

# Cytotoxicity and ion release of alloy nanoparticles

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**Abstract** It is well-known that nanoparticles could cause toxic effects in cells. Alloy nanoparticles with yet unknown health risk may be released from cardiovascular implants made of Nickel–Titanium or Cobalt–Chromium due to abrasion or production failure. We show the bio-response of human primary endothelial and smooth muscle cells exposed to different concentrations of metal and alloy nanoparticles. Nanoparticles having primary particle sizes in the range of 5–250 nm were generated using laser ablation in three different solutions avoiding artificial chemical additives, and giving access to formulations containing nanoparticles only stabilized by biological ligands. Endothelial cells are found to be more sensitive to nanoparticle exposure than smooth muscle cells. Cobalt and Nickel nanoparticles caused the highest cytotoxicity. In contrast, Titanium, Nickel–

Iron, and Nickel–Titanium nanoparticles had almost no influence on cells below a nanoparticle concentration of 10  $\mu$ M. Nanoparticles in cysteine dissolved almost completely, whereas less ions are released when nanoparticles were stabilized in water or citrate solution. Nanoparticles stabilized by cysteine caused less inhibitory effects on cells suggesting cysteine to form metal complexes with bioactive ions in media.

**Keywords** Nanoparticles · Nickel–Titanium · Cobalt · Endothelial cells · Smooth muscle cells · Ligands · Environmental and health effects

## Introduction

Nickel–Titanium (NiTi) and Cobalt–Chromium (CoCr) alloys are important materials for biomedical applications and are widely used for cardiovascular implants (Vogel et al. 2003; Shen et al. 2009; Huang et al. 2009). Owing to failure during production (e.g., residual particles from laser cutting), or wear, or abrasion of metallic implants nanoparticles might be unintentionally released into the body and are known to cause adverse effects (Webster 2007; Case et al. 1994; Papageorgiou et al. 2007; Borm et al. 2006). The properties of these unintentional released particles are not qualified up to now and particle-size distribution is expected to be relatively broad (Case et al. 1994). Furthermore, the lifetime and dissolution behaviour of

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the nanoparticles might be different depending on the type and local environment of the implant.

As of now, a variety of studies dealing with the cytotoxicity of nanoparticles exist (Oberdörster et al. 2004; Dahl et al. 2009; Studer et al. 2010; Borm and Kreyling 2004; Bhabra et al. 2009; Gojova et al. 2007; Horie et al. 2011), but statements on nanoparticle toxicity should be viewed carefully due to the complexity of the mechanisms determining the interactions at the bio-nano interface (Nel et al. 2009). The concentration and size of nanoparticles, their up-take mechanism, their bonding characteristics on cell surfaces, or even the characteristics of the exposed tissue influence cytotoxicity. Hence, it is substantial that nanoparticle toxicity needs to be determined by choosing an appropriate nanoparticle material and a relevant cell type with regard to the present in vivo conditions (Neuss et al. 2008). Thus, the bio-response of human endothelial cells (EC) and smooth muscle cells (SMC) derived from coronary arteries has yet to be determined in particular for alloy nanoparticles when considering stents as one example of cardiovascular implants. In particular, nanoparticles released from stents made of alloys-like NiTi might cause adverse effects since Nickel (Ni) compounds are known to be toxic (Costa et al. 1981; Schwerdtle and Hartwig 2006). Cell response to Ni-nanoparticles and -alloy nanoparticles should be examined as well, with the intention to determine the toxic potential of Ni as alloying element in nanoparticles.

