Cytocompatibility of Medical Biomaterials Containing Nickel by Osteoblasts: a Systematic Literature Review

Marcin Mikulewicz · Katarzyna Chojnacka

Abstract The present review is based on a survey of 21 studies on the cytocompatibility of medical biomaterials containing nickel, as assessed by cell culture of human and animal osteoblasts or osteoblast-like cells. Among the biomaterials evaluated were stainless steel, NiTi alloys, pure Ni, Ti, and other pure metals. The materials were either commercially available, prepared by the authors, or implanted by various techniques to generate a protective layer of oxides, nitrides, acetylides. The observation that the layers significantly reduced the initial release of metal ions and increased cytocompatibility was confirmed in cell culture experiments. Physical and chemical characterization of the materials was performed. This included, e.g., surface characterization (roughness, wettability, corrosion behavior, quantity of released ions, microhardness, and characterization of passivation layer). Cytocompatibility tests of the materials were conducted in the cultures of human or animal osteoblasts and osteoblast-like cells. The following assays were carried out: cell proliferation and viability test, adhesion test, morphology (by fluorescent microscopy or SEM). Also phenotypic and genotypic markers were investigated. In the majority of works, it was found that the most cytocompatible materials were stainless steel and NiTi alloy. Pure Ni was rendered and less cytocompatible. All the papers confirmed that the consequence of the formation of protective layers was in significant increase of cytocompatibility of the materials. This indicates the possible further modifications of the manufacturing process (formation of the passivation layer).

Keywords Cytocompatibility · Nickel · Osteoblasts · Cell proliferation · Cell viability

M. Mikulewicz
Department of Dentofacial Orthopedics and Orthodontics, Medical University of Wrocław, Wrocław, Poland

K. Chojnacka
Institute of Inorganic Technology and Mineral Fertilizers, Wrocław University of Technology, Wrocław, Poland

M. Mikulewicz (✉)
ul. Krakowska 26, 50-425 Wrocław, Poland
e-mail: mmikulewicz@poczta.onet.pl

Abbreviations

OB	Osteoblasts
OB-like	Osteoblast-like cells
FB	Fibroblasts
ALP	Alkaline phosphatase activity
OC	Osteocalcin
CICP	Type I collagen
PICP	Pro-collagen I
NO	Nitric oxide
MTT	Enzymatic reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
BCA	Protein assay/quantification of total protein amount
WST-1	Cell proliferation reagent
FBS-DMEM	Fetal bovine serum–Dulbecco's modified Eagle's medium
AAS	Atomic absorption spectroscopy
FAAS	Flame atomic absorption spectroscopy
FTIR	Fourier transform infrared spectroscopy
GFAAS	Graphite furnace atomic absorption spectroscopy
ICP-MS	Inductively coupled plasma–mass spectroscopy
DSC	Differential scanning calorimetry
VASE	Variable angle spectroscopic ellipsometry
WLI	White-light interferometry
XPS	X-ray photoemission spectroscopy
XRD	X-ray diffractometry
AFM	Atomic force microscopy
SEM	Scanning electron microscopy
EPMA	Electron probe microanalysis

Introduction

There are certain requirements towards all the materials (implants or dental appliances) which are inserted in human body. The following problems are considered: biological safety tissue response, biofunctionality and corrosion resistance. Also, technological and economic aspects are important [1]. Many of those aspects are covered by biocompatibility approach. It seems that the applied biomaterials should possess two significant features. The first aspect is integrity and degradation of the material and the second is the reaction of the host organism (toxic effects and allergic response) [2]. Alloys should be biocompatible and present required mechanical properties (resistance to stress and wear) [3].

The application of orthodontic devices (brackets, bands, and wires) in the oral cavity requires evaluation of biocompatibility of the materials. The latter is related with the susceptibility for ion release, corrosion resistance, and ability to create a passivation layer. Valid definitions of biocompatibility were given by Black (1984) [4] and Williams (1998) [5]. The term was defined as the response of a host organism to the presence of potentially inert biomaterials. Biocompatibility studies usually include investigation of histopathological changes in host which was exposed to the material for an extended period of time. Host response is a measure of biocompatibility [6]. In order to make the results of biocompatibility studies comparable, the guidelines and procedures were included in ASTM and ISO standards [7, 8].

Previously, the problem of corrosion of orthodontic appliances and resulting ions release in in vitro and in vivo conditions was discussed [9, 10]. The objective of the present work was to review systematically cytocompatibility of the materials by cell culture experiments on osteoblasts (OB) and osteoblast-like cells. In the available literature, no systematic review discussing this topic was found.

Materials and Methods

The following search criteria were selected to find papers describing the cytocompatibility of metal alloys containing Ni applied for medical purposes by osteoblast and osteoblast-like cell cultures. The search consisted of the following criteria: cytocompatibility (or biocompatibility) and osteoblast and nickel (or Ni–Ti or NiTi or Nitinol or "stainless steel"). The combinations of keywords and search results are presented in Table 1. To find articles which can match the mentioned criteria, a search in PubMed database was conducted (from 1966 to April 2010).

The keyword "cytocompatibility" was extended with "biocompatibility," because cytocompatibility is a particular case of biocompatibility, which is the wider term. To the keyword "nickel," additional keywords "or Ni–Ti or NiTi or Nitinol" or "stainless steel" were added because not all papers which discussed cytocompatibility of materials potentially release nickel, the words "nickel" and "stainless steel" were included in title and abstract section, and Ni–Ti, NiTi, and Nitinol are the most frequently used terms for superelastic alloys containing nickel, applied in orthodontics and orthopedics. The keyword "nickel" was selected, because this metal and its potential toxicity are of particular concern. All the articles that met the inclusion criteria of the systematic review were selected for the study. Eligibility of the selected studies was determined by reading the abstracts of papers identified by the search. The abstracts of related articles were reviewed to search for any similar studies. Following exclusion criteria were applied: other than English papers, exposure to nickel from materials other than those used in orthodontic and orthopedic treatment, reviews, case reports, or papers concerning topics other than cytocompatibility tests by osteoblasts.

Table 1 PubMed search strategy

No.	Word or phrase	Results
1a	Cytocompatibility	535
1b	Biocompatibility	9,142
2	Osteoblast	25,016
3a	Nickel	24,687
3b	Ni–Ti	1,436
3c	NiTi	688
3d	Nitinol	1,810
3e	"Stainless Steel"	10,745
5	(1a or 1b)	9,142
6	(1a or 1b) and 2	598
7	(3a or 3b or 3c or 3d or 3e)	36,155
8	(1a or 1b) and 2 and (3a or 3b or 3c or 3d or 3e)	43

Results

The PubMed search identified 43 studies [11–53]. No additional search in other databases was performed. From 43 identified studies, 21 met the selection criteria [33–53]. Excluded studies [11–32]—with the reported reason of exclusion—are presented in Table 2. Included studies are listed in Table 3.

Tables 3, 4, and 5 present the materials, methodology, and results. Because of severe differences in applied methodology, it was impossible to conduct quantitative statistical elaboration of the results reported in various papers. The main characteristics investigated in the evaluation of the biomaterials included physical and chemical properties, biocompatibility by culturing OB or fibroblasts (FB; proliferation and viability tests) and by phenotypic biomarkers, e.g., ALP (alkaline phosphatase activity), OC (Osteocalcin), CICP (Type I C-terminal collagen propeptide), as well as bioadhesion.

Table 3 discusses the properties of evaluated materials. The main biomedical applications investigated were orthopedic implantology, surgery, and orthodontics.

