Implication of Crystal Orientation in Controlling Cell Attachment

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Statement of Purpose: The ability to control the interactions between cells and biomaterials is crucial to address fundamental questions relevant to tissue engineering and medical implants. When there is little or no control over these interactions, scar tissue may form and the regeneration of tissue with the same function and behaviour is inhibited. Cell-substrate interactions are influenced by surface characteristics including nano-scale topographies [1], hydrophilicity [2] and composition [3]. We reported recently on the effect of the grain size of polycrystalline Ti substrates on cell behavior. It is evident that crystal orientation, which can influence protein adsorption and consequently the cell behavior, also is an important factor in the bioperformance of biomaterials [4,5]. In this study, the effect of different crystal orientation on cell attachment is investigated using pre-osteoblast and fibroblast cell lines.

Methods: Titanium single-crystal substrate with crystallographic orientations of (100), (110) and (001) at the substrate surface (MaTecK GmbH Co., Im Langenbroich 20, D-52428 Juelich, Germany) was used for cell culture experiments. Surface roughness and topography was assessed by atomic force microscopy (AFM) and aqueous contact angle measurements were performed to determine surface wettability of the substrate. Cell culture experiments were conducted using mouse pre-osteoblast MC3T3-E1 subclone 14 and rat fibroblast Rat1 cell lines (American Type Culture Collection, Manassas, VA USA). Disks of titanium substrate with a diameter of 10 mm were placed in 24-well culture plates and incubated in alpha minimum essential medium (α–MEM, Invitrogen Corporation, USA) and Dulbecco's modified Eagle's medium (DMED, Provider) supplemented with 10% fetal bovine serum, 100 U/ml penicillin containing 100 µg/ml streptomycin at 37 ºC in a humidified atmosphere of 5% CO_2 and 95% air. Controlled experiments were carried out using conventional culture well plates for each set of experiments.

Fluorescent labeling of nucleic acids was performed to assess the number of attached pre-osteoblasts and fibroblasts on the surface of the titanium substrates with different grain orientation following the procedure described in [5,6].

Results and Discussion: Cell attachment analysis of the two cell lines on the Ti substrates with different crystallographic orientation show significant variation in the attachment pattern after two hours of incubation (Fig 1). The number of attached osteoblast cells on the (110) surface is statistically higher than on the (001) and (100) Ti substrates. While the osteoblasts show very poor attachment to the surface of the (100) Ti substrate, the fibroblast cells are attached with significantly higher frequency to the (100) surface. Cellular response to the substrate is controlled by the physico-chemical properties of the surface of the substrate and the structure of the macromolecules (proteins) involved in the cell-substrate interactions.

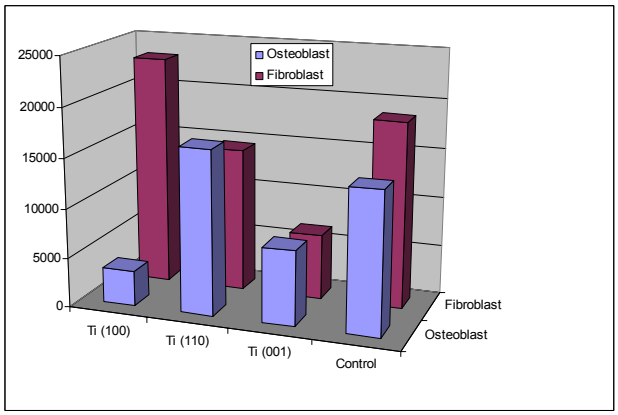

Figure 1. Histogram showing the cellular density of pre-osteoblast and fibroblast cells on the Ti substrates with different grain orientation after two hours of incubation.

The crystallographic orientation on the surface of the Ti substrate affects cell density suggesting that the cell-substrate interaction is controlled by the atomic order of the structure of the exposed Ti surface. It is, therefore, possible that the specific atomic configuration of the macromolecules involved in cell attachment such as fibronectin matches the atomic structure of specific crystal surface of titanium substrate. This is in agreement with the results reported for mineral-protein interactions. For example, it has been shown that osteocalcin can only interact with calcium ions on specific crystallographic planes in the hydroxyapatite lattice [7]. While there has been extensive emphasis in the literature on the importance of surface roughness, topography, grain size and wettability for the fabrication of titanium-based biomaterials, the importance of atomic structure at the cell-substrate interface as documented in this study has not been explored. Further study is required to understand the mechanism of cell-substrate interaction at the molecular level.

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