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Early implant healing: promotion of platelet activation and cytokine release by topographical, chemical and biomimetical titanium surface modifications *in vitro*

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Abstract

Objectives: Platelet releasate has been shown to promote osteogenetic cell proliferation and differentiation. Topography and chemistry of biomaterials have high impact on platelet activation. More specifically, the bioactive cell adhesive peptide sequence Arg–Gly–Asp (RGD) triggers platelet activation mediated by the $\alpha_{IIb}\beta_3$ integrin receptor. Accordingly, topographical, chemical and biomimetical (immobilized RGD peptide) modifications of titanium (Ti) surfaces may enhance early platelet activation and bony healing of implants. Therefore, the aim of the study was to evaluate platelet activation with subsequent platelet-derived cytokine release by accordingly modified Ti surfaces.

Materials and methods: Pre-treated (PT; mean roughness [R_a] = 0.04 μm, contact angle [CA] = 91°), acid-etched (A, R_a = 0.83 μm, CA = 106°), large grit-sandblasted, acid-etched (SLA, R_a = 3.2 μm, CA = 109°) as well as hydrophilically modified acid-etched (modA, R_a = 0.83 μm, CA = 0) and modified large grit-sandblasted, acid-etched (modSLA, R_a = 3.2 μm; CA = 0°) titanium surfaces were investigated. Additionally, RGD peptides were chemically immobilized on PT, A and SLA surfaces (PT-RGD [CA = 18°], A-RGD [CA = 0°], SLA-RGD [CA = 0°]). The different Ti surfaces were incubated with platelet concentrate of three healthy volunteers at room temperature for 15 min and for 30 min. High thrombogenous collagen served as the control group. Out of the supernatant, platelet consumption was assessed via platelet count (PC). Cytokine release was quantified via the level of platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF).

Results: After 15 min, especially the rough SLA surface showed a strong decrease in PC and a strong increase in VEGF and PDGF levels. After 30 min, high platelet consumption as well as high levels of VEGF and PDGF were measured for unspecifically modified (modA) and especially for biomimetic, specifically modified (PT-RGD, A-RGD) surfaces, indicating a delayed effect of the surface modifications on platelet activation.

Discussion: Modifications of surface roughness modifications appear to influence early platelet activation and cytokine release after 15 min whereas surface chemistry modifications with increased hydrophilic properties and surface modifications via RGD peptide on plainer surfaces lead to a further, more specific promotion of platelet activation and degranulation after 30 min. The observed effect could be valuable for critical clinical situations like compromised bone sites.

For biomaterial surfaces, protein adsorption and platelet interactions are pivotal events for the early healing cascade including important subsequent cellular responses (Tanaka et al. 2009) (Klein et al. 2010). After insertion of a titanium graft, the wound site is occupied by a blood clot, which forms immediately around the implant (Davies 1996; Park et al. 2001). Both endothelial cells as well as osteogenic cells have to migrate through the remnants of this clot in order to initiate vascularization and new bone formation. Cell activation and proliferation is mediated via blood-borne cell activities such as platelet activation. When activated, platelets aggregate and release growth factors such as vascular endothelial growth factor A (VEGF-A) and platelet-derived growth factor (PDGF) (Harrison & Cramer 1993). Measurement of platelet consumption out of cellular supernatant after activation – a decrease in platelet count (PC) is the equivalent of platelet consumption – is a reliable indirect method to quantify platelet activation (Klein et al. 2010).

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VEGF, a 34–46 kDa glycoprotein, is involved in early angiogenesis and osteogenesis by attracting relevant haematopoietic and osteogenic cells (Kanczler & Oreffo 2008). Furthermore, it is promoting osteogenic cell differentiation (Samee et al. 2008; Grellier et al. 2009).

PDGF, a dimeric 30 kDa protein, has a chemoattractive function towards several cell types. Besides, it stimulates the proliferation of endothelial and osteogenic cells as well as cell differentiation (e.g. osteoprotegerin production) (Sanchez-Fernandez et al. 2008; McCarthy et al. 2009).

