Testing the cytotoxicity of metal alloys used as magnetic prosthetic devices

M. HOPP*
Ernst-Moritz-Arndt-Universität Greifswald, Zentrum für Zahn-, Mund-und Kieferheilkunde, Abt. Prothetik und Werkstoffkunde, Rotgerberstraße 8, 17489 Greifswald, Germany

S. ROGASCHEWSKI
Department of Physics, Humboldt University, Invalidenstrasse 110, 10115 Berlin, Germany

TH. GROTH*
Institute of Chemistry, Department Biomaterials, GKSS Research Centre, Kantstrasse 55, D-14513 Teltow, Germany
E-mail: Thomas.Groth@gkss.de MDR.Hopp@t-online.de

Technical magnetic materials are increasingly used for the development of magnetic retained dental prosthetic and orofacial epithetic devices. Since most of the magnets based on rare earth metals, such as samarium–cobalt based alloys have a high tendency for corrosion they were first coated by tin and then encapsulated by titanium. However, the high mechanical load particularly on dental devices may cause a rupture of the titanium capsule and the alloys contact directly biological fluids. Hence, it is important to know the cytotoxicity of these magnets to assess their potential effects on the surrounding tissue. In this study, the cytotoxicity of neodymium–iron–boron and samarium–cobalt (plain, tin and titanium coated) magnets was tested. First, magnets were incubated up to 7 days in culture medium to prepare extracts for cytotoxicity measurements. Changes in the surface morphology due to corrosion were visualized by scanning electron microscopy and analysis of the elemental composition. 3T3 mouse fibroblasts were cultured in the presence of extracts and their viability measured by neutral red and metabolic assays. To learn more about a possible toxic activity of the main components of magnets, salt solutions of different concentrations resembling those elements, which are main constituents of the magnets, were used. 3T3 fibroblasts were also cultured in direct contact with the materials and material induced effects on cell morphology and growth monitored by microscopy. As a result of this study it was found that samarium–cobalt magnets have a strong tendency for corrosion and exert a considerable cytotoxicity. Neodymium–iron–boron magnets have a lesser tendency for corrosion and are only moderate cytotoxic. Coating of samarium–cobalt magnets with tin or titanium makes the materials non-toxic. Application of salt solutions shows that cobalt has a tendency to be cytotoxic at higher concentrations, but enhances cell metabolism and proliferation at lower concentrations while the other magnet constituents had a lower or negligible cytotoxic potential.
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1. Introduction

Prosthetic constructions retained by magnets play an increasing role in the application of dental implants [1], for dental combination prosthesis [2], and orofacial epithesis [3]. Rare earth metal alloys like samarium–cobalt, neodymium–iron–boron and others have a considerable high magnetic strength [4, 5]. Therefore, the magnetic force necessary for dental and other applications can be obtained with very small magnets. Consequently, these materials have been introduced recently for the construction of dental magnets [6, 7]. One of the issues during the introduction of these magnets into clinical practice was if the high magnetic field strength applied might exert some negative impact on the surrounding tissue. However, it was found that the exposure of dental pulp and gingival tissue, e.g. did not result in any adverse effect on the cells [8]. On the other hand, it is known that rare-earth metal alloys have a rather low corrosion resistance [9, 10]. Hence, particularly dental applications pose a significant problem since

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the environment in the mouth provides unfavorable conditions with regard to corrosion stability [11]. In fact, earlier investigations have shown that magnetic materials composed of samarium–cobalt and neodymium–iron–boron have rather weak corrosion stability [9, 10, 12]. Negative effects that were observed in the surrounding tissue were consequently attributed to a possible toxic effect of magnet corrosion products and not to the static magnetic fields [13]. One of the possibilities to avoid the corrosion of rare earth metal alloys is the encapsulation of magnets. Previous experiments with polymer coatings were quite successful [14], however not suitable for long-term applications because of the hydrolysis of polymers. Recently, magnets were covered by metal coatings with higher corrosion stability, such as tin or encapsulated by titanium [15]. Nevertheless these coatings can prevent a strong and fast corrosion of the magnet materials, the mechanical stability of the coatings is still an important issue. The high mechanical load in dental applications can cause a rupture of the titanium capsule resulting in an exposure of the surrounding tissue to corrosion products of magnets [15].

The *in vitro* testing of the cytotoxicity of biomaterials has to address certain effects of possible material-derived toxins on the viability of cells in terms of membrane integrity, metabolic and functional activity, but also regarding their morphology and potential to proliferate [16–18]. The possible toxic effect of dental and other biomaterials has to be tested according to the ISO standard 10993-5 [19] using filter [20] and agar overlay techniques [21] or applying material extracts in contact with cell cultures [22]. These techniques exclude any direct physical contact between the test material and cells, but allow the testing of a possible toxic activity of any leachable substances that reach the cells via diffusion (agar or filter method) or being solubilized in the extraction medium added to the cell culture (extraction method). Direct contact between the test specimen and cells as a further possibility combines possible toxic material effects with the influence of the physicochemical nature of the substratum on the cell directly [17, 18]. This allows an insight into the intimate interaction of tissue cells with the test materials that is closely related to the application of materials in dentistry and medicine. Hence to obtain a comprehensive view about the toxicity and biocompatibility of materials it is desirable to combine different methods directed to different cellular targets and resulting in different responses [17–19].

In this paper we have investigated the possible toxic effects of rare earth metal alloys, such as neodymium–iron–boron and samarium–cobalt used as dental magnets. The samarium–cobalt magnet was also obtained with a tin coating or encapsulated in titanium. The viability and growth of 3T3 mouse fibroblasts were tested in direct contact with the test specimen or using material extracts with a number of colorimetric and microscopic techniques. To learn more about the role of the different metal components of the magnets the cytotoxicity of salt solutions comprising main constituents of the magnets was investigated as well. As a result it was found that uncoated samarium–cobalt magnets have a high cytotoxic potential in contrast to neodymium–iron–boron, which is due to the activity of cobalt. However, cobalt expressed also a high stimulating activity on the cells if the concentration was low. Results are reported herein.

2. Materials and methods

2.1. Materials

The materials used in this study were supplied from Steco-System-Technik, Lemgo, Germany. They comprised metal alloys with high magnetic field strength made for the application as dental prosthetic support holders or for cranofacial applications, such as for the attachment of epithesis. One of the magnets was a neodymium–iron–boron ($Nd_2Fe_{14}B$) alloy while the second type was made of a samarium–cobalt alloy (Sm_2Co_{17}). Both types were delivered as plain materials or coated with Tin. The samarium–cobalt magnet was also delivered as commercial device for medical application covered by a polished titanium capsule. The magnets had a cylindrical shape with a diameter of 5 mm and a height of 3 mm. Furthermore, samples made of brass and titanium were used as positive (cytotoxic) and negative control materials. All samples were first washed with an ethanol/acetone mixture (1 : 1, vol/vol), then sterilized in 70% ethanol and stored dry until use.