The Type of Materials and Additional Processing

The type of the material included stainless steel (316 L [33, 34, 40, 42, 43], 316LS [37, 52], P558 [39, 40, 44], Stst not specified [35, 38, 44, 51]), NiTi (different types) [34, 36, 41, 43,

Table 2 Studies that fulfilled the selection criteria but were excluded from the "Results" section [11–32]

	Authors, date	Reason for exclusion
[11]	Arslan et al., 2008	Different topic
[12]	Berger-Gorbet et al., 1996	Different material—NiTi screws
[13]	Bogdanski et al., 2002	In German
[14]	Bombonato-Prado et al., 2009	Different topic—expression of genes
[15]	Bosetti et al., 2002	Different material—silver coated
[16]	Brors et al., 2002	Different topic
[17]	Brunot et al., 2007	Different topic
[18]	Diaz et al., 2008	Different topic
[19]	González-Carrasco et al., 2005	Different topic
[20]	Gough and Downes, 2001	Different topic
[21]	Kapanen et al., 2002	Different topic—surface stresses
[22]	Kapanen et al., 2002	Different topic—surface stresses
[23]	Li et al., 2006	In Chinese
[24]	Macnair et al., 1997	Different material—orthopedic polymers
[25]	Misra et al., 2010	Different material—nanograined/ultrafine-grained
[26]	Morais and Pereira, 2000	Different topic
[27]	Nicula et al., 2007	Different topic
[28]	Petrolati et al., 1999	Different topic
[29]	Santavirta et al., 1992	Different topic
[30]	Shahryari et al., 2009	Different topic
[31]	Tschon et al., 2005	Different topic—soft tissue response
[32]	Woodruff et al., 2007	Different topic

Table 3 Studies that fulfilled the selection criteria and were included for the review in the "Results" section—characterization of the studied materials, methodology, and results

References	Example application of the material	Type/additional material processing	Determination of physical and chemical properties of the materials	Results
Human osteoblasts (OB)				
Bordji et al. [33]	Orthopedic implants	Stst AISI 316 L implanted with (1) N-implanted, (2) C-doped, (3) nitrided, (4) untreated	Chemical composition (surface layer) EPMA, microhardness (Vickers), wear, and corrosion resistance	Surface treatment improved the mechanical properties; surface characterization—corrosion, wear resistance, and microhardness improved (by ion implantation or carbon coating)
Ryhänen et al. [34]	Orthopedic implants	Nitinol (Unitec), Stst AISI 316 LVM, ASTM Grade 2 commercially pure Ti, composite material Silux Plus®, white soft paraffin	Determination of corrosion rate by GFAAS	Corrosion rate: Ni initial release order: Nitinol> Stst; after 2 days: Nitinol=Stst
Schmidt et al. [35]	Dental, orthopedic implants	Pure Ti (cpTi), Ti–6Al–7Nb, Stst, Thermanox (control)	Material surface characterization—roughness (profilometer AFM) and SEM	SEM: Thermanox—very smooth surface with almost no elevations and depressions; stainless steel—smooth surface with some humps uniformly distributed; traces from surface polishing; pure titanium—depressions of different depths resulting from the manufacturing process; edges of the holes smooth; scratches on the material surface; titanium alloy—prominent ridges of about 5–10 mm
Bogdanski et al. [36]	Orthodontic wires, implants, bone substitute materials	Pure Ni, NiTi alloy (with variable Ni and Ti content, 10 samples), pure Ti	Microscope characterization of biomaterials; XRD	The composition of ten different sections was consistent with the phase diagrams—different intermetallic phases
Hao et al. [37]	Internal fixation devices	Stst 316 LS modified by CO_2 laser treatment	Surface analysis (SEM), XPS, surface profiling/surface roughness	Wettability positively correlated with proliferation of cells; roughness depended on electric field intensity of laser CO_2 treatment, at 1,500 W, which was higher than when treated with 2,400 W

Table 3 (continued)

References	Example application of the material	Type/additional material processing	Determination of physical and chemical properties of the materials	Results
Human osteoblast-like cells				
Riccio et al. [38]	Biomaterials	Stst, Ti alloy, Co–Cr–Mo alloy, carbon fiber-reinforced polybutylene terephthalate, hydroxyapatite	–[b]	–[b]
Bogdanski et al. [36]	–[a]	–[a]	–[a]	–[a]
Torricelli et al. [39]	Orthopedic implants	Stst P558, Ti6Al4V (Ti alloy), polystyrene wells (control)	Surface roughness, chemical composition	Surface roughness of Ti alloy was higher than Stst P558
Montanaro et al. [40]	Orthopedic implants	Stst Böhler P558 (Ni-free), Stst AISI 316 L	Surface roughness, chemical composition	Ra values below 0.2 μm
Michiardi et al. [41]	Dental (orthodontics), orthopedic implants	NiTi alloy oxidized at 400°C at subatmospheric pressure, untreated NiTi, cpTi	–[b]	–[b]
Animal osteoblasts				
Morais et al. [42]	Implants	AISI Stst 316L	Determination of released metal ions—AAS	The concentration of metal ions released from Stst (μg/ml): Fe(III), 500; Cr(III), 122; Ni(II), 101. Diluted 10^3, 10^4 10^5 times
Kapanen et al. [43]	Dental wares and gastrointestinal surgery	Nitinol (Ni–Ti alloy), Stst AISI 316LVM, ASTM Grade 2—pure Ti, Tisto, pure Ni	–[b]	–[b]
Fini et al. [44]	Dental, orthopedic implants	Stst P558, SSt, Ti6Al4V, polystyrene wells (control)	Surface roughness (laser profilometer), chemical composition	Ti6Al4V—the highest roughness values (in vivo test)
Cortizo et al. [45]	Orthodontic appliances	Pure metals: (1) Ag, (2) Au,	Measurement of concentration of released ions—FAAS; electrochemical experiments	Anions and proteins interfered in the corrosion process—by voltammograms; fetal bovine serum (FBS) influenced electrochemical

Reference	Application	Material	Methods	Results
		(3) Pt, (4) Pd, (5) Cu, (6) Ni/Ti alloy (Nitinol), (7) FBS-DMEM (control)		process by decrease of the oxidation rate of the metal
Yeung et al. [46]	Orthopedic implants	NiTi alloy implanted with (1) NiTi—not implanted, (2) NiTi-N, (3) NiTi-O, (4) control (empty well)	Chemical composition and depth profile (XPS)	Near-surface Ni content in the treated materials was reduced. PIII treatment improved the surface properties of NiTi alloys; better corrosion resistance was achieved together with the reduced released of Ni
Yeung et al. [47]	Orthopedic implants	NiTi alloy implanted with (1) NiTi, (2) NiTi, (3) NiTi, (4) NiTi—not implanted	Depth profile (XPS), microhardness, surface morphology (SEM), ion release (ICP-MS), corrosion resistance	Leaching of Ni and Ti was reduced by implanting
Wu et al. [48]	Medical implants	Ni and Ti powder fabricated into porous alloy oxidized at 6 different temperatures and untreated porous NiTi, wells without any metal disks (control)	Oxygen plasma immersion ion implantation, compression test, XPS, immersion tests—ICP-MS	Good mechanical properties and superelasticity; the quantity of nickel released from the material was lower than from the untreated samples; XPS—nickel-depleted surface layer predominantly composed of TiO_2 is produced by O-PIII and was a barrier against release of nickel
Wu et al. [49]	Surgical implants, material for bone grafts	Ni and Ti powder fabricated into porous alloy oxidized at 6 different temperatures 300°C, 400°C, 450°C, 550°C, 600°C, 800°C for 1 h and untreated (control)	Roughness, Ni release behavior (ICP-MS), transformation temperatures, superelasticity, morphology (SEM), XPS, immersion test, DSC, and compression test	Lower Ni release, best superelasticity, austenite transition temperature below 37°C for the material fabricated at 450°C
Liu et al. [50]	Orthopedic implants	NiTi with the surface modified by nitrogen plasma immersion ion implantation (N-PIII) at various voltages	Chemical composition XRD; topography and roughness before and after N-PIII—AFM; immersion test (ICP-MS), SEM; three-point bending tests	Near-surface Ni concentration—reduced by PIII; the surface TiN layer suppressed nickel release
Yeung et al. [51]	Orthopedic implants	NiTi—nitrogen plasma ion implantation (N-PIII);	Chemical composition XPS, corrosion resistance—immersion tests	The corrosion resistance and release of Ni ions were improved by ion implantation as

Table 3 (continued)

