Bacterial adherence on fluorinated carbon based coatings deposited on polyethylene surfaces

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Abstract. Development of intrinsically antibacterial surfaces is of key importance in the context of prostheses used in orthopaedic surgery. In this work we present a thorough study of several plasma based coatings that may be used with this functionality: diamond like carbon (DLC), fluorine doped DLC (F-DLC) and a high fluorine content carbon-fluor polymer (CFX). The study correlates the surface chemistry and hydrophobicity of the coating surfaces with their antibacterial performance. The coatings were deposited by RF-plasma assisted deposition at room temperature on ultra high molecular weight polyethylene (UHMWPE) samples. Fluorine content and relative amount of C-C and C-F bond types was monitored by X-ray photoelectron spectroscopy and hydrophobicity by water contact angle measurements. Adherence of Staphylococcus aureus and Staphylococcus epidermidis to non-coated and coated UHMWPE samples was evaluated. Comparisons of the adherence performance were evaluated using a paired t test (two materials) and a Kruskall Wallis test (all the materials). S. aureus was statistically significant (p ≤ 0.001) less adherent to DLC and F-DLC surfaces than S. epidermidis. Both bacteria showed reduction of adherence on DLC/UHMWPE. For S. aureus, reduction of bacterial adherence on F-DLC/UHMWPE was statistically significant respect to all other materials.

1. Introduction.

Bacterial adherence is the process whereby bacteria attach themselves to cells or other surfaces before proliferating. Development of intrinsically antibacterial surfaces is of key importance in the context of prostheses used in orthopaedic surgery. Bacterial adherence on implant surfaces is the first step in the infection process that can eventually lead to implant removal. Septic failure of prostheses represents severe consequence not only because morbidity and mortality of patients, but also for the society, which faces high economical burdens.

The bacterial adherence process is a complex series of physical and chemical interactions between the substratum and the microbe, and thus can be modified with changes in the biomaterial under risk.
One approach to provide anti-adherent functionality is to manipulate the properties of biomaterials’ surface by coating them with specific chemical functionalities. Diamond like carbon (DLC) and other coatings (as, for example, F-DLC, Si-DLC, or Ag-DLC) have been proposed to improve the antibacterial performance of biomaterials [1]. It is also worth mentioning that DLC coatings have shown good performances regarding hardness, wear resistance, friction, chemical inertness and good biocompatibility, which make them a good option for implants in biomedical applications [2].

Attending to the previous hypothesis, we have 1) selected the microorganisms to be tested from two staphylococci species, *Staphylococcus aureus* and *Staphylococcus epidermidis*, both usual gram positive bacteria which are involved in the most of biomaterial-related infections and frequently isolated from orthopaedic infections; and 2) set the aims of the present study in the investigation of these two bacterial species adherence to carbon and fluorinated carbon based coatings on UHMWPE, compared to uncoated material.

2. Materials and methods.

2.1. UHMWPE.

The raw material used in the first part of the study was a compression moulded sheet of GUR 1050 UHMWPE (Orthoplastic Ltd., Lancashire UK). Slices of a size of 10x10 mm and ~600 microns thick were used as reference samples for the adhesion studies and as substrates for the coatings as described below. The roughness of the UHMWPE slices was in the level of the tenth of micron.

2.2. DLC, F-DLC, and CFX coatings.

All the UHMWPE coatings were deposited at room temperature using a RF capacitive coupled plasma reactor. Details about the physical characteristics of the DLC deposited material can be found elsewhere [3]. Besides, two types of C-F functionalities were incorporated to the UHMWPE surface. A first one consisted of a DLC based material with a certain amount of F incorporated in its structure (F-DLC in the following). This was achieved by letting a certain amount of C$_4$F$_8$ gas in the reactor chamber while the DLC growth was taken place. A second one consisted of fluorine rich amorphous carbon structure obtained by simple decomposition of C$_4$F$_8$ precursor in a Ar plasma (CF$_X$ in the following). Process time to coat the UHMWPE surface was about 10 minutes.

2.3. Surface chemical characterization.

Identification of the functional groups at the surface of the UHMWPE samples before and after surface processing was performed by X-ray Photoelectron Spectroscopy (XPS). The measurements were performed using a VG-ESCALAB100 electron spectrometer with unmonochromatised Mg Kα radiation. Pass energies of 20 eV or 100 eV were used for high resolution or survey spectra acquisition, respectively.

2.4. Contact angle measurements.

Contact angle (CA) measurements were performed in the last specimens with a CAM100 optical system (KSV Instruments, Helsinki, Finland) using deionised water.

