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H. J. Mathieu Surface Characterization in Biomaterials Applications

Invited Paper

Surface Characterization in Biomaterials Applications

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Applications biomaterials are illustrated as examples of functionalized surfaces in the growing field of synthetic materials for medical applications. After a brief introduction into the main surface analytical tools such as X-ray photoelectron spectroscopy (XPS), Time-of-flight secondary ion mass spectrometry (Tof-SIMS) and contact angle measurements two examples, endotracheal tubes for with anti-bacterial characteristics and the preparation of neural biochips are chosen to illustrate recent achievements.

1. Introduction

Surface Characterization has added a new chapter, i.e. that of biomaterials. Protein chemistry, structured biology and part of DNA technology are joined by biomaterials characterization to reveal the molecular mechanisms of fundamental biological processes at the top nanometers at the surface. Many of our former dreams a few years ago can now be realized and the surface chemistry of functionalized synthetic polymers and biochips can be studied [1]. We are starting to visualize proteins, peptides and amino-acids attached to the outer surface are identified by sophisticated spectrometers available in your laboratory. A molecular ballet is coming into view [2]. Indeed, we can see and control the steps of molecules and atoms during their adhesion steps to the surfaces. However, this is only the beginning of our control of biomaterials. Before going on to present the machines that we were using, let me refer to D. Williams "The biomaterial has the ability to perform with an appropriate host response in a specific application".

2. Characterization methods

In this paper I will restrict myself to present the major tools applied to the characterization of biomaterials surfaces within the first nanometers. Typical tools used for the chemical characterization are X-ray Photoelectron Spectroscopy (XPS), Time-of-Flight Secondary Ion Mass Spectrometry (Tof-SIMS), Electron Energy Loss Spectroscopy (EELS) as well as contact angle analysis (CA), Atomic Force Microscopy and Electron scanning Microscopy (EM). Important parameters for the chemical analysis of surfaces are the ability to detect building blocks of biomaterials such as atoms and molecules, ions and electrons together with their molecular fragments. Qualitative and quantitative analysis and, if possible, structural information is searched for.

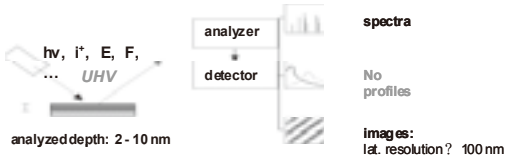

Fig. 1. Principle of surface analysis.

The principle of chemical surface analysis is illustrated in Fig. 1 (in-out) indicating in addition the lateral information available for XPS and Tof-SIMS applied under UHV conditions, which certainly is a limiting factor, because the vacuum might change the composition and structure of the biomolecules.

Photoelectrons first observed by H. Hertz [3] and later explained by A. Einstein [4] are created when X-rays provoke the emission of electrons carrying as information the binding energy. The number of the photoelectrons can be used for quantification. ESCA was developed by K. Siegbahn in Upsala and Berkeley [5]. The

principle of photoelectrons based on the electronic structure of a atoms is illustrated in Fig. 2.

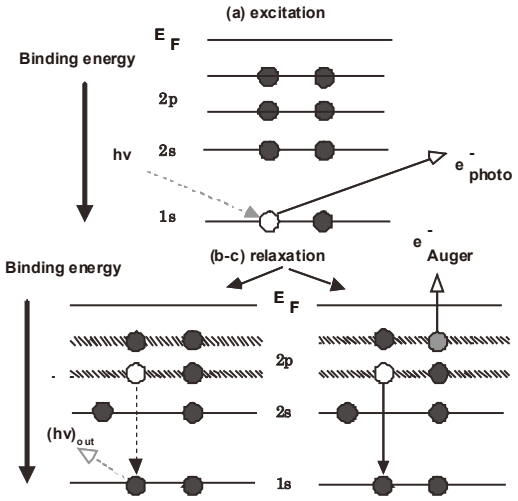

Fig. 2. Creation of a photoelectron (schematic).

