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How do wettability, zeta potential and hydroxylation degree affect the biological response of biomaterials?

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ABSTRACT

It is well known that composition, electric charge, wettability and roughness of implant surfaces have great influence on their interaction with the biological fluids and tissues, but systematic studies of different materials in the same experimental conditions are still lacking in the scientific literature. The aim of this research is to investigate the correlations between some surface characteristics (wettability, zeta potential and hydroxylation degree) and the biological response (protein adsorption, blood wettability, cell and bacterial adhesion) to some model biomaterials. The resulting knowledge can be applied for the development of future innovative surfaces for implantable biomaterials. Roughness was not considered as a variable because it is a widely explored feature: smooth surfaces prepared by a controlled protocol were compared in order to have no roughness effects. Three oxides (ZrO_2, Al_2O_3, SiO_2), three metals (316LSS steel, Ti, Nb) and two polymers (corona treated polystyrene for cell culture and untreated polystyrene for bacteria culture), widely used for biomedical applications, were considered. The surfaces were characterized by contact profilometry, SEM-EDS, XPS, FTIR, zeta potential and wettability with different fluids. Protein adsorption, blood wettability, bacterial and cell adhesion were evaluated in order to investigate the correlations between the surface physiochemical properties and biological responses.

From a methodological standpoint, XPS and electrokinetic measurements emerged as the more suitable techniques respectively for the evaluation of hydroxylation degree and surface charge/isoelectric point. Moreover, determination of wettability by blood appeared a specific and crucial test, the results of which are not easily predictable by using other type of tests.

Hydroxylation degree resulted correlated to the wettability by water, but not directly to surface charge. Wetting tests with different media showed the possibility to highlight some differences among look-alike materials. A dependence of protein absorption on hydroxylation degree, charge and wettability was evidenced and its maximum was registered for surfaces with low wettability in both water based and protein containing media and a moderate surface charge. As far as bacterial adhesion is concerned, no effect of surface charge or protein adsorption was evidenced, while the presence of a high acid component of the surface energy appeared significant. Finally, the combination of hydroxylation degree, wettability, surface charge and energy (polar component) emerged as a key parameter for cell adhesion and viability.

1. Introduction

A thorough knowledge of interaction between the different surface features and biological response to biomaterials is required both for a better understanding of in-vivo behaviour of implants and design of innovative biomaterials and surfaces.

It is qualitatively well known that surface properties (roughness, chemical composition, charge, wettability and hydroxylation degree)

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can determine interaction of the biomaterials with the biological environment [1–3] and some general rules are reported in literature [4,5], but they are not proved on a quantitative scale and it is not clear if the same rules apply for materials with different chemical nature (metals, oxides, polymers).

On a time scale, the first contact (during some nanoseconds) is between the implant surface and the water molecules of the biological fluids, then ions are adsorbed, and, after few seconds, proteins cover the surface. Finally, in a time interval typically comprised between some minutes and few hours, different kinds of cells will approach the material, already covered by a protein layer [1]. At the same time, bacteria can compete with the cells for surface colonization: a sort of "race for the surface" has been described between cells and bacteria upon biomaterials implantation in the human body [6]. Surface characteristics, such as topography, chemistry and surface energy, affect the material ability to adsorb water and proteins and consequently to interact with cells and bacteria. Numerous studies have been focused on the effects of surface topography (both at the micro and nanoscale) on cellular and bacterial adhesion [2,3,7–15], that is why it has been decided not to go ahead on this side.

The importance of surface wettability, surface energy and hydroxylation degree on cellular and bacterial adhesion has been highlighted in the scientific literature [16–22], but a more systematic approach is needed. The majority of the cited papers are focused on titanium substrates [23], but the techniques used for the characterization of the surfaces vary from paper to paper and a comparison between the materials investigated by different research groups is difficult. Therefore, a systematic investigation of the effect of the surface characteristics of different biomaterials on their biological response, performed with a coherent experimental protocol, is still lacking.