Laser ablation of solid targets in liquids is a method to generate nanoparticles of a variety of materials in different liquids without impurities or precursors (Kazakevich et al. 2004; Kabashin and Meunier 2003; Mafuné et al. 2003). No alternative method is able to provide alloy nanoparticles made of the same material than the implant, in particular alloy nanoparticles-like NiTi. Moreover, nanoparticles generated via this method have a relatively broad-size distribution (Menendez-Manjon and Barcikowski 2011) similar to the undefined size distribution expected from particles released during implant abrasion or wear. Another advantage of this method is the flexible choice of the carrier fluid which might vary from pure distilled water to organic solvent doped with silicone (Petersen and Barcikowski 2009a, b; Hahn and Barcikowski 2009). Hence, the generation of stabilised nanoparticles as well as ligand-free nanoparticles is possible (Petersen et al. 2009). In situ stabilisation of

nanoparticles while adding ligands to the carrier fluid enables the design of the nanoparticles surface (Besner et al. 2006; Petersen and Barcikowski 2009b). This technique is suitable to provide a variety of nanoparticulate materials for toxicity assessment in particular alloy nanoparticles like NiTi (Barcikowski et al. 2010). Overall, this method allows therefore a screening of nanoparticulate materials as well as the carrier fluid.

In fact, the aggregation state of nanoparticles in vivo is unknown and might change during their lifetime. If nanoparticles are unintentionally released into the body, the aggregation state is different from that of synthesised nanoparticles. It is unlikely that only colloidal stable and monodisperse nanoparticles are present near the implant. Furthermore, aggregation state depends also on the nanoparticle concentration and the surrounding fluid (Stebounova et al. 2011) and is yet unknown for nanoparticles released from alloy implants. Furthermore, nanoparticles taken up into cells are usually detected as aggregates or as nanoparticle complexes (Pujalté et al. 2011). Laser ablation aims to provide nanoparticles having primary particle sizes in the range of nanometres, but an aggregation of nanoparticles might also occur before their contact with cells during their transfer into cell culture media. We therefore focus on the impact of the nanoparticles with regard to their concentration and not on their aggregation state.

In our study, we demonstrate the bio-response of EC and SMC with five different colloidal metal nanoparticles (NiTi, Ni, Ti, NiFe, Co) generated in three different media (H<sub>2</sub>O, cysteine, and citrate solution). Proteins like bovine serum albumin are known to adsorb on the surface of nanoparticles via amino acid groups such as cysteine (Tsai et al. 2011). Cysteine and citrate are known to stabilise nanoparticles (Park et al. 2010; Brewer et al. 2005). Nanoparticles coated with cysteine and citrate may therefore be appropriate to imitate in vivo conditions since cysteine and citrate are components occurring naturally in the blood and might therefore adsorb to the unintentional released nanoparticle. Under in vivo conditions, a protein corona will form on the particle surface. Hence, the influence of the nanoparticle material is investigated together with the effects of the stabilizing agent.

The aim of this study is to evaluate the impact of alloy and metal nanoparticles which might be

unintentionally released from alloy implants. Since nanoparticles might dissolve *in vivo*, the metal ion release is investigated in addition. Although cytotoxicity of nanoparticles often is determined by the particle number or the surface of the nanoparticles (Oberdörster 2001), we investigated the molarity (molar mass per volume) in order to allow a comparison of nanoparticle doses with those of metal ions solely and furthermore with known toxic metal ion doses. Following this approach this study may contribute to the evaluation of the risk of an unintended release of alloy nanoparticles *in vivo*.

## Experimental

### Generation of nanoparticle colloids

The generation of nanoparticles was performed by femtosecond laser ablation of solid targets in liquids. Laser ablation of Ni, Ti, Ni<sub>50</sub>Ti<sub>50</sub>, Co, and Ni<sub>48</sub>Fe<sub>52</sub> was carried out using a femtosecond laser (Spitfire Pro, Spectra-Physics), emitting 150 fs laser pulses at 800 nm with a pulse energy of 200  $\mu$ J. A 4-axis positioning station (3D-Micromac) was used for sample movement with a speed of 1 mm s<sup>-1</sup>. The laser focus (focal length: 150 mm) was determined in air and the focus position in liquid was set as  $\Delta z = -4.6$  mm due to non-linear effects occurring during laser ablation in liquids (Petersen and Barcikowski 2009b). The target was placed in the centre of a vessel with a liquid layer of  $3.10 \pm 0.6$  mm. The vessel was filled with double distilled water (6.9 pH) or doubled distilled aqueous solution of cysteine or citrate with a concentration in the range of 0.01–20 mM. The pH of 5 mM L-cysteine solution (Sigma Aldrich) was 6.0 and pH of 5 mM sodium citrate tribasic dihydrate solution (Sigma Aldrich) was 7.5, respectively. The difference in the pH value of the solutions is expected to have no influence on the stability of the nanoparticles (Tsai et al. 2011).

