J Mater Sci (2009) 44:6786–6794
DOI 10.1007/s10853-009-3565-x

MESOSTRUCTURED MATERIALS

Cultivation of human fibroblasts and multipotent mesenchymal stromal cells on mesoporous silica and mixed metal oxide films

D. Böcking · J. Fiedler · R. E. Brenner · N. Hüsing

Received: 30 November 2008 / Accepted: 12 May 2009 / Published online: 2 June 2009

Abstract The application of mesoporous silica and silica–titania-mixed metal oxide films prepared via sol–gel processing as substrates for cell growth was investigated. A deliberate tailoring of the chemical composition of the porous substrates with different Si:Ti ratios was achieved by using a single-source precursor based on a titanium-coordinated alkoxysilane, resulting in mesoporous silica–titania films with hydrophilic surfaces. The different coatings were investigated with respect to their applicability in the cultivation of human cells such as human fibroblasts and multipotent mesenchymal stromal cells. It was found that they promoted cell adhesion and proliferation of human fibroblasts up to a period of 14 days. After 2 weeks only single apoptotic cells could be detected on silica–titania mixed oxide films in contrast to a somewhat higher amount on silica coatings. Furthermore, none of the films inhibited osteogenic differentiation of multipotent mesenchymal stromal cells.

Introduction

The application of mesoporous materials, e.g. M41S-phases [1], in bio-related fields is drastically increasing in the recent years due to their attractive structural characteristics such as high specific surface areas, high pore volumes, tailorable pore sizes, connectivity and high thermal stability. The incorporation of whole cells into porous inorganic sol–gel matrices has been demonstrated over 15 years ago by Carturan et al. [2], and the progress in this area as seen by the successful sol–gel immobilization of bacteria, protozoa, and mammalian cells for applications in cell-based sensors, bioreactors and artificial organs was recently reviewed by Avnir [3–5]. In the last few years, reports on mesoporous materials as hosts for drug delivery systems and substrates for tissue engineering and bone formation establish a new field of application in biomaterial science [6–9]. As a new approach to cell immobilization, Brinker et al. reported the in situ incorporation of yeast and bacterial cell lines within nanostructured silica matrices formed by evaporation-induced self-assembly [10].

Especially for bone replacement applications, mesoporous materials seem to be very promising candidates. Here, the major goal is to optimize the interface between biomaterial and bone tissue in order to promote osseointegration [11]. Therefore, commercially available metallic implants used for endoprothesis have been coated with ceramics to improve the integration into the tissue of the host [12]. In this context, sol–gel-derived titania (TiO_2) coatings may be of special interest because of their ability to induce calcium phosphate formation [13]. Sol–gel-derived non-porous silica–titania and titania coatings have recently been investigated in detail regarding cell adhesion, cell proliferation and cell response [14, 15], but no special emphasis was given to the influence of the structural characteristics, such as porosity, chemical homogeneity, crystallinity, of the coating.

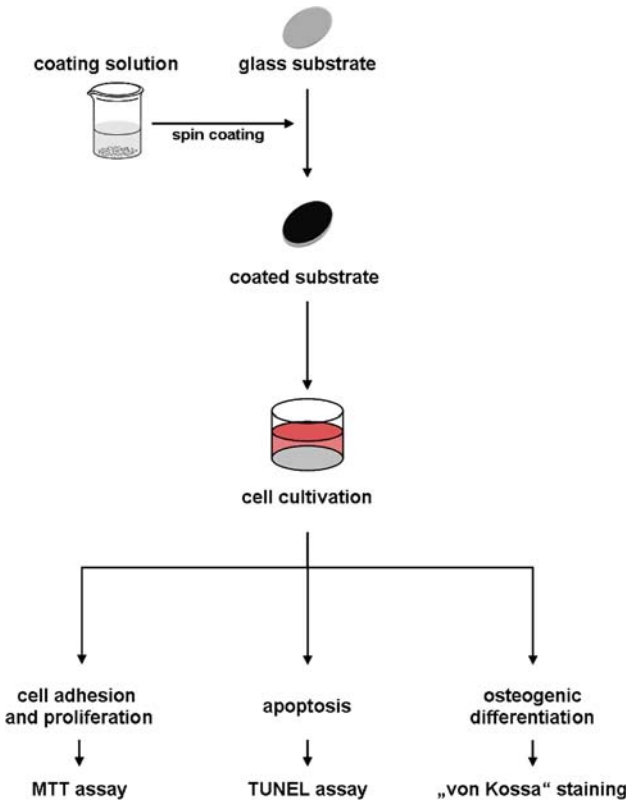
Evaporation-induced self-assembly approaches combined with the "chimie douce" hydrolysis and condensation reactions of metal alkoxides allow for the preparation

D. Böcking · N. Hüsing (✉)
Institute of Inorganic Chemistry I, Ulm University, Albert- Einstein- Allee 11, 89081 Ulm, Germany
e-mail: nicola.huesing@uni-ulm.de

J. Fiedler · R. E. Brenner
Department of Orthopaedics, Division for Biochemistry of Joint and Connective Tissue Diseases, Ulm University, Oberer Eselsberg 45, 89081 Ulm, Germany

of periodically organized mesoporous coatings with deliberately tailored chemical and structural properties [16]. These mesoporous coatings offer great advantages when compared to materials commonly used for bone replacement: (a) the mesoporous structure can serve as drug reservoir, e.g. for growth factors, antibiotics, etc.; (b) silica-based coatings are in principle biodegradable, but the possibility to prepare mixed metal oxide coatings allows for stable matrices with specific functions, e.g. by incorporation of titanium oxide into the silica matrix a silica-releasing sol–gel coating is prepared; and (c) these coatings can easily be spatially defined in the upper nanometre range by different techniques, such as lithography or stamping [17, 18]. This is of special interest for applications as interface with bio-environments, since nanostructuring is considered an important factor for cell–material interactions and cell viability [19].

