Bacterial adhesion driven by mechanical properties of DMAEMA plasma polymer coatings

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Abstract: The control of surface mechanical properties is a new approach to prevent bacterial adhesion. In this work, plasma polymerization of dimethylaminoethyl methacrylate (DMAEMA) is investigated to obtain hydrogel deposited thin films with a wide range of mechanical properties. Microbiological experiments were conducted using *Escherichia coli* K12 to observe antibacterial effects.

Keywords: DMAEMA, plasma polymerization, hydrogel, antibacterial surface

1. Introduction

The development of antibacterial materials that prevent bacterial colonization addresses many social issues. Different strategies are described in the literature and consist mainly on surface modifications by playing with topographical, chemical or hydrophobic properties. Recently, a new approach was proposed based on a judicious design of surface mechanical properties [1]. Here, we investigate the potential of using a plasma polymer functionalization step to finely tune mechanical surface properties of materials. Dimethylaminoethyl methacrylate (DMAEMA) was selected because of its capacities to undergo hydrogen bonding or van der Waals bonding in the plasma polymerized thin film leading to hydrogel properties [2]. By playing with the plasma parameters it was possible to obtain plasma polymer surfaces with a wide range of mechanical properties when immersed in liquid bacteria suspension.

2. Materials and methods

Plasma polymerization was performed on silicon wafer in an electrodeless cylindrical glass reactor (6 cm diameter, 680 cm^3 volume, base pressure of 5.10^{-4} mbar, and with a leak rate better than $1.0x10^{-10}$ kg.s^{-1}) enclosed in a Faraday cage. An L-C matching network (Dressler,VM1500W-ICP) was used to match the output impedance of a 13.56 MHz RF power supply (Dressler, Cesar 133) to the partially ionized gas load by minimizing the standing wave ratio of the transmitted power. The DMAEMA (Sigma Aldrich) vapour was introduced into the reactor by sublimation under evacuation using different reactor pressures (0.15 - 0.3 mbar) and different flow rates ($3.18x10^{-7}$ - $2.08x10^{-6}$ mol.s^{-1}). The plasma was initiated with different deposition conditions by varying the power (5 – 60 W) and the duty cycle of discharges (2 - 100%). Variation in the aforementioned parameters allowed *i)* to elucidate the growing mechanisms of DMAEMA under plasma discharges, and *ii)* to determine the experimental window in which

hydrogel properties could be tailored to obtain different mechanical properties [2]. The plasma polymer thin films were characterised by atomic force microscopy (AFM), Fourier transform infrared spectroscopy (FTIR), X-ray photoelectron spectroscopy (XPS), contact angle measurements and capillary rise.

The antibacterial properties were evaluated by using *Escherichia coli* K12 SCC1 [3]. Bacteria were cultivated in a selective medium at 30 °C. Selective nutritive medium (M63G, pH 6.8 [4]) was then inoculated with one bacterial colony and incubated overnight at 30 °C. This culture was used to inoculate a second preculture (10% vol. of first preculture) which was grown for about 4 h. Finally the culture was used for experiments with an absorbance at 600 nm of 0.01, measured by UV spectrophotometry (containing about 5×10^6 bacteria/mL).

Bacteria were directly observed in the last rising medium by confocal microscopy (Zeiss LSM 700) using a 9.1mm long focal objective (LD EC « Epiplan-Neofluar » 50x/0,55 DIC M27) after 2 hours.

Two samples were observed for each studied condition and experiment was repeated three times. For each sample, 10 randomly drawn fields were considered. Quantity of adherent bacteria was determined using Image J 1.49n software (National Institute of Mental Health, Bethesda, MD) and home-made plug-ins. Two different principles were applied for the determination of bacterial quantity, based on direct enumeration of bacteria or calculation of the – surface fraction covered by bacteria. Both plug-ins were validated by comparison with manual counting prior to use.