### Analysis of nanoparticles

The nanoparticle concentration was estimated gravimetrically (R160P, Sartorius) by weighing the target before and after ablation. The zeta potential of nanoparticles was measured in triplicates using a Zetasizer ZS (Malvern Instruments Ltd). From measurements

mean values and standard deviation were calculated and given in the graph.

The particle-size distribution was determined using field emission scanning electron microscopy (FE-SEM) images. For the latter, a drop of the nanoparticle dispersions was placed on a polished graphite target, gently dried, and investigated by FE-SEM using a Quanta 400 FEG system (FEI, The Netherlands). The most probable Feret diameter was determined by taking the maximum of a log normal fit of each size distribution.

### Determination of metal ion concentration

In parallel to the preparation of the nanoparticle dilution subjected to the cytotoxicity tests, nanoparticle colloids were diluted 1:40 in endothelial cell growth medium MV (Promocell, see below) and incubated for 72 h. Nanoparticles were separated by centrifugation of nanoparticle suspensions for 30 min at 120,000 g. The supernatant containing the ions from nanoparticles was analysed by inductively coupled plasma optical emission spectrometry (ICP-OES, Varian).

### Evaluation of cell viability

Human coronary artery endothelial cells (hCAEC) and human coronary artery smooth muscle cells (hCASMC) were obtained from Promocell (Heidelberg, Germany). According to the recommendations of the manufacturer, the cells were cultured in endothelial cell growth medium MV and smooth muscle cell growth medium 2 (Promocell), respectively.

The cells were seeded into 96-well-plates. After 24 h they were treated with nanoparticle colloids in the concentration range of 0.1–1,000  $\mu$ M for 72 h. The dose exposed to the cells consists therefore of the solid nanoparticles and the metal ions. The stabilisation agents (water, 5 mM citrate, and 5 mM cysteine without nanoparticles served as negative control (100% viability)). The metabolic activity was determined using the CellTiter 96 Aqueous One reagent (MTS, Promega, Mannheim, Germany). Cell viability was carried out following ISO 10993-5/12 (2009a, b). The experiments were performed in triplicates and repeated three times. From measurements mean values and standard deviation were calculated and given in

**Table 1** The Feret diameter as well as the zeta potential of the laser-generated NiTi, Ti, Ni, Co, and NiFe nanoparticles

Nanoparticle material	Solvent	Feret diameter (FE-SEM) (range) (nm)	Feret diameter (FE-SEM) (most probable) (nm)	Zeta potential (mV)
Ti	Citrate	13–200	28	−46
	Cysteine	14–250	40	−35
	H <sub>2</sub> O	7–110	25	−50
Co	Citrate	14–75	28	−43
	Cysteine	14–120	44	−16
	H <sub>2</sub> O	5–30	13	+13
Ni	Citrate	10–150	45	−39
	Cysteine	20–110	32	−25
	H <sub>2</sub> O	25–100	36	+1
NiTi	Citrate	15–140	40	−45
	Cysteine	20–185	34	−32
	H <sub>2</sub> O	13–105	24	−12
NiFe	Citrate	15–175	34	−47
	Cysteine	30–200	100	−17
	H <sub>2</sub> O	14–125	58	+9

the graphs. The mean values were given in % of the negative control (“relative metabolic activity”) Table 1.

