

Analysis of the adhesion protein expression of osteoblasts and fibroblasts cultured on NiTi surfaces of different roughness

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INTRODUCTION: The surface properties of biomaterials have a direct *in vitro* and *in vivo* influence on a cell's morphology, migration, orientation and protein synthesis^{1,2}. We propose to study the protein expression of rat osteoblasts and human fibroblasts on two different Nickel-Titanium (NiTi) surfaces. The NiTi samples were mechanically polished to obtain two kinds of surface roughness (Ra=0.07 μm for "NiTi 2400" and Ra=0.15 μm for "NiTi 400"). RT-PCR was performed to evaluate the expression of type I collagen and fibronectin for both osteoblasts and fibroblasts; and osteocalcin, osteopontin and osteonectin for osteoblasts.

METHODS:

Cell Culture: Osteoblasts were obtained after collagenase digestion of neonatal rat calvaria. Fibroblasts were obtained from biopsies of clinically healthy human gingiva.

RT-PCR: Total RNA was isolated from cells after 7 days of culture on substrates using the "Qiagen RNeasy" kit. First-Strand cDNA synthesis was performed by RT with 2 μg of total RNA using the Superscript II enzyme with oligo dT12-18 (Invitrogen). cDNA was amplified by PCR with oligonucleotide primers for type I collagen, fibronectin, osteocalcin, osteopontin, osteonectin and GAPDH. The standard program was: initial incubation of 2 min at 94°C and 25-30-35-40 cycles (94°C for 45 s, Tm for 30 s, and 72°C for 90 s), final incubation of 5 min at 72°C, and storage at 4°C. PCR products were visualized after electrophoresis in a 1.5% agarose gel containing ethidium bromide by ultraviolet light transillumination. Relative expressions were evaluated by detection of specific bands.

RESULTS: Figures 1 and 2 present agarose gel analysis of RT-PCR products. Fig. 1: Specific bands have the same intensity on NiTi 400 and NiTi 2400. Protein expression in fibroblasts is not affected by NiTi surface roughness. Fig. 2: Levels of type I collagen, fibronectin, osteopontin and osteonectin were not different on NiTi 400 and NiTi 2400. Band intensity of osteocalcin was

higher on NiTi 2400 than on NiTi 400. Expression of this protein is affected by NiTi surface roughness.

Fig. 1: Effect of NiTi 400 and NiTi 2400 on protein expression in human fibroblasts (R: rough surface, NiTi 400; S: smooth surface, NiTi 2400; Col: type I collagen; F: fibronectin).

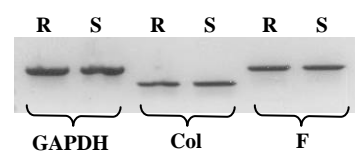
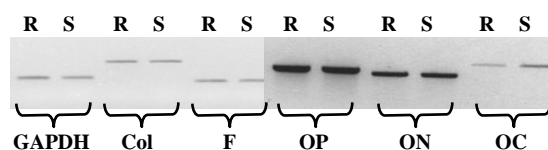


Fig. 2: Effect of NiTi 400 and NiTi 2400 on protein expression in rat osteoblasts (R: rough surface, NiTi 400; S: smooth surface, NiTi 2400; Col: type I collagen; F: fibronectin; OP: osteopontin; ON: osteonectin; OC: osteocalcin)



CONCLUSION: Our results indicate that the nickel-titanium alloy studied influenced expression of osteocalcin. This protein expression was improved by a smooth surface. No significant difference between the synthesis of type I collagen, fibronectin, osteonectin and osteopontin on smooth and rough surfaces was obvious through RT-PCR.

REFERENCES: ¹XF. Walbommers, JA. Jansen (2001), *Odontology* 89:2-11. ²K. Anselme (2000) *Biomaterials* 21:667-81.

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