Photoelectrons e_{photo}^- are excited from their electronic level as indicated in (a). The relaxation (b-c) results either in an X-ray fluorescence $(hv)_{out}$ or the creation of an Auger electron e_{Auger}. The measurement of the kinetic analyzer E_{kin} and its work function Φ_A under X-ray radiation with an energy hv leads to the Einstein equation

$$E_b = hv - E_{kin} - \Phi_A \qquad (1)$$

The electron binding energy, E_b, in the Ultra High Vacuum chamber (UHV) measured with respect to the Fermi energy, E_F, allows identifying different binding states of the emitting atom with an energy resolution up to $\Delta E \cong 0.5$ eV. Quantification is achieved by determining the area underneath a transition after appropriate background subtraction. Obtained information from the first 5-10 nm and a sensitivity limit at approximately 1% of a monolayer which corresponds to 10^{14}-10^{15} at/cm^2. The lateral XPS resolution depends on the spectrometer used and is typically > 3 μm. Insulators and conducting samples can be analyzed with an optional electron charge compensation for all elements and molecules from Li-U. Time-of-flight Secondary Ion Mass Spectrometry (Tof-SIMS) developed by A. Benninghoven *et al*. [6] allows one to determine molecular fragments and structures with high sensitivity. Its principle is illustrated in Fig. 3.

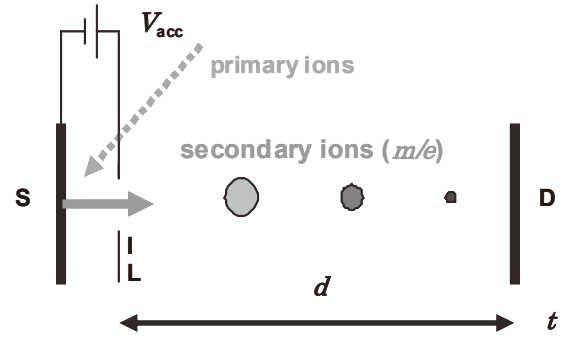

Fig. 3. Time-of flight Secondary Ion Mass Spectrometry: S sample, I immersion lens, D detector, *d* flight distance, *t* flight time, *m* mass, and e electronic charge.

A pulsed ion beam with the choice of various ions (Ga^+, In^+, Cs^+, Au_n^+, C_{60}^+, etc) is focused on the sample surface with a lateral resolution down to approximately 0.1 μm. Under ion bombardment at low doses the emitted secondary ions are accelerated by applying the energy of eV_{acc}. Typical flight times over a distance d = 2m are a few μs for a molecular mass 100 amu. The flight time, t, can be converted into a mass, m, by equalizing the potential and kinetic energy (speed $v=d/t$)

$$eV_{acc} = \frac{m}{2}v^2 \qquad (2)$$

, from which the flight time, t, is obtained

$$t = \sqrt{\frac{m}{2eV_{acc}}} \qquad (3)$$

If ion pulses in the ps - range are applied as primary irradiation a mass resolution of $m/\Delta m = 10^3$- 0^4 are obtained. Charge compensation can be applied for positive charging of the sample surface. Imaging of the surface binding states is obtained by scanning the primary ion beam over a solid surface for molecular fragments and isotopes from H-U. In general, quantification is difficult, because of the strongly varying ionization probability within the sample matrix over several orders of magnitude. Sensitivity is in general much better than in XPS, and is observed at 10^7 – 10^{11} at/cm^2.

Other surface characterization and imaging methods like Electron Microscopy (EM), Atomic Force Microscopy (AFM), Contact angle measurements (CA), Electron Energy Loss Spectroscopy (EELS) and others are referred to the appropriate literature [7-8].