2.2. Preparation of material extracts and standard salt solutions

Extracts were prepared from the materials immersing the sterilized specimen in Dulbeccos modified Eagle medium (DMEM, Sigma, St. Louis, MO, USA) without phenol red for 1, 3 and 7 days at 37 °C. The surface to volume ratio was adjusted at $1.25\,cm^2/ml$. Then, the supernatant medium was removed and applied in the toxicity measurements. For these measurements extracts were applied directly to the cells or used as a stock solution to prepare a number of dilutions ranging from 50%, 25%, 12.5% up to 6.25%.

For a better understanding of the possible toxic effects of extracts from the different magnets or control materials, different salt solutions were prepared. These salt solutions comprised main elements of which the magnets or controls consisted. The following salts were solved in distilled pyrogen-free water: $FeCl_3$, $CoCl_2$, $NdCl_2$, $SmCl_2$, $TiCl_4$, $SnCl_2$, $ZnCl_2$, B_2O_3 and $CuCl_2$ (all from Merck, Germany). 50 µl stock solutions of these salts were added to 2 ml DMEM to obtain final salt concentrations of 1, 0.5, 025 and 0.125 mM. 50 µl distilled water was added to DMEM and used as a non-toxic control solution. These solutions were sterilized by membrane filtration and applied during the toxicity studies as well.

2.3. Scanning electron microscopy and EDS analysis of samples

The investigation of surface structure and composition of the magnets before and after the extraction procedure can yield information about the corrosion of metal alloys and has implications for the subsequent biological reactions. Therefore, samples were investigated with a scanning electron microscope (SEM, S360, Cambridge Instruments, UK) equipped with an energy-dispersive

X-ray spectrometer (EDS, EDR288, Roentec GmbH, Germany) to study surface structure and chemical composition of the new magnets or after 7 days extraction.

2.4. Cell culture

NIH-3T3 mouse fibroblasts were used as an established cell line for testing the cytotoxicity of extracts and salt solutions. Cells were cultivated in tissue culture flasks with DMEM containing 10% foetal bovine serum (FBS, Sigma). Cells were harvested with trypsin/EDTA (Sigma) from nearly confluent cultures. Trypsin was neutralized with FBS. According to the tests, cells were seeded in 96-well tissue culture plates at 50 000 cells/well or in six well plates at 25 000 cells/well.

2.5. Cytotoxicity assays with extracts and salt solutions

96-well plates with almost confluent 3T3 fibroblasts were washed with DMEM to remove any remnant serum components from the wells. Then, 200 μl of the mixture of DMEM and extracts was added. The cells were incubated for 24 h at 37 °C in an incubator with 5% CO_2. At the end of incubation, the supernatant test solutions were aspirated; the wells were washed once with DMEM. These steps were carried out very carefully to avoid the detachments of damaged, loosely attached cells. Then, two different cytotoxicity assays were carried out addressing membrane integrity and metabolic activity of cells.

The neutral red assay was employed to differentiate viable from dead cells since only living cells are able to take up and concentrate this dye intra-cellular. The assay was carried out according to the literature [23]. Neutral red (Sigma) was solved in DMEM with 10% FBS at a concentration of 5 mg NR/ml DMEM. 200 μl of the dye solution were added to each well and incubated for 3 h at 37 °C. Thereafter, the dye solution was removed and discarded, the wells were washed with DMEM, and emptied. Then, the neutral red stained cells were lysed by the addition of 100 μl ethanol acetic acid mixture (50% ethanol/1% acetic acid in distilled water). The adsorption of the resulting cell lysate was measured with a micro plate reader (Anthos Reader, Austria) set at a wavelength of 540 nm.

The metabolic activity of cells after incubation with the different extracts or salt solutions was measured with the XTT assay (Boehringer Mannheim, Penzberg, Germany). This assay measures the activity of mitochondrial dehydrogenases by the conversion of the tetrazolium salt sodium 3′-[1-(phenylaminocarbonyl)-3,4tetrazolium]-bis(4-methoxy-6-nitro)benzyl-sulfonate to a colored reaction product [24]. The apparent advantage of the XTT assay in comparison to other assays like MTT assay (Boehringer Mannheim) is the generation of a water-soluble reaction product that can permeate the cell membrane. Thus, the generated dye is released into the surrounding medium without cell lysis necessary in many other assays. Cells were incubated with a 2 : 1 mixture of DMEM and XTT reagent according to the description of the producer for 4 h. Then, the optical density of the resulting colored supernatant was measured with a plate reader set at a wavelength of 450 nm.

2.6. Cytotoxicity of magnets measured by culture of cells in direct contact

Sterile magnets or control materials were placed on the bottom of 6-well tissue culture plates. 2 ml suspension of 3T3 cells (50 000 cells/ml) was added. After 24 h and 72 h incubation at 37 °C in a CO_2 incubator, neutral red (see above) was added to stain all viable cells. Plates were then investigated with light microscopy to visualize the distribution of cells in vicinity of the test specimen. A qualitative evaluation of the micrographs taken was done denoting the occurrence of cell death and the degree of cell proliferation around the sample. A further observation was the visibility of gross material corrosion by the release of large particles and change of surface morphology.

3. Results

Fig. 1 shows the changes in the surface morphology of neodymium–iron–boron magnets as results of the incubation in DMEM. It is visible that the surface of the magnet has a certain roughness *per se* (Fig. 1(a)) which further increased after 7 days incubation (Fig. 1(b)). Table I shows the elemental composition of the magnet surface before and after extraction. The