2.5. Bacterial adherence study.

For bacterial adherence studies, two collection staphylococci strains were used: *S. aureus* 15981 [4] and *S. epidermidis* ATCC 35984. An inoculum of $10^{7}$-$10^8$ colony former units (CFU) / mL was prepared according to a previously described method [5] as follows. Overnight-grown cultures in Tryptic-Soy Broth (bioMerieux Spain S.A., Madrid, Spain) at 37 ºC were centrifuged and the concentrates washed three times with Phosphate Saline Buffer (PBS, Sigma-Aldrich Inc., St. Louis, USA). Then, inoculum was prepared from concentrate, making a bacterial suspension in PBS of 0.5 Mc Farland turbidity. Inoculum size was confirmed with serial bacterial counts on Tryptic-Soy Agar supplemented with 5 % sheep blood (bioMerieux Spain S.A., Madrid, Spain).
Six well-tissue culture treated plates (Iwaki, Asahi Glass Co. LTD., Japan) were used as solid support for fixing the different accretion surfaces, which were previously sterilized by mean of gas plasma treatment. The inoculum was added in a final volume of 4 mL / well and plates were then incubated 90 minutes at 37 ºC. After this incubation period, inoculum was removed and surfaces washed with PBS three times to elute non-adhered bacteria. Surfaces were incubated 24 hours at 4 ºC.

Adhered bacteria were visualized by fluorescence microscopy (Laborlux D, Leitz, Germany) after Acridine Orange staining (AO, Becton Dickinson and Company, Sparks Maryland, USA). Surfaces were exposed to 4 mL of AO 2 minutes at room temperature. Excess stain was removed with distilled water washing for two times, and then, surfaces were air-dried. Microscopy visualization was performed at 40x magnification and images of 10-20 fields / surface were photographed.

The percentage of surface covered by bacteria was determined using Image J software [6]. Differences among surfaces as well as between strains were analysed by ANOVA, Kruskal-Wallis, Mann-Withney, unpaired and paired t tests. A statistical difference was considered for p < 0.001.

Assays were made in triplicates. Number of observations was of twelve in the case of UHMWPE surfaces and twenty for all of the other tested materials.

3. Results.

3.1. Chemical characteristics of the UHMWPE coated surfaces.

The chemical nature of the species present at the surface of the samples was analysed by XPS. Figure 1 shows the C 1s spectra acquired for the studied samples. Uncoated and DLC coated UHMWPE samples presented a single C 1s peak, that was located at 285.0 eV binding energy (BE). It corresponds to the aliphatic C bond type of polyethylene and also to the amorphous structure of sp²/sp³ bond types that compose the DLC material.

![Figure 1](image-url)  
**Figure 1.** High resolution C 1s spectra of the samples obtained from the XPS analysis. The signal corresponding to the different C bond types present at the surface of the samples was deconvoluted by peak fitting analysis.
The addition of the fluorinated volatile precursor to the plasma process gas used for the DLC growth has the effect of incorporating some F content in the films (table 1) up to a [F]/[C] concentration ratio of 0.32. The F incorporated this way is mainly in the -C-CF form (21%), but also some minor contributions of -CF (5%) and -CF₂ (1%) is also detected.

The direct decomposition of the C₄F₈ precursor in a Ar plasma induces a completely different C-F based polymer. In this case, the F content increase considerably to a [F]/[C] ratio of 1.25, with the presence of all the possible -C-F bond types with a more or less even contribution of all the possible -C-F bond types (12% of -C-C, 23% of -C-CF, 24% of -CF, 26% of -CF₂, and 15% of -CF₃).

Table 1. Quantification of C 1s peaks of UHMWPE surface before and coating with DLC, F-DLC and CFₓ functionalities. Relative [F]/[C] atomic concentration of the samples is also included.

| Sample                  | [F]/[C] | -C- bond types (%) |
|-------------------------|---------|---------------------|
|                         |         | -C-C      | -C-CF      | -CF      | -CF₂     | -CF₃     |
| Raw UHMWPE              | 0       | 100       | 0          | 0        | 0        | 0        |
| DLC/UHMWPE              | 0       | 100       | 0          | 0        | 0        | 0        |
| F-DLC/UHMWPE            | 0.32    | 73        | 21         | 5        | 1        | 0        |
| CFₓ/UHMWPE              | 1.25    | 12        | 23         | 24       | 26       | 15       |

3.2. Surface wettability.

Water contact angle (WCA) measurements were performed to probe the hydrophobic character of the samples. The measurements were performed 24 hours after surface treatment to minimize the well-known aging effects after plasma surface treatment. The results of this analysis are compiled in table 2. The uncoated UHMWPE material presented a WCA of 100°, the DLC coated material 79°, the F-DLC coated 98° and the CFₓ coated surface resulted to be superhydrophobic, i.e., with a WCA larger than 170°.