3. Example I: inhibition of bacteria

In this paper two examples of the preparation of a biomaterial are illustrated. The first one concerns the functionalization of endotracheal tubes applied in hospitals.[9-10] Mechanically ventilated tubes are an elevated risk for acquiring pulmonary infections caused by bacteria residing in the hospital [11-13]. The duration of the intubation period increases the risk of infection and the mortality rate attributable to pneumonia has been shown to be particularly high when the pathogen is *Pseudomonas aeruginosa*. Bacterial colonization of surfaces begins with an attachment of the bacteria to the surface. After the bacteria have attached, they may excrete extracellular substances, divide to form colonies, and form a biofilm. After the biofilm is formed, the bacteria tend to be somewhat protected from antibiotics. Consequently, it is preferable to prevent bacterial adhesion – the step before biofilm formation – rather than to treat an already formed biofilm. Surface modification of the tubes is therefore a good way to prevent ventilator-associated disease and also to reduce the usage of antibiotics. A very large portion of commercially available endotracheal tubes is made from poly(vinyl chloride) (PVC) containing large amounts of the plasticizer diethylheylphthalate (DEHP) or dioctylphtalate (DOP) that increase the thermal stability and transparency of the polymer. Studies of D. J. Balazs *et al.* [9,14] have shown that incorporation of silver into the tube surface could inhibit bacterial colonization of the tube surfaces. The antibacterial properties are due to several processes. Silver ions form bonds with DNA and with bacterial membrane proteins, and, thus damage different cell and membrane functions. Silver ions are bioactive in the range of nano – micro molar concentrations. The work by M. Ramstedt *et al* [11] presents a faster, simpler method to attach silver to the surface of PVC tubes. The treatment avoids NAOH soaking and leaches $AgNO_3$ directly from solution to the PVC directly in shorter times. Fig. 4 shows the $AgNO_3$ reaction with PVC.

Both spectra show the Cl2p doublet spectra demonstrating that the Cl concentration in the $AgNO_3$ – treated cases is much lower. Despite the changes in C and O spectra after $AgNO_3$ immersion, XPS could not detect any significant increase in silver at the surface. However, from the intensity of the Cl(PVC) with time while the Cl(salt) remains almost constant [11]. Leaching of silver of samples treated for 43 days in $AgNO_3$ a slow release of silver Ag+ ions. Simultaneous to the decrease with Ag, the amount of Cl(salt) decreased and the components form the bulk, C(Cl) and Cl(PVC), increased. The ratio between Cl(NaCl) and Cl(PVC) decreased from 6.7 initially to 1.5. The initial ratio reflects the depletion on Cl in the polymer structure in favor of AgCl formation.

To screen the bacterial approach to the different wet chemical treatment, an aerosol technique was used to deposit the bacteria. This mimics air-borne deposition that one expects inside ventilator tubes. To study growth inhibition of the P. aeruginosa wild type strain by Ag-ions, the colonies were determined in liquid and solid Langmuir-Blodgett media. It was found that 23 μm of Ag^+-ions inhibits bacterial growth in liquid medium while 18 mm was sufficient on the solid medium. Fig. 5 shows Petri dish results under different conditions with respect to the non-treated native sample.

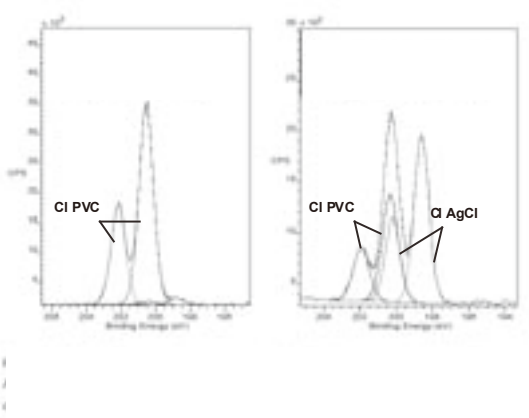

Fig.4. XPS Cl 2p spectra of (a) nontreated tube and (b) tube treated with $AgNO_3$. Both spectra show Cl 2p doublets, but the low energy doublet is higher in samples treated. After [11].

In a recent study, antibacterial properties of silver have been studied with using polymer sulfonate brushes as illustrated in Fig. 6.

