

Biocompatibility of nickel-titanium shape memory metal and its corrosion behavior in human cell cultures

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Abstract: Nickel-titanium alloy (Nitinol) is a metallic biomaterial that has a unique thermal shape memory, superelasticity, and high damping properties. Nitinol is potentially very useful in orthopedic surgery, for example. At present, there are not enough confirmative biocompatibility data available on Nitinol. The aim of our study was to clarify the primary cytotoxicity and corrosion rate of Nitinol in human cell cultures. Comparisons were made with stainless steel (Stst), titanium (Ti), composite material (C), and control cultures with no test discs. Human osteoblasts (OB) and fibroblasts (FB) were incubated for 10 days with test discs of equal size, 6 × 7 mm. The cultures were photographed and the cells counted. Samples from culture media were collected on days 2, 4, 6, and 8, and the analysis of metals in the media was done using flameless atomic absorption spectrophotometry. The proliferation of FB was 108% (Nitinol), 134% (Ti) ($p < 0.02$), 107% (Stst), and 48% (C) ($p < 0.0001$) compared to the

control cultures. The proliferation of OB was 101% (Nitinol), 100% (Ti), 105% (Stst), and 54% (C) ($p < 0.025$) compared to the controls. Initially, Nitinol released more nickel (129–87 µg/L) into the cell culture media than Stst (7 µg/L), but after 2 days the concentrations were about equal (23–5 µg/L versus 11–1 µg/L). The titanium concentrations from both Nitinol and Ti samples were all <20 µg/L. We conclude that Nitinol has good *in vitro* biocompatibility with human osteoblasts and fibroblasts. Despite the higher initial nickel dissolution, Nitinol induced no toxic effects, decrease in cell proliferation, or inhibition on the growth of cells in contact with the metal surface. © 1997 John Wiley & Sons, Inc. *J Biomed Mater Res*, 35, 451–457, 1997.

Key words: biocompatibility; nitinol; shape memory alloy; corrosion; human cell cultures

INTRODUCTION

Nickel-titanium alloys of near equiatomic parts of nickel and titanium, known as Nitinol, exhibit three characteristic properties not commonly observed in metallic materials: thermal shape memory, superelasticity, and high damping properties.^{1–3} All these properties seem to suggest that it is an excellent surgical implant material.

The word “superelasticity” refers to the ability of an alloy specimen to return to its original shape upon unloading after substantial deformation. Nitinol can be strained several times more than ordinary metal

alloys without being plastically deformed. Superelastic alloys undergo a thermoelastic martensitic transformation, which is also a prerequisite for the shape memory effect. Although Nitinol is flexible in its lower-temperature form (martensite), it resumes its original shape and stiffness when heated to its higher-temperature form (austenite). The temperature at which the alloy regains its high-temperature form when heated can be adjusted by slight changes in the alloy composition and through heat treatment. These properties can also be modified by mechanical working. The austenite-to-martensite phase transformation causing the shape recovery is a result of the need of the crystal lattice structure to accommodate to the minimum energy state for a given temperature. In these particular alloys, the relative symmetries between the two phases lead to a highly ordered transformation, where the displacements of individual atoms can be accurately predicted and eventually lead to a shape change on a macroscopic scale.^{1,4–6} Nitinol also has suitable mechanical

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properties for surgical implantation, as well as good corrosion resistance.^{1-3,7-10}

Nitinol could open up completely new prospects in the field of biomedical engineering and surgery. However, its *in vivo* performance and biocompatibility must be very well confirmed before it can be safely used as an implant material. At present, there are not enough confirmative biocompatibility data available on Nitinol.

The purpose of this study was to assess the primary acute cytotoxic effect of Nitinol in cell cultures of human fibroblasts and osteoblasts. We also wanted to determine the rate of metal dissolution of this alloy in a simulated physiologic environment *in vitro* and to compare it to stainless steel and titanium.

MATERIALS AND METHODS

Test materials

The materials tested were vacuum-melted, drawn, and fully annealed Nitinol (54 wt % Ni, 46 wt % Ti, $A_f = -10^\circ\text{C}$; Unitec), stainless-steel AISI 316 LVM (12% Ni, 18% Cr, 68% Fe, 2% Mo; Sandvik, Sweden), ASTM Grade 2 commercially pure titanium (TISTO GmbH; Dusseldorf, Germany), white soft paraffin (a semisolid mixture of hydrocarbons obtained from petroleum and bleached), and a composite material known as Silux Plus® (Bisfenol-A-diglycidylmetacrylate 15–20%, triethylglycoldimetacrylate 15–20%, aluminoxide <1%, amorphous siliconoxide 50–60%, 3-metacryloxypropyltrimetoxysilan <7%; 3M).