Our primary goal in this study was to determine the applicability of sensitive, thin mesoporous silica and mixed oxide coatings prepared via an evaporation-induced self-assembly approach as substrates for adhesion and growth of human cells such as fibroblasts and multipotent mesenchymal stromal cells. The mixed oxide coatings were prepared with a specially designed single-source precursor, linking a silicon and titanium centre within one molecule via covalent Si–C or coordinative Ti–O bonds, respectively, to obtain maximum homogeneity in the coating (see Scheme 1). Coatings with different Si:Ti ratios of 1:0 up to 1:1 were prepared and applied in different cell cultures: (a) of human fibroblasts to investigate cell adhesion, proliferation and apoptosis and (b) of human multipotent mesenchymal stromal cells to test the influence on the osteogenic differentiation potential (Scheme 2).



Scheme 2 Overview of the substeps of the biocompatibility testing of silica films. Films were prepared with a spin-coating method on glass substrates and used in cell culture. After cell seeding and cultivation, cell adhesion, proliferation and apoptosis was investigated. The influence of the films on the osteogenic differentiation potential of human multipotent mesenchymal stromal cells was tested

Experimental

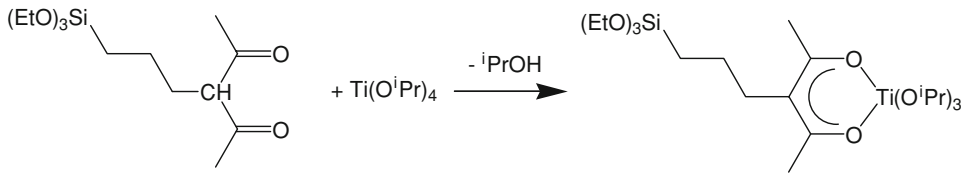
Materials

Glass substrates (d = 18 mm) were supplied by Menzel (Braunschweig, Germany). Ethanol (96%), toluene (99%), acetone (99%), titanium isopropoxide (98%), acetylacetone (99%), tetraethoxysilane (99.9%), sodium iodide (99%) and potassium carbonate (99%) were purchased from Merck KgaA (Darmstadt, Germany). The non-ionic surfactant Pluronic P123^{TM} and 3-(chloropropyl)-triethoxysilane (97%) were supplied by Sigma-Aldrich (Munich, Germany). All chemicals were used without further purification. DMEM, heat-inactivated fetal calf serum, L-glutamine, 0.05%/0.02% trypsin/ethylenediaminetetraacetic acid, penicillin and streptomycin (all Biochrom AG, Berlin, Germany) were stored at −20 °C. Dexamethasone (Merck), phosphate buffered saline (Biochrom), ascorbic acid and β-glycerophosphate (both Sigma-Aldrich) were used as supplied by the manufacturer.

Synthesis of the titanium-complexed silane

The titanium-complexed silane was synthesized in a two-step procedure via 3-acetyl-6-triethoxysilylhexane-2-one according to Puchberger et al. [20, 21]. Sodium iodide was

Scheme 1 Precursor molecule for the synthesis of mixed metal oxide coatings



dissolved in acetone under argon atmosphere and an equimolar ratio of 3-(chloropropyl)-triethoxysilane was added. After refluxing overnight potassium carbonate and acetylacetone were added and refluxed again. The reaction mixture was filtered and the solvent was removed under reduced pressure. The resulting 3-acetyl-6-triethoxysilylhexane-2-one was reacted dropwise with titanium isopropoxide in an equimolar ratio under argon atmosphere. Released isopropanol was removed under reduced pressure after 15 min of stirring at room temperature.

Preparation of the coating sol for silica coatings

The silica precursor was prepared as described by Brinker et al. [22]. Tetraethoxysilane was stirred in a solution of ethanol, 0.07 M hydrochloric acid and deionized water in molar ratios of $1:3.8:0.98:5.09 \times 10^{-5}$ (TEOS:ethanol:deionized water:hydrochloric acid) at 60 °C for 90 min. The stock solution was stored at −20 °C, and 5 mL of the stock solution was dissolved in 10 mL of ethanol. To this solution 0.4 mL deionized water and 0.4 mL of 0.07 M hydrochloric acid were added under stirring and 5 wt% Pluronic P123^{TM} was dissolved in this solution.

Preparation of the coating sol for mixed metal oxide coatings

For the preparation of mixed metal oxide films, tetraethoxysilane in the coating solution was replaced by a defined amount of the titanium-coordinated silane. For 25% titania, 2.1 mmol of the single-source precursor was added to a TEOS sol prepared as described above (with a 50% reduction in the molar amount of TEOS). For coatings with a Si:Ti ratio of 1:1, TEOS was completely substituted by the single-source precursor (4.2 mmol SSP) and the sol was also prepared as described above.

