

MICROFABRICATED SURFACES TO STUDY CELL-SURFACE INTERACTIONS

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INTRODUCTION: Most artificial materials, once implanted in the patient's body, induce a cascade of reactions with the biological environment through interaction of the biomaterial with body fluid, proteins and various cells. The specific surface interactions determine the attitude the body takes towards the foreign material, the path and speed of the healing process and the long-term development of the biomaterial-body interface. As regards surface properties, both the chemical composition and the topography (structure, morphology) are known or believed to be important in bone, since they regulate type and degree of the interactions that take place at the interface. Microfabrication has proved to be a very valuable tool for producing geometric patterns of well-defined surface chemistry and/or topography. Such surfaces are ideal to study—in separate experiments—the influence of chemical composition and surface morphology and to learn how cells sense surfaces of biomaterials, in both in vitro and in vivo assays.

METHODS: Silitronix (100)-surface polished silicon 2" wafers were coated with 20nm of either Ti, Al, Nb or V in a thin film deposition system. Wafers were then spin-coated with 1.5µm thick photoresist (Shipley S-1813 Microposit). The samples were exposed to UV light (400 nm; Mercury lamp; 3.5 mWatt/cm²) with a contact printer through a chromium mask. Then developed (Shipley MF322 Microposit) to remove exposed photoresist. A second evaporation with a 20nm layer of either Ti, Al, Nb, or V was carried out. The remaining resist was then removed with acetone. Finally the wafer surface consisted of spatially patterned regions (Figure 1) of Ti, V, Al and Nb, representing models of heterogeneous titanium alloy surfaces currently used in orthopaedic and maxillofacial implants. The dimension of the patterns was varied between 0.5 and 150 µm.

Prior to testing and characterizing all samples were cut to 11x9 mm size, extensively washed in acetone-isopropanol-DI water, treated in an O₂-

plasma for 5 min and then studied by AFM, XPS, ToF-SIMS, SEM measurements.

Studies of the attachment and spreading behaviour of human osteoblasts on these well-defined chemically patterned model surfaces have been carried out in collaboration with University of Nottingham.

Protein adsorption studies were done on the structured (and also non-structured) metal-oxide surfaces either by fluorescent microscopy or by OWLS. For the experiments we used either fresh human serum, or single proteins like fibronectin, human serum albumin, fibrinogen and human IgG. In microscopy the visualisation of proteins was done by using labelled antibodies.

RESULTS: AFM, XPS, ToF-SIMS, SEM indicated a well defined surface structure, corresponding to the expected/designed one. The metal film surfaces have been shown to be protected by natural oxide layers upon exposure to air. The most prominent stoichiometries have been found to be: TiO₂, Al₂O₃, V₂O₅ and Nb₂O₅.

Cell Culture Tests show no significant difference between the long term (>18 h) adhesion/spreading behaviour of the osteoblasts on the metal-oxide surfaces. However, on the short time scale (90 min – 6 h) cells clearly preferred the V and Nb surfaces instead of Al on the structured Al/Nb, Nb/Al and Al/V, V/Al surfaces. The Ti/Ti showed no such differences.

Protein Adsorption Studies were performed in order to explain the observed cell behaviour. The results show that single protein studies are unable to solve the problem, more complex experiments using protein mixtures or full serum should be used. Preliminary results indicate that the amount (ratio) of Fibronectin compared to other serum proteins (like IgG) is higher on the V, Nb surfaces than on the Al surfaces.