Metal test discs 6×7 mm in size were taken from a large bar, using a lathe. The composite material was prepared using a rubber mold. The surface preparation was made following the manufacturers' instructions: electrolytic polishing for stainless steel and water sanding (Orfix Ltd., Raahe, Finland) for titanium and Nitinol (waterproof silicon carbide paper FEPA P No. 2400; Struers, Denmark). The test discs were degreased with 70% ethanol, washed with an ultrasonic vibrobath, and autoclaved.

Cell cultures

The cells used for the assessment of the materials were human fibroblasts taken from a healthy palatal gingiva and osteoblasts taken from an alveolar bone. Both cell lines had been derived in our laboratory. Histochemical analysis of osteoblasts was done by staining them for alkaline phosphatase using Sigma Diagnostics Procedure No. 85, in which only osteoblasts stained blue.¹¹

Both cell lines were plated on three 60×15 -mm-diameter culture flasks (Nunc®; Dern Intermed, Denmark). The culture medium used was Dulbecco's modified Eagle's medium (DMEM) (Life Technologies Ltd., Paisley, Scotland) containing 5% newborn calf serum, 1% ascorbine (Merck, Darmstadt, Germany), 100 IU/mL penicillin, 100 µg/mL streptomycin, 1% L-glutamine, and 0.1% amphotericin B (Fungizone®; Life Technologies Ltd.). The cultures were maintained in an atmosphere of humidified air and 5% carbon dioxide at 37°C . They were passaged routinely with medium changes every second day. They were allowed to reach confluency before subculturing. Cells between the sixth and 10th passages were used for the study. Cells from the three flasks were removed with trypsin (Life Technologies Ltd.), collected in one plastic tube, and counted. Equal amounts of cells (1×10^5 cells) were then seeded to 40-mm-diameter test flasks, each containing one test disc applied in the middle of the plastic flask and glued to the bottom by using sterile white soft paraffin. There were also control flasks without test discs and flasks with only sterile white soft paraffin of the same size as the test discs. Each flask contained 1 mL of culture medium as described in detail above. The incubation conditions were also the same as above. The cells were then allowed to grow with medium changes every second day. At the end of the study, the cells were trypsinised and counted using a hemocytometer. The total number of cell culture flasks was 18 for fibroblasts and 18 for osteoblasts.

The culture flasks were also photographed after a week of incubation, using a Wild MPS 51 camera (Heerburg, Switzerland) and a photoautomat MPS 45. The magnification was $\times 108$.

Determination of corrosion rates

Media collected every second day were stored in plastic tubes and kept at -20°C until analysis. The media were collected from three flasks containing the same type of test material and mixed in a single tube. Corrosion analysis was done from this sample. Before the sample was analyzed, the media were burned with acid. Both nickel and titanium concentrations were assessed from the Nitinol media. Nickel was determined from the stainless steel and titanium from the titanium flask media.

The analyses were performed using a flameless atomic absorption spectrophotometer (GFAAS; Perkin Elmer SIMAA 6000, Rautaruukki oy, Raahe, Finland). Atomic absorption is a technique based on the unique spectrum of each element. For every element analyzed, characteristic wavelengths are generated in a discharge lamp (hollow cathode lamp) and then absorbed by a cloud or vapor of that element. The amount of absorption is proportional to the concentration of the element

that is vaporized into the light beam. The resolution with nickel was $0.7 \mu\text{g/L}$, and with titanium, $7 \mu\text{g/L}$ (1 SD).

Statistical analysis

The data were analysed using a two-sample *t* test.

RESULTS

Cell proliferation and contact to test material

In the control group flask of fibroblast cultures, the number of cells at the end of the study was 1.6×10^5 (SD 1.2×10^4). The average proliferation of fibroblasts was 134.1% ($p < 0.02$) in the flasks containing titanium, compared to the control flasks. It was 108.3% ($p < 0.136$) and 106.8% ($p < 0.174$) in the Nitinol and stainless-steel flasks. The proliferation of cells was only 63.2% ($p < 0.002$) in the white soft paraffin flasks and 48.3% ($p < 0.0001$) in the composite material flasks, compared to the control group (Fig. 1).