Coating and powder synthesis

The solutions were first coated on glass substrates (d = 18 mm) by spin-coating (4000 rpm, 30 s). The residual sol was casted in Petri dishes and the resulting films scratched out to prepare powders for nitrogen sorption measurements. Films were calcined at 350 °C for 3 h with a heating rate of 1 °C/min, and powders at 550 °C for 3 h (ramp rate 1 °C/min).

Structural characterization

X-ray diffraction patterns were recorded on a PANalytical MPD PRO with a CuK_α source. All samples were measured in a range between 0.6° and 10° 2θ. Nitrogen sorption measurements were performed on a Quantachrome Autosorb MP1 and a NOVA 4000e after heating the samples at 350 °C for 3 h under vacuum. The surface area was calculated according to Brunauer, Emmett and Teller (BET) via 5-point analysis in a pressure range 0.05–0.3 p/p_0 and the pore size distribution was obtained as described by Barrett, Joyner and Halenda (BJH) from the desorption branch of the isotherm. TEM images were recorded on a Philips EM 400 with a tungsten source (80 kV). Contact angle measurements were performed with a Krüss goniometer (Krüss GmbH, Germany) using water. On each sample two droplets were measured ten times over a period of 10 s giving the contact angle as average value of left and right contact angle. Film thickness was evaluated using a Dektak 150 surface profiler (Veeco Instruments) with a 2.5 μm tip and a tipping force of 2 mg.

Cell culture

Human multipotent mesenchymal stromal cells (hMSC) were obtained from human bone marrow derived from pelvic osteotomies in accordance with the ethics committee of the University of Ulm. The hMSCs were isolated as described earlier [23]. After isolation the cells were cultured in a basal medium consisting of DMEM with 10% heat-inactivated fetal calf serum (FCS), 2 mM L-glutamine and 100 U/mL penicillin/streptomycin at 37 °C, 5% CO_2, in 95% humidity. To promote osteogenic differentiation 10^{-7} M dexamethasone, 50 μg/mL ascorbic acid and 216 μg/mL β-glycerophosphate was added to the basal medium. The culture medium was changed twice a week.

Human dermal fibroblasts were cultivated under the same conditions in DMEM with 10% FCS, 1% L-glutamine and 1% penicillin/streptomycin. The cells were passaged with 0.05%/0.02% trypsin/ethylenediaminetetraacetic acid in phosphate-buffered saline (PBS).

MTT assay

In order to investigate the number of vital fibroblasts on the mesoporous films a MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) (Merck KgaA, Darmstadt, Germany) assay was performed. After cultivating human fibroblasts over a period up to 14 days, the cells were incubated in 1.5 mL of MTT-medium (12 mmol MTT/L basal medium) at 37 °C, 5% CO_2 and 95% humidity for 3 h. After removing the medium the formed formazan-crystals were dissolved during 5 min of cultivation at 37 °C, 5% CO_2 and 95% humidity in 400 μL 0.04 M hydrochloric acid in isopropanol (Ölfabrik Schmid, Ulm, Germany). Absorption was measured with an ELISA-reader (Dynatech Laboratories, USA) at a wavelength of 530 nm.

TUNEL-staining

Apoptotic cells were visualized using the terminal deoxynucleotidyl-transferase mediated nick-end labelling method. Fibroblasts were washed with PBS and then fixed on the substrates with 4% formaldehyde. Afterwards the fibroblasts were incubated in permeabilization solution (0.1% Triton X-100 in 0.1% sodium citrate) at room temperature for 8 min. The nuclei of apoptotic cells were labelled by addition of 5 µL TUNEL-enzyme in 45 µL TUNEL-label per slide and incubation in a humidified chamber in the dark at 37 °C for 60 min (all Roche Diagnostics GmbH, Mannheim, Deutschland). Cells were washed twice with PBS and counterstained with 0.5 µg/mL propidiumiodide for 3 min at room temperature.

"Von Kossa" staining

Mineralized matrix was stained using the "von Kossa" method. Cells were fixed in 4% formaldehyde for 15 min, incubated in aqueous silver nitrate solution (50 g/L) for 60 min, rinsed with PBS and incubated in pyrogallic acid (10 g/L) for 10 min. Excessive silver ions were removed by incubating the cells in sodium thiosulphate solution (50 g/L). The nuclei were counterstained with nuclear fast red (5 g aluminium sulphate and 0.1 g nuclear fast red per 100 mL deionized water). A specimen slide was mounted with Kaiser's glycerol gelatine to avoid drying-out.

Microscopy

Fluorescence and optical microscopy was done by using a Zeiss "Axioskop mot plus" with a Zeiss AxioCam MRC and Zeiss Axio Vision (v. 3.1) software.

Results and discussion

Mesoporous coatings

Sol–gel-based titania and mixed oxide silica–titania coatings have already been applied in studies of cell adhesion and cell response [14, 15]. However, these coatings are prepared via a time-consuming two-step hydrolysis procedure, resulting in heterogeneously mixed oxide phases, without any templating agent and, thus, show a low degree of porosity. Up to our knowledge, highly porous mixed silica-titania coatings have not been applied for studies on the cultivation of cells.