## Results

### In situ stabilisation of nanoparticles

To find a suitable concentration of ligands for electrosteric stabilization of the colloids, different concentrations of L-cysteine and sodium citrate were added prior to laser ablation of NiTi. Stability of NiTi nanoparticles is evidenced by an absolute zeta potential value higher than 30 mV. The zeta potential of NiTi nanoparticles is shown in Fig. 1.

The electrosteric stability of NiTi-nanoparticles generated in citrate solution is higher compared to cysteine due to the higher negative charge of the citrate anion compared to the amino acid cysteine.

The highest absolute zeta potential of nanoparticles was achieved using a cysteine and citrate concentration of 5 mM during laser ablation. Consequently, solutions having these ligand concentrations were

used to generate nanoparticles during laser ablation for the cytotoxicity tests.

As shown in the FE-SEM images in Fig. 1, the morphology of the nanoparticles is spherical. Alloy particles released from implants have been found to have similar morphologies (Case et al. 1994; Papa-georgiou et al. 2007). Laser-generated NiTi-nanoparticles show therefore good qualities as material for cytotoxicity tests.

However, the transfer of the nanoparticles from cysteine and citrate solution into the cell culture media might provoke a change in the properties of the nanoparticles, e.g., its aggregation state. This is not further investigated since the focus lays on the material screening having primary particle sizes in the nanoscale.

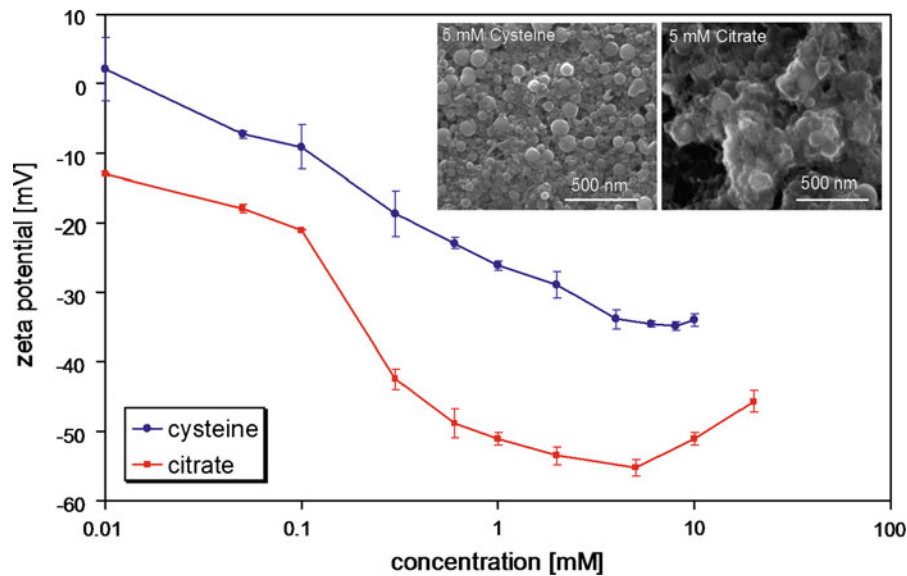
### Nanoparticle properties

The primary particle size ranged from 5 to 250 nm for all nanoparticles. All most probable primary particle diameters are below 100 nm. The smallest nanoparticles were generated during laser ablation of Co in H<sub>2</sub>O with a size distribution of 5–30 nm. The polydispersity is typical for laser-generated nanoparticles when the growth is not quenched (Petersen and Barcikowski 2009a). With the exception of Ni nanoparticles, all primary particles were smaller in water than in media containing ligands.

With the exception of Ti nanoparticles, all nanoparticles agglomerated (positive zeta potential) when generated in H<sub>2</sub>O. Overall, the highest absolute zeta potential (highest stability), for all metals except Ti, was achieved using citrate as stabilization agent.

It needs to be noted that the aggregation state from each material is different depending on the material and the ligand. Since the aggregation state of nanoparticles due to an unintentional release is not known, the molar mass was taken as reference value to allow a quantitative comparison during material screening.