References	Example application of the material	Type/additional material processing	Determination of physical and chemical properties of the materials	Results
		control—untreated NiTi, Stst, Ti–6Al–4V	(ICP-MS), hardness measurements; surface roughness (AFM)	compared with Stst and NiTi, comparable with alloy containing Ti
Ochsenbein et al. [52]	Orthopedic implants	Pure Ti coated by the sol–gel process, oxidized with TiO_2, SiO_2, Nb_2O_5, $SiO_2–TiO_2$, uncoated, 316 SL Stst as a positive control	FTIR, X-ray diffraction, VASE, dual-beam focused ion beam/SEM, WLI, AFM, contact angle measurement for surface energy	Physicochemical characterization of the oxide coatings showed a nanoporous structure in the TiO_2 and Nb_2O_5 layers, the SiO_2 and $SiO_2–TiO_2$ layers appeared almost smooth; the absence of organic residues; the thickness of layers was 100 nm
Liu et al. [53]	Orthopedic implants	NiTi with the surface modified by nitrogen plasma immersion ion implantation (N-PIII) at 0 (control), 50, 100, 200 Hz	XPS, AFM	XPS—implantation depth of nitrogen increased with higher pulsing frequencies; AFM—nanoscale surface roughness increased and surface features are changed from islands to spiky cones with higher pulsing frequencies

[a] The results are discussed in the section "Human Osteoblasts" (OB)

[b] Not found

Table 4 Studies that fulfilled the selection criteria and were included for the review in the "Results" section—cell culture experiments

References	Type of cells	Type of assay	Control	Results	Statistics
Human osteoblasts (OB)					
Bordji et al. [33]	OB from trabecular bone	Cell proliferation and viability test (after 14, 18, 21 days), cell protein content test	Yes	OB viability after 14, 18, 21 days (%): (1) Stst N-implanted: 94.4, 94.4, 93.4; (2) Stst C-doped: 94.3, 94.4, 93.9; (3) Stst nitrided, 70.0, 66.9, 66.4; (4) Stst untreated, 93.7, 91.8, 93.5	t test
Ryhänen et al. [34]	OB from alveolar bone	Cell proliferation and viability test (after 2, 4, 6, 8 days)	Yes	Cell culture of OB revealed no toxic effect, no decrease in cell proliferation, and no inhibition on the growth of cells. Cell proliferation vs. control group (%): (1) Nitinol, 100.5; (2) Stst, 104.7; (3) Ti, 99.5; (4) Composite, 53.6	Two-sample t test
Schmidt et al. [35]	OB from femur or femoral head	Cell proliferation and viability test (after 3, 7, 11, 15, 19 days)	Yes	After 19 days, 300% increase of cell number; Thermanox, Ti–6Al–7Nb, cp Ti—the culture reached stationary phase, in culture on Stst—growth curve was linear, stationary phase was not reached. Cell counts after 19 days[c]: (1) pure Ti (cpTi), 95,000; (2) Ti–6Al–7Nb, 95,000; (3) Stst, 120,000 (linear growth); (4) Thermanox (control), 109 000; (1, 2, 4) stationary phase reached after 15 days	Tukey–Kramer test
Bogdanski et al. [36]	Primary OB	Cell proliferation and adhesion test (after 3 days)	—[b]	Good biocompatibility for a nickel content up to 50%; the lack of biocompatibility at high nickel contents may be ascribed to the	—[b]

Table 4 (continued)

References	Type of cells	Type of assay	Control	Results	Statistics
Hao et al. [37]	OB-hFOB 1.19	Proliferation test (after 7 days), cell attachment, morphology—by SEM	Yes	presence of elemental nickel or nickel-rich intermetallic phases; the released nickel rapidly reached cytotoxic concentrations within 1 day. Significant increase in cell proliferation	One-way ANOVA, Scheffe's post hoc multiple-comparison test
Human osteoblast-like cells					
Riccio et al. [38]	Embryonic OB-like	Viability, morphology, osteogenic capacity	Yes	Studied materials did not exert any significant cytotoxic effects on cultured osteoblasts; plating efficiency, adhesion, and morphology of OB; ability of cells to proliferate around the tested materials was confirmed	—[b]
Bogdanski et al. [36]	OB-like osteosarcoma cells MG63 and SAOS-2	—[a]	—[a]	—[a]	—[a]
Torricelli et al. [39]	OB-like cells-MG63	Cell proliferation and viability test (after 3 days) with use of WST-1, morphology—SEM images	Yes	Stst P558—no negative effects on cell proliferation, activation, and differentiation compared to alloy of Ti or control; SEM images—no changes in morphology	One-way ANOVA, Scheffe's post hoc multiple-comparison test
Montanaro et al. [40]	OB-like cells MG63	Cell proliferation test (NR uptake assay, AB staining assay)	Negative and positive control	The extracts did not reduce viability or cell growth potential and therefore did not have toxic effects. Cell viability index (%)[c]: (1) Stst Böhler P558 (Ni-free), 100;	t test

Reference	Cells	Test	Control	Results	Statistical analysis
Michiardi et al. [41]	OB-like cells MG63	Cell proliferation and viability test (after 1, 3, 6, 9 days) with use of WST-1, adhesion test with use of WST1 test (after 1, 4, 8 h)	Positive (polystyrene surface) and negative (Teflon surface) control	(2) Stst AISI 316 L, 95. Cell growth index (%)[c]: (1) Stst Böhler P558 (Ni-free), 110; (2) Stst AISI 316 L, 102 Proliferation—untreated and oxidized NiTi surfaces are not cytotoxic; the differences of initial adhesion did not affect the proliferation; adhesion test—oxidation treatment delays cell adhesion (no stat. sign. vs. control); proliferation study—the cells continually proliferated, except for the positive control (the difference is not significant; the negative control—stat. sign. higher number of cells at each time of culture	t test, one-way ANOVA with Fishers and Tukey's multiple-comparison tests

Animal osteoblasts

Reference	Cells	Test	Control	Results	Statistical analysis
Morais et al. [42]	Rabbit OB from bone marrow	Cell proliferation and viability test (after 7, 14, 21, 28 days)	Yes	Metal ions stimulate proliferation vs. control; cell proliferation increased in the presence on Ni	Double-sided t test/yes
Kapanen et al. [43]	Rat osteosarcoma cell line ROS—17/2.8	Viability test (after 2 days) with use of LIVE/DEAD® Viability/Cytotoxicity Kit	—[b]	Ratio of dead to live cells significantly higher in Ni and Stst; Ti culture—lower death rate comparing to Stst culture. Amount of dead cells/1,000 cells: NiTi, 4; Stst, 21; Ti, 4.8; Ni, 51. Stat. sign. NiTi and Ti<Ni	ANOVA, t test with Bonferroni correction
Fini et al. [44]	Rat OB from trabecular bone	Cell proliferation and viability test (after 3 days) with use of WST-1	Yes	Stst P558 enhanced osteoblast differentiation. WST1 OD at 450 nm: (1) control, 1.016; (2) P558, 1.028; (3) Ti6Al4V, 0.966	Multiple-way ANOVA
Cortizo et al. [45]	Rat OB osteosarcoma derived cells UMR106 and MC3T3E1 cells	Cell growth (mitotic index) and differentiation (after 2 days)	Yes	Cu and Ag are most toxic elements; other metals are biocompatible with OB (cell survival (%), mitotic index)[c]: pure metals: (1) Ag 75, 0.005;	t test; correlation by Pearson's correlation coefficient

Table 4 (continued)

References	Type of cells	Type of assay	Control	Results	Statistics
				(2) Au 96, 0.01; (3) Pt 100, 0.016; (4) Pd 100, 0.012; (5) Cu 3, 0; (6) Ni/Ti alloy (Nitinol) 110, 0.025; (7) control 100, 0.018	
Yeung et al. [46]	Mice OB from calvarial bone	Cell proliferation, viability, and adhesion test (after 2, 4, 6, 8 days)	Yes (empty wells)	Cell proliferation (×10,000) after 2, 4, 6, 8 days[c]: (1) NiTi 4.9, 10, 15.1, 17; (2) NiTi–N 4.9, 4.8, 10, 18; (3) NiTi–O 4.9, 4.2, 8, 12; (4) control 2, 6, 20, 24;	t test
Yeung et al. [47]	Mice OB from calvarial bone	Cell proliferation, viability, and adhesion test (after 2, 4, 6, 8 days)	Yes (not implanted material)	The best biological effect—material implanted with nitrogen	Unpaired two-sample t test
Wu et al. [48]	Mice OB from calvarial bone	Cell proliferation test (after 8 days), morphology—fluorescent microscopy	Yes	No immediate cytotoxic effects were found; the treated and untreated materials were well tolerated by the EGFP-expressing osteoblasts; the cells attached and proliferated	–[b]
Wu et al. [49]	Mice OB from calvarial bone	Cell proliferation test (after 8 days), morphology—fluorescent microscopy	Yes	Cell cultures showed that NiTi oxidized at 450°C—no cytotoxicity (cell proliferation and growth); the cells attached to and proliferated on the entire surface of NiTi (oxidized at 450°C and untreated); only a small amount attached to the material fabricated at 600°C	–[b]
Liu et al. [50]	Mice OB from calvarial bone	Cell proliferation and viability test (after	Yes	The surface TiN layer favored osteoblast proliferation; this concerned materials	Unpaired two-sample t test