A number of different synthesis protocols towards periodically arranged mesoporous coatings have been developed by now. The first detailed mechanistic study on dip-coated silica-based samples was performed by Brinker and his coworkers, and they termed the process "evaporation-induced self-assembly" (the EISA-process) [16, 24]. In principle, these porous coatings can be modified with e.g. metal oxides by several different approaches such as direct synthesis [25], post-treatment [26] or by one-pot synthesis, e.g. by coordinating the metal alkoxides to the ethylene glycol moiety of the structure-directing surfactant to name just a few [27–30]. However, the main problem of deliberately avoiding or inducing phase separation or clustering of one species with increasing amounts of the modifying species, e.g. a transition metal oxide in a silica matrix, is still not sufficiently solved up to date. Inorganic-organic single-source precursors for oxide network formation have been reported in the synthesis of monolithic materials, e.g. titanium isopropoxide complexed by a chelating ligand, such as the silane (3-acetyl-trialkoxysilylhexane-2-one) coordinated to a metal alkoxide, e.g. titanium isopropoxide [20, 31, 32]. This precursor seems to be an ideal candidate to increase the concentration of metal centres in the otherwise inert silica matrix to even higher ratios than was reported for the metal-complexed surfactant systems, since the inherent ratio of Si:metal in these precursors is already set to 1:1.

In this study, different mesoporous silica and silica-titania coatings were synthesized by spin-coating using a pre-hydrolysed silica sol and the 3-acetyl-6-triethoxysilylhexane-2-one coordinated titanium alkoxide as single-source precursor (SSP) (see Scheme 1) in different ratios, thus resulting in silica films with different contents of titania with a maximum of 50 wt% TiO_2. Homogeneous transparent coatings with a thickness between 100 nm and 150 nm were obtained on glass substrates for all precursor mixtures. The increasing amount of the SSP, and thus the increasing content of titanium dioxide in the matrix, however has a distinct influence on the porosity and the degree of mesostructural ordering in the coating.

X-ray diffraction patterns of pure silica films showed a sharp (10) diffraction peak with a *d*-spacing of 4.35 nm (Fig. 1). Moreover, the pattern indicates a long-range pore ordering (the 20 reflection is also visible) which could be confirmed by TEM images (Fig. 2a), where a hexagonally ordered mesoporous system was observed.

As expected, the addition of the SSP is strongly disturbing this hexagonal arrangement of the pore system. Coordination of the titanium alkoxide to the silane results in a lowering of the reactivity of the titanium centre; however, still different reaction rates in the condensation can be found. Nevertheless, macroscopic phase separation is avoided owing to the stable link between the different alkoxides. This results in the formation of small titania particles surrounded by a shell of organosilane ligands. Diffraction patterns showed one single diffraction peak for

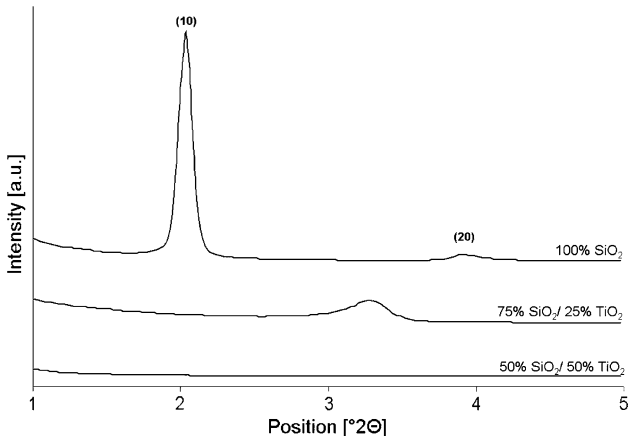


Fig. 1 X-ray diffraction pattern of calcined samples. Films were synthesized using a pre-hydrolysed coating sol and a titanium-complexed 3-acetyl-6-triethoxysilylhexane-2-one as titanium source

silica films with 25% TiO_2—however strongly shifted to larger angles—and no ordering for films prepared solely with the titanium-complexed silane as single-source precursor was observed. In agreement with the XRD results, TEM images showed a wormhole-like mesoporous system for silica–titania films containing 25% titania (Fig. 2b) and a less porous system for films with a ratio of 1:1 for Si:Ti (Fig. 2c). A better insight into the pore structure is obtained from nitrogen sorption experiments (Fig. 3).

With an increasing content of the single-source precursor in the coating solution, the specific surface area decreases from 467 $m^2\ g^{-1}$ to 170 $m^2\ g^{-1}$ (see also Table 1). The lack in porosity is correlated to the loss of mesopores and pore ordering. This is also reflected in the hysteresis of the ad- and desorption isotherms. The adsorbed volume is drastically reduced and the hysteresis changes from a H4 type to H1, indicating a lower accessibility of the pores. As described earlier, this can probably be related to the special condensation mechanism of the SSP. As seen from previous SAXS measurements on bulk samples, first small titania particles grow, limited in size by the steric hindrance of the alkylalkoxysilanes moieties covering the primary particle surface. Only upon calcination the organic groups are destroyed and phase separation can occur on a larger length scale resulting in titania-rich and silica-rich nanodomains in the films [21, 31]. However, crystallization of titania polymorphs such as anatase or rutile is not observed in any of the described coatings.

For application of these coatings in cell cultivation in an aqueous environment, the surface properties are of major importance. Therefore, the wetting behaviour was investigated by contact angle measurements according to the sessil-drop method (Table 1) on calcined films. On pure silica films and on silica films with a content of 25% of titania, water droplets fully spread on the surface, whereas silica-titania coatings prepared from the SPP alone (1:1) showed a slightly higher contact angle, which is equivalent to a less hydrophilic surface. In both cases calcination resulted in dehydroxylation of vicinal hydroxyl groups on the metal dioxide surfaces, starting at temperatures higher than 150–200 °C for silica and higher than 200 °C for titania. Hence, the density of hydroxyl groups on silica-titania films with a ratio 1:1 (silicon:titanium) is lower than that on pure silica and on silica films doped with 25% titania [33].