Light-microscopic examination revealed no clear differences in the cell growth behavior near the surfaces of different metallic test discs. Marked inhibition of cell growth was seen near the white soft paraffin. Near the composite material discs, inhibition was even stronger.

The proliferation of osteoblasts in the control group was 1.7×10^5 (SD 1.6×10^4). The values for titanium, Nitinol, and stainless steel were almost the same as those for the control group: 99.5% ($p < 0.483$), 100.5% ($p < 0.475$), and 104.7% ($p < 0.334$), respectively. In the white soft paraffin group, the number of cells was 81.5% ($p < 0.058$), compared to the control group. In

the composite material flasks it was only 53.6% ($p < 0.025$) (Fig. 1).

Microscopy revealed that the cells had grown very close to the surfaces of titanium and Nitinol, but slightly less close to stainless steel. Inhibition of cell growth around the composite was also prominent [Fig. 2(a-f)].

Corrosion rate

In the fibroblast cell culture media, the amount of nickel in the Nitinol group was $129 \mu\text{g/L}$ on the second day (first analysis), $23 \mu\text{g/L}$ on the fourth day (second analysis), $9 \mu\text{g/L}$ on the sixth day (third analysis), and $8 \mu\text{g/L}$ on the eighth day (fourth analysis). The titanium concentration was below $20 \mu\text{g/L}$ in all cases, and there were no measurable concentration variations.

Nickel release from stainless steel was $7 \mu\text{g/L}$ on the second day, $3 \mu\text{g/L}$ on the fourth day, $2 \mu\text{g/L}$ on the sixth day, and $2 \mu\text{g/L}$ on the eighth day (Fig. 3).

The titanium concentrations assessed from the titanium cell culture media were below $20 \mu\text{g/L}$ in all samples and showed no variations.

Metal dissolution measured from osteoblast media correlated quite well with the results of the fibroblast media. Again, the most pronounced increase in nickel ion concentration in the media of the osteoblast cultures was observed in the first Nitinol medium analysis: $87 \mu\text{g/L}$. It then decreased rapidly: $14 \mu\text{g/L}$ (sample 2), $5 \mu\text{g/L}$ (sample 3), and $5 \mu\text{g/L}$ (sample 4). The titanium concentrations were all below $20 \mu\text{g/L}$ in both the Nitinol and the titanium medium samples. The nickel concentrations in stainless steel-exposed culture media were $7 \mu\text{g/L}$ (sample 1), $1 \mu\text{g/L}$ (sample 2), $11 \mu\text{g/L}$ (sample 3), and $1 \mu\text{g/L}$ (sample 4) (Fig. 3).

Although the amount of nickel in both fibroblast and osteoblast cell culture media was found to be higher in the Nitinol than in the stainless-steel cell culture media at the baseline, it had no effect on, or correlation to, cell proliferation or cell growth near the implant surface at the concentration levels measured in this study.

DISCUSSION

At present, there are few biocompatibility data available on Nitinol. These *in vitro* and *in vivo* animal studies have so far revealed few or no biocompatibility problems.^{2,8,12-17} Nitinol has also been successfully used in some prefatory experimental clinical studies.¹⁸⁻²⁰

In this study, we investigated the *in vitro* response of human fibroblasts and osteoblasts to Nitinol. Although cell culture studies cannot directly mimic the cellular

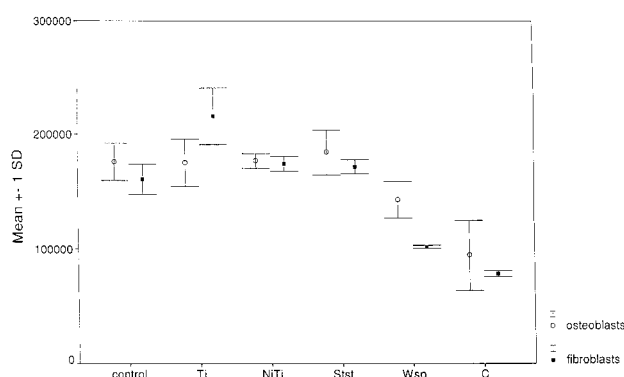


Figure 1. Final amount of osteoblasts and fibroblasts (cells per milliliter) at the end of the trial (10th day) in different test disc groups: mean \pm 1 SD. control = no test disc; Ti = titanium; NiTi = Nitinol; Stst = stainless steel; Wsp = white soft paraffin; C = composite material Silux plus®.