These brushes consist of polymer chains that are tethered to a surface at sufficiently high grafting densities to force the polymer chains to exhibit a stretched confirmation that is rarely found in bulk polymers. By growing brushes from the surfaces, a higher density of polymer chains per surface area can be achieved compared to grafting. When the brushes are loaded with Ag^+-ions it was shown that the inhibited growth of both gram

positive and gram negative bacteria. [15] The silver loaded-loaded brushes were able to maintain silver at the surface and indicated a slower leaching into water in a NaCl medium. Thus, silver loaded brushes sulfonate brushes exhibit ion-exchange properties highly desirable for antibacterial surfaces. Further studies are envisaged to create antibacterial surfaces by exploiting the supramolecular chemistry of ion-exchange reactions.

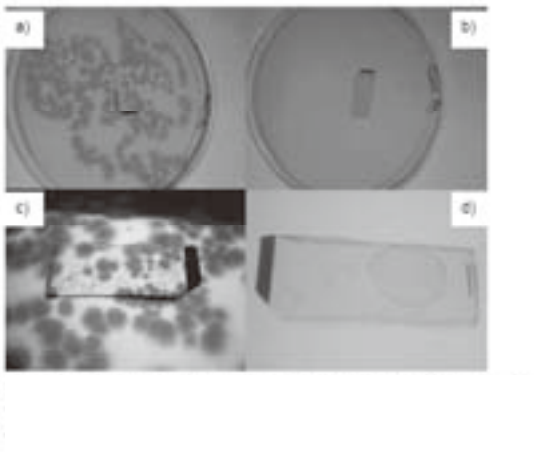

Fig. 5. Petri dish (a,c) with nontreated tube (native) and (b,d) tube pieces treated for half a day in $AgNO_3$. Colonies forming in the agar are larger than colonies formed on the tube pieces. In the treated samples (b,d) samples did not show any growth. After [11]

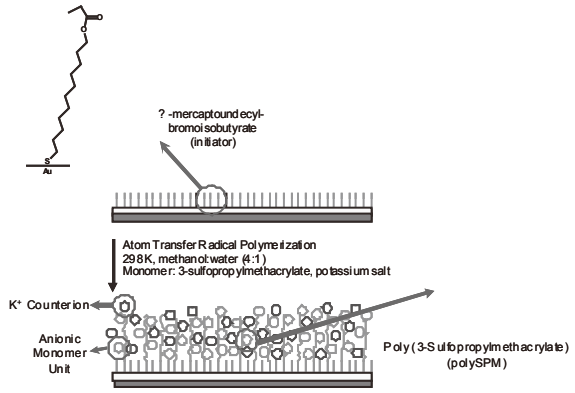

Fig. 6. Schematic description of the synthesis of polySPM (sulfonate brush). After [15]

4. Example II: biochips

The second example illustrates the application of Tof-SIMS to characterize and evaluate the surface of a biochip prototype consisting of an array of gold microelectrodes on which the laminin–derived oligopeptide CDPGYIGSR-NH_2 was immobilized by S. A: Makohliso *et al* [16]. The microelectrodes for purposes of biopattering neural cell adhesion were isolated from each other via a thin film of amorphous Teflon® [17]. Prior to studying biochip surfaces, characterization of gold surfaces supported on oxidized silicon wafers incorporating the oligopeptide was carried out, to serve as reference standards. The amino species could only be detected by Tof-SIMS, because XPS results were not specific or sensitive enough. In such an application, the outermost surface (max 3 monolayers) forms an interface through which the device can associate with or interrogate the surrounding environment. As the application ultimately requires an association of the synthetic system with a biological element, the surface has to be tailored and controlled for foreign species. Furthermore, if living cells are to contact this surface, even the smallest quantities of material contamination can pose an appreciable cytotoxic hazard. This assessment required the highest level of detail feasible, Tof-SIMS proved to be the ideal tool for probing the surface chemical details of this system. In addition, the imaging feature provided the possibly of mapping the distribution of surface species and check of homogeneity of the functionalized surfaces.