Cell adhesion and proliferation

Modifications of the coatings were achieved by doping mesoporous silica films with different contents of titania (25% and 50%). To estimate the quality of the synthesized films in cell culture, 10.000 human fibroblasts (hFb) were seeded on each sort of film and cultivated under standard conditions for several days. Within 3 h of cultivation the cells began to adhere on the substrates. Due to the hydrophilicity of all tested films, adsorption of organic compounds, in particular proteins of the supplemented serum, was supported. The hFb interacted with the deposited protein layer and adhered on it. Cell spreading and proliferation started after 24 h of cultivation on all substrates (Fig. 4), which indicates the presence of a high number of binding sites on the material [34]. For quantification of cell proliferation an MTT assay was performed. Human fibroblasts were cultivated over a period of 14 days and the

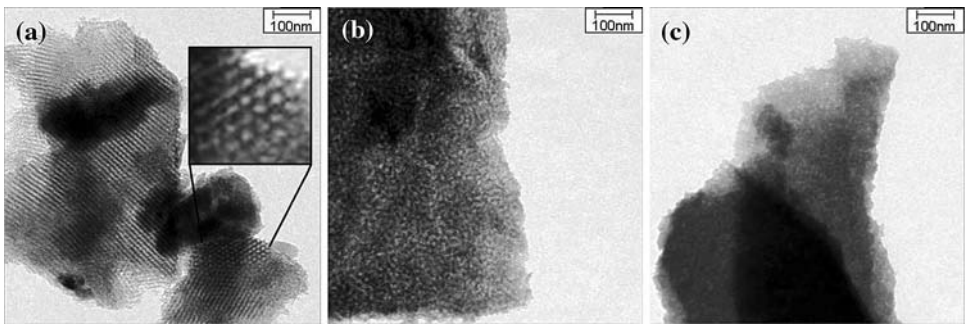


Fig. 2 TEM images of calcined silica films (**a**), 75% silica/25% titania films (**b**) and 50% silica/50% titania films (**c**)

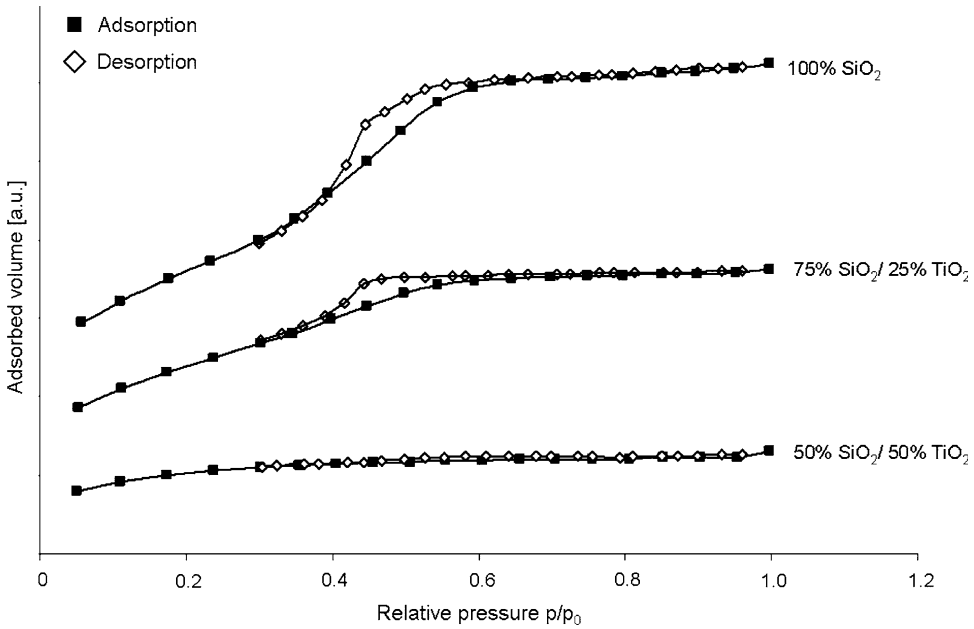


Fig. 3 Nitrogen adsorption-desorption isotherms of mesoporous silica, 75% silica/25% titania and 50% silica/50% titania

Table 1 Specific surface area (SSA), pore size distribution (PSD), film thickness and contact angle measurements

Content of TiO_2 (%)	0	25	50
SSA[a] (m^2 g^{-1})	467	354	170
PSD[b] (nm)	3.45	3.45	2.66
Contact angle (°)	9.7	7.2	21.6
Film thickness (nm)	140 ± 25	142 ± 20	100 ± 23

[a] The specific surface area was calculated according to the BET method

[b] The pore size distribution was calculated using the desorption branch of the isotherm and calculated by the BJH model