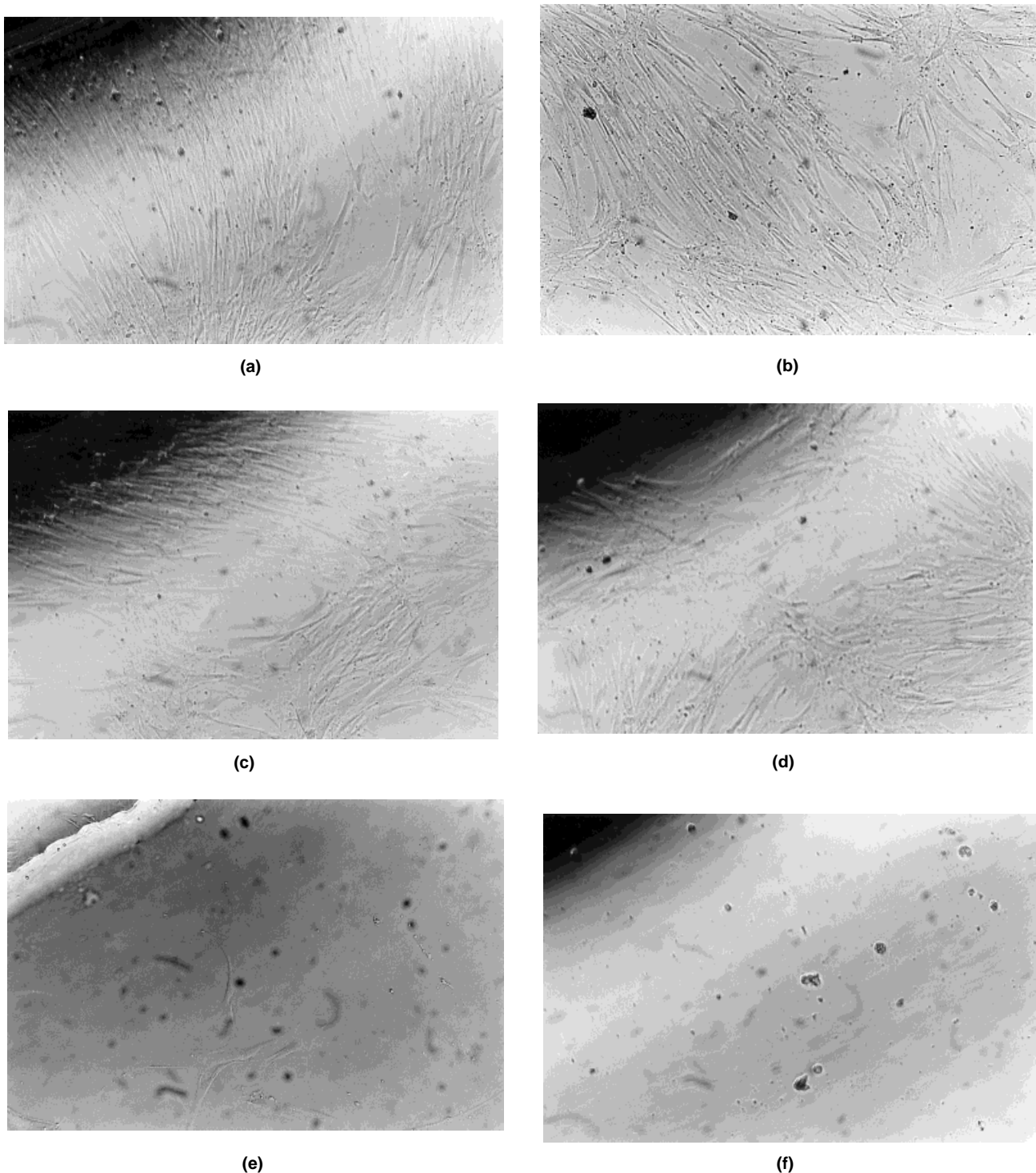


Figure 2. Growth of osteoblasts near the test discs after 1 week of incubation: (a) Nitinol, (b) control, (c) titanium, (d) stainless steel, (e) white soft paraffin, and (f) composite. Black areas at the corners of the photographs are the borders and shadows of the test discs (original magnification $\times 108$).

response and environment *in vivo*, the toxic effects of different metals can be well quantified *in vitro*, because the cell culture method is a sensitive method for toxic screening.²¹ We were interested in finding out whether the amount of nickel dissolved from the alloys in this study was high enough to affect cell proliferation in a cell culture.