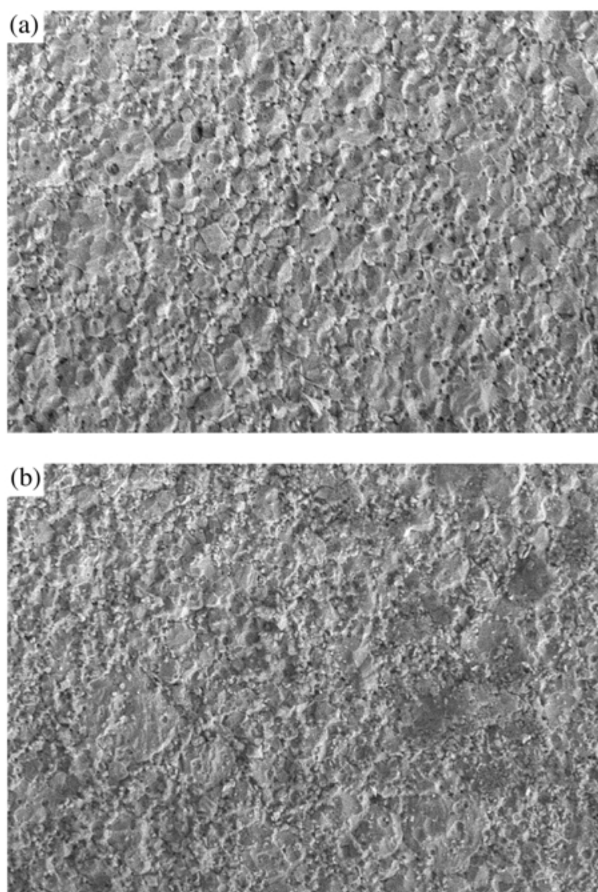


Figure 1 Scanning electron micrographs of neodymium–iron–boron magnets before (a) and after 7 days (b) incubation in tissue culture medium at 37 °C. Magnification 200 × .

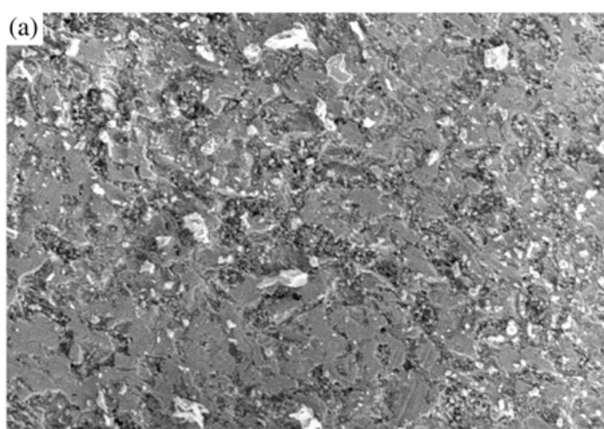
TABLE I EDS analysis of elemental composition in atom% of neodymium–iron–boron magnets before and after extraction in DMEM

Element	New magnet	Magnet after 7 days extraction
Fe	84.56	40.33
Al	2.26	0.72
Si	0.58	0.05
Nd	10.76	5.69
Dy	1.83	1.17
P		1.15
Ca		0.78
S		0.23
Na		18.37
Cl		2.08
O		29.45

TABLE II EDS analysis of elemental composition in atom% of samarium–cobalt magnets before and after extraction in DMEM

Element	New magnet	Magnet after 7 day extraction
Co	35.42	31.24
Fe	12.52	10.83
O	21.33	38.10
P	2.12	1.67
Cu	4.23	2.94
Ca	0.54	1.01
Si	0.53	0.28
Al	1.15	0.11
Sm	6.68	4.94
S	0.34	0.35
C	15.15	2.79
Na		5.38
Cl		0.34

presence of iron and neodymium was confirmed. Boron could not be detected with the EDS device used. Therefore no boron is shown in the elemental composition. In addition small quantities of aluminum, silicon and dysprosium were detected. After the incubation, elements that are constituents of the extraction medium, such as sodium, chlorine, calcium and phosphate were detected as well. Particularly, a strong increase in oxygen was observed which points to the oxidation of the surface. Fig. 2 shows the changes of the surface of samarium–cobalt magnets. The new material is characterized by the presence of sintered granules. This structuring of the surface was maintained over 7 days incubation though an increasing number and size of precipitates from the surrounding DMEM solution was visible. It was also found in the later investigations that these granules tended to detach from the materials surface. If the surface compositions of samarium–cobalt magnets was assessed by EDS analysis as shown in Table II the complex composition of the magnets was obvious. Not only cobalt and samarium were contained but also large quantities of iron and oxygen, carbon, some copper and other elements in minor amounts. It was evident from the comparison that after 7 days incubation again a strong rise in the concentration in oxygen was observed accompanied by a decrease of all other elements except sodium and chlorine. If the samarium–cobalt magnets were coated with tin or encapsulated with titanium no changes in the very smooth morphology of the surfaces were observed after 7 days incubation in DMEM (not shown here). If the EDS analysis was carried out for both magnets, no significant changes of the elemental composition were observed for tin coated magnets shown in Table III. However, with titanium-encapsulated magnets again a strong rise in the content in oxygen was observed indicating the oxidation of the material surface (Table IV).



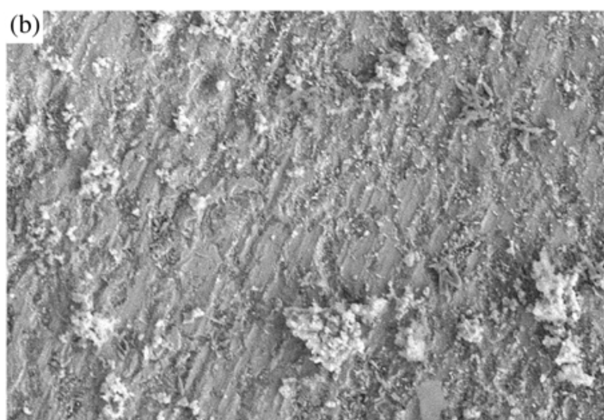


Figure 2 Scanning electron micrographs of samarium–cobalt magnets before (a) and after 7 d (b) incubation in tissue culture medium at 37 °C. Magnification 200 ×.

Investigations of material extracts were carried out

TABLE III EDS analysis of elemental composition in atom% of tin-coated samarium–cobalt magnets before and after extraction in DMEM

Element	New magnet	Magnet after 7 days extraction
Sn	100	100

TABLE IV EDS analysis of elemental composition in atom% of samarium–cobalt magnets encapsulated in titanium before and after extraction in DMEM

Element	New magnet	Magnet after 7 days extraction
Ti	45.01	42.23
N	35.62	27.72
Al	0.11	0.18
O	19.26	27.36
Na		1.93
Cl		0.58