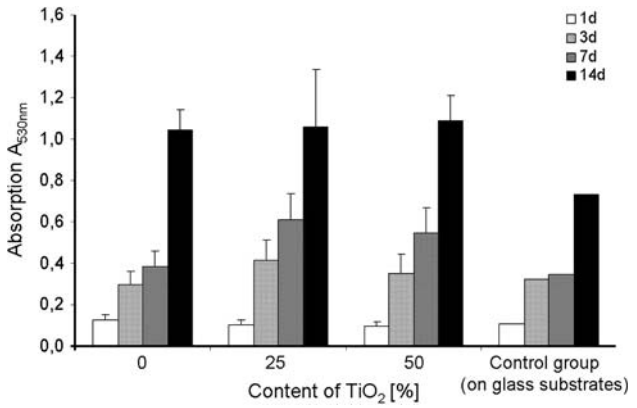


Fig. 4 MTT assay to investigate the relative proliferation rate of human fibroblasts on silica and silica-titania films. As a control, cell growth was determined on uncoated glass substrates. Absorbance was measured after 1, 3, 7 and 14 days of cultivation under standard conditions. Bars represent the average value and the standard deviation of six independent measurements

number of viable cells was measured as a proportional function of absorbance of the MTT-test solution after 1, 3, 7 and 14 days (Fig. 5). Uncoated glass substrates were used for control experiments.

After adherence, proliferation of the hFb started on all tested coatings. It is obvious that an increasing duration of cell cultivation led to higher absorbance values and to a higher cell count. In comparison to uncoated glass substrates the number of viable cells was increased after 7 days for 25% and 50% TiO_2 and after 14 days for all coatings. The modification of silica films with titania therefore resulted in a better cell proliferation after 7 days of cultivation. Our results on fibroblast adhesion and proliferation on mesoporous mixed metal oxide films are in accordance with those of Areva et al. on sol–gel-based thick and less porous TiO_2–SiO_2 coatings showing that fibroblasts exhibit the highest proliferation activity on coatings with a ratio of 70:30 (silica:titania) [15]. In contrast to mixed metal oxide films, seeding on pure TiO_2 films resulted in less cell attachment and proliferation [15]. This indicates that mesoporosity had no negative influence. In our experimental setting, hFb grew to confluence and partly in multilayers until day 14 on the coated but not on native glass surfaces. At this last time point studied, no further influence of the different silica substrates on cell proliferation was detectable.

Apoptosis

The influence of the mesoporous silica and silica–titania films on cell viability and apoptosis of hFb was studied by the TUNEL (terminal deoxynucleotidyl-transferase

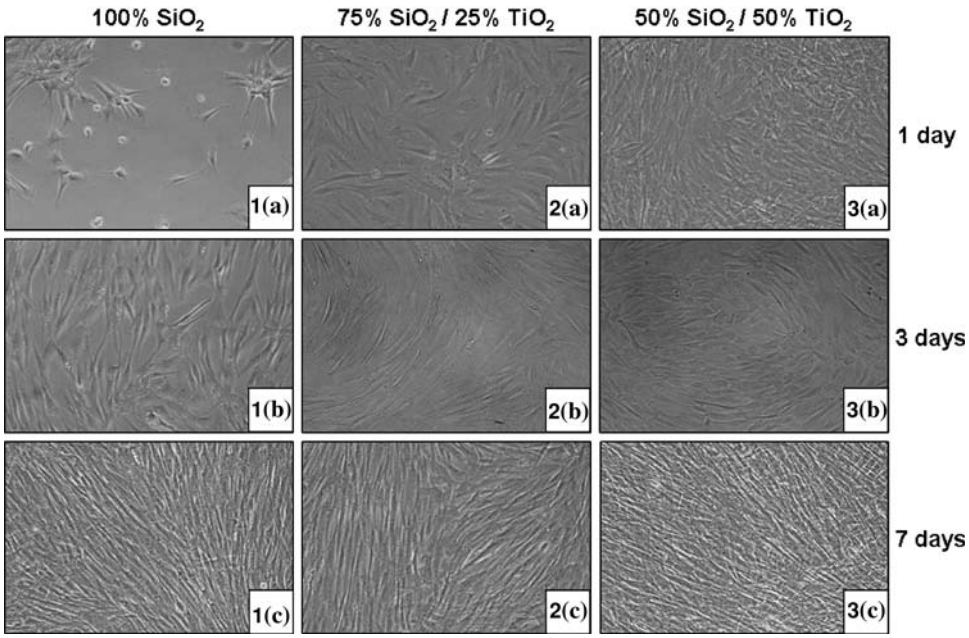


Fig. 5 Influence of titania in mesoporous silica films on the adhesion and the proliferation of human fibroblasts (hFb). Optical microscopy was performed after 1 day (**a**), 3 days (**b**) and 7 days (**c**) of cultivation on pure silica (1), 75% silica/25% titania (2) and 50% silica/50% titania (3) films. Magnification 100×

