J Mater Sci: Mater Med (2007) 18:465–473
DOI 10.1007/s10856-007-2006-0

The influence of implant surface properties on cell adhesion and proliferation

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Received: 14 April 2005 / Accepted: 24 October 2005
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Abstract Interactions of the foreign material of implant and the living tissue on the cell level can cause prolonged healing or, worse, loss of the implant. The cell response to the presence of some implant materials was studied under *in vitro* conditions.

The influence of physicochemical surface parameters on the response of the cells in the immediate vicinity of implants, namely on adhesion, proliferation and synthetic activity of fibroblasts, and on the blood coagulation were compared. The direct contact of tested materials (titanium and Ti6Al4V alloy with various surface treatments, Cr Co Mo alloy, hydroxyapatite-coated titanium, zirconium oxide ceramics, polyethylene and carbon composite) on cell spreading was monitored and the presence of TNF-α and IL-8 was evaluated in the cultivation medium. The formation of blood clots was investigated on samples immersed in a well with freshly drawn whole rabbit blood using a scanning electron microscope. The surface free energy was estimated using the measurement of static contact angle. Both the advancing and receding contact angles were measured by the dynamic Wilhemy plate method.

Two main groups with extremes in cell viability were established. In the first group the increased polar component of surface free energy, the highest cell density, the lowest inflammatory cytokine production, but no fibres in the clotting blood were found. On the contrary, the second group of materials with a very low polar component of the surface free energy showed distinctly higher expression of inflammatory mediators, low cell proliferation, but faster formation of fibres in the blood coagulum.

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1 Introduction

In recent years, various types of artificial materials as implants have been widely used in all medicine fields. Implants replace quite often bones and bone-embedded structures. For load-bearing bone replacements, traditional natural materials (bone grafts) or semisynthetic materials (bone substituent materials) may be used [1]. Synthetic metal and nonmetal materials have recently replaced these materials, mainly for mechanical reasons. Basic mechanical requirements that have to be met by synthetic implant materials are resistance, elasticity and resistance to abrasion. It is also necessary to pay attention to their biological tolerance.

The improvement of the contact between bone and the implant, speed-up of the healing process as well as prolongation of the functional period of implants are still primary tasks of the tissue healing management. Interactions between the foreign material (implant) and the living tissue on the cell level are one of the causes leading to prolonged healing or, worse, to oss of the implant [2]. These interactions are difficult to avoid completely as there has been no material, which would not initiate any tissue reactions. In short, the tissue responds to every implant as to a foreign body. The intensity of the reactions is proportional to the level of mechanical and physicochemical irritation. The interaction between the implant and the surrounding tissue depends also on characteristics of the

Table 1 Implant materials for testing

Material		Surface treatment	Manufacturer
Metals	titanium	polished	Beznoska, Kladno, CR[a]
		sand-blasted	Beznoska, Kladno, CR
		plasma-sprayed	Beznoska, Kladno, CR
		hydroxyapatite	Lasak, Prague, CR
		etched	Fopos, Prague, CR
	Ti6Al4V alloy	sand-blasted	Beznoska, Kladno, CR
	CoCrMo alloy	polished	Beznoska, Kladno, CR
Nonmetals	polyethylene	—	Beznoska, Kladno, CR
	ZrO_2 ceramics	non-polished	Saint-Globain, Turnov, CR
	C/C composite	pyrolytic carbon	Institute of Rock Structure and Mechanics Academy of Sciences, Prague, CR
Control	TCPS[c]	—	Nunc A/S, Roskilde, Denmark

[a]Czech Republic, [b]carbon/carbon composite, [c]tissue-culture-grade polystyrene.

implant surface. The change of physical and chemical surface properties often leads to a different number of adhered cells and thus initiates a different cell response [3, 4].

Other possible reasons for the implant failure are an inappropriate surgical procedure, insufficient primary implant retention, too wide gap between the implant and the bone bed [5] or insufficient blood clot formation [6, 7]. Processes during the fracture healing as well as during the iatrogenic bone damage caused prior to implant placement proceed by similar mechanisms. Fibrin fibres serve as a way for migration of osteogenic cells, platelets release a number of growth factors and cytokines, which play an important role in the wound healing cascade [7].

The aim of our work is to investigate the cell response to the presence of a wide range of some commonly used and newly developed implant materials under *in vitro* conditions. In the present preliminary study, an established fibroblast line preferred for biomaterial testing, was used as a model system in order to describe basic correlations between the cell response and biomaterial surface. First, the investigated materials were ordered according to the response of cells growing in the immediate vicinity of the implant. Particularly, adhesion and proliferation and cytokine expression of fibroblasts were investigated and compared. Second, implants with the best and the worst cell adhesion and proliferation were used for more detailed study of selected surface properties and for blood clot formation experiments.