Accordingly, increased platelet activation via modified surfaces and subsequent promotion of the healing cascade offer new therapeutic potential, especially in critical clinical situations. These can be found in patients with bisphosphonate therapy anamnesis, in older patients, in patients with smoking habits, with history of diabetes or head and neck radiation as well as in patients that are postmenopausal and on hormone replacement therapy (Moy et al. 2005; Kasai et al. 2009; Klein, et al. 2009). Approaches to increase platelet activation by titanium surfaces can be subdivided into modifications of surface topography and chemistry (hydrophilicity) as well as specific biomimetic surface modifications (Park, et al. 2001; Al-Nawas & Gotz 2003; Li, et al. 2009). Especially extracellular matrix-like biomimetic surface modifications are a promising approach for further regulation of platelet activation (Li et al. 2009). In prior studies, a positive influence of unspecific biomaterial surface topography and chemistry on platelet activation could be shown (Park, et al. 2001; Kikuchi, et al. 2005; Chuang & Lin 2007; LaFayette, et al. 2007; Klein, et al. 2010). However, the use of clinical applicable specific biomimetical surface modifications still has not quit experimental levels and remains exciting.

The function of integrin receptor $\alpha_{IIb}\beta_3$ is crucial in platelet activation and aggregation with consecutive cytokine release. It is the most commonly expressed membrane protein on platelets (60,000–100,000 per thrombocyte) (Gawaz et al. 1991; Harker 1998). 70% of the receptors are located on the surface; the rest is expressed during activation. When activated, the receptor also undergoes a change of conformation into a more reactive form (Coller 1995). The major function of this receptor is binding of fibrinogen (also von-Willebrand-factor and fibronectin) via specific Arg–Gly–Asp (RGD) binding sites. As pharmacological $\alpha_{IIb}\beta_3$ antagonists such as abciximab (Saltzman et al. 2010) are also using RDG sequences in order to inhibit platelet aggregation, the importance of αIIbβ3-RGD interactions is obvious.

In this study, modified titanium surfaces were examined. Besides topographical and chemical surface alterations, RGD sequences were covalently immobilized on respectively pre-treated titanium surfaces. The aim was to evaluate the influence of various unspecific and specific surface properties on platelet activation by means of platelet consumption (PC) and release of relevant cytokines (VEGF, PDGF). To the best of our knowledge, this is the first study examining platelet activation dependent on topographical, chemical and additionally biomimetical-modified titanium surfaces.

Material and methods

Topographical and chemical preparation of titanium (Ti) discs

Cylindrical discs with a diameter of 15 mm and a cylinder height of 1 mm have been stamped out of Ti sheets of grade 2 unalloyed Ti. Pre-treated (PT), acid-etched (A), large grit-sandblasted acid-etched (SLA) as well as acid-etched, hydrophilic (modA) and large grit-sandblasted acid-etched, hydrophilic (modSLA) titanium surfaces were created according to Fig. 1.

Data of mean surface roughness (R_a) were determined in prior studies (Al-Nawas & Gotz 2003). Table 1 provides information concerning R_a of the different surfaces.

Biomimetical preparation of Ti discs

Silanization of Ti samples (PT, A, SLA) was performed in 5% Aminopropyl-triethoxysilane (APTES; Sigma, Deisenhofen, Germany) in toluene at room temperature over night. After cleaning in toluene and ethanol, samples were cured at 80°C for 1 h. Subsequently, aminosilanized Ti-plates were immersed in a 5% solution of diethylenglycol diglycidylether (diepoxide) in 50 mM carbonate-buffer (pH 9) for 2 h. The excess was removed by repeated rinsing with water. RGD-immobilization was then immediately performed by incubating the activated samples in 0.5 mg/ml RGD-peptide (c-RGD, Anaspec, Fremont, CA, USA) in carbonate-buffer over night in a wet chamber. Followed by a washing step with water, the samples were stored dry and sterilized with 70% ethanol for 30 min before experimental use (Gabriel et al. 2006). In preliminary investigations, successful coating was analysed by means of the sulpho-SDTB-assay (Interchim, Mannheim, Germany) according to the manufacturer's instructions.

Table 1. **Mean roughness (R_a) of mechanical modified surfaces and contact angle (CA, °) with standard deviation (SD) of all surfaces**

Sample	R_a	CA (SD)
PT	0.04	90.8 (3.1)
A	0.83	106 (4.4)
SLA	3.2	108.7 (6.6)
modA	0.83	0
modSLA	3.2	0
PT-RGD		18.1 (4.5)
A-RGD		0
SLA-RGD		0

PT, pre-treated; A, acid-etched; SLA, large grit- sandblasted, acid-etched; modA, hydrophilically modified acid-etched; modSLA, modified large grit- sandblasted, acid-etched.