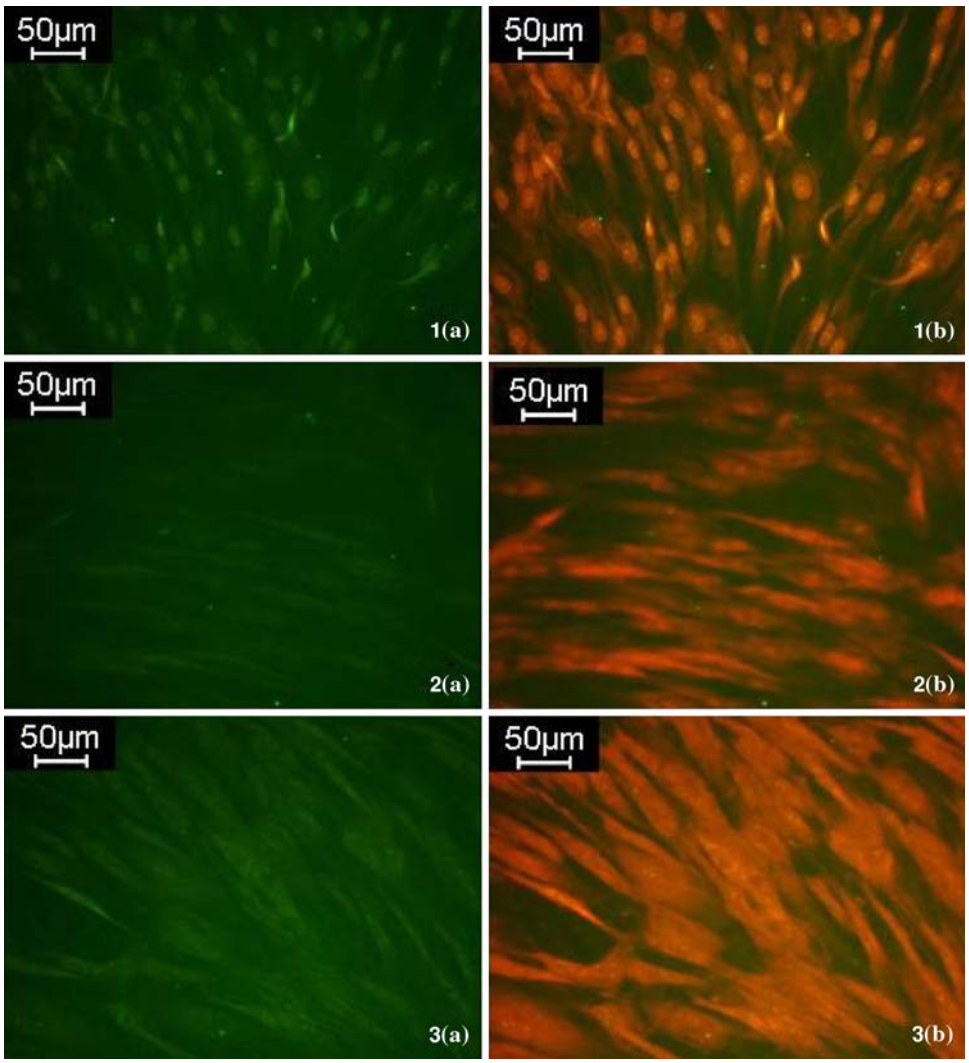


Fig. 6 TUNEL-staining of human fibroblasts after 10 days of cultivation under standard conditions. Images show few green-labelled nuclei of hFb grown on pure silica (**1a**) and the green background fluorescence of cells seeded on 75% silica/25% titania (**2a**) and 50% silica/50% titania (**3a**) films. Counterstaining with propidiumiodide led to red fluorescent cells on silica (**1b**), 75% silica/25% titania (**2b**) and 50% silica/50% titania (**3b**) films

mediated nick-end labelling) method. All cells were counterstained with propidiumiodide. Fluorescence microscopy showed several apoptotic cells on pure silica substrates (Fig. 6), whereas only single apoptotic fibroblasts were detectable on silica films doped with titania. In combination with our results on cell adhesion and proliferation this indicates good in vitro biocompatibility of silica-titania-mixed metal oxide films.

Osteogenic differentiation

To investigate the effect of the films on the differentiation potential of human multipotent mesenchymal stromal cells (hMSC), these cells were cultured under conditions that induce osteogenic differentiation. After 14 days of cell cultivation, the hMSC formed typical matrix producing nodules. The mineralization nodule formation could be visualized as black spots by "von Kossa" staining (Fig. 7, 1a and 1b). After one additional week of cultivation the osteogenic differentiation of the hMSC proceeded representing the ongoing mineralization of the surrounding matrix (Fig. 7, 2a and 2b). Therefore, pure silica films as well as 75% silica/25% titania films do not inhibit the differentiation of hMSC to osteoblasts. Previously, it has been shown that solid TiO_2–SiO_2-coatings support the formation of calcified nodules by differentiated osteoblasts presumably by releasing silica [15], which was observed as a stimulative factor for osteogenesis [35]. Therefore, silica–titania-mixed metal oxide films enable osteogenic differentiation and osteoblastic function of cells derived from different stages of the osteogenic lineage.

Conclusion

Mesoporous silica and silica-titania mixed metal oxide films were successfully employed as substrates for the cultivation of human fibroblasts and human multipotent mesenchymal stromal cells in vitro. They allowed cell adherence and cell proliferation and did not lead to high rates of apoptosis. With respect to cell proliferation and cell viability, mesoporous silica–titania metal oxide films seem to have some advantage. Human multipotent mesenchymal stromal cells showed mineralized matrix deposition on all coatings, which is a result of osteogenic differentiation. We conclude that the investigated films are biocompatible in vitro and represent promising tools to improve biological processes at the bone implant interface by using the mesoporous properties for local drug release.