2 Material and methods

2.1 Implant materials

2.1.1 A list of implant materials selected for testing is given in Table 1. The tissue-culture-grade polystyrene (TCPS) was used as a positive control in order to compare cultivation properties of the tested materials with this "gold" standard. TCPS represents the material with properties optimized for cultivation of eukaryotic cells in vitro.

2.1.2 Preparation, coating and sterilization of materials

For tissue culture and blood clot formation assays, small round plates (diameter 30 mm, thickness 1 mm) were prepared from the tested materials. Square plates ($20 \times 10 \times 1$ mm) were used for the measurement of the surface free energy and wettability of the implant surface. The mechanical surface treatment of metal materials (polishing, sand-blasting, etching, plasma-spraying) and the hydroxyapatite coating was provided by the material producer (see Table 1). All materials were washed in deionized water and in ethanol in an ultrasonic bath and subsequently autoclaved before use.

2.2 Tissue culture

Human embryonal lung fibroblasts (LEP 17th passage, Sevapharma, Prague, Czech Rep.) were cultivated up to the 19th passage [8]. LEPs, human stable diploid stem cells corresponding to CCL 75 (WI-38) are recommended for biomaterial testing by ISO 10993-5:1992(E). The tested materials were put into a six-well dish (Nunc A/S, Roskilde, Denmark), LEP cells were inoculated (80000 cells/cm^2) on implants and cultured under standard conditions for 3 days [9]. For further estimations, the culture medium and harvested cells were used.

2.3 In vitro cell proliferation and viability tests, cytokine detection

The proliferation activity of LEP cells was characterized by density and distribution of fibroblasts on implant materials. LEP cells were fixed with paraformaldehyde (0.4%), treated

with 0.2% Tween (Sigma-Aldrich, Prague, Czech Rep.) and subsequently stained with a 1% aqueous solution of propidium iodide (Sigma, Prague, Czech Rep.) in the medium (10 μl solution/ 6 ml Dulbecco Essential Medium (D-MEM), overnight, 4°C). Samples were inspected with a fluorescence microscope (Olympus, Prague, Czech Rep.). The viability of cells was estimated by the mitochondrial oxidation activity of the cell monolayer using MTT tests according to Laughton [10].

The medium from cultivation experiments was also utilized for cytokine detection (TNF-α, IL-8) using the Immulite analyzer (DPC, Los Angeles, USA). This system utilizes an assay-specific antibody or antigen-coated plastic beads as the solid phase, an alkaline phosphatase-labeled reagent and chemiluminescent enzyme substrate.

2.4 Blood clot coagulation kinetics

Based on preliminary experiments, samples of each tested implant were randomly divided into four time related groups with incubation in the clotting blood for 2, 3, 4 and 5 min. Samples were put into a 24-well dish (Nunc A/S, Roskilde, Denmark) under sterile conditions. The wells were infused with 3 ml of a freshly drawn whole rabbit blood, covered with a lid and left standing. After selected incubation time (2–5 min), samples were taken out, rinsed in the saline solution, fixed in glutaraldehyde, dried and coated with gold for evaluation by scanning electron microscopy (JSM 5500 LV, Tokyo, Japan) [11].

2.5 Characterization of the surface free energy γ and wettability

The surface free energy (SFE) γ was estimated by the measurement of static contact angle (Θ) in three different solvents (water, formamide, diiodomethane) using the drop sessile method (video-camera based instrument OCA 20, Dataphysics, Filderstadt, Germany). An average value of Θ obtained from Young-Laplace fitting approach was used for calculation of the surface free energy γ and its polar γ^{p} and dispersive γ^{d} component according the OWCK method. The advancing (Θ_A) and receding (Θ_R) contact angles were estimated by the dynamic Wilhemy plate method (tensiometer Kruss K12, Hamburg, Germany) in water. For selected samples, the measurement was performed also in the cultivation medium. The surface tensions γ of water and the cultivation medium were estimated using the drop sessile method. Samples were cleaned and sterilized before the measurement in the same way as samples for cell experiments.

2.6 Statistical evaluation

Cell proliferation on the materials was determined in eight independent experiments. For a single experiment, four parallel samples of each material were evaluated. Values of the population cell density represent an average mean and an average ± standard deviation of eight assays. Statistical evaluation was performed by the Kruskal-Wallis one-way analysis of variance and Mann-Whitney rank-sum test. The calculation of the statistical significance of cell viability was performed for tested materials (No. 1–10) against TCPS control (Table 2).

The cytokine detection was performed by the standard procedure using one ELISA test plate. The sample of the culture medium, used for the ELISA test was a mixture of three parallel runs of one tested surface. The two parallel determinations were performed. Using this method, statistical evaluation could not be made.

For calculation of the surface free energy (SFE) γ, five plates were tested with six drops for each solvent. For

Table 2 Tested materials ordered according to the number of cells growing on their surface.

