

CELL BEHAVIOUR OF RAT CALVARIA BONE CELLS ON NITI WITH DIFFERENT SURFACE ROUGHNESSES

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INTRODUCTION: Nickel-titanium alloys (NiTi) are metallic biomaterials known for their superelastic and shape memory properties [1]. These properties suggest that it could be used for medical purposes such as surgical implants. The interactions between cells and implants are influenced by a number of physical and chemical processes, among which, a major factor is the implant surface roughness [2,3]. The purpose of this study was to examine the behaviour of rat bone cells cultured *in vitro* on NiTi with different surface roughnesses.

METHODS: NiTi plates were mechanically polished with wetted metallographic polishing (grade 400 and 2400) SiC papers. According to the paper grade used, the samples will be referred to NiTi 400 and NiTi 2400. To determine the surface topography, prepared NiTi disks were observed with scanning electron microscopy (SEM) and examined for average surface roughness (Ra) with a profilometer. The cells were obtained after collagenase digestion of neonatal rat calvaria as described by Nefussi *et al.* (1985). The cell morphology, the proliferation and the synthesis of extracellular matrix protein (type I collagen and fibronectin) was studied. The morphology of the cells was analysed after 120 min and 15 days of culture with a Hitachi S800 scanning electron microscope. The proliferative activity of cultured cells was determined with the MTT colorimetric assay as described by Mossman (1983), at 2, 4 and 7 days seeding. After 7 days, cultured bone cells were prepared for immunofluorescence staining.

RESULTS: The average value of roughness profile (Ra) was equal to 0.07 μm for NiTi 2400 and 0.15 μm for NiTi 400. The SEM micrographs (Figure 1) showed the difference in density size of the grooves between these two substrates. Figure 2 showed cellular morphology after 120 min of culture on NiTi. The cells adhered to their support by thin cytoplasmic digitations or filopodia. After 15 days of culture, cells had multilayered and organized in nodules of various sizes and shapes. No particular orientation of the cells was observed for all the samples. The MTT assay revealed that

cells cultured on NiTi 400 showed high rates of proliferation. After 7 days, an intracellular labeling was obtained with anti-fibronectin and type I collagen antibodies. Fluorescence was intense around the nucleus and more diffuse in the rest of the cytoplasm.

Fig. 1: SEM images of NiTi 2400 (left) and NiTi 400 (right).

Fig. 2 : SEM images of osteoblastic cells cultured on NiTi 2400 (left) and NiTi 400 (right) at 120 min after seeding.

DISCUSSION & CONCLUSIONS: Rat'embryo osteoblastic cells were shown to respond specifically to two closely related biomaterials, NiTi 400 and NiTi 2400. The cell proliferation was significantly greater on NiTi 400 than on NiTi 2400. No significant difference between the synthesis of extracellular matrix protein (collagen and fibronectin) was obvious through immunofluorescence staining.

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