Yeung et al. [51]	Mice OB from calvarial bone		1 day), morphology—fluorescent microscopy	implanted at higher voltages (30 and 40 kV), which adhere better than unimplanted and 20 kV PIII. Cell proliferation after 8 days, cell number[c]: (1) empty well, 1,100; (2) control, 700; (3) 20 kV, 740; (4) 30 kV, 825; (5) 40 kV, 775	
		Yes	Cell proliferation (after 2,4, 6, 8 days) and cell viability test	Number of viable cell ($\times 10,000$) after 2, 4, 6, 8 days[c]: (1) NiTi 4.9, 6, 8, 12.5; (2) NiTi–N 4.9, 5, 10, 15; (3) Stst –[b], 2.5, 7, 9; (4) Ti–6Al–4V 2, 2.3, 7.5, 10.2	Two-sample t test
Ochsenbein et al. [52]	Mice OB from calvarial bone—MC3T3-E1	Yes	Cell proliferation (after 3–6 days) and viability test (Alamar-blue dye by intracellular respiratory activity), morphology—SEM	SEM—good cell attachment for all the materials; higher cell proliferation rates—SiO_2–TiO_2 and TiO_2, and lower in Nb_2O_5 and SiO_2; the vitality rates increased for cpTi and Nb_2O_5. Proliferation rate with regard to control after 6 days (%)[c]: (1) cpTi, 120; (2) Nb_2O_5, 105; (3) SiO_2, 100; (4) SiO_2–TiO_2, 130; (5) TiO_2, 125; (6) 316 L 18. Vitality test with regard to control after 6 days (%)[c]: (1) cpTi, 105; (2) Nb_2O_5, 105; (3) SiO_2, 98; (4) SiO_2–TiO_2, 101; (5) TiO_2, 103; (6) 316 L, 20	One-way ANOVA

Table 4 (continued)

References	Type of cells	Type of assay	Control	Results	Statistics
Liu et al. [53]	Mice OB from calvarial bone	Cell proliferation and viability (after 1 day), morphology—fluorescent microscopy	Yes	More cells were attached to the materials treated with 50 and 100 Hz than in the control and 200 Hz; similar results were obtained for proliferation; the majority of OB—polygonal shape and the plasma membranes—extended to all sides; many OB in the control and at 200 Hz polarized shape and elongate in opposite directions (partially spreading behavior)	_b

[a] The results are discussed in the section "Human Osteoblasts" (OB)

[b] Not found

[c] Read from the graph

45–51, 53], pure Ni [36, 43] and pure Ti [34–36, 43, 52], Ti alloy (Ti–6Al–4V [39, 44, 51], Ti–6Al–7Nb [35]), Co–Cr–Mo alloy, carbon fiber-reinforced polybutylene terephthalate, ultra-high molecular weight polyethylene, ceramic, calcium phosphate, and hydroxyapatite [38]. The following materials served as the control: composite material Silux Plus® [34], Thermanox [35], polystyrene [39, 44], growth medium (e.g., FBS-DMEM) [45], and white soft paraffin [34]. In many studies, the properties of untreated and treated (high voltage, oxidization, nitridization, and ion implantation) materials were conducted.

Physical and Chemical Properties of the Materials—Methods

In the majority of papers, physical/chemical properties of the materials were evaluated. Different methodological approaches have been applied. The basic characteristics were as follows: the chemical composition of the materials (XRD [36, 50, 52], XPS [37, 46–49, 51, 53], EPMA [33], and not specified [39, 40, 44]), the quantity of ions released (immersion tests—ICP-MS, FAAS, GFAAS, AAS [34, 42, 45, 47–51]), corrosion resistance [33, 47, 51], wear resistance [33], microhardness [33, 47, 51], surface roughness (profilometry, AFM [35, 37, 39, 40, 44, 49–53]), surface morphology (SEM [35, 37, 47–50, 52]), three-point bending test [50], compression test [48, 49], transformation temperature [49], superelasticity [49], DSC [49], FTIR [52], VASE [52], and WLI [52]). The expected properties of the materials depend on their application, e.g., higher surface roughness is requested in orthopedic implants (better osteointegration), but lower in orthodontic wires (lower friction).

Physical and Chemical Properties of the Materials—Results

The main conclusion arising from the performed studies was that the applied treatment improved the formation of the passivation layer (oxides, nitrides, and acetylides) which was protective against the release of metal ions from the material and consequently improved the biocompatibility. Practically, in all the studies, the initial release of metal ions was confirmed. If the materials were treated, this phenomenon was not so significant. This initial release was followed by the formation of the passivation layer. In the case of treated materials, this layer was formed under controlled conditions during the manufacturing process.

Table 4 discusses cell culture experiments—materials, methods, and results.

Cell Culture Experiments—Type of Cells

Mainly three types of cells were investigated (Table 4): human OB (bone, primary, cell line) [33–37], human osteoblast-like cells (OB-like; osteosarcoma cells MG63, SAOS-2, embryonic) [38–41], and animal OB (rabbits, mice, rats; osteosarcoma, bone—mainly calvaliar) [42–53]. Since the cell culture experiments were carried out on different types of cells (human OB and OB-like cells, as well as animal OB), it is difficult to compare the experimental results. However, it is possible to relate interpretations and conclusions.

Cell Culture Experiments—Type of Assay

Cells were cultivated under laboratory conditions, and the following types of assays were performed: cell proliferation (hemacytometer), cell viability (cell counts), and morphology by SEM [33–53].

Table 5 Studies that fulfilled the selection criteria and were included for the review in the "Results" section—results of experiments of phenotypic and genotypic markers

References	Tests	Results
Human osteoblasts (OB)		
Bordji et al. [33]	ALP, OC production (OSTK-PR kit with rabbit polyclonal antibody against human OC)	ALP was diminished in OB culture on nitrided Stst, as compared with untreated Stst. OC was 30% less than for Stst which was not treated. Stst nitrided at low temperature strongly influenced expression of phenotypic markers of OB. Although, those markers were not influenced by N-implantation or C-doping of Stst as compared to untreated Stst
Ryhänen et al. [34]	ALP	—[a]
Schmidt et al. [35]	ALP, CICP, OC	Control Thermanox, Stst, cpTi—moderate increase of alkaline phosphatase activity; Ti–6Al–7Nb—no significant change of ALP; OC levels—generally higher on implant material than in the control
Bogdanski et al. [36]	—[a]	—[a]
Hao et al. [37]	MTT	MTT optical density after 7 days of cell culture of osteoblast cells[b]: (1) untreated, 0.06; (2) mechanically roughened, 0.08; (3) CO_2 laser 1,500 W, 0.022; (4) CO_2 laser 2,400 W, 0.048
Osteoblast-like cells		
Riccio et al. [38]	ALP, CICP	Elevated alkaline phosphatase 1,25(OH)2D3 response activity associated with plasma membranes and matrix vesicles; production of thick extracellular matrix (CICP) mineralized in the environment of beta-glycerophosphate
Bogdanski et al. [36]	—[a]	—[a]
Torricelli et al. [39]	ALP, NO, PICP, IL-6, OC, TGF-β1	No differences in IL-6 production; OC, and TGF-β1 were higher compared to control and Ti group
Montanaro et al. [40]	ALP, CICP, and OC; genotoxicity tests	ALP, CICP, and OC production showed that the materials support the expression of these phenotypic markers
Michiardi et al. [41]	ALP, BCA protein assay, OC	ALP and OC increased on oxidized surface
Animal osteoblasts		
Morais et al. [42]	MTT, ALP, ALP staining (ALP positive cells), phosphate and calcium deposits	Disadvantageous and slight alteration in the levels of ALP in the presence of ions; Ca and P deposits—the process of mineralization retarded in the presence of ions; lower ALP production ability vs. control