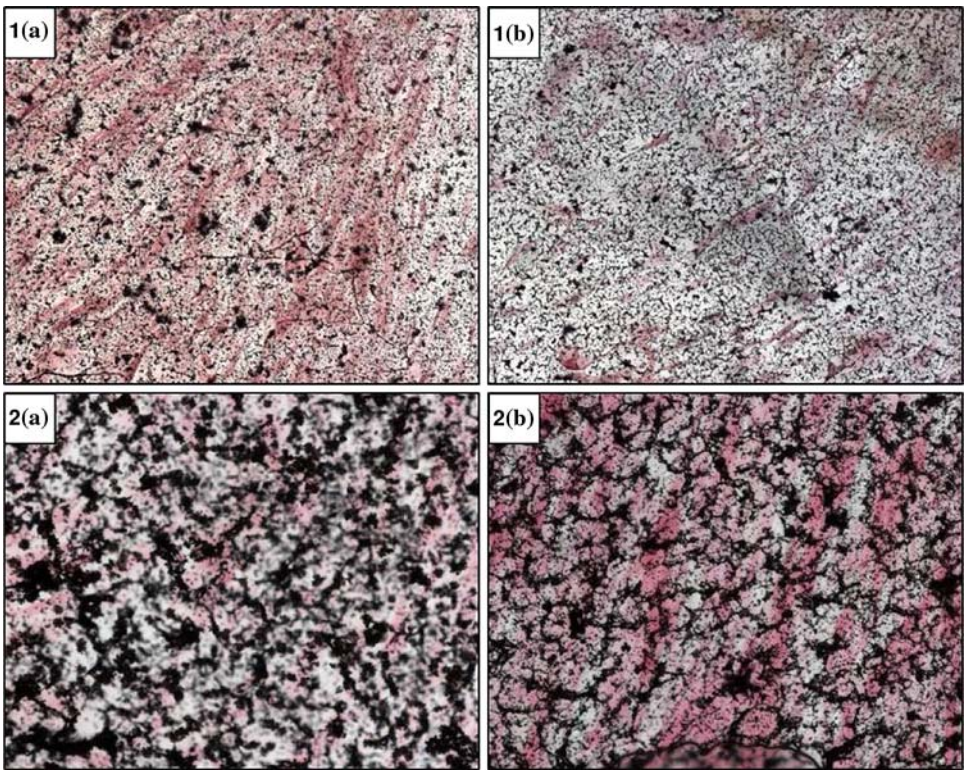


Fig. 7 "Von Kossa" staining of human multipotent mesenchymal stromal cells (hMSC) cultured for 14 (1) and 21 days (2) on mesoporous silica films (**a**) and 75% silica/25% titania films (**b**). The cells were counterstained using nuclear fast red. Mineralized matrix is visualized by black dots

References

1. Kresge CT, Leonowicz ME et al (1992) Nature 359:710
2. Carturan G, Campostrini et al (1989) J Mol Catal 57:L13
3. Avnir D, Coradin T, Lev O, Livage J (2006) J Mater Chem 16:1013
4. Rooke JC, Léonard A et al (2008) J Mater Chem 18:2833
5. Yang XY, Li ZQ et al (2006) Adv Mater 18:410
6. Vallet-Regi M (2006) Chem Europ J 12:5934
7. Coffer JL, Whitehead MA et al (2005) Phys Stat Sol A Appl Mater Sci 202:1451
8. Yan X, Yu C et al (2004) Angew Chem Int Ed 43:5980
9. Balas F, Manzano M et al (2008) Acta Biomater 4:514
10. Baca HK, Carnes E et al (2006) Science 313:337
11. Puleo DA, Nanci A (1999) Biomaterials 20:2311
12. Vallet-Regi M (2006) Dalton Trans 44:5211
13. Viitala R, Jokinen M et al (2002) Biomaterials 23:3073
14. Ääritalo V, Areva S et al (2007) J Mater Sci Mater Med 18:1863
15. Areva S, Ääritalo V et al (2007) J Mater Sci Mater Med 18:1633
16. Brinker CJ, Lu Y et al (1999) Adv Mater 11:579
17. Doshi D, Hüsing N et al (2000) Science 290:107
18. Hozumi A, Kimura T (2008) Langmuir 24:11141
19. Spatz JP (2004) Nanobiotechnology 53
20. Puchberger M, Rupp W et al (2004) New J Chem 28:1289
21. Torma V, Peterlik H et al (2005) Chem Mater 17:3146
22. Brinker CJ, Raman NK et al (1995) J Sol-Gel Sci Technol 4:117
23. Fiedler J, Brill C et al (2006) Biochem Biophys Res Commun 345:1177
24. Lu Y, Ganguli R et al (1997) Nature 389:364
25. Garcia C, Zhang Y et al (2003) Angew Chem Int Ed 42:1526
26. Matsura V, Guari Y et al (2004) J Mater Chem 14:3026
27. Tura C, Coombs N et al (2005) Chem Mater 17:573
28. Supplit R, Hüsing N et al (2006) J Mater Chem 16:4443
29. Hüsing N, Launay B (2003) Appl Catal 254:297
30. Supplit R, Hüsing N et al (2007) Europ J Inorg Chem 18:2797
31. Koehler J, Cai J et al (2007) Mater Res Soc Symp Proc 1007:S04–S12
32. Andrianainarivelo M, Corriu R et al (1996) J Mater Chem 6:1665
33. Kanta A, Sedev R et al (2005) Langmuir 21:2400
34. Lebaron RG, Athanasiou KA (2000) Tissue Eng 6:85
35. Hench LL (2000) Key Eng Mater 192–195:575