Of course, a cytotoxicity study with identical particle size, aggregation state and mass concentration may be interesting from the point of view of identical dose. However, to the best of our knowledge, creating this kind of materials would be impossible without the use of strong binding, artificial chemical ligands (not present in vivo), with unknown effects of those ligands on the cytotoxicity of the bare material. In our approach, we tried to avoid the use of those size-controlling



**Fig. 1** Zeta potential of NiTi nanoparticle colloids generated during laser ablation in aqueous solutions with different concentrations of ligands; FE-SEM images of nanoparticles generated in 5 mM cysteine (*left*) and citrate (*right*) solution (*insets*)

ligands, with higher purity but less defined aggregation state. We have the feeling that these conditions may represent the nanotoxicity of implant abrasion in a better way from the point of view of purity of the nanoparticle material, avoiding artificial nanoparticle surface modifications.

#### Cell viability

The effect of metal and alloy nanoparticles on EC viability is shown in Fig. 2a. A 50% reduction of EC vitality was observed at 1–10  $\mu\text{M}$  for all Ni-containing nanoparticles. Overall, the EC metabolic activity decreased to zero in contact with 1 mM Co, Ni, NiTi, or NiFe nanoparticles. In contrast, the highest concentration of Ti (1 mM in water and citrate) caused only a mild reduction of the metabolic activity by less than 25%.

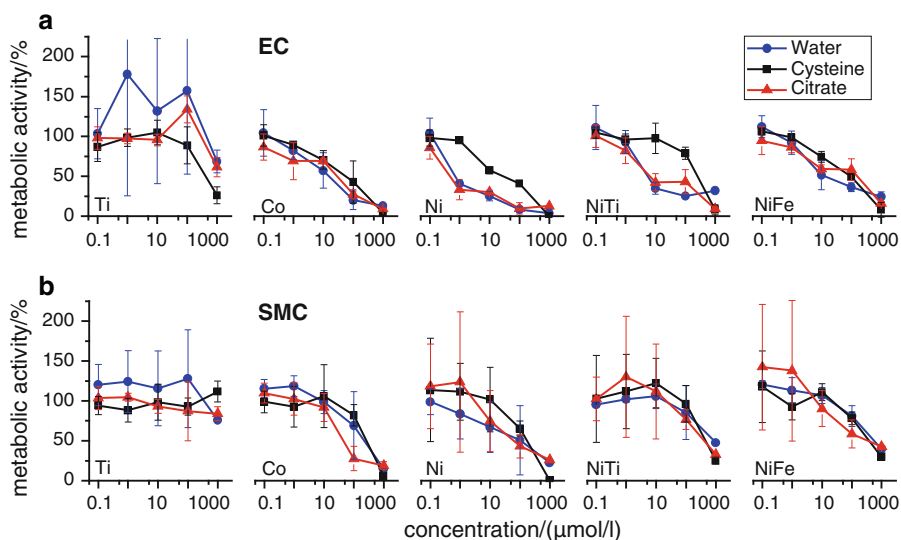
Dose–response curves of Ti, Co, Ni, NiTi, and NiFe for SMC viability are shown in Fig. 2b. Co and Ni nanoparticles had the highest cytotoxic effects among the nanomaterials tested, followed by the nanoparticle alloys NiTi and NiFe. At the highest concentration (1 mM) of NiFe and NiTi nanoparticles the residual metabolic activity of SMC was still 25%. Remarkably, 1 mM Ti did not show any significant influence on SMC viability.

In comparison to SMC, EC were more sensitive to the investigated nanoparticulate materials. Concentrations of 0.1  $\mu\text{M}$  (Ni, Co), 1  $\mu\text{M}$  (NiFe, NiTi), or 100  $\mu\text{M}$  (Ti) revealed a reduction of the EC metabolism by more than 20% (<80% metabolic activity). SMC reacted with a reduced metabolic activity (Fig. 2b) when incubated with nanoparticle concentrations at least one magnitude higher compared with the corresponding concentrations revealing the same effect on EC.