Kapanen et al. [43]	Apoptosis tests—DNA laddering, TUNEL assay, immunofluorescence microscopy of focal contacts	TUNEL assay (rate of apoptosis): Ti>NiTi>Stst>Ni. Comparison of cytotox. and TUNEL: % apoptotic cells in dead cells: (1) NiTi, 48; (2) Stst, 5.6; (3) Ti, 62; (4) Ni, 1.8. Focal contacts: NiTi>Ti>Stst>Ni
Fini et al. [44]	ALP, PICP, OC, NO, TGF-β1, IL-6	OC (ng/ml): (1) control, 9.65; (2) P558, 19.58; (3) Ti6Al4V, 19.43. P558 enhanced OC levels and reduced IL-6 production ALP (IU/l): (1) control, 14.44; (2) P558, 17.18; (3) Ti6Al4V, 15.42
Cortizo et al. [45]	ALP	Metal ions caused induction of cell death by early mitosis arrest, apoptosis, and necrosis
Yeung et al. [46]	—[a]	
Yeung et al. [47]	—[a]	
Wu et al. [48]	—[a]	
Wu et al. [49]	—[a]	
Liu et al. [50]	—[a]	
Yeung et al. [51]	—[a]	
Ochsenbein et al. [52]	Actin and vinculin, immunolabeling	All materials induced a normal cytoskeleton and well-developed focal adhesion contacts
Liu et al. [53]	—[a]	

[a] Not found

[b] Read from the graph

Cell Culture Experiments—Results

Cell proliferation was usually investigated by counting with the use of hemacytometer grid and expressed as cell number per square centimeter. The cells were then used in cell viability assay, and measurement of the content of cell protein was performed. Cell viability was assessed by staining and counting the number of cells. Stained cells were dead cells, because the dye penetrated into their interior. The results are presented as percent of living cells (not stained). Evaluation of cell protein content is based on dissolution of cell membrane with detergent. This enables to obtain protein lysates from cells. In the method, a calibration curve is prepared. The curve is based on colorimetric determination of bovine serum albumin (Table 4).

The results of viability and proliferation tests showed the following biocompatibility order: Stst>NiTi>Ti and Ti alloys>Ni. The determination of dead vs. live cells revealed the following sequence: NiTi>Ti>Stst>Ni. Cell surviving showed the best properties of Nitinol>control, Pt, Pd>Au>Ag>>Cu [45]. No changes in morphology and no strong negative effect on proliferation and differentiation of OB were detected (Table 4).

Table 5 reports descriptive results of phenotypic/genotypic markers.

Phenotypic/Genotypic Markers—Tests and Results

In the papers discussed in the present systematic review, the response of OB to the materials was determined by investigation of phenotypic markers, including biochemical (ALP, CICP, OC, MTT (enzymatic reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), NO (nitric oxide), immunoassays) and histochemical (identification of ALP positive cells, as well as calcium and phosphates deposits) tests [42]. ALP is used to determine cell viability by the measurement of conversion of p-nitrophenylphosphate into p-nitrophenol which is then analyzed colorimetrically. OC was assessed to determine its production capacity by OB and was determined by competitive radioimmunoassay with the use of polyclonal antibodies. ALP and OC are the measures of cell viability. CICP was a biochemical indicator of collagen production. In some works, transforming growth factor beta (TGF-β1) was evaluated. This is a protein that controls proliferation, cellular differentiation, and other functions in cells. Cytochemical assays (actin and vinculin labeling) were measured to determine cytoskeleton development. Enzymatic reduction of MTT assay was used to evaluate both cell viability and proliferation [42]. Also, genotypic markers were determined: DNA laddering and TUNEL assay [43].

Among phenotypic markers, practically in all the studies, ALP was determined, frequently OC and CICP [33–35, 38–42, 44, 45]. Less often, interpreted markers included MTT [37], PICP (pro-collagen I) [39, 44], TGF-β1 [39, 44], IL-6 [39, 44], actin, and vinculin [52]. In some cases, also genotoxicity tests were conducted [40], including DNA laddering and TUNEL assay [43]. Also, immune assays were performed [52]. The results of measurements of phenotypic markers were in accordance with the results obtained in cell culture experiments (proliferation and viability).

The measured values of phenotypic markers (ALP, OC, and CICP) were usually better for implanted materials, as compared with the not implanted ones [33, 35, 37, 38, 40, 41]. In particular, this concerned implanted NiTi. The results for Stst were not so clear—one paper showed the improvement of biocompatibility [35], another, the decrease [33]. It was confirmed that the presence of metal ions in the growth medium in which OB were cultivated had inhibitory effect also on phenotypic markers [42, 45].

Adhesion of Cells

Adhesion of cells was found to be related with the properties of the materials, in particular with their morphology, roughness, and wettability [53]. It is thought that the main role in the initial adhesion of cells was played by wettability [53].

Cell Culture Experiments on Fibroblasts

Bordji et al. [33] investigated FB and determined fibronectin and type I collagen (CICP). High content of fibronectin in cells cultivated on untreated materials, N-implanted, and C-doped was detected. Ryhänen et al. [34] also carried out cell culture experiments on human fibroblasts and evaluated cell proliferation and contact with the tested material. The results of cell culture of FB were similar to OB. Bogdanski et al. [36] studied fibroblasts from murine 3 T3 (proliferation and adhesion). Montanaro et al. [40] carried out experiments on FB from mouse (FB L929). Cells cultured on oxidized surfaces showed higher ALP and OC levels. Osteoblasts appeared more sensitive than fibroblasts in cytocompatibility tests [33].

General Conclusions from the Studies

Solution Containing Ions

It was found that parameters of biocompatibility were correlated with the level of ions released from the materials [45]. Morais et al. [42] identified decrease in the expression of the osteoblast phenotypic markers if the cells were cultivated in the solution of metal ions. Slight effects of released ions on osteoblast phenotypic markers and negative impact on tissue mineralization ability were confirmed [42]. The strongest effect was found for Ni ions [42]. ALP was most rapidly produced on the 14th day of culture in the solution containing Ni ions [42]. The percentage of simulation of MTT reduction was higher than the percentage of increase of ALP activity in the presence of metal ions vs. control [42].

Growth of MG63 on Stst was not negatively influenced as compared to alloy of Ti and the control [39]. This confirmed good biocompatibility of Stst with orthopedic application [39]. Biocompatibility of P558 and osteointegration were better in P558 than in other materials [44]. However, Yeung et al. found that among the tested materials, the worst properties were showed by Stst [51].

Good biocompatibility up to 50% of Ni content was found—50:50% Ni/Ti [36]. NiTi was well tolerated by osteoblastic type ROS-17 cells [43]. Also, other studies showed good biocompatibility of NiTi and modified NiTi [48, 49]. Biocompatibility of Nitinol with human osteoblasts and fibroblasts was also good according to Ryhänen et al. [34].

Pure Ti showed the best biocompatibility among the tested materials (proliferation and enzymatic activity) [35].

Good in vitro biocompatibility of Ni-free alloy was confirmed. Therefore, it was concluded that the material can be potentially used in orthopedics [40].

The Effect of Treatment on Biocompatibility

Practically in all the studies, the advantageous effect of various treatment techniques on biocompatibility of the materials was proved [41]. Yeung et al. found that biocompatibility was improved by the implantation with N_2, C_2H_2, and O_2 [47]. Yeung et al. [53] confirmed that the release of Ni ions was reduced, as compared with NiTi which was not treated.

The most biocompatible materials were those treated by nitrogen implantation [46]. Dramatic cellular reactions were observed in contact with nitrided Stst [33]. N-PIII significantly improved the biocompatibility and mechanical properties [50]. Nitridization resulted in increased proliferation of cells [51]. Oxide layers were thin, pure, and nanostructured [52].