Succession No.	Material	Surface treatment	Cell number[a] $\times 10^3$ (ml^{-1})	Standard deviation[b]	Statistical significance[c]
1	ZrO_2 ceramics	—	160	±68.2	NS
2	titanium	polished	156	±64.2	NS
3	titanium	sand-blasted	148	±55.5	NS
4	titanium	etched	138	±48.4	NS
5	titanium	plasma-sprayed	129	±42.8	NS
6	Ti6Al4V alloy	sand-blasted	121	±32.4	NS
7	CoCrMo alloy	polished	107	±45.4	NS
8	titanium	hydroxyapatite	92	±76.6	NS
9	polyethylene	—	85	±42.3	NS
10	C/C composite	—	49	±41.0	**
Control	TCPS	—	235	±108	—

[a]Average from 8 subsequent tests; [b]The mean standard deviation of eight assys, always with four parallel runnings of the sample; [c]** $P \leq 0.01$ versus control; NS—nonsignificant - samples No. 1–9.

dynamic measurement, five parallel samples for each type of the material were tested.

3 Results

3.1 Proliferation, viability and synthetic activity of fibroblasts

The viability of cells was estimated from the mitochondrial oxidation activity of the cell monolayer. Table 2 shows a list of implants ordered according to cell viability from materials with the best cell viability (ZrO_2 ceramic, Ti-polished) to the worst ones (hydroxyapatite-Ti, polyethylene (PE), C/C composite). The assay was repeated eight times, every independent experiment was performed with a new passage of cells and these facts correspond with a high standard deviation. The materials in the list are numbered from 1 for the highest cell viability to 10 for the lowest one. (The numbers are used in all figures). The population cell density on all the tested materials is lower in comparison with the TCPS control. However, there are no significant statistical differences either between the tested materials or the TCPS control. An exception is the C/C composite with the statistical difference $P \leq 0.01$ versus the TCPS control.

Proliferation of LEP cells was also characterized by evaluation of the fibroblast density and their distribution on implant materials obtained by fluorescence microscopy. The character of the fibroblast proliferation depended on the material type and on its surface treatment as presented on selected examples in Fig. 1. Fibroblasts well colonized the surfaces where the highest mitochondrial oxidation activity of fibroblasts was detected, such as ZrO_2 ceramics (Fig. 1a) or polished Ti (Fig. 1b). As expected, significantly lower numbers of adhered cells were observed on the surfaces with the lowest population density, such as PE (Fig. 1c) or C/C composite (Fig. 1d).

