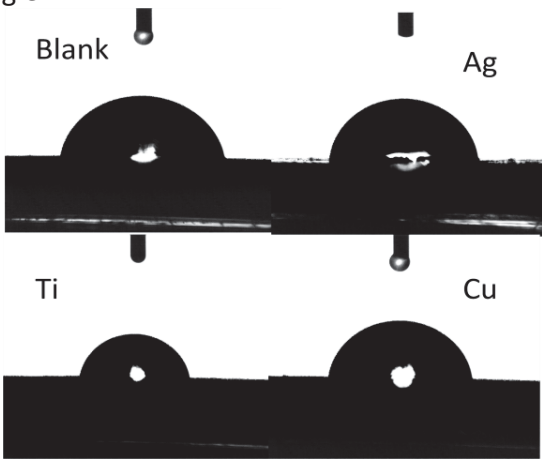


Fig. 7: Water drops used for the estimation of the wettability through the contact angle method

The measurements, whose values are reported in Table II, show that the process does not significantly alter the hydrophilic nature of the UHMWPE surface.

	Blank	Ag	Cu	Ti
θ (deg)	81.7	81.2	79.6	81.4

Table II: *contact angles on the samples*

Conclusions

In this work we used ion implantation technique to improve the antibacterial proprieties of the biocompatible UHMWPE. The ion beams utilised for this goal were made respectively of Ag, Cu and Ti ions, accelerated up to a maximum of 60 kV. After this treatment, the UHMWPE samples were challenged with *Staphylococcus aureus* in bacterial adhesion assays. The results demonstrated that the adherence of bacteria showed a reduction of 90%, 21% and 7% for Ti, Ag and Cu implanted samples with respect to non-implanted control one. The technique presented in this work seems to be interesting, since it can open the way to an easier realization of antibacterial biofilms. Higher ion doses could improve the results presented here.

References

[1] Lee, H. B. (1989). Application of synthetic polymers in implants. In T. Seagusa, T. Higashimura, & A. Abe (Edited by), Frontiers of Macromolecular Science. Oxford: Blackwell Scientific Publications.

[2] Li, B. Z., Zhang, W., & Yan, Q. (1997). Plastic Sci. Technol. (6), 16.

[3] Huang, Z. J. (1999). J. Mater. Rev. (13), 35.

[4] Nakamura, S., Ookubo, N., & Miyakusu, K. (1997). Nisshin Steel Tech. Rep. (76), 48.

[5] Zhang, W., Chu, P. K., Ji, J., Zhang, Y., Liu, X., Fu, R. K., et al. (2006). Biomaterials (27), 44.

[6] Kenaway, E. R., Abdel-Hay, F. I., El-Shanshoury, A. E., & El-Newehy, M. H. (2002). J. Polym. Sci. Part. A: Polym. Chem (40), 2384.

[7] Quintavalla, S., & Vicini, L. (2002). Meat. Sci. (62), 373.

[8] Lorusso, A., Velardi, L., Nassisi, V., Paladini, F., Visco, A. M., Campo, N., et al. (2008). Nucl. Instrum. Methods B (266), 2490.

[9] Noli, F., Lagoyannis, A., & Misaelides, P. (2008). Nucl. Instrum. Methods Phys. Res. B (266), 2437.

[10] Roe, D., Karandikar, B., Bonn-Savage, N., Gibbins, B., & Roullet, J. B. (2008). J. Antimicrob. Chemother. (61), 869-76.

[11] Li, X. Z., Nikaido, H., & Williams, K. (1997). J. Bacteriol. (179), 6127–32.

[12] Baker, J., Sitthisak, S., Sengupta, M., Johnson, M., Jayaswal, R. K., & Morrissey, J. A. (2010). Appl Environ Microbiol. (76), 150-60.

[13] Z. Huang, P. – C. Maness, D. M. Blake, E. J. Wolfrum, S. L. Smolinski and W. A. Jacoby, J. Photochem. Photobiol. A 130, 163-170 (2000).

[14] Allongue, P., Delamar, M., Desbat, B., Fagebaume, O., Hitmi, R., Pinson, J., et al. (1997). J. Am. Chem. Soc. (119), 201-207.

[15] Vittorino, E., Giancane, G., Manno, D., Serra, A., & Valli, L. (2012). J. Colloid Interface Sci. (368), 191-196.

[16] Lorusso, A., Siciliano, M. V., Velardi, L., & Nassisi, V. (2010). Nucl. Instrum. Methods A (614), 169.

[17] Ziegler, J. F. SRIM. Available at http://www.srim.org

[18] Nassisi V., Velardi L. and Delle Side D., App. Surf. Sci. 272, p. 114-118 (2013).