Role of surface nickel content on human cell cytoskeleton formation on Nitinol

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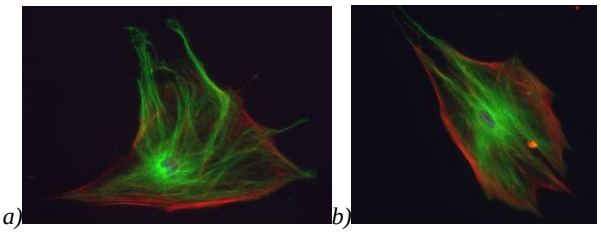
INTRODUCTION: Cell activity on an implant surface can be modulated by cues such as topography, chemistry or stiffness[(1,2)]. For Ni-Ti alloy this is achieved mainly by alteration in chemistry. However, high nickel concentrations may be a concern in the use Nitinol on a larger scale. Current reports on Nitinol bring contradictory data[(3-5)] suggesting that high nickel content is not particularly dangerous and nickel-titanium alloys are safe to be used. On the other hand it was shown that nickel has a toxic effects on cells[(6)]. Nevertheless, shape memory effects and pseudo-elasticity could support different treatments (e.g. scoliosis) and currently, Nitinol is used to produce porous foams and coatings (Actipore™), pins, clamps and intramedullary nails. In this paper authors investigated a role for nickel surface concentration on influencing cell behaviour e.g. cytoskeleton formation and organization *in vitro*.

METHODS: NiTi samples were ground on SiC paper to a mirror finish and washed. Three groups of samples were prepared: thermally oxidised at 400°C in air for 1 h (TO); alkali treated in 10M NaOH (24 h, 80°C), then heat treated at 600°C in air for 1 h (BNT); and plasma cleaned for 1 h (PC). To assess chemical composition the XPS analysis were conducted. To investigate role of the nickel (high *vs.* low surface content) on formation and organization of the cytoskeleton samples were cultured with primary human cells (osteoblasts) for 3 days and stained for actin, tubulin and nuclei.

RESULTS: Surface chemistry tests showed that TO samples had surfaces composed of TiO_2 and nickel oxides – *Table 1*. The chemical treatment resulted in significant increase in Ni concentration on the top layer, and drop in Ti content. Both elements were oxide forms. Relatively high level of carbon contamination (28%) was observed after the chemical treatment. Plasma cleaning resulted in similar content of Ni and Ti that was observed for TO samples and both elements were observed in oxide states. Fluorescent staining showed that the cells cytoskeleton was well developed on all tested samples. Actin stress fibers were formed primarily in the cell periphery (Fig. 1). For TO samples dense actin stress fibre network was also observed in the cytoplasm region. Tubulin appeared well organized, forming radiating fibres out from the organizing centre beside the nucleus. No significant difference in cell spreading was observed for all tested samples.

Table 1. Nickel and titanium content (XPS.

	Ni (wt. %)	Ti (wt. %)
TO	6.12±3.01	20.07±0.28
BNT	20.23±0.46	1.37±0.1
PC	7.60±0.57	17.36±0.14



a) *b)*

Fig. 1 Fluorescence images of actin (red) and tubulin (green) cytoskeleton: a) 400, b) BNT.

DISCUSSION & CONCLUSIONS:
Concentration of nickel causes the main concern for wider applications of Nitinol in medicine. The present study showed that high nickel content and different types of oxide layers on Nitinol do not alter cytoskeletal formation and organisation. This suggested that cell behaviours on Nitinol are not directly linked to the nickel surface content or its release, which was different for the tested samples[(5)]. Furthermore, prolonged test are currently undergoing to confirm the findings.

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