Plasma-treated materials showed better cytocompatibility, improved adhesion, and proliferation of OB [46]. Plasma implantation caused reduced delamination. Consequently, N-PIII significantly improved biocompatibility of NiTi [51]. Ion-implanted and carbon-coated materials were more biocompatible [33]. Cells adhered better and grew faster on the materials treated with CO_2 laser [37].

Conditions of Treatment

Conditions of treatment had substantial effect on the properties of the materials. Cytocompatibility diminished if the alloys were oxidized at higher temperatures [48, 49]. Cell cultures (no cytotoxicity) and results of additional experiments showed the best cytocompatibility of NiTi alloys oxidized at 450°C [50]. Nanoscale surface morphology altered by the implantation frequencies affected the surface free energy and wettability of the NiTi surfaces. Osteoblast adhesion behavior and proliferation were affected [53].

Cell Culture Experiments—Proliferation, Viability, Phenotypic, and Genotypic Markers

Different responses of osteoblasts to the materials were due to the mutual action and coadjustment of different interrelated surface parameters [52]. The biomaterials did not exert any significant deleterious effects on the osteoblasts. Adhesion and matrix mineralization were not modified [38]. Consequently, the conclusion was that the tested materials did not interfere with physiological functions of OB [38]. No alteration in the production of ALP, NO, and PICP was observed [39]. Also, Montanaro et al. found no significant cytotoxicity and genotoxicity of the alloys [40]. P558 enhanced osteoblast differentiation, as confirmed by ALP [44]. The effect of P558 on osteoblast viability, PICP, TGF β-1, and tumor necrosis factor-α production did not significantly differ from Ti6Al4V and controls [44].

Discussion

Despite the potential toxicity of metals, alloys are widely used in, e.g., orthodontics and orthopedics, because of their unique properties: elasticity and shape memory, stiffness, hardness, endurance to tensile stresses, which prevail their application over other materials [1]. Many of metals, which are components of alloys of which orthodontic devices are made, have been identified as mutagenic, cytotoxic, and allergenic [54–68]. It is widely accepted that ions of toxic metals released in measurable amounts can cause cytotoxic effects. At present, it has not been justified, if, e.g., orthodontic treatment is related with exposure to metal ions in toxic doses from biomaterials used in therapy [9, 10].

Generally, two classes of biocompatibility tests are distinguished: screening and specific toxicity tests. The first category is rather qualitative and the latter quantitative. Screening tests included investigation of biological effects under extreme testing conditions. The second group of assays studied multiple doses in order to determine the threshold level and also the measures of acute, sub-chronic, chronic, and lifetime exposure to a given material

or toxic substances released from them. Studies which investigate biocompatibility of materials used in orthodontics often include cytotoxicity assays in cell cultures, where proliferation, viability, morphology, lysis, propagation, and enzymatic activity were assessed [6]. Also, the molecular mechanisms were checked by investigation of increased expression and DNA binding activity of transcription factor which is critical for differentiation of OB [69]. Cytotoxicity tests should follow the requirements of ISO 10,993 guidelines and are divided into extract, direct contact, and indirect contact methods [7, 8].

In the papers discussed in the present systematic review, tests included the cultivation of OB in the medium in which the material was immersed or the cultivation on the surface of the material. However, many papers underlined the importance of biological corrosion caused by the attack of oral cavity flora on the materials. This is the cause that the quantity of metal ions released does not reflect the conditions of oral cavity. Actually, the contribution of microbial corrosion in the overall corrosion of dental materials has not been established [70].

The conclusion from practically all the studies was that the effect of material treatment on biocompatibility was advantageous. Better results (in some cases increased biocompatibility as compared with control) were obtained if the materials were implanted—the best results were obtained for oxidation (of, e.g., NiTi) and nitridation (of, e.g., NiTi, Ti). The formation of surface oxides or nitrides hindered the release of ions and increased proliferation and adhesion. Modification of the materials using high voltages, high frequencies, or high intensity CO_2 laser treatment improved the biocompatibility of the materials, however, up to a threshold value.

The desired properties of the materials were connected with their application. Adhesion (cell attachment) of OB cells to surfaces of different materials, such as titanium, titanium alloys, or Co–Cr alloys, seemed to be more relevant in the clinical assessment of orthopedic/dental implants than in orthodontics. Physical properties of the surface (roughness, grooves) play an important role in osteointegration of orthopedic devices [71]. Adhesion of cells was found to be related with the properties of the materials, including their morphology, roughness, and wettability [53]. It is thought that the main role in the initial adhesion of cells was played by wettability.

The objective of the performed in vitro experiments on osteoblasts was to simulate the conditions occurring in an organism of human, whereby a given material (implants) or solution containing ions released from the material (orthodontic devices) stayed in contact with OB. However, it is necessary to bear in mind that the conditions of OB cultivation under artificial laboratory conditions substantially differ from in vivo environment. For instance, in the cell culture experiments, evaluated human OB and OB-like, and animal OB, undergo shear stress effects throughout laboratory restricted cultivation techniques [69]. Also, the experiments were carried out under the regime of sterile environment, meaning that microorganisms were absent. While these conditions were consistent with testing orthopedic materials, they differ from the conditions of oral environment, whereby microbial corrosion plays an important role in the deterioration of biocompatibility of the materials [72]. So far, no one has evaluated the total dose to which a patient is exposed during the whole orthodontic treatment. This is related with difficulties of using invasive biomarkers of exposure from human patients.

Studies have been performed to investigate the possible connection between changes in clinical chemistry patterns in blood after the removal of dental amalgams [73] or changes of trace elements in erythrocytes/plasma, also after amalgams/other metal alloy removal [74]. Other studies concerning caries susceptibility with the relation to trace metal concentration in saliva of primary school children have been performed [75]. Additional field of the

assessment of exposure to trace elements discussed in the literature were teeth, evaluated to reveal the relation of tooth element content of diabetics and hypertensives [76]. There is the lack of long-term studies or papers discussing effects/consequences after the treatment had been finished. Therefore, so far in the available literature, unknown are toxicological aspects related with orthodontic treatment, including dose–response relationship or estimation of the effect of the duration of the treatment and the type of the appliance used on the dose of metals released in an organism of a patient.

Conclusions

The conclusions which arose from the studies included in the present systematic review were that a variety of measures of OB function, including enzyme production, synthesis of collagen, and non-collagenous proteins, mineral production, DNA synthesis were not strongly affected by the tested materials. The conclusions from the papers are recommendations for the manufacturing process which should include the formation of artificial passivation layer. This would eliminate the stage of initial release of metal ions immediately after placement of the material in an organism of human, a phenomenon which was confirmed practically in all the papers.

The general conclusions on cytocompatibility of the materials are as follows:

1. No negative dramatic effect of the materials on proliferation, viability, or morphology (with the exception of Cu in one study) was detected.
2. Viability and proliferation rate were the highest in Stst, subsequently, NiTi, Ti, and its alloys, Ni. Such conclusions arose in the majority of papers, with some exceptions which appeared in experiments on animal osteoblasts.
3. Generally, covering the materials with the layer of oxides or nitrides by using different techniques and different conditions improved all biocompatibility indicators, by decreasing the susceptibility of the materials to deterioration.