3. Discussion

Based on the variation of thickness of plasma polymer thin films, the growth kinetics were quantified to explore the different regimes as a function of plasma polymerization conditions (Fig. 1). Each condition was characterised by the well-known Yasuda's parameter

determined by the following formula (Eq. 1):

$$Y = \frac{W \times DC}{F \times M} \qquad (1)$$

where W is the power discharge (J/s), DC is duty cycle, F the gas flow rate (mol/s) and M the molecular weight of the precursor (mol/g).
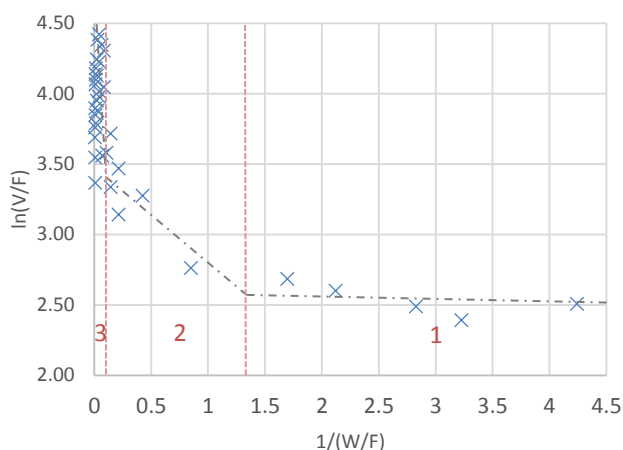

Fig. 1. Kinetics of the plasma polymer growth as a function of plasma polymerization conditions. V, growth rate ($nm.min^{-1}$).

Three different regimes were hypothesized: Regime of homogenous growth (1) where the precursor was partially fragmented during the plasma process with a high degree of retention of its structure in the plasma polymer layer; Regime (3) with heterogeneous growths where the precursor was highly fragmented with almost totally lost of its structure in the plasma polymer layer; Regime of transition (2) where the structure of precursor was more or less preserved as function of the plasma parameters.

Hypotheses were confirmed by FTIR measurements. Peaks at 3100 cm^{-1} (C=C), 2820 cm^{-1} (>NCH$_3$), 2775 cm^{-1} (>NCH$_3$), 1720 cm^{-1} (C=O) and 1160 cm^{-1} (C-O) are the main characteristics IR bands of DMAEMA. Fig. 2 shows the evolution of the IR intensities according to Yasuda parameters.
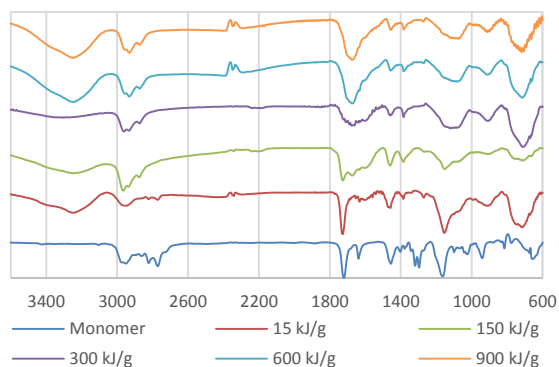

Fig. 2. FTIR spectra of plasma polymer thin films obtained for different Yasuda's parameter.

Disappearance of the peak at 3100 cm^{-1} indicates that plasma polymerization was initiated by the C=C bonds. For low Yasuda parameter values, the characteristic IR bands of DMAEMA were clearly identified confirming good retention of the precursor structure in the plasma polymer thin film (Fig. 2). For high values of Yasuda's parameter, the characteristic peaks of >NCH$_3$ disappeared, while new peaks were identified, showing that new functions were created in the plasma polymer layer. For example, the intensity of the peak at 1670 cm^{-1} belonging to amide functions increases with Yasuda's parameters (mainly up to 150 kJ.g^{-1}) until reaching a plateau. These results were explained by (poly-)recombination(s) of fragments of precursor in the plasma phase during deposition.

All the aforementioned results were also confirmed by XPS analysis. They clearly showed lost in the precursor structure when Yasuda parameter increased (Fig. 3). For the highest values of Yasuda's parameter, new functionalities were detected such as O-C-O or C-OR.
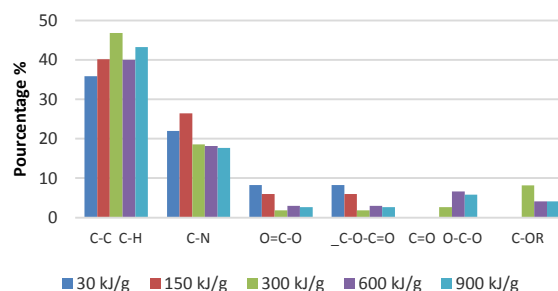

Fig. 3. Deconvolution of C(1s) XPS spectra of plasma polymer thin films obtained for different Yasuda's parameter.