The toxic effect of the nanoparticles on EC and SMC was different depending on the ligand used for stabilisation. In general, the reduction of metabolic activity of SMC was slightly higher if the cells were exposed to nanoparticles stabilized with water or citrate compared to those stabilized with cysteine. For instance, Co nanoparticles generated in cysteine solution only slightly reduced SMC viability (by 20%) whereas Co nanoparticles in citrate solution reduced SMC metabolism by more than 70% when applied in a concentration of 100  $\mu\text{M}$  each. The water, cysteine and citrate solution without nanoparticles showed no significant influence on cells (data not shown) and served as the corresponding negative controls (100% viability).

The influences of ligands were even more obvious on metabolic activity of EC. The overall difference in

**Fig. 2** Metabolic activity of EC (a) and SMC (b) after  $72 \pm 2$  h incubation with different concentrations of Ti, Co, Ni, NiTi, and NiFe nanoparticles generated in water, citrate, or cysteine solution



the reduction of metabolic activity caused by the ligand ranged from 10 to 70%. In addition, the material-derived effects on metabolic activity are more distinctive in EC than in SMC. Overall, cysteine tended to reduce the adverse effect of all tested nanoparticles on metabolic activity of SMC and EC, except in case of Ti. This demonstrates that the surface modification of nanoparticles (by ligands present almost everywhere in the biological system) affects the toxicity of nanomaterials.

#### Metal ion release

It may be possible that the ligand influences the release characteristics of nanoparticles in physiological solutions. In order to study the effect of corrosion- and complexation-induced metal release, the metal ions released from the nanoparticle colloid with ligands were investigated depending on the ligand.

Thus, we analyzed the supernatant after centrifugation of 100  $\mu\text{M}$  nanoparticles incubated in cell culture media.

Figure 3 shows the ions of single elements released from nanoparticles with varying ligand. Results show that the dissolution of nanoparticles is highest when stabilised with cysteine, except in the case of Ti.

Co and Ni show the highest ion release. This means that Co nanoparticles stabilized by cysteine dissolve almost completely into ions (90%). More than 70% of

nanoparticulate Ni dissolves in the culture media when generated in cysteine.

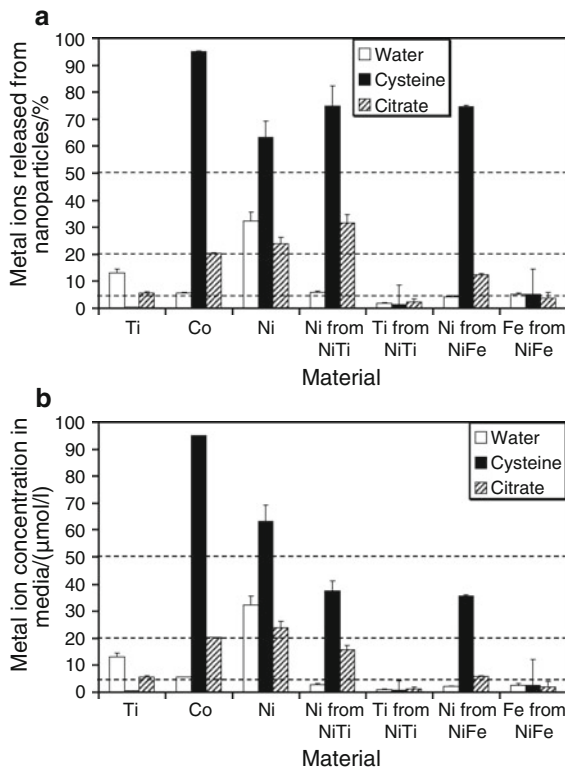
Nanoparticles generated in pure water release lower amounts of metal ions into culture media compared to cysteine-stabilized nanoparticles.