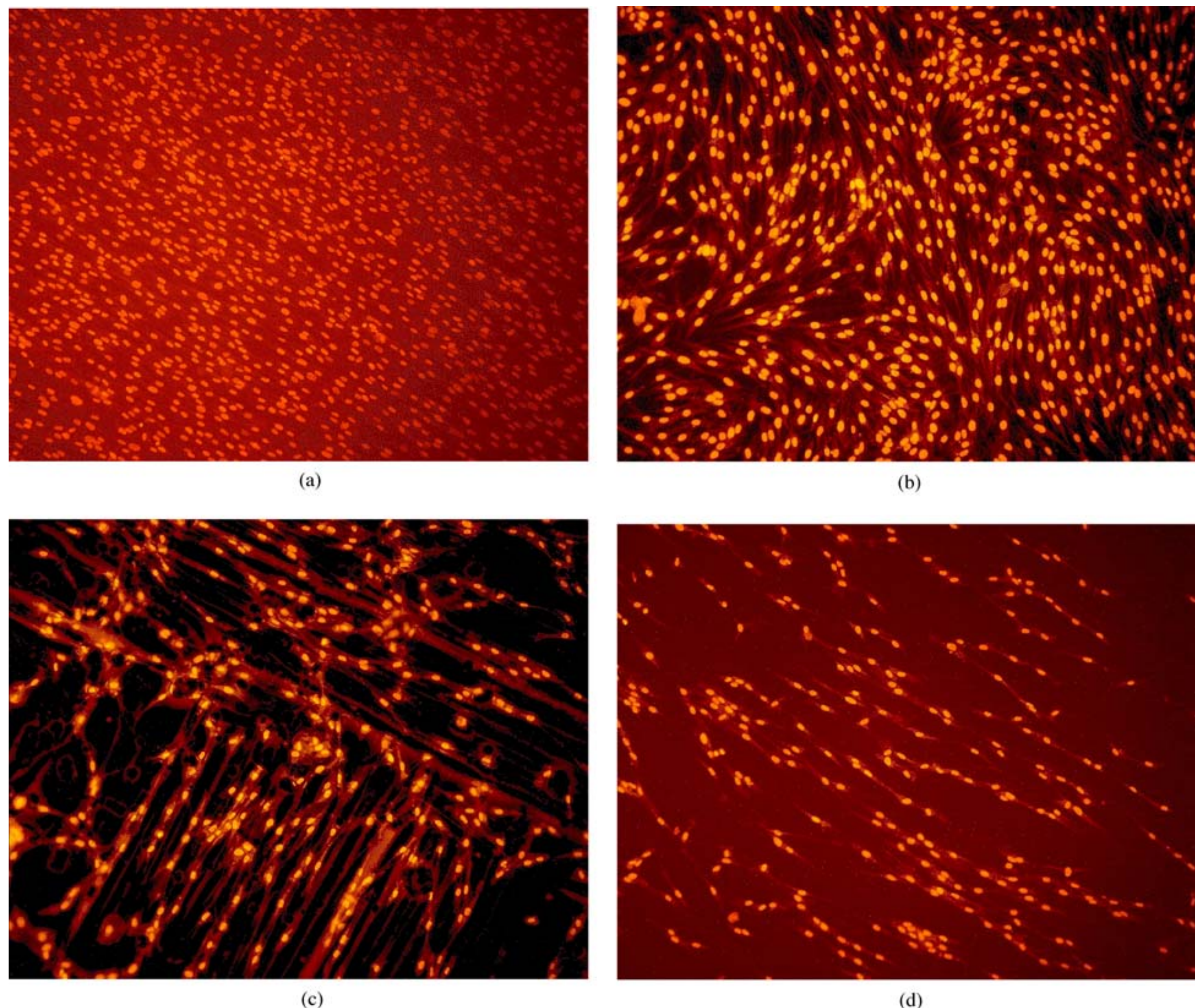

Fig. 1 LEP fibroblasts after 2 days of cultivation on selected implant materials: a) a uniform cell monolayer on ZrO_2ceramics material; b) a well colonized polished titanium surface; c) the character of cell adhesion respects orientation of carbon fibres on the C/C material; d) a thin cell monolayer on polyethylene. Propidium iodide staining, magnification 160×.

Table 3 Blood clot coagulation on selected implant material surfaces

Material	Incubation time with blood (min)			
	2	3	4	5
ZrO_2 ceramics	—	—	elements	clusters
Polished Ti	elements	elements and clusters	individual fibers	network
Polyethylene	elements and clusters	individual fibers	individual fibers	network
C/C composite	network/clots	—	—	—

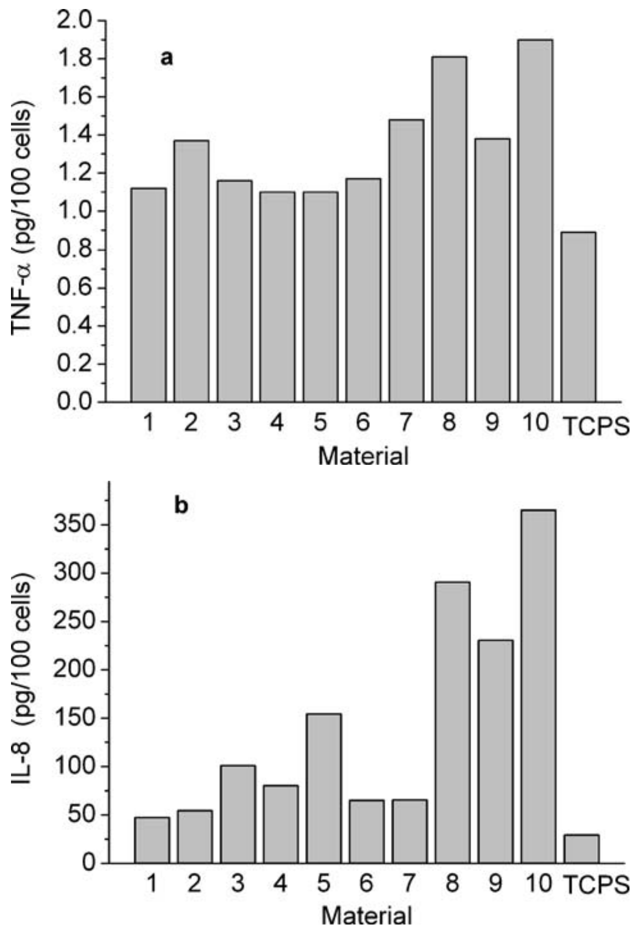

Fig. 2 Cytokines produced by LEP fibroblasts growing on tested material: (a) production of TNF-α; (b) production of IL-8; 1-ZrO_2 ceramics; 2-polished Ti; 3-sand-blasted Ti; 4-etched Ti; 5-plasma-sprayed Ti; 6-sand-blasted Ti6A14V; 7-CoCrMo alloy; 8-hydroxyapatite-Ti; 9-polyethylene; 10-C/C composite; TCPS–a control.