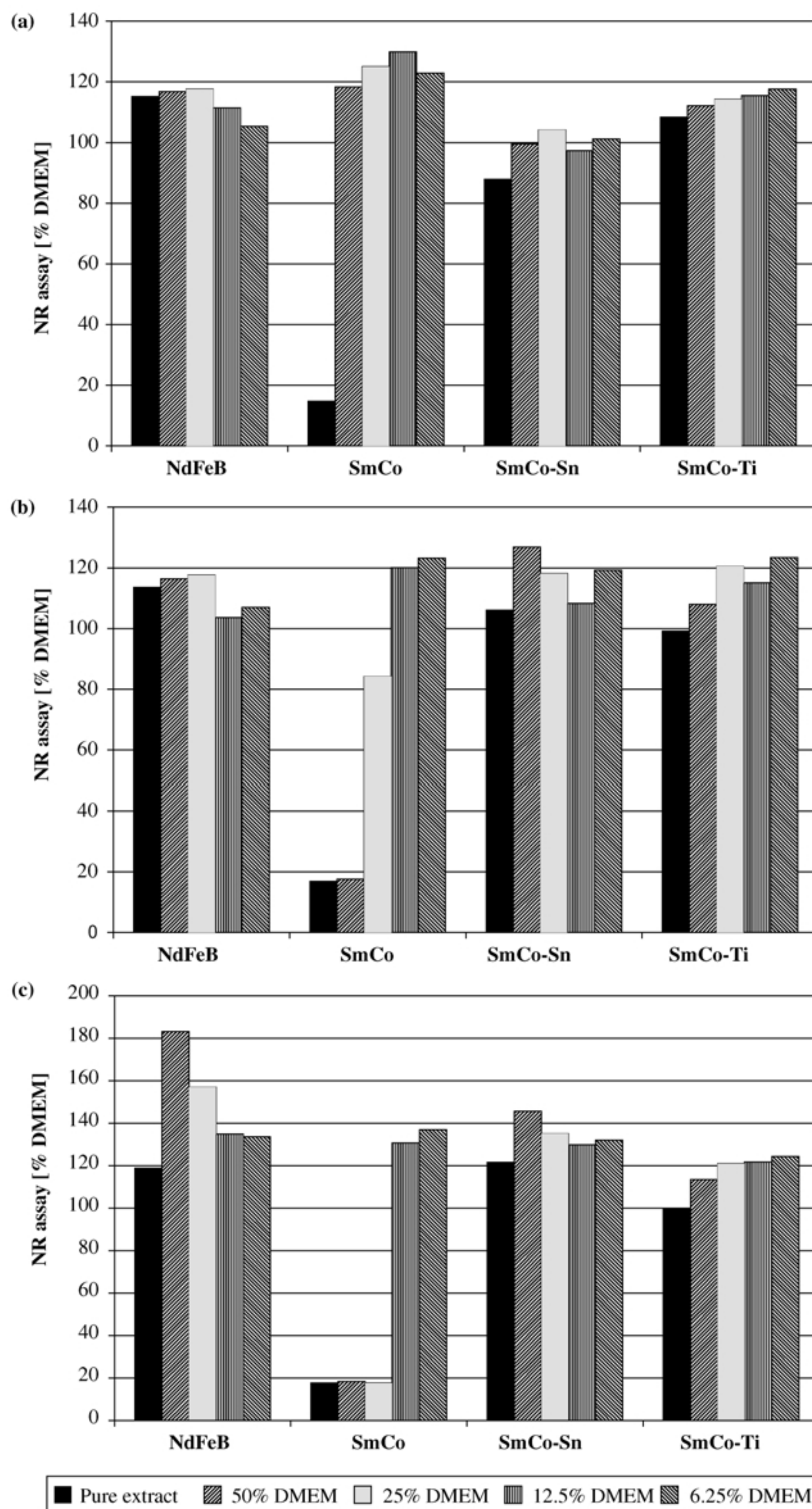


Figure 3 Uptake of neutral red (NR) of 3T3 fibroblasts after 24 h exposure to extracts prepared after 1 (a), 3 (b) and 7 days (c) incubation of neodymium–iron–boron (NdFeB), samarium–cobalt (SmCo), tin coated samarium–cobalt (SmCo–Sn) and titanium encapsulated samarium–cobalt (SmCo–Ti) magnets at 37 °C in DMEM in comparison to NR uptake of cells in pure medium. Results shown are medians of four experiments.

with two different assay, such as NR and XTT assay. Materials were incubated in DMEM for 1 days, 3 days and 7 days and these extracts were applied directly to pre-confluent 3T3 mouse fibroblasts or diluted with DMEM up to 6.25%. Pure DMEM was applied in these investigations to establish non-toxic control conditions whereas in the later studies of cell growth and morphology brass was used as a toxic and pure titanium as a non-toxic metallic control material. Fig. 3(a) shows that after 1 day extraction some slight increase in the NR uptake was observed for extracts prepared from neodymium–iron–boron magnets. Highly diluted extracts however, did not cause any change in growth in comparison to DMEM. In contrast, a strong inhibition of NR uptake was observed for undiluted extracts prepared from samarium–cobalt magnets. However, if these extracts were diluted 1 : 1 and more, than an enhanced uptake of NR by the cells was observed.