Table 2. Water contact angle (WCA) of virgin and coated UHMWPE samples. The standard deviations of the obtained mean are indicated with parenthesis.

| Sample             | WCA (degrees) |
|--------------------|---------------|
| Raw UHMWPE         | 100 (5)       |
| DLC/UHMWPE         | 79 (3)        |
| F-DLC/UHMWPE       | 98 (4)        |
| CFₓ/UHMWPE         | >170 (5)      |

3.3. Bacterial adherence studies.

Bacterial inocula were of 7.73 ± 0.18 and 7.84 ± 0.03 Log₁₀UFC/mL for S. aureus and S. epidermidis, respectively. After inoculum exposure, both bacteria were able to adhere to all of the accretion surfaces tested in the study. Figure 2 summarizes the percentage of bacterial-covered surface on the four materials. Between species, S. epidermidis was a much more adherent microorganism than S. aureus, because of the higher extent of covertures obtained in three out of the four tested surfaces. Statistically significant differences (p ≤ 0.001) where found when compared percentages of covered surface by S. aureus and S. epidermidis in DLC/UHMWPE (2.18 ± 0.47 and 4.46 ± 1.03, respectively) and F-DLC/ UHMWPE (1.02 ± 0.27 and 7.00 ± 1.76, respectively).
In the comparison of bacterial adherence to each surface, the two strains showed a diminution in their adherence on DLC/UHMWPE surfaces. Statistically significant differences in *S. aureus* adherence were found in DLC/UHMWPE versus UHMWPE (2.18 ± 0.47 vs. 4.43 ± 1.50) and versus CFx/UHMWPE (2.18 ± 0.47 vs. 4.28 ± 1.47). Significant differences in *S. epidermidis* adherence were also found in UHMWPE versus DLC/UHMWPE (7.21 ± 2.95 vs. 4.46 ± 1.03) and CFx/UHMWPE versus F-DLC/UHMWPE (4.05 ± 1.14 vs. 7.00 ± 1.76).

The lowest percentage of bacterial adherence was registered for F-DLC/UHMWPE surfaces exposed to *S. aureus* inocula (1.02 ± 0.27). On the contrary, *S. epidermidis* adherence to F-DLC/UHMWPE was similar to that observed in UHMWPE.

### Figure 2

% Covered surface by *S. aureus* 15981 and *S. epidermidis* ATCC 35984 on UHMWPE and modified UHMWPE surfaces (the error bars indicate the standard deviation of the corresponding mean values).

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### 4. Discussion.

Bacterial adherence and subsequent biofilm formation are a spontaneous, natural phenomenon which depends on multiple factors including the microorganism, inoculum size, environmental physicochemical conditions (atmosphere, pH, temperature), nutrient availability, hydrodynamic forces, or the nature of accretion surface [7]. Bacterial adherence is a complex phenomenon which involves two different phases where different relationships are established between the organism and the surface. Because no changes can be made on bacteria, modifications of surfaces have been suggested to be an interesting strategy for preventing bacterial adherence and biofilm development. *In vitro* studies involving the surface coating with biological molecules or compounds [8,9] and, further, *in vivo* experimental assays using coating medical devices [10,9], have been previously described with promising results. One of the explanations about the properties of these compounds for preventing bacterial adherence is based on the changes in accretion surface’s charge and thus, the changes in interaction between bacteria and surface [11]. According to this premise, here we show that the inert DLC coatings are more hydrophilic that the uncoated polyethylene surfaces. It has been reported that nutrient concentration could be favored by apolar surfaces, and such phenomenon could be associated to an increase of attached bacteria [12] in a nutrient deprived medium (like PBS). It is worth mentioning that the DLC coatings have recently been proposed to improve the wear performance of UHMWPE implants [2]. The lower adherence performance of this surface with respect to the uncoated surface represents a preliminary important result.

On the other hand, the incorporation of a small amount of F in the inert DLC structure improves the antiadherent performance of the UHMWPE coated surfaces. Although it is expected that the wear
performance of F-DLC coating on UHMWPE be worst, it is also true that only a very thin layer of this compound is necessary to handicap the infection of this surface during surgery.

Superhydrophobic surfaces can be obtained with a combination of high fluorine content C-F polymer, with approximately even composition of all possible C-F bond type functionalities and a surface roughness in the range of tenths of microns. However, this not diminishes bacterial adherence for any of the strains used in this study. Thus, note that not necessarily the most hydrophobic surface presents the lowest adherence performance.

5. Summary and conclusions.

DLC and other fluorinated carbon based functionalities can successfully be incorporated on UHMWPE surfaces at room temperature by RF plasma deposition. Several type and amount of C-F chemical bonds can be added to the UHMWPE surface varying process parameters. Thus, incorporation of increasing amount of Fluorine atoms to an initial DLC structure results in the presence of more oxidized CFX species. Incorporation of F atoms in the C matrix is correlated to an increase of water contact angle of the corresponding surfaces.

In general, S. epidermidis was more adherent than S. aureus in all the tested surfaces. S. aureus and S. epidermidis showed less bacterial adherence on DLC/UHMWPE than the raw UHMWPE, with statistically significant difference. The lowest bacterial adherence was registered for F-DLC/UHMWPE surfaces exposed to S. aureus. However, this surface showed similar adherence than the raw UHMWPE material to S. epidermidis.

The most hydrophobic surface (CFX/UHMWPE) showed similar adherence of both bacteria, with the same performance of that obtained for raw UHMWPE (S. aureus) and F-DLC/UHMWPE (S. epidermidis).

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