There is a clear advantage in using human mesenchymal cells in cultures, because they are the cells present at the site of implantation. As far as we know, Nitinol has been tested only once with human fibroblasts. The results of that study showed that Nitinol was well tolerated.¹² To the best of our knowledge, Nitinol has never been tested with human osteoblasts.

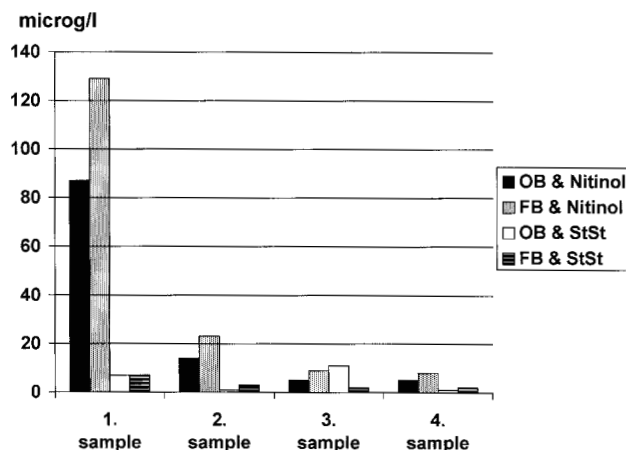


Figure 3. Nickel release from Nitinol and stainless-steel test discs (micrograms per milliliter) due to corrosion in osteoblast (OB) and fibroblast (FB) cell culture media. The first, second, third, and fourth samples were measured on the second, fourth, sixth, and eighth days from the beginning of the assay.

Osteoblasts were used as an *in vitro* model to determine the cytotoxicity of Nitinol as an orthopedic material. We also wanted to compare the behavior of fibroblasts and osteoblasts in the presence of test materials.

Our study showed that fibroblasts appear to be more sensitive to the growth-inhibiting effect of composite material and white soft paraffin or the growth stimulation of titanium than osteoblasts. The responses of both cells were similar, however. Parallel findings have been made in another study, where certain polymers in orthopedic use were tested with both fibroblasts and osteoblasts.²²

Stainless steel is the most commonly used implant material in surgery. It has good mechanical and corrosion properties. It is very well tolerated by human tissues, although it contains nickel (10–14 wt %). At high concentrations, nickel itself is known to have toxic effects in cell cultures and in tissues, but less so than cobalt or vanadium, which are also routinely used in implant alloys.²³ As Nitinol also contains nickel, we wanted to determine the way in which it dissolves in a simulated physiologic environment comparable to stainless steel.

Nickel is the major cause of allergic contact dermatitis.²⁴ Allergic contact dermatitis is caused by Ni^{2+} ions, which bind with a carrier protein. This nickel–protein complex activates Langerhans cells in the skin, which present an antigen to T lymphocytes. Memory T cells are developed. When circulating in the body, these memory cells can start cell-mediated immune reactions upon meeting the same allergen again. Control of the allergic reaction also requires inhibitory systems which prevent the immune response from causing systemic damage. To control the reactions, several kinds of suppressor T cell are generated at different levels.²⁵ Lack

of responsiveness to oral exposure (oral tolerance) to nickel is due to the action of these suppressor cells.^{26,27} This is also presumably why sensitization and immune disorders from metallic prostheses are very unusual, although, for example, the stainless steel used for implants contains perceptible amounts of nickel.^{28,29}

Titanium is the other main component of Nitinol. It was chosen as one of the experimental materials because it has been widely used in traumatology and orthopedics. It is known to be well tolerated by nonosseous tissue and it forms a close connection with bone.^{30,31}

The composite material is known to have shown some toxicity in *in vitro* studies. It is used as a tooth-filling material in dentistry.³² In this study, it was chosen to serve as a toxic-positive control of the other tested materials.