With the help of parallel extraction of spectral data from user-defined regions within the imaged area, it is possible to obtain the complete chemical composition of the various biochip areas studied as illustrated in Fig. 7a-b. Fig. 7a shows the Tof-SIMS imaging of a microelectrode of a biochip. Fig. 7b illustrates positive ion images of a peptide-derived ion used for the "region-of-interest" (ROI) analysis indicating the selected regions where spectral information was obtained (data not shown). Another spectral region is reproduced showing the complete oligopeptide CDPGYIGSR-NH_2^+ (Fig. 8).

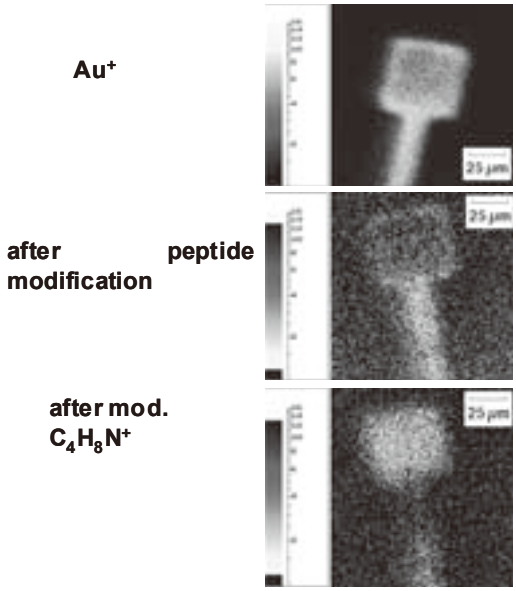

Fig. 7a. Positive-ion Tof-SIMS image of a defective microelectrode from a biochip. The Au^+-ion is shown prior to surface modification with the peptide, (b) after peptide modification and (c) the distribution of a peptide-derived ion $C_4H_8N^+$.After [16]

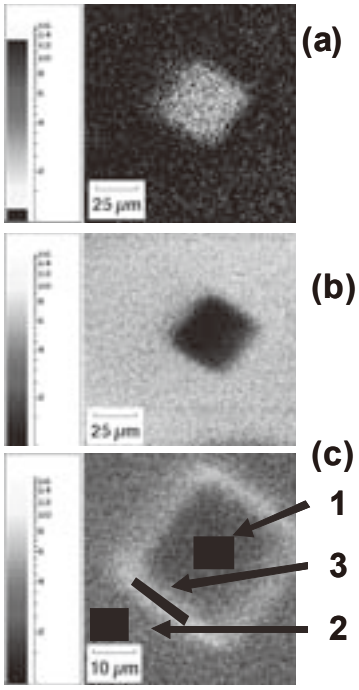

Fig. 7b Typical positive-ion Tof-SIMS images of a microelectrode from a biochip: (a) $C_4H_8N^+$, (b) ion derived from Teflon® AF, (c) image used for the 3 region-of-interest (ROIs) for the selection of spectral information. After [16]

5. Conclusions

The objective of this paper was to illustrate the tremendous possibilities that surface analysis offers to the development of the fast growing field of biomaterials. Today we are only at the beginning of an interdisciplinary research bringing together know-how from the materials side, chemistry, biology and medicine. The two examples from different applied areas illustrated the power of surface characterization for the control of the first few nanometers of the surface. However, there are also other applications for implantable biomaterials reported in the literature. It can be predicted that in the near future a remarkable progress will be available for better chemical control of biomaterials applied in orthopedic surgery as well in dental applications.

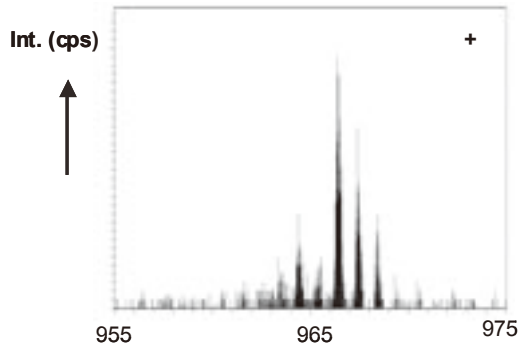

Fig. 8. Tof-SIMS spectrum of CDPGYIGSR-NH_2^+ showing the intensity (cps) as a function of *m/e*. After [16]

6. Acknowledgements

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