The amount of cytokines TNF-α and IL-8 produced by cultivated cells is presented in Figs. 2a and 2b. In comparison with fibroblasts cultivated on control TCPS dishes, fibroblasts on all the tested materials exprimed both cytokines to a higher extent. Interestingly, materials from the end of the list in Table 2, i.e. C/C composite, polyethylene and hydroxyapatite-Ti, induced the highest expression of cytokines.

3.2 Blood clot coagulation

The clot formation was evaluated on selected samples for which, in our opinion, most significant differences in the cell proliferation and production of cytokines were observed. Thus, the first materials (i.e. ZrO_2 ceramics, polished Ti) and the last ones (PE and C/C composite) in the line according to Table 2 were tested.

The mean clotting time of blood in the syringe was approximately from 6 to 8 min. Blood elements adhered to the implant surface at different times depending on the used material. In general, the individual sitting blood element, or elements aggregated in the cell clusters were visible using the SEM technique in samples with a short exposure (2 and 3 min) to the clotting blood. Clusters and developing fibres were observed with samples remaining longer in blood (3 and 4 min) and a network of fibres with entrapped blood elements in samples with the longest exposition (4 and 5 min). Results for particular tested implant materials are presented in Table 3.

The rate of the blood clot formation as well as the fiber development was faster on C/C composite and PE surfaces. On the C/C composite, a network of fibres with entrapped blood elements already formed after 2 min (Fig. 3a). On the contrary, only individual sitting elements on the polished titanium and no elements on ZrO_2 ceramics were observed after 2 min. In the case of PE surfaces, individual sitting elements were widely spread on the whole surface, starting to aggregate to clusters after 2 min; short fibres grew up after 3 min. Interestingly, a similar though slower trend was observed for the polished titanium. The slowest clot formation was observed on the ZrO_2 ceramics; only some blood elements were visible even after 4 min of incubation (Fig. 3b).

3.3 Wettability and the surface free energy of tested surfaces

The advancing (Θ_A) and receding (Θ_R) contact angles of dry materials are plotted in Fig. 4. The materials are placed in the graph according to their position in Table 2. It follows from Fig. 4 that Θ_A (mainly characterizing total surface wettability) increased with increasing position of the material in Table 2. For ZrO_2 ceramics and most titanium surfaces,

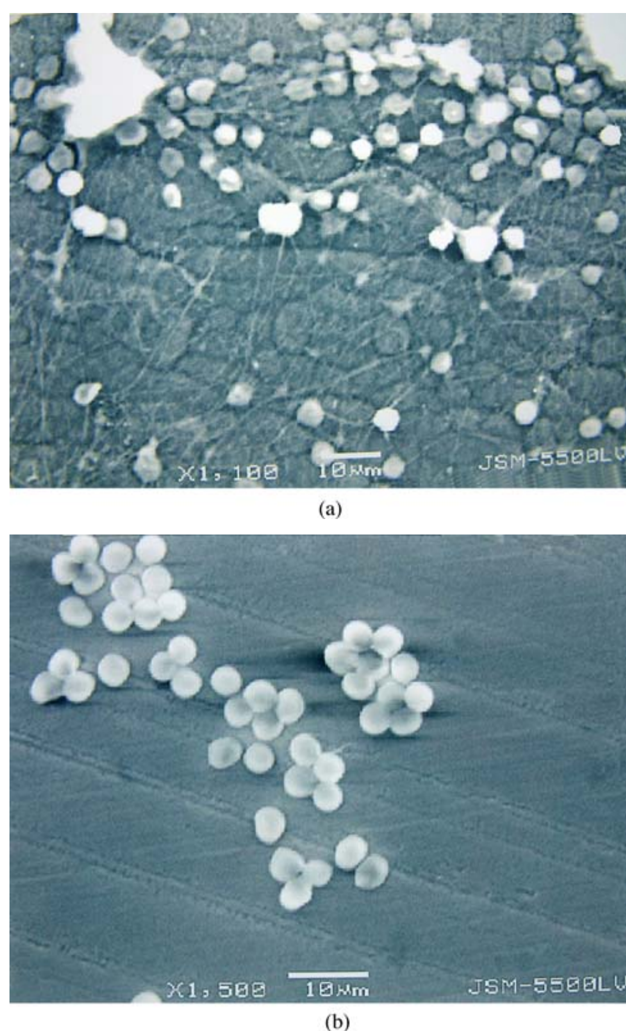

Fig. 3 Blood clot coagulation on selected materials: (a) network of fibres with entrapped blood elements on C/C composite material observed after 2 min; (b) no blood coagulum on ZrO_2 ceramics material after 4 min.