The rate of Ni ions released from Ni nanoparticles does not vary remarkably compared to those released from Ni alloy nanoparticles (Fig. 3a). However, the absolute mass concentration of Ni ions in solution was lower in case of Ni alloy nanoparticles and is half of the ion concentration of pure Ni (Fig. 3b).

#### Discussion

The properties of nanoparticle colloids are affected when entering a biological system (Gojova et al. 2007, Horie et al. 2009a, b). The nanomaterial-bio-interface is determined by several dynamic physicochemical interactions, kinetics, and thermodynamic exchanges between the surface of nanoparticles and biological systems (Gojova et al. 2007). Consequently, toxicity has to be evaluated with regard to effects caused by the solid nanoparticulate material together with the metal ions released from the nanoparticles. The risk from the nanoparticles is therefore attributed to a nanoparticle-metal ion complex.

Our results showed a higher sensitivity of EC to nanoparticle exposure compared to SMC. But, we observed similar order of sensitivity of EC and SMC



**Fig. 3** Ion release into cell culture media (incubation time: 72 h) depending on nanoparticle material (100 µM) and ligand (5 mM): relative ion release of each element from total element concentration in nanoparticles (a) and absolute released metal ion concentration (b)

on the nanoparticle material: Ni ≈ Co > NiFe ≈ NiTi > Ti.

At a concentration of 500 µM, Ti nanoparticles do not inhibit metabolic activity of both EC and SMC, whereas Co strongly inhibits metabolic activity. In agreement with our findings, Limbach et al. observed that at approximately the same nanoparticle concentration (30 µg mL<sup>-1</sup> ≈ 560 µM) the bio-response of lung epithelial cells depends on the nanomaterial it was exposed to. They found the following order of sensitivity: Co > Fe > Ti (Limbach et al. 2007).

Toxicity of Co and Ni is mainly attributed to the ion release of the metals (Papageorgiou et al. 2007; Schwerdtle and Hartwig 2006; Limbach et al. 2007; Hanawa 2004). Hence, the nanoparticles of metals releasing ions (Co, Ni) are more toxic than those which do not dissolve (Ti). Our results show that the cytotoxic concentration thresholds of alloy nanoparticles containing Ni and Co contents are one magnitude higher than that of the pure nanoparticles of these

elements. One might conclude that this is also attributed to the solubility of nanoparticles since the absolute amount of ions (Co, Ni) are lower in case of the alloy compared to the pure materials.

Ti nanoparticles show overall the lowest effect on cell viability. Similar to our results, recent studies showed that Ti nanoparticles compared to Ni or other nanoparticles are less toxic (Pujalté et al. 2011; Limbach et al. 2007; Horie et al. 2009b). TiO<sub>2</sub> nanoparticles are known to adsorb high concentrations of proteins on the surface without toxic effects on cells and without releasing ions into cell culture medium (Horie et al. 2009b).

Since the effects of Ti nanoparticles are comparatively low for all cells, NiTi was expected to be in between the toxicity range of single Ti and Ni nanoparticles. This hypothesis is confirmed by our results and this shows that Ni was the toxicity-determining element of the metal alloy nanoparticle constituents.

Metallic nanoparticle cytotoxicity is always divided into two determining effects: (a) the nanoparticle-derived effect due to its high mass-specific surface and (b) the effect of the metal ions dissolved from nanoparticles. A further step would be to identify the role of the ligand in relation to (a) and (b), since our results show ligand-dependent differences in cytotoxicity.

Little is known about the relation between the stabilization additive (nanoparticle ligand) and the cytotoxicity of the nanoparticles. We found a change of nanoparticles cytotoxicity depending on the ligands. Accordingly, Yin et al. (2005) examined the exposure of NiFe nanoparticles on neuroblastoma cells with and without Oleic acid used as stabilizer observed a similar effect. Horie et al. (2009b) found that the bioactivity of nanoparticles change by adsorption effects on the nanoparticles surface.