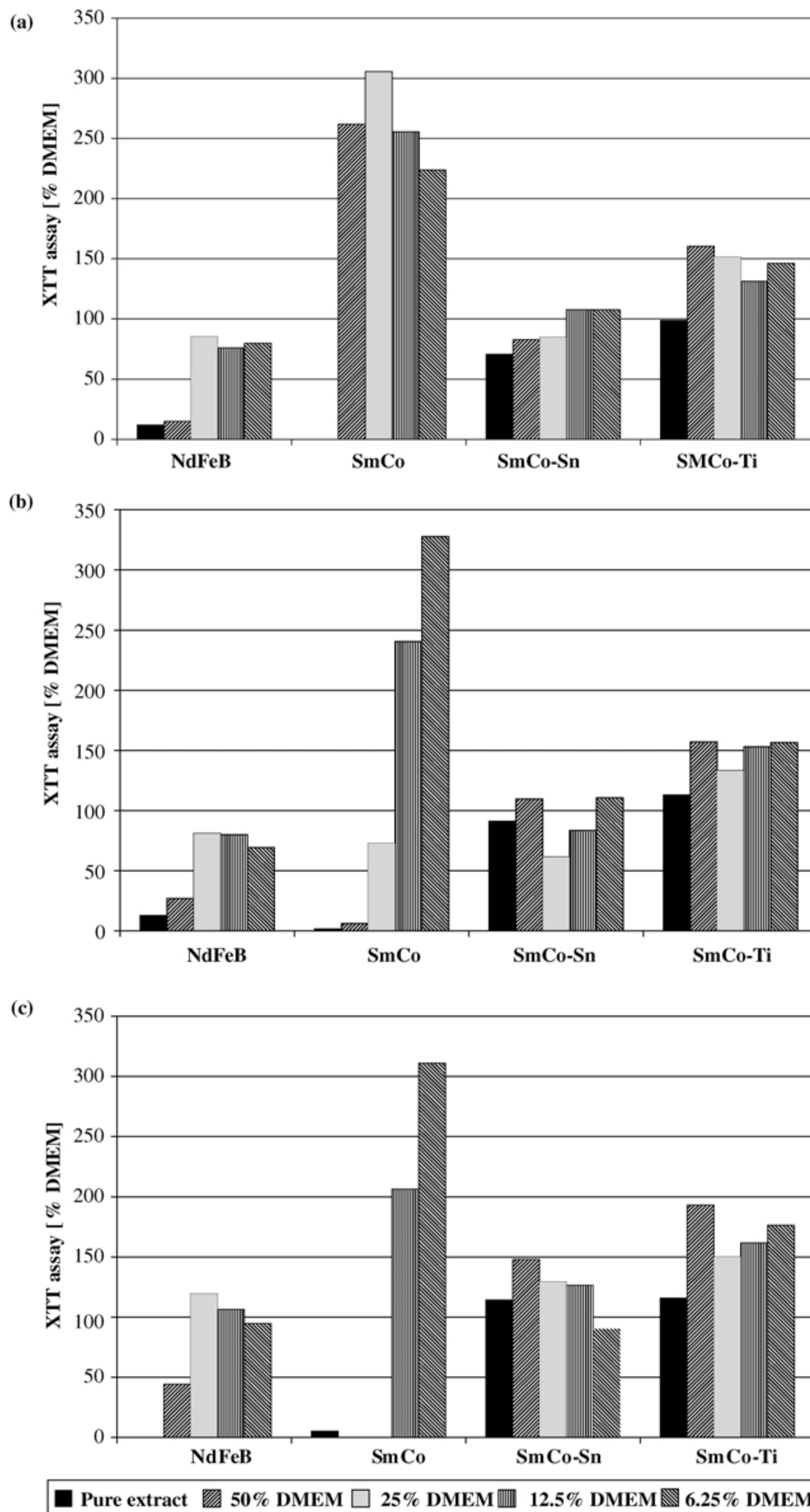


Figure 4 Conversion of the tetrazolium dye XTT by 3T3 fibroblasts after 24 h exposure to extracts prepared after 1 (a), 3 (b) and 7 days (c) incubation of neodymium–iron–boron (NdFeB), samarium–cobalt (SmCo), tin coated samarium–cobalt (SmCo–Sn) and titanium encapsulated samarium–cobalt (SmCo–Ti) magnets at 37 °C in DMEM in comparison to cells in pure medium. Results shown are medians of four experiments.

Coating of samarium–cobalt magnets with tin reduced the toxicity though some small inhibition of NR uptake was still observed for undiluted extracts. Encapsulation of samarium–cobalt magnets with titanium did not show any inhibiting effects of the extracts on the uptake on NR red. After 3 days extraction (Fig. 3(b)) no decrease of NR uptake was observed for neodymium–iron–boron magnets. The toxic effect of extracts prepared from samarium–cobalt magnets however was greatly enhanced because almost complete inhibition of NR uptake was seen for undiluted and 1 : 1 diluted extracts, while 1 : 4 diluted extracts still exerted 20% inhibition. No inhibition of NR uptake was observed for extracts prepared from tin or titanium coated samarium–cobalt magnets. Fig. 3(c) demonstrates that extracts prepared from neodymium–iron–boron magnets had a stimulating

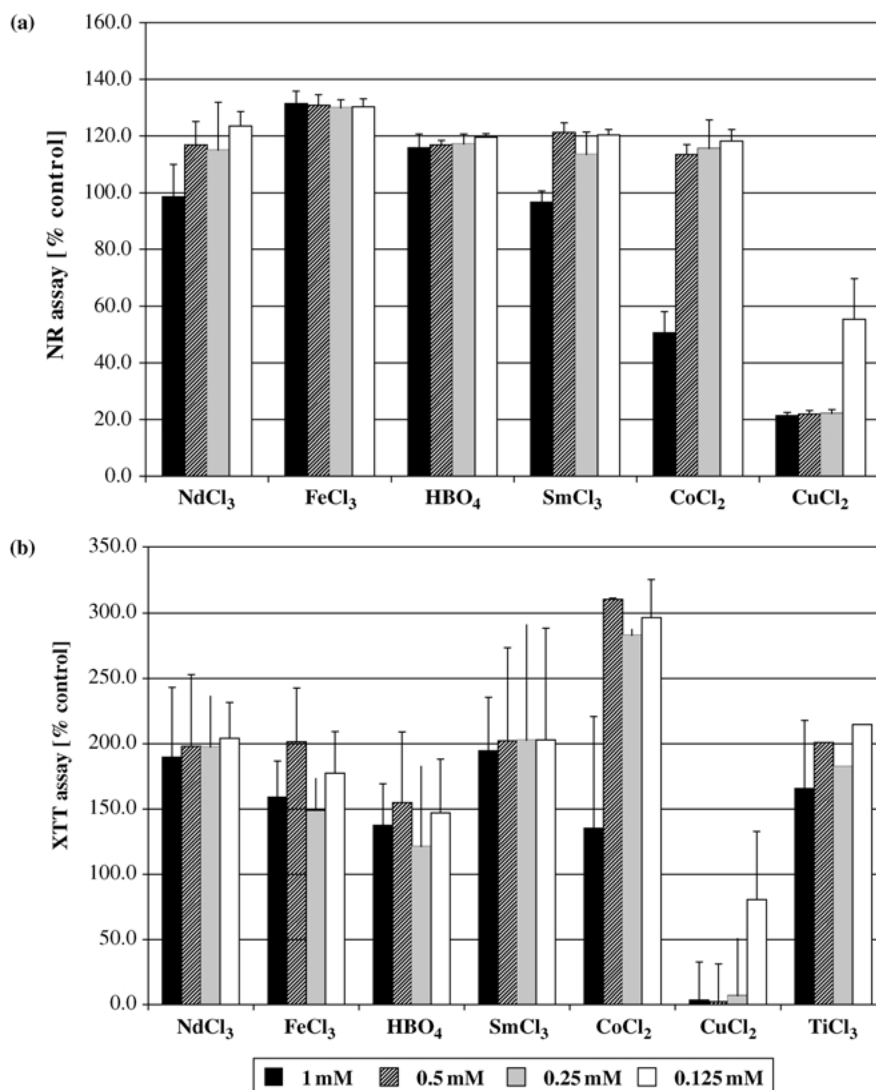


Figure 5 Uptake of neutral red (NR, (a)) and conversion of tetrazolium salt XTT (b) by 3T3 fibroblasts after 24 h exposure to salt solutions. Results shown are means with standard deviations of eight estimates.

effect on the neutral red uptake of cells, particularly if they were diluted 1 : 1 or 1 : 2. The inhibiting effect of extracts from samarium–cobalt magnets was more pronounced in comparison to 1 or 3 days extraction. In comparison, tin coated or titanium encapsulated magnets exerted some stimulating effect as it was described above.