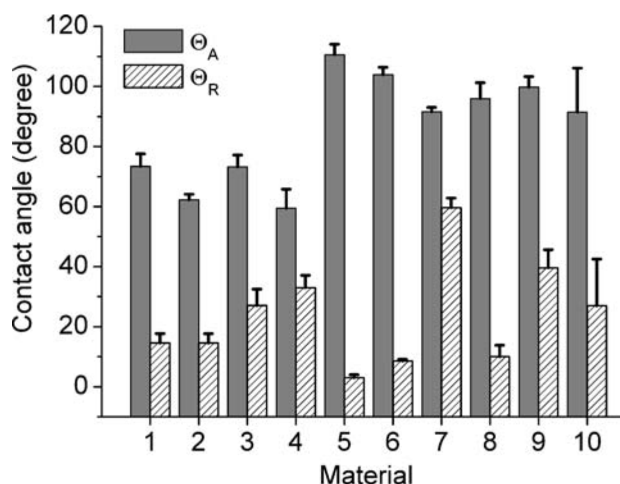

Fig. 4 Advancing and receding contact angles of tested materials in water: 1-ZrO_2 ceramics; 2-polished Ti; 3-sand-blasted Ti; 4-etched Ti; 5-plasma-sprayed Ti; 6-sand-blasted Ti6A14V; 7-CoCrMo alloy; 8-hydroxyapatite-Ti; 9-polyethylene; 10-C/C composite.

Table 4 Dynamic advancing Θ_A and receding Θ_R contact angles of selected surfaces (Wilhemy plate method)

	Contact angle (°) in water[a]		In cell medium[b]	
Surface	Θ_A	Θ_R	Θ_A	Θ_R
Polished Ti	62 ± 1.8	18 ± 3.1	69 ± 2.1	30 ± 1.2
ZrO_2 ceramics	73 ± 4.2	13 ± 3.1	87 ± 2.6	32 ± 1.5
Polyethylene	102 ± 3.5	43 ± 2.5	100 ± 4.8	26 ± 2.5
C/C composite	70–100[c]	16–51[c]	77–102[c]	25–45[c]

[a]Surface tension of water $\gamma = 72.8$ mN/m.
[b]Surface tension of cell medium $\gamma = 62.4$ mN/m.
[c]Variance due to surface heterogeneity.

lower Θ_A values (below 75°) were obtained. Polished and etched Ti samples are the most hydrophilic substrates of the tested materials ($\theta_A = 60°$). Starting with position 5 in Fig. 4, Θ_A exceeds 95°. Thus, materials from the end of the list in Table 2, such as PE or C/C composites, are highly hydrophobic substrates with Θ_A values close to 100°.

The calculated differences between Θ_A and Θ_R values (hysteresis) should be considered, in the first approximation, as basic parameters for characterization of the surface heterogeneity, particularly the surface roughness. Indeed, plasma-sprayed (No. 5) or hydroxyapatite (No. 8) titanium surfaces, which are well known for their high surface roughness and Θ_A values close to 100° due to the particular surface treatment, exhibit high hysteresis ($\Theta_A - \Theta_R$). In the case of C/C composite surface, high hysteresis together with a high standard deviation of contact angle values also confirmed a high surface roughness, which can be observed with naked eye.

The surface free energy (SFE) and wettability in cultivation medium were evaluated for selected samples for which, in our opinion, the most significant differences in the cell proliferation and production of cytokines were observed. Thus, the first (i.e. ZrO_2 ceramics, polished Ti) and the last materials (PE and C/C composite) as order in Table 2 were tested. Contact angles in water and in the cell medium are listed in Table 4. The surface free energy and its polar (γ^p) and dispersive (γ^d) components are presented in Fig. 5.

The highest values of γ were observed for Ti and C/C composite surfaces, whereas the lowest for the PE surface. The polished Ti surface exhibits also the highest value of γ^p out of tested surfaces. The decrease in the surface tension of the cell medium ($\gamma_{medium} = 62.4$ mN/m), when compared with water ($\gamma_{water} = 72.8$ mN/m), is caused by a decrease in its polar component due to the presence of inorganic salts. The polished Ti and ZrO_2 surfaces reflected this fact when an increase in Θ_A and Θ_R was observed in the cell medium. Actually, the polar component γ^p of the surface free energy affected the surface wettability in the used liquids: Ti surface with higher γ^p was better wetted in a less polar cell medium

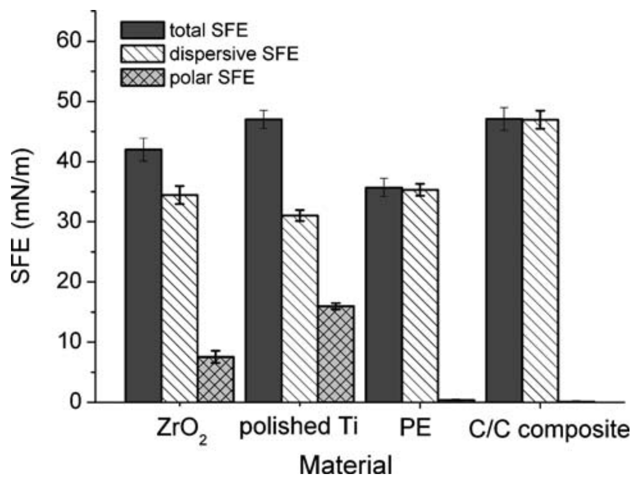

Fig. 5 The surface free energy γ and dispersive (γ^{d}) and polar (γ^{p}) part of ZrO_2 ceramics, polished Ti, polyethylene (PE) and C/C composite.