In conclusion, it was possible to finely tune the chemical composition of the plasma polymer thin film according to the plasma parameters used. This readily underwent changes in the proportion of hydrogen bonding or van der Waals bonding in the plasma polymerized thin layer leading to different hydrogel properties.

Hydrogel properties of the plasma polymer thin films were evaluated by contact angle measurements and capillary rises. The results clearly show two different wetting behaviors (Fig. 4). The first one was obtained with low Yasuda's parameter values (typically less than 150 kJ.g^{-1}) where the equilibrium contact angle values were below 10°. In this domain, the equilibrium contact angle value was never reached, which results in a competition between spreading over the surface and absorption through the plasma polymer layer. The second regime of wetting let to finite equilibrium contact angle values (ranging typically from 30° to 60°). Here, spreading and absorption were negligible. Interestingly the wetting transition was found in the same range of Yasuda's parameters that the chemical transition detected by infrared spectroscopy or XPS.
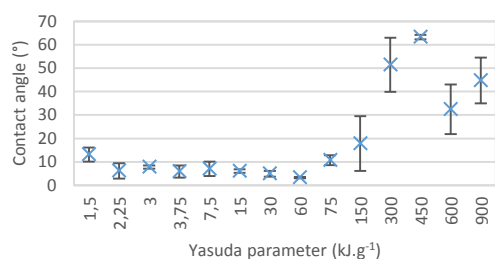
Fig. 4. Water contact angle values of DMAEMA thin films as a function of Yasuda's parameter.

The capillary rise measurements were performed with capillary tubes of 1mm in diameter. The internal walls were coated with constant thickness of plasma polymer layer but by using different plasma polymerization conditions. Once again, the results showed two domains (Fig. 5), one domain where the capillary rise height decreased with increasing Yasuda's parameter and a second domain where the capillary rise height increased with the Yasuda's parameter. These differences were explained by the balance between i) water uptake capacity of the hydrogel layer (mainly driven by hydrogen bonding), and ii) physical forces involved in capillary rise phenomenon.
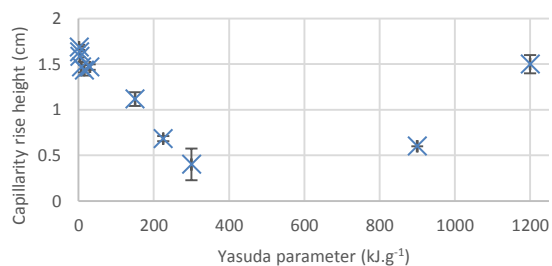

Fig. 5. Capillarity rise measurement as a function of Yasuda's parameter.

Both domains showed differences in terms of bacterial adhesion after 2 hours of culture (Fig. 6). For high Yasuda's parameter value (900 kJ.g^{-1}), number of adherent bacteria was similar to on the reference surface (i.e., silicon wafer used as internal control of the experiments). In contrast, bacteria number was significantly less on the surface type with low Yasuda's parameter value (i.e., 1.5 kJ.g^{-1}). These results confirm that it is possible to considerably reduce bacterial adhesion by playing with the plasma polymerized hydrogel properties (i.e., mechanical properties). More details will be given during oral presentation, especially regarding changes in Young's modulus values of the plasma polymer thin films.
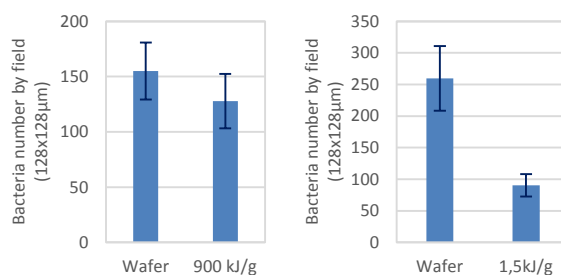

Fig. 6. Bacteria number measured by microscopic field as a function of Yasuda's parameter.

4. Conclusion

This work demonstrates the potential of plasma polymerization process to adapt surface mechanical properties of materials for driving their antibacterial behaviours. This strategy appears to us as one of the most promising route to fight against bacterial colonization.

5. References

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