Fig. 4 shows the result of tests on the metabolic activity of cells in contact with the material extracts assessed with XTT test. Generally speaking the inhibiting, but also stimulating effect of some material extracts was much more pronounced then the effects observed with the NR assay. Fig. 4(a) shows the effect of materials extracts prepared after 1 day incubation on the metabolic activity of 3T3 cells. It can be seen that undiluted and 1 : 1 diluted extracts prepared from neodymium–iron–boron exerted a high cytotoxic potential as visible by more than 50% inhibition of XTT conversion. A further dilution of the extracts reduced the cytotoxic potential though the activity of XTT assay was still below 100%. If extracts prepared from samarium–cobalt were tested than undiluted extracts caused a complete inhibition of metabolic activity. However, it was observed that a further dilution of the material extracts caused a tremendous increase in the metabolic activity because the conversion level exceeded the 100% level of DMEM reaching up to 300%. The coating of samarium–cobalt magnets with tin reduced the toxic potential of uncoated samarium–cobalt magnets though still some slight inhibition was observed up to a dilution of 25%. The encapsulation of samarium–cobalt into titanium abolished any toxic activity. However, some increase of metabolic activity above the 100% level was observed for the different extract dilutions. If the magnets were extracted 3 days (Fig. 4(b)) then the toxic potential of neodymium–iron–boron was not increased in comparison to 1 day extraction resulting in an almost identical pattern of metabolic activity. However, it was evident that the toxic activity of extracts prepared from samarium–cobalt magnets had increased since dilutions up to 25% exerted still a high toxic potential indicated by the absence or decrease of XTT conversion. No gross changes were observed for tin-coated or titanium encapsulated samarium–cobalt magnets in comparison to 1 day extraction. If the magnets were incubated for 7 day in DMEM (Fig. 4(c)) then undiluted extracts from neodymium–iron–boron inhibited the conversion of XTT totally and 1 : 1 diluted extracts caused a 50% inhibition. Higher diluted extracts however, did not cause any inhibition of the assay. The

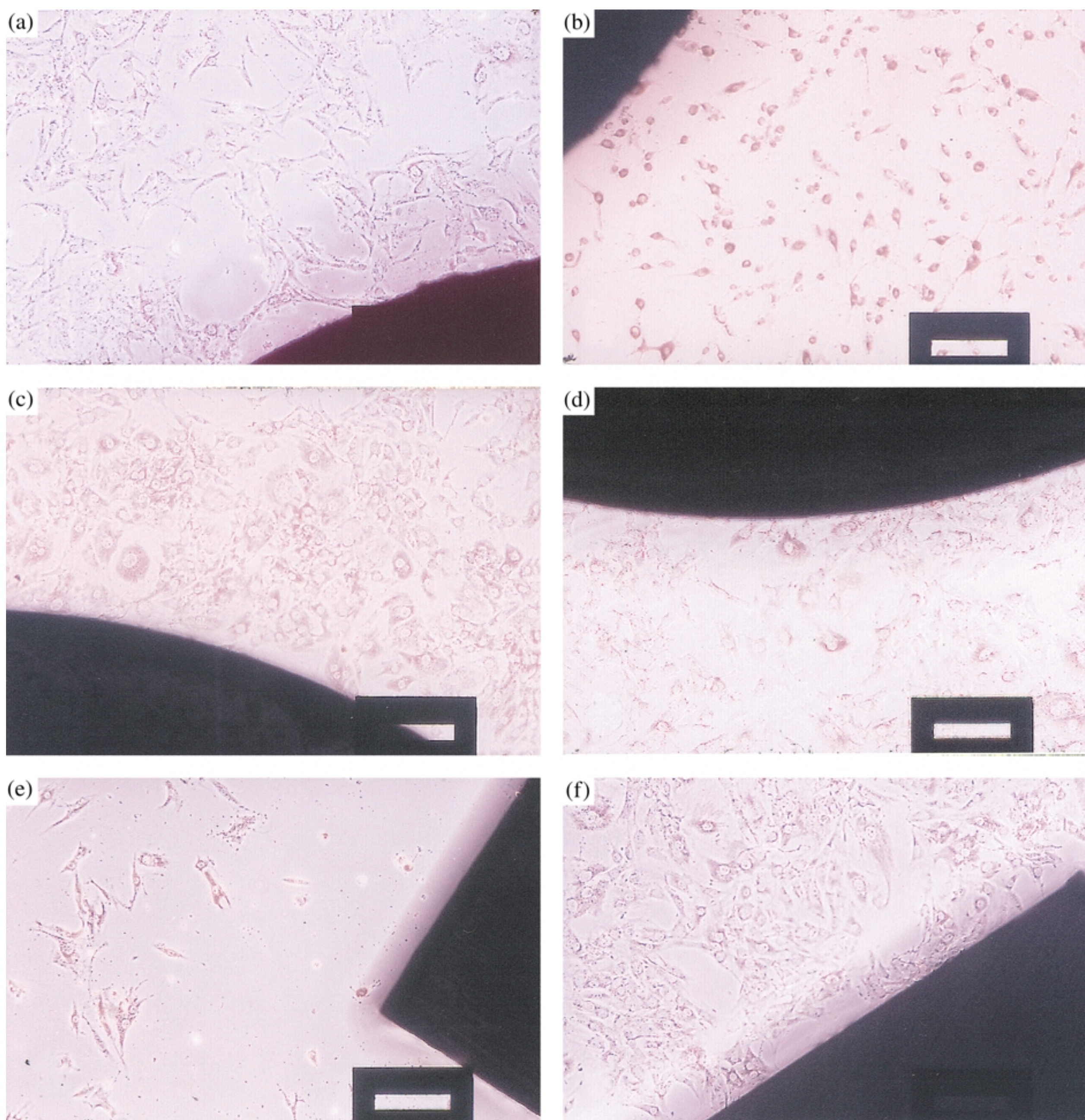


Figure 6 Culture of 3T3 fibroblasts close to magnets or control materials. Cells were incubated in DMEM with 10% serum for 24 h in 6-well tissue culture plates. Then neutral red was added and cells visualized 2 h later with light microscopy. Black areas show the test specimen which are neodymium–iron–boron (a), samarium–cobalt (b), samarium–cobalt coated with tin (c), samarium–cobalt encapsulated in titanium (d), brass (e) and titanium (f). Magnification 150 × .

toxicity of extracts prepared from uncoated samarium–cobalt magnets was higher then after 3 days extraction. This was evident by the complete inhibition of XTT conversion up to a 25% concentration. However, a further dilution increased the XTT conversion above the 100% value (see above). The coating of samarium–cobalt with tin and titanium resulted in a similar picture as observed for the other extraction times. There were no signs for any toxic activity of the extracts. Rather we observed increased values as shown for high dilutions of extracts from uncoated samarium–cobalt.