than ZrO_2 surface. On the contrary, in the case of PE and C/C composite surfaces, no significant changes in wetting in both liquids were observed since both surfaces exhibit almost zero polar component γ^{p} of SFE.

4 Discussion and conclusions

Many implant materials available on the market differ in material and/or its surface treatment, but there is no detailed information about how the type of the surface treatment helps osteointegration [12]. All the modifications changing surface topography lead to an enhancement of a bone-to-implant contact and to an increase the mechanical interlocking with bone in vivo [13, 12]. Many of *in vitro* or *in vivo* studies are focused on the effect of mechanical or chemical surface treatment of the implant surface (e.g. sand-blasting, etching, plasma-spraying, HA coating, etc.) on cell adhesion and, form a long-term point of view, on osteointegration processes [14]. However, even though the implant materials used are commercial materials, surface modifications are often performed by researchers; also experimental procedures are different. Therefore the results are hardly comparable [15]. The present study was designed with the purpose to align commonly used implant materials with various surface modifications already established on the market (Table 1) from the early healing point of view in *in vitro* experiments.

First, adhesion and proliferation of cells as basic parameters of biotolerance were investigated and synthetic activity of cells (production of TNF-α, IL-8) reflecting cell viability was evaluated. The tissue mediator TNF-α belongs to main basic indicators of inflammation whereas IL-8, an inflammatory cytokine, acts also as a chemokine and has a stimulation effect on released cytokines. In this study, IL-8 has been considered mainly an inflammatory mediator.

Since all the materials under study are already used in implantology and considered as at least biotolerant materials [16, 17], it is not surprising that the difference in cell proliferation on almost all tested materials is not statistically significant when compared with TCPS (Table 2). Our observations summarized in Table 2 are generally in accordance with data from the literature. A similar biocompatibility of ZrO_2 ceramics and pure Ti [18] and better acceptance and biological properties of neat Ti than Ti and/or CrCoMo alloys were observed [16, 19]. Surface modifications of Ti samples (sand-blasting, etching, plasma spraying) did not lead to significant changes in fibroblast proliferation compared with polished materials in this study. This is in accordance with observations of Ponnsonet [20] and Boyan [21].

Polyethylene is mostly used for hip or knee-joint replacement components where low or possibly no cell adhesion and optimal friction properties are required. Thus it was expected that cell proliferation activity would be also lower on the PE substrate [17]. On the other hand, the lowest proliferation observed on hydroxyapatite-Ti and C/C composite is in contrast with data in the literature [8, 17]. In the case of C/C composite used in this study, the reason should consist in inappropriate manufacturing processes during a pyrolytic carbon deposition on the carbon substrate. Concerning hydroxyapatite-Ti, most of the literature shows a very positive effect of hydroxyapatite coating on cell proliferation [14, 17]. The observed contradiction may be related to the unsuitable chemical composition and/or crystallinity of hydroxyapatite deposited on the Ti surface used in our study [14, 22] resulting in poor cell proliferation.

Cells synthesized both cytokines (TNF-α, IL-8) to a greater extent on implant materials with lower cell proliferation (Fig. 2, Table 2). This indicates that these surfaces also cause a higher inflammatory reaction of fibroblasts.

In conclusion, an ablation surface treatment (sand-blasting, polishing, etching) lead to materials exhibiting a higher cell proliferation and lower cytokine production than deposition surface treatment (plasma spraying on Ti, HA spraying on Ti, pyrolytic carbon deposition). A possible reason for this contradiction could be alteration of the original material used for coating during deposition [23].

The surface wettability is assumed to respond especially to the extent of cell adhesion [20, 24]; therefore hydrophilicity of the used implant materials was evaluated. It was not the intention to compare the obtained Θ_A values Fig. 4 with Θ_A data for particular materials published in the literature since absolute values could differ depending on metallurgical and processing conditions used by implant manufacturers. However, in accordance with the literature, titanium-based smooth surfaces are moderately wettable surfaces ($\Theta_A \cong 65^\circ$) when compared with the tested alloys [20]. As also expected, sand-blasting or plasma-spraying treatments resulted in a higher surface hydrophobicity of the original Ti surface, whereas

etching treatment led to a moderate increase in hydrophilicity due to the formation of a hydrated TiO_2 layer on the original Ti surface [25].