Moreover, our results show that except for Ti nanoparticles, all other metal nanoparticles released the most ions in cysteine solution. Cysteine is not only known to bind to colloidal particles via thiol linkage (Ding et al. 2009) which mechanism is used to stabilise the laser-generated nanoparticles, but it also acts as a heavy metal adsorber due to its ability to form metal ion complexes (Disbudak et al. 2002). This explains the different results for Ti as light metal (hard Lewis-acid) from those of the heavy metals (soft Lewis-acid) with thiolic ligand (soft Lewis-base).

Hence, a complexation-induced metal ion release seems to take place.

In contrast to the high-ion released caused by cysteine, nanoparticles generated in pure water might corrode and form an even more distinctive passivated surface which inhibits metal ion release (Lewis et al. 2005).

Interestingly, the higher absolute release of metal ions from nanoparticles does not result in stronger reduction of metabolic activity in cells. For instance, the exposure of 100  $\mu\text{M}$  Co nanoparticles to EC results in nearly similar metabolic activity of 25–50% for all three solutions (water, citrate, cysteine). However, the amount of ions released from Co nanoparticles in cysteine solution is 90% of the whole Co concentration. This means that almost no solid nanoparticles are present in media. Here, the Co ions cause a decrease in the metabolic activity of EC. However, in case of Co nanoparticles in water only 5% of the particulate matter does dissolve. Since the metabolic activity is comparable to that of nanoparticles in cysteine, the higher metal ion concentration does not result in a higher reduction of metabolic activity. Hence, it is obvious that the bioactivity of the metal ions depends on the ligands in the solution and therefore the ligands determine the cytotoxicity of nanoparticles containing soluble ligand-coupling metal ions.

Since the metabolic activity of EC and SMC at nanoparticle concentrations of 100  $\mu\text{M}$  is higher for cysteine than for citrate and water, cysteine is assumed to enable an elimination of toxic ions to a certain extent. This cysteine-metal complex is already in use to clean water (Sljukic et al. 2006).

Overall, we found that the ion release from nanoparticles and the bio-response of the colloids depend on the ligand. The ions in solution have an influence on the cytotoxicity together with a nanoparticle-derived effect. Both effects have to be considered and are influenced by the ligand.

## Conclusions

For the first time we have screened EC and SMC cytotoxicity with colloidal metal and related alloy nanoparticle material. Laser-generated Ti-nanoparticles showed no toxic effects on EC and SMC below a concentration of 100  $\mu\text{M}$ . In contrast, Ni and Co nanoparticles caused the highest toxicity on EC and SMC starting at concentrations of 1–10  $\mu\text{M}$ . NiFe alloy

nanoparticles show the same effects on cells as the NiTi alloy nanoparticles probably due to the same Ni content which primarily determines the cytotoxic effect.

EC are found to be more sensitive to nanoparticles than SMC. This is critical with regard to stent applications where EC viability is important. Concentrations of 10  $\mu\text{M}$  NiTi nanoparticles unintentionally released into this environment are critical for EC. This concentration would correspond to a release of more than 40 billions of nanoparticles (50 nm average diameter) from a stent (1 mm in diameter, 20 mm length) into the blood. This quantity of nanoparticles is most unlikely to occur due to wear, abrasion, or product failure.

In addition, we have shown that cytotoxicity changes depending on the ligand or additive that is used to stabilise the nanoparticle colloids. Cytotoxic threshold concentrations differ up to two orders of magnitude when varying the ligand. The presence of the additive in the media influences the metal ion release from the nanoparticles and changes the bioactivity and thus the toxic effect of the nanoparticle-metal ion complex. Up to 30% of nanoparticles generated in pure water or citrate solution dissolve when generated and incubated in culture medium. In contrast, nanoparticles in cysteine solution dissolve by 60–80%. Higher Ni ion concentrations in cysteine compared to citrate does not lead to a decreased metabolic activity. This may be due to cysteine forming metal ion complexes. Consequently, an unintended release of nanoparticles might be accompanied by a faster dissolution *in vivo* due to the presence of cysteine in the blood.

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