To control the possible toxic effect of the extracts main constituents of the different alloys were applied as salt solutions ranging from 1 to 0.125 mM. Copper chloride was used as a positive (toxic) control. Fig. 5(a) shows that 1 mM $CoCl_2$ yielded a 50% inhibition of neutral red uptake. None of the other main constituents of the magnets of coatings exerted any toxic activity in the concentration range applied here. One exception was copper chloride used as a control solution. Here a strong toxic action was observed over the whole concentration range. A similar observation was made with the XTT assay as shown in Fig. 5(b). Here $CuCl_2$ concentrations up to 0.25 mM inhibited the conversion of XTT completely. $CoCl_2$ did not show any inhibition, however, it was visible that there was a tremendous rise in the XTT conversion from 1 to 0.5 mM. None of the other salts solutions showed any sign for a toxic activity.

Furthermore, 3T3 fibroblasts were cultured in the presence of test specimen made of the different magnets or brass and titanium as positive and negative control materials for 24 h and 72 h. Fig. 6(a) shows that after 24 h there were no signs of an inhibition of cell attachment in the vicinity of neodymium–iron–boron. Cells were also well spread and viable as visible by the uptake of neutral red. A different observation was made for samarium–

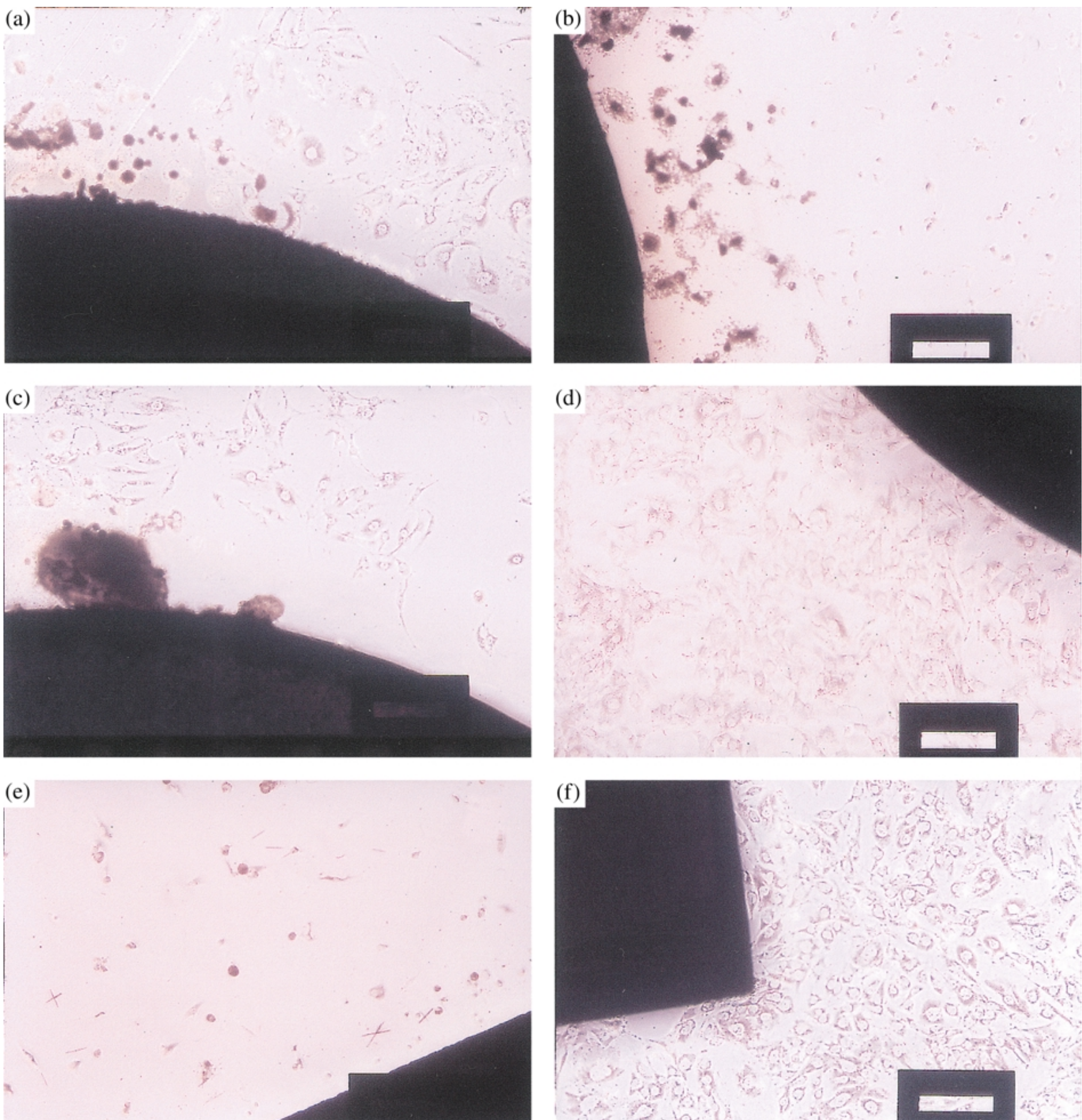


Figure 7 Culture of 3T3 fibroblasts close to magnets or control materials. Cells were incubated in DMEM with 10% serum for 72 h in 6-well tissue culture plates. Then neutral red was added and cells visualized 2 h later with light microscopy. Black areas show the test specimen which are neodymium–iron–boron (A), samarium–cobalt (B), samarium–cobalt coated with tin (C), samarium–cobalt encapsulated in titanium (D), brass (E) and titanium (F). Magnification 150 × .

cobalt as shown in Fig 6(b). Less cells were attached in the surrounding of samarium–cobalt and cells looked more compact, i.e. less cell spreading was observed. In Fig. 6(c) it is demonstrated that cells attached and spread well close to tin coated, but also to titanium-encapsulated samarium–cobalt magnets (see Fig. 6(d)). In contrast, very few cells attached close to brass (Fig. 6(e)) while very good cell attachment and spreading was observed in vicinity of titanium (Fig. 6(f)). After 72 h cultivation of 3T3 fibroblasts in contact with the different test materials it was observed that cell growth was slightly diminished in the vicinity of neodymium–iron–boron as shown in Fig. 7(a). There were also a number of particles observed which could resemble corrosion products released from the magnet surface. A strong inhibition of cell growth was also observed in the surrounding of samarium–cobalt magnets (see Fig. 7(b)). Some cells could be still observed. However they looked shrunk and non-viable. Again, corrosion products were observed close to the magnet surface. Also coating with tin caused an inhibition of cell growth close to the material surface as visualized by the small number of cells in Fig. 7(c). There were also some indications for accumulation of cellular debris on the magnet surface. In contrast, encapsulation of the magnet with titanium produced a very good biocompatible milieu. In Fig. 7(d) is shown that cell grew up to confluence close to the magnet that was also the case for the titanium control (see Fig. 7(f)). Cells looked also well spread and viable. On the other hand, culture of 3T3 cells in contact with bras was not possible. Only cell debris and non-viable cells were observed (see Fig. 7(e)).