References

1. Breme J, Biehl V (1998) Metallic biomaterials. In: Black J, Hastings G (eds) Handbook of biomaterial properties. Chapman & Hall, London, pp 135–137
2. Weber JH (2001) Nitinol as a biomedical material. In: Buschow KH, Jürgen KH, Cahn RW, Flemings MC, Ilschner B, Kramer EJ, Mahajan S (eds) Encyclopedia of materials—science and technology, vol 1–11. Elsevier, Amsterdam, pp 6155–6160
3. Williams DF (2001) Biomedical and dental materials: introduction. In: Buschow KH, Jürgen KH, Cahn RW, Flemings MC, Ilschner B, Kramer EJ, Mahajan S (eds) Encyclopedia of materials—science and technology, vol 1. Elsevier, Amsterdam, pp 584–592
4. Black J, Hastings G (1998) Handbook of biomaterial properties. Springer, New York
5. Williams DF (1998) In: de Putter C, de Lange K, de Groot K, Lee AJC (eds) Advances in biomaterials. Elsevier, Amsterdam, pp 11–16

6. Es-Souni M, Es-Souni M, Fischer-Brandies H (2005) Assessing the biocompatibility of NiTi shape memory alloys used for medical applications. Anal Bioanal Chem 381:557–567

7. ISO 10993-5:2009 Biological evaluation of medical devices—Part 5: tests for in vitro cytotoxicity

8. American Society for Testing and Materials (ASTM) F136. Accessed 1 June 2010

9. Mikulewicz M, Chojnacka K (2010) Trace metal release from orthodontic appliances by in vivo studies: a systematic literature review. Biol Trace Elem Res. doi:10.1007/s12011-009-8576-6

10. Mikulewicz M, Chojnacka K (2010) Release of metal ions from orthodontic appliances by in vitro studies: a systematic literature review. Biol Trace Elem Res. doi:10.1007/s12011-010-8670-9

11. Arslan E, Iğdil MC, Yazici H, Tamerler C, Bermek H, Trabzon L (2008) Mechanical properties and biocompatibility of plasma-nitrided laser-cut 316 L cardiovascular stents. J Mater Sci Mater Med 19:2079–2086

12. Berger-Gorbet M, Broxup B, Rivard C, Yahia LH (1996) Biocompatibility testing of NiTi screws using immunohistochemistry on sections containing metallic implants. J Biomed Mater Res 32:243–248

13. Bogdanski D, Köller M, Bram M, Stöver D, Buchkremer HP, Choi J, Epple M, Muhr G (2002) Rapid analysis of biocompatibility with graded test samples exemplified by Ni–NiTi–Ti. Biomed Tech 47 (1):500–502

14. Bombonato-Prado KF, Bellesini LS, Junta CM, Marques MM, Passos GA, Rosa AL (2009) Microarray-based gene expression analysis of human osteoblasts in response to different biomaterials. J Biomed Mater Res A 88:401–408

15. Bosetti M, Massè A, Tobin E, Cannas M (2002) Silver coated materials for external fixation devices: in vitro biocompatibility and genotoxicity. Biomaterials 23:887–892

16. Brors D, Aletsee C, Schwager K, Mlynski R, Hansen S, Schäfers M, Ryan AF, Dazert S (2002) Interaction of spiral ganglion neuron processes with alloplastic materials in vitro. Hear Res 167:110–121

17. Brunot C, Ponsonnet L, Lagneau C, Farge P, Picart C, Grosgogeat B (2007) Cytotoxicity of polyethyleneimine (PEI), precursor base layer of polyelectrolyte multilayer films. Biomaterials 28:632–640

18. Díaz M, Sevilla P, Galán AM, Escolar G, Engel E, Gil FJ (2008) Evaluation of ion release, cytotoxicity, and platelet adhesion of electrochemical anodized 316 L stainless steel cardiovascular stents. J Biomed Mater Res B Appl Biomater 87:555–61

19. González-Carrasco JL, Ciapetti G, Montealegre MA, Pagani S, Chao J, Baldini N (2005) Evaluation of mechanical properties and biological response of analumina-forming Ni-free ferritic alloy. Biomaterials 26:3861–3871

20. Gough JE, Downes S (2001) Osteoblast cell death on methacrylate polymers involves apoptosis. J Biomed Mater Res 57:497–505

21. Kapanen A, Danilov A, Lehenkari P, Ryhänen J, Jämsä T, Tuukkanen J (2002) Effect of metal alloy surface stresses on the viability of ROS-17/2.8 osteoblastic cells. Biomaterials 23:3733–3740

22. Kapanen A, Kinnunen A, Ryhänen J, Tuukkanen J (2002) TGF-beta1 secretion ofROS-17/2.8 cultures on NiTi implant material. Biomaterials 23:3341–3346

23. Li Q, Zhang Q, Sun Z (2006) The study on biocompatibility of diamond-like carbon coated nickel–titanium shape memory alloy with osteoblasts cultured in vitro. Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi 20:5–8

24. Macnair R, Rodgers EH, Macdonald C, Wykman A, Goldie I, Grant MH (1997) The response of primary rat and human osteoblasts and an immortalized rat osteoblast cell line to orthopaedic materials: comparative sensitivity of several toxicity indices. J Mater Sci Mater Med 8:105–111

25. Misra RD, Thein-Han WW, Mali SA, Somani MC, Karjalainen LP (2010) Cellular activity of bioactive nanograined/ultrafine-grained materials. Acta Biomater 6:2826–2835

26. Morais S, Pereira MC (2000) Application of stripping voltammetry and microelectrodesin vitro biocompatibility and in vivo toxicity tests of AISI 316 L corrosion products. J Trace Elem Med Biol 14:48–54

27. Nicula R, Lüthen F, Stir M, Nebe B, Burkel E (2007) Spark plasma sintering synthesis of porous nanocrystalline titanium alloys for biomedical applications. Biomol Eng 24:564–567

28. Petrolati M, Abbiati G, Delaria G, Soffiatti R, Robotti P, Guerriero C (1999) A new prosthesis for the metacarpophalangeal joint. Study of materials and biomechanics. J Hand Surg Br 24:59–63

29. Santavirta S, Gristina A, Konttinen YT (1992) Cemented versus cementless hiparthroplasty. A review of prosthetic biocompatibility. Acta Orthop Scand 63:225–232

30. Shahryari A, Azari F, Vali H, Omanovic S (2009) The positive influence of electrochemical cyclic potentiodynamic passivation (CPP) of a SS316LS surface on its response to fibronectin and pre-osteoblasts. Phys Chem Chem Phys 11:6218–6224

31. Tschon M, Fini M, Giavaresi G, Borsari V, Lenger H, Bernauer J, Chiesa R, Cigada A, Chiusoli L, Giardino R (2005) Soft tissue response to a new austenitic stainless steel with a negligible nickel content. Int J Artif Organs 28:1003–1011

32. Woodruff MA, Jones P, Farrar D, Grant DM, Scotchford CA (2007) Human osteoblast cell spreading and vinculin expression upon biomaterial surfaces. J Mol Histol 38:491–499

33. Bordji K, Jouzeau JY, Mainard D, Payan E, Delagoutte JP, Netter P (1996) Evaluation of the effect of three surface treatments on the biocompatibility of 316L stainless steel using human differentiated cells. Biomaterials 17:491–500

34. Ryhänen J, Niemi E, Serlo W, Niemelä E, Sandvik P, Pernu H, Salo T (1997) Biocompatibility of nickel–titanium shape memory metal and its corrosion behavior in human cell cultures. J Biomed Mater Res 35:451–457

35. Schmidt C, Ignatius AA, Claes LE (2001) Proliferation and differentiation parameters of human osteoblasts on titanium and steel surfaces. J Biomed Mater Res 54:209–215

36. Bogdanski D, Köller M, Müller D, Muhr G, Bram M, Buchkremer HP, Stöver D, Choi J, Epple M (2002) Easy assessment of the biocompatibility of Ni–Ti alloys by in vitro cell culture experiments on a functionally graded Ni–NiTi–Ti material. Biomaterials 23:4549–4555

37. Hao L, Lawrence J, Phua YF, Chian KS, Lim GC, Zheng HY (2005) Enhanced human osteoblast cell adhesion and proliferation on 316 LS stainless steel by means of CO_2 laser surface treatment. J Biomed Mater Res B Appl Biomater 73:148–156

38. Riccio V, Della Ragione F, Marrone G, Palumbo R, Guida G, Oliva A (1994) Cultures of human embryonic osteoblasts. A new in vitro model for biocompatibility studies. Clin Orthop Relat Res 308:73–78

39. Torricelli P, Fini M, Borsari V, Lenger H, Bernauer J, Tschon M, Bonazzi V, Giardino R (2003) Biomaterials in orthopedic surgery: effects of a nickel-reduced stainless steel on in vitro proliferation and activation of human osteoblasts. Int J Artif Organs 26:952–957

40. Montanaro L, Cervellati M, Campoccia D, Arciola CR (2006) Promising in vitro performances of a new nickel-free stainless steel. J Mater Sci Mater Med 17:267–275