Eight different substrates (i.e. alumina, silica zirconia, titanium, steel, niobium and treated/untreated polystyrene) have been chosen for this research, according to the following rationale. They are widely employed for biomedical applications (e.g. dental and orthopaedic prostheses), they cover a wide range of materials with different chemistry (metals, oxides and polymers) and crystallographic structure (crystalline, amorphous), they are known to be non-toxic and they show a negligible ion release (at least at short times). That is why they are suitable in order to verify the influence of some surface chemical and physical parameters on the biological response of biomaterials. A protocol for samples surface preparation has been developed in order to obtain comparable roughness and cleaning on all the tested materials, allowing the determination of the effects of the other surface characteristics, on the biological response. In fact, it has already been evidenced that surface properties (e.g. wettability) can vary in a considerable way depending on the sample preparation procedure [16].

Surface chemical composition, hydroxylation degree, wettability by different fluids (i.e. water, Simulated Body Fluid (SBF), Foetal Bovine Serum (FBS), cell culture medium, bacterial culture medium, human blood and organic solvents), zeta potential, protein adsorption, bacterial adhesion and cell adhesion have been determined for all the selected materials in the present research work. Eventual relationships between the physicochemical surface characteristics of the various substrates and their biological response (blood wettability, protein adsorption, cell adhesion, bacterial adhesion and biofilm formation) are discussed in this paper.

2. Materials and methods

2.1. Specimens

Eight different materials (Table 1) have been selected for this characterization and samples of comparable area were obtained for each one.

All the oxide and metal samples were mirror polished with SiC abrasive papers (up to 4000 grit); a final polishing suspension (OP-U suspension, Struers, SiO_2 0.04 μm) was used on metals in order to obtain uniform and comparable surfaces. Roughness measurements, obtained by contact profilometry, are reported in Table 2.

In order to obtain clean and comparable surfaces for analyses, the oxide and metal samples were washed in an ultrasonic bath for 5 min in acetone and subsequently two times in ultrapure water for 10 min. At the end of the washing steps, samples were dried in a laminar flow cabinet (FASTER CYTOSAFE-N 2000) and decontaminated with UV irradiation for 1 h under the same cabinet. Samples were then packed in aluminium foils and closed in plastic-paper bags for sterilization until use.

Polystyrene substrates for cells and bacteria cultures were used without carrying on any further surface modifications or cleaning, their roughness is reported in Table 2 as well.

2.2. Characterization

In order to investigate surface topography and cellular shape after cell culture tests, samples were observed by means of Scanning Electron Microscopy equipped with Energy Dispersive Spectroscopy for chemical analyses (SEM – FEI, QUANTA INSPECT 200, EDS - EDAX PV 9900). The oxide samples were sputter coated with a thin Cr layer (5–10 nm) before observation.

Surface chemical composition and hydroxylation degree were evaluated by means of XPS analyses (XPS, PHI 5000 VERSA PROBE, PHYSICAL ELECTRONICS). Both the survey spectra and the high resolution spectra of carbon and oxygen regions were acquired. All the high resolution spectra were referenced by setting the hydrocarbon C1s peak to 284.80 eV for charging effect compensation.

Fourier Transformed InfraRed Spectroscopy (FTIR) (FT-IR, IR Hyperion 2000, Alpha, Bruker Optics) measurements were performed for a further characterization of surface chemical composition and hydroxylation

Table 1
Materials.

Material	Symbol	Class	Surface treatment	Structure	Source
Silica	SiO_2	Oxide	–	Amorphous	Heraeus HSQ300
Alumina	Al_2O_3	Oxide	–	Crystalline: gamma	Expert System Solutions S.r.l.
Zirconia	ZrO_2	Oxide	–	Crystalline: cubic and tetragonal, TZP-A (95% ZrO_2, 5% Y_2O_3, 0.25% Al_2O_3)	Metoxit
Titanium	Ti-commercially pure grade 2	Metal (pure)	–	Crystalline: hexagonal	Titanium Consulting and Trading
Niobium	Nb	Metal (pure)	–	Crystalline: body centred cubic	New Tech
Steel	316L	Metal (alloy)	–	Crystalline: face centred cubic	Tresoldi Metalli
Polystyrene for cells culture	PS-cells	Polymer	CORONA treatment for eukaryotic cells culture	Amorphous	Nunclon Delta Surface, Thermo Fisher Scientific, Roskilde, Denmark
Polystyrene for bacteria cultures	PS-bact	Polymer	–	Amorphous	Sterilin, PBI-VWR International, Milan, Italy