Various studies have shown that some metallic components of the alloys used in orthopedics are toxic and dissolve in body fluids owing to corrosion.³³ The corrosion resistance of the alloy and the toxicity of individual metals in the alloy are the main factors determining its biocompatibility. The physical properties of the metal surface and the microparticles that dissolve from it also have an important role.^{34–36}

The corrosion properties of metals depend on the surface reaction taking place in the respective environments. In many practical applications, corrosion resistance is due to passivation of the metal surface, i.e., the metal atoms at the surface react with the oxygen (and nitrogen) present in the environment to form a stable, nonreactive surface layer of oxide. This *in situ* process protects the metal from further reaction. In the presence of oxygen, rapid self-healing takes place if the surface film is mechanically damaged.^{37,38}

Oxides with a high dielectric constant have been hypothesized to cause less protein denaturation than surfaces with a low dielectric constant. Titanium, which forms a stable oxide with a high dielectric constant and exhibits intimate bonding to bone, is consistent with this suggestion.³⁹ The surface layer of stainless steel is mainly composed of Cr and Ni oxides.

The surface characteristics of Nitinol have not been properly studied. It is evident that the surface of Nitinol is composed of titanium and nickel oxides, while nickel-titanium constitutes the inner layer. Nickel may dissolve more easily than titanium because its oxide is not as stable. Surface layers of nickel-titanium archwires have been observed to have irregular features characterized by lengthy islandlike structures where selective dissolution of nickel may occur. The surface can also be electrochemically modified to selectively etch nickel away, leaving a titanium-enriched surface layer and forming a uniformly distributed porous surface.⁴⁰

Our study revealed that nickel concentrations in both osteoblast and fibroblast cell culture media were

significantly higher for Nitinol than for stainless steel in the first samples (second day), but decreased rapidly to the same levels in the later samples (fourth, sixth, and eighth days). Titanium concentrations were at the same level in both the Nitinol and the titanium media. It may thus be that after some initial dissolution of nickel from the surface, the nickel-titanium alloy may form a surface containing mainly titanium oxides in the outer layer and nickel-titanium in the inner layer. This may be a rapid process, and the decreasing concentration might have been found earlier in our study if the first sample had been taken earlier than on the second day.

In our study, the surface preparation was different for stainless steel and Nitinol. Nitinol was polished by mechanical water sanding, while stainless steel was electrochemically prepared. Electrolytic polishing is known to have a strongly passivating effect on stainless steel and is always applied in the manufacturing of implants. The difference in surface preparation may cause some difference in the corrosion behaviors of the alloys. Mechanical polishing also leaves some microscopic roughness, which increases the effective surface area in contact with the surrounding. Consequently, it may be assumed that electrochemical polishing can be used to minimise the initial release of nickel from the surface of Nitinol specimens, i.e., to passivate the metal surface. In other words, the performance of Nitinol in physiologic environment is likely to be even better if an electrochemical polishing method applicable to this alloy can be developed.

Another way to improve corrosion resistance could be, for example, thermal oxidation or some coatings. It has been demonstrated that titanium nitride coatings of nickel-titanium alloy can improve corrosion resistance.⁴¹ Considerably more studies are needed to clarify their possible effects on mechanical or shape memory behavior.

The higher nickel concentrations observed for Nitinol at the beginning of the test had no effect on the proliferation and the total amount of fibroblasts and osteoblasts. Cells formed in quite close connection with the Nitinol test discs, which is also a sign of good biocompatibility. A clearly different reaction was seen in the case of composite material and white soft paraffin. The observed toxic influence of white soft paraffin on cultured cells was surprising.

We found that the osteoblast proliferation with titanium was not higher than that of Nitinol, stainless steel, or control, but it provided some proliferatory stimulus to fibroblasts. The same phenomenon has been reported previously in some other studies. Enhanced fibroblast proliferation is not always good. It may contribute to the fibrous membrane, which may act as a conduit for the transport of polymeric debris produced at the articulating surface of hip prostheses and contribute to loosening or osteolysis.³⁴ In our

study, Nitinol caused no inhibitory or stimulatory effect with fibroblasts.

CONCLUSIONS

We conclude that Nitinol has good *in vitro* biocompatibility with human osteoblasts and fibroblasts. In the beginning, Nitinol releases more nickel in cell culture media than stainless steel, but after 2 days the concentration decreases to about the same level. The amount of dissolved nickel is so low that no toxic effects or decrease in cell proliferation can be observed with this sensitive method. Nor is there any inhibiting effect on the growth of cells in contact with the metal surface. Further studies are needed to show whether electrochemical passivating treatment or another surface treatment as a finishing treatment in nickel-titanium implant fabrication can be used to minimize nickel release at the beginning.

On the basis of the present study, Nitinol appears to have an excellent potential for clinical use, as its biocompatibility is very good.

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