41. Michiardi A, Engel E, Aparicio C, Planell JA, Gil FJ (2008) Oxidized NiTi surfaces enhance differentiation of osteoblast-like cells. J Biomed Mater Res A 85:108–114

42. Morais S, Sousa JP, Fernandes MH, Carvalho GS (1998) In vitro biomineralization by osteoblast-like cells. I. Retardation of tissue mineralization by metal salts. Biomaterials 19:13–21

43. Kapanen A, Ilvesaro J, Danilov A, Ryhänen J, Lehenkari P, Tuukkanen J (2002) Behaviour of Nitinol in osteoblast-like ROS-17 cell cultures. Biomaterials 23:645–650

44. Fini M, Nicoli Aldini N, Torricelli P, Giavaresi G, Borsari V, Lenger H, Bernauer J, Giardino R, Chiesa R, Cigada A (2003) A new austenitic stainless steel with negligible nickel content: an in vitro and in vivo comparative investigation. Biomaterials 24:4929–4939

45. Cortizo MC, De Mele MF, Cortizo AM (2004) Metallic dental material biocompatibility in osteoblast like cells: correlation with metal ion release. Biol Trace Elem Res 100:151–168

46. Yeung KW, Poon RW, Liu XY, Ho JP, Chung CY, Chu PK, Lu WW, Chan D, Cheung KM (2005) Investigation of nickel suppression and cytocompatibility of surface-treated nickel–titanium shape memory alloys by using plasma immersion ion implantation. J Biomed Mater Res A 72:238–245

47. Yeung KW, Poon RW, Liu XY, Ho JP, Chung CY, Chu PK, Lu WW, Chan D, Cheung KM (2005) Corrosion resistance, surface mechanical properties, and cytocompatibility of plasma immersion ion implantation-treated nickel–titanium shape memory alloys. J Biomed Mater Res A 75:256–267

48. Wu S, Liu X, Chan YL, Ho JP, Chung CY, Chu PK, Chu CL, Yeung KW, Lu WW, Cheung KM, Luk KD (2007) Nickel release behavior, cytocompatibility, and superelasticity of oxidized porous single-phase NiTi. J Biomed Mater Res A 81:948–955

49. Wu SL, Chu PK, Liu XM, Chung CY, Ho JP, Chu CL, Tjong SC, Yeung KW, Lu WW, Cheung KM, Luk KD (2006) Surface characteristics, mechanical properties, and cytocompatibility of oxygen plasma-implanted porous nickel titanium shape memory alloy. J Biomed Mater Res A 79:139–146

50. Liu XM, Wu SL, Chan YL, Chu PK, Chung CY, Chu CL, Yeung KW, Lu WW, Cheung KM, Luk KD (2007) Surface characteristics, biocompatibility, and mechanical properties of nickel–titanium plasma-implanted with nitrogen at different implantation voltages. J Biomed Mater Res A 82:469–478

51. Yeung KW, Poon RW, Chu PK, Chung CY, Liu XY, Lu WW, Chan D, Chan SC, Luk KD, Cheung KM (2007) Surface mechanical properties, corrosion resistance, and cytocompatibility of nitrogen plasma-implanted nickel-titanium alloys: a comparative study with commonly used medical grade materials. J Biomed Mater Res A 82:403–414

52. Ochsenbein A, Chai F, Winter S, Traisnel M, Breme J, Hildebrand HF (2008) Osteoblast responses to different oxide coatings produced by the sol–gel process on titanium substrates. Acta Biomater 4:1506–1517

53. Liu XM, Wu SL, Chu PK, Chung CY, Chu CL, Chan YL, Lam KO, Yeung KW, Lu WW, Cheung KM, Luk KD (2009) Nano-scale surface morphology, wettability and osteoblast adhesion on nitrogen plasma-implanted NiTi shape memory alloy. J Nanosci Nanotechnol 9:3449–3454

54. Lewis CG, Sunderman FW (1996) Metal carcinogenesis in total joint arthroplasty. Animal models. Clin Orthop 329(suppl):264–268

55. Kasprzak KS, Bialkowski K (2000) Inhibition of antimutagenic enzymes, 8-oxo-dGTpases, by carcinogenic metals. J Inorg Biochem 79:231–236
56. Pereira ML, Silva A, Tracana R, Carvalho GS (1994) Toxic effects caused by stainless steel corrosion products on mouse seminiferous cells. Cytobios 77:73–80
57. Veien NK, Bochhorst E, Hattel T, Laurberg G (1994) Stomatitis or systemically-induced contact-dermatitis. Contact Dermat 30:210–213
58. Al-Waheidi EM (1995) Allergic reaction to nickel orthodontic wire: a case report. Quintessence Int 26:385–387
59. Dunlap CL, Kirk Vincent S, Barker BF (1989) Allergic reaction to orthodontic wire: report of a case. J Am Dent Assoc 118:449–450
60. Basketter DA, Briatico-Vangosa G, Kaestner W, Lally C, Bontinck WJ (1993) Nickel, cobalt and chromium in consumer products: a role in allergenic contact dermatitis? Contact Dermat 28:15–25
61. Bass JK, Fine H, Cisneros GJ (1993) Nickel hypersensitivity in the orthodontic patient. Am J Orthod Dentofac Orthop 103:280–285
62. Greig DGM (1983) Contact dermatitis reaction to a metal buckle on a cervical headgear. Br Dent J 155:61–62
63. Hayes RB (1997) The carcinogenicity of metals in humans. Cancer Causes Control 8:371–385
64. Janson GRP, Dainesi EA, Consolaro A, Woodside DG, de Freitas MR (1998) Nickel hypersensitivity reaction before, during, and after orthodontic therapy. Am J Orthod Dentofacial Orthop 113:655–660
65. Jensen CS, Menné T, Lisby S, Kristiansen J, Veien NK (2003) Experimental systemic contact dermatitis from nickel: a dose response study. Contact Dermat 49:124–132
66. Kolokitha O, Kaklamanos EG, Papadopoulos MA (2008) Prevalence of nickel hypersensitivity in orthodontic patients: a meta-analysis. Am J Orthod Dentofacial Orthop 134:722.e1–722.e12
67. Kerosuo H, Kullaa A, Kerosuo E, Kanerva L, Hensten-Pettersen A (1996) Nickel allergy in adolescents in relation to orthodontic treatment and piercing of ears. Am J Orthod Dentofacial Orthop 109:148–154
68. Oller AR, Costa M, Oberdorster G (1997) Carcinogenicity assessment of selected nickel compounds. Toxicol Appl Pharmacol 143:152–166
69. Ethieer CR, Simmons CA (2007) Cellular biomechanics, biomechanics—from cells to organisms. Cambridge University Press, Cambridge, pp 97–98
70. Fors R, Persson M (2006) Nickel in dental plaque and saliva in patients with and without orthodontic appliances. Eur J Orthod 28:292–297
71. Swee HT (2004) Engineering materials for biomedical applications. World Scientific, Singapore
72. Perego P, Fabiano B (1999) Corrosion, microbial. In: Flickinger MC, Drew SW (eds) Encyclopedia of bioprocess technology: fermentation, biocatalysis and bioseparation. Wiley, New York, pp 717–726
73. Frisk P, Lindvall A, Hudecek R, Lindh U (2006) Decrease of trace elements in erythrocytes and plasma after removal of dental amalgam and other metal alloys. Biol Trace Elem Res 113:247–259
74. Frisk P, Danersund A, Hudecek R, Lindh U (2007) Changed clinical chemistry pattern in blood after removal of dental amalgam and other metal alloys supported by antioxidant therapy. Biol Trace Elem Res 120:163–170
75. Shigemi T, Tanaka T, Hayashida Y, Maki K (2008) Study of salivary strontium and silver concentrations in primary school children related to dental caries. Biol Trace Elem Res 123:80–90
76. Nagaraj G, Sukumar A, Nandlal B, Vellaichamy S, Thanasekaran K, Ramanathan AL (2009) Tooth element levels indicating exposure profiles in diabetic and hypertensive subjects from Mysore, India. Biol Trace Elem Res 131:255–262