4. Discussion

The aim of the present study was to show with a number of different methods that the cytotoxic potential of magnets made of neodymium–iron–boron and samarium–cobalt can be eliminated by coating with tin and encapsulation with titanium. Indeed, it was observed that magnets produced from samarium–cobalt, but to a lesser extent also from iron–neodymium–boron have a considerable high cytotoxic potential. The cytotoxicity of these materials however, can be diminished by coating of magnets with tin or completely abolished by encapsulation with titanium. Since the cytotoxic potential particularly of samarium–cobalt magnets was quite high, a safe and stable encapsulation with titanium is one of the basic requirements particularly for dental applications.

Corrosion of dental materials and implants poses a serious problem to the medical application of metallic biomaterials. Particularly the conditions in dental applications where the materials are exposed to high mechanical load, but rapid changes in temperature and pH can contribute to a rapid corrosion of metallic materials [25]. Hence it is not surprising that metal ions are released from dental materials *in vitro* and *in vivo* [26, 27]. Released metal ions in dental and other applications can cause staining of the surrounding tissue, mild to severe local inflammation up to systemic effects, such as sensitivity and allergic reactions [28, 29]. We have shown in this paper that neodymium–iron–boron magnets have a tendency for corrosion, which leads to an increased surface roughness. There was also a strong rise in oxygen and formation of precipitates with the surrounding medium components. The incubation of fibroblasts close to the magnets did not show any strong toxic effect within 24 h, which was however the case if the cells were cultured in the presence of material extracts. The direct culture of fibroblasts on top of the materials indicated some beginning destruction of cells within 24 h and the cell shape was irregular. After 72 h culture of fibroblasts in close contact to the magnets, a stronger toxicity was noted because cells in the vicinity became round shaped [17, 18]. Also extracts prepared from these neodymium–iron–boron magnets (3rd and 7th day of extraction) showed a strong toxic activity in the XTT assay. The NR assay seemed to be less sensitive because in comparison to XTT assay no decrease below the control value of DMEM set at 100% was observed here. The use of metal salts comprising the main constituents of the magnets revealed no toxic effects of neodymium, iron or boron in comparison to copper as positive and titanium chloride as negative control at the concentrations applied. However a certain *in vitro* toxicity of neodymium was reported recently applying dye exclusion tests at lower concentrations using alveolar macrophages [30]. Also boron is known to have a general and reproductive toxicity [31] but at much higher concentrations than those applied in the present study. A recent study on the toxicity of plain and parylene coated neodymium–iron–boron magnets has also shown a certain *in vitro* toxicity applying different test systems [32].

Samarium–cobalt magnets corroded quickly within 24 h, which led to the generation of material particles. The surface of the material possessed a high roughness a priory and oxidized very easily during incubation in the medium. The response of fibroblasts cultured in close contact to the samarium–cobalt magnets showed the deleterious effect of this material on cell viability and proliferation. This was visible already after 24 h incubation, indicated by the presence of round cells in close neighborhood to the test specimen or by dead cells cultured on top of the magnets. Here no spreading of cells was observed which was very similar to the results obtained for brass. If extracts were prepared from samarium–cobalt magnets then it was obvious that undiluted extracts were very toxic for the cells. This was disturbing their metabolic activity as shown with XTT test, but also membrane integrity as visible by the uptake of neutral red. The longer the incubation of the magnets was, the stronger became this effect since the toxic activity was then noticeable also at higher dilutions. Indeed, the experiments with salt solutions have shown that this toxicity was due to the presence of cobalt and not samarium since a 1 mM $CoCl_2$ solution caused about 50% inhibition of NR uptake. A strong toxicity was also reported in a recent paper where the toxicity of cobalt was quite similar to that of copper [33]. On the other hand, extracts at higher dilutions or $CoCl_2$ solutions with a concentration of less than 1 mM caused a very strong increase in the metabolic activity which was much above compared to all other materials or salt solutions applied. This increase was not due to some interaction of cobalt with the XTT assay reagents, since there was no effect on the conversion of the dye in the absence of cells in control experiments (not shown here). It rather indicates some interaction of cobalt with the mitochondrial dehydrogenases responsible for the conversion of XTT. In fact, an increased reduction of nitroblue tetratzolium dye in rabbit alveolar macrophages due to cobalt was described by Johansson *et al*. [34]. This enhanced metabolic activity, however, is due to a direct interaction of cobalt with the mitochondrial respiratory chain which leads to an increased oxygen consumption and generation of ATP as found in recent investigations [35, 36]. Hence, lower concentrations of cobalt released into the surrounding tissue have some stimulating activity on cells which has to be considered cautious.

An unexpected observation was that most of the salt solutions provoked a higher response of both XTT and NR assay in comparison to pure DMEM where non-pyrogenic water was added. This effect indicates that the presence of salt ions at lower concentrations had some promoting effect on the cell metabolism and growth since the extracts were added to pre-confluent cultures. On the other hand there was no obvious interference of the salt ions with the assay reagents since in control experiments (not shown here) carried out in the absence of cells no stimulating effect of the different ions was observed.

The biocompatibility of magnets prepared from neodymium–iron–boron or samarium–cobalt could be substantially improved by coating with tin. There was no detectable corrosion of the coating within 7 days incubation. Also no noticeable cytotoxicity was detected by cell culture close or on top of the materials and little effects with materials extracts. This could be further

improved by the encapsulation of tin-coated magnets with titanium. Neither the culture of cells with the magnets nor the use of extracts gave any hints for a possible cytotoxic activity of these magnets. That means if a stable and safe encapsulation of magnets is carried out with titanium then an application of these magnets does not impose any cytotoxic risk to the surrounding tissue.

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