Adsorption of proteins (also present in cultivation medium) always precedes to cell adhesion. Proteins adsorb at significantly lower concentrations to hydrophilic than to hydrophobic substrates whereas cells prefer hydrophilic surfaces [26]. Indeed, there is a visible correlation between the decreasing tendency in the population cell density (Table 2) and increasing hydrophobicity (Fig. 4) of tested biomaterials. We assume that proteins adsorb on the implant surface in higher concentrations with increasing Θ_A of the surface thus weakening the cell adhesion and proliferation.

In the second part of the project, implants with the best and the worst cell adhesion and proliferation (i.e. Ti-polished, ZrO_2 ceramic, PE and C/C composite) were chosen for more detailed study of selected surface properties and were utilized for blood clot formation experiments in this preliminary study.

The data of SFE and wettability of four selected materials, Fig. 5 and Table 4, correlate with the results of cell viability and synthetic activity (Table 2, Fig. 2) and also with to the blood clot coagulation test (Table 3).

There are two groups with extremes in cellular adhesion. In the group of ZrO_2 ceramic and the polished Ti with the polar component γ^P (7.55 mN/m and 15.97 mN/m, respectively), the highest cell density and the lowest inflammatory cytokine production, but no fibers in the clotting blood were found. In contrast, low cell proliferation and distinctly higher expression of inflammatory mediators but also faster formation of fibers in blood coagulum were observed in the group of materials with a very low polar component γ^P of SFE (0.39 mN/m for polyethylene and 0.12 mN/m for the C/C composite). Thus, it seems that a good cellular adhesion and proliferation and an acceptable inflammatory response observed in our experiment is supported by the effect of the polar component γ^p of SFE, i.e. the interaction energy of the material with water, and an appropriate surface hydrophilicity (here 60–75 degrees in water). These findings correlate well with the previously published data about the fibroblast proliferation on Ti surfaces [27], or about the osteoclast adhesion and activity on biomaterials [28]. Hallab *et al.* also observed that poly(tetrafluoroethylene) and silicone rubber substrates with a low polar component γ^p (close to 0.1 mN/m) exhibited very low adhesion shear strength, and that the adhesion shear strength of adhered fibroblasts increased with increasing γ^p [29].

For a successful use of bone facing implant materials and surfaces, the fast formation of the blood clot is often highly desired [11]. The blood clot forms a natural shield preventing infection of the wound [30], platelets play an important role in the wound healing cascade and fibrin serves as a way for the osteogenic cell migration [7]. Our results imply that more hydrophilic materials (a higher polar part γ^p of SFE) favorable to cell adhesion and proliferation with an acceptable inflammatory response do not seem to be sufficiently suitable substrates for adhesion and eventually for the activation of platelets when compared with hydrophobic materials. This is consistent with findings of Jones [4] who observed that materials with low contact angles showed smaller quantities of adhered platelets. Jones also [4] found platelets with a high state of activation and spreading on Ti surfaces, which is in an agreement with our finding about polished Ti. On the other hand, the platelets were isolated and round, so less activated on Ti surfaces coated with diamond-like carbon, which is the situation comparable with ZrO_2 ceramics but in contrast to C/C composite tested in our study. The highest number of platelets with a high state of activation present on the C/C composite sample was attributed to a high degree of the surface hydrophobicity in combination with a high surface roughness of the C/C composite as well as to higher expression of inflammatory mediators. A significant effect of surface roughness and "microtexture" on platelet activation and formation of fibrin mesh was observed by Park [31] on treated Ti surfaces.

To summarize, the present pilot study demonstrates a strong mutual dependence of cell adhesion and proliferation and platelet activities on the surface free energy of tested implants, particularly on its polar component γ^P. The most favorable surface treatment should be an optimal compromise of investigated parameters. The clotting factor is important in cases where a bigger distance exists between the bone bed and implant (very coarse surfaces have more space than polished ones, discoid implants show not so tight contact to the bone as cylinders). The right proportion of these parameters partly depends on the implant type (blade, cylinder, discoid), surgical procedure (submerged/non submerged dental implants) and loading protocol (immediate or delayed loading). For example, polished Ti seems to be a most suitable material out of tested samples for press-fit dental implants regarding high proliferation activity, low production of cytokines and sufficiently rapid blood clot formation. However, the effect of roughness on the blood clot formation should not be omitted as well. Further more detailed study of the effect of surface properties on the fibroblast and, consequently, on the osteoblast response and on the blood clot coagulation is in progress. We expect that the obtained results will allow us to better understand the influence of implant materials on the contact quality between the bone and implant, on the speed up of the healing process as well as on the prolongation of the functional period of implants.

Acknowledgment The authors are indebted to the Internal Grant Agency of Ministry of Health of the Czech Republic for financial support (project No. NK7573-3/2003).

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