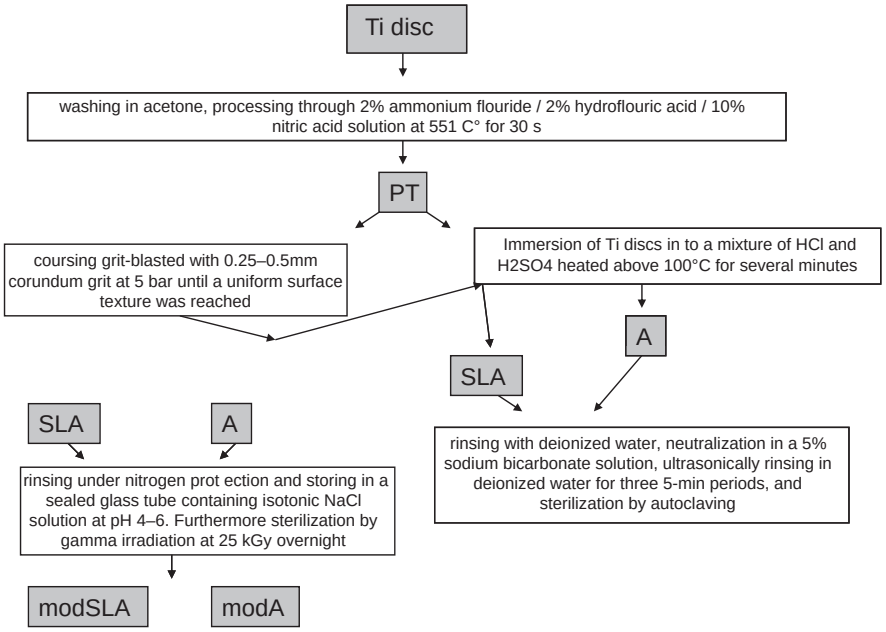

Fig. 1. Fabrication of pre-treated (PT), acid-etched (A), large grit- sandblasted acid-etched (SLA) as well as acid-etched, hydrophilic (modA) and large grit- sandblasted acid-etched, hydrophilic (modSLA) titanium surfaces.

Static contact angle (CA) microscopy

Before experimental use, the hydrophilic properties of all Ti samples were examined by static water CA microscopy (Jones & Richards 1999). In cases of maximal wetting, the CA is 0°, in cases of minimal wetting, it goes towards 180°. Smaller CA are also usually detected when the zwitterionic RGD moieties are immobilized (Hersel et al. 2003). All measurements were performed in triplets. Mean values were given.

Preparation of blood samples and incubation of Ti discs

Platelet-rich blood-concentrate platelet-rich plasma (PRP) was obtained from three healthy volunteers in a previously described manner (Klein, et al. 2010). In brief, from each volunteer, peripheral blood was taken, carefully mixed with sodium citrate (nine parts blood, one part sodium citrate 3.13%) and centrifuged for 15 min at 160 g. In accordance to the regulations of the local ethics committee, no patient-related data were recorded and the platelet samples were pseudonomized (PRP samples A–C). Informed consent was obtained from each subject. In sample A, $n = 363{,}000/\mu l$, in sample B, $n = 433{,}000/\mu l$ and in sample C, $n = 401{,}000/\mu l$ platelets were measured. As described before by Park et al. (2001), Ti discs were inserted almost press-fit into the bottom of 24-well plates (Falcon, BD Labware, Franklin Lakes, NJ, USA). and secured with a large diameter piece of Silastic® tubing. Two hundred microlitres of freshly prepared PRP were added to each well. The plate was gently mixed at 37°C in order to simulate surface–platelets interactions. One well with highly thrombogenic collagen type 1 served as the reference and an additional well with PRP only (without titanium disc) served as the control group. The supernatant was pipetted off after 15 and 30 min for further investigations.

PC measurement

Out of 20 µl of each supernatant, the number of remaining, non-aggregated platelets was determined using a haematology analyser (KX21, SysmexEurope GmbH, Norderstedt, Germany).

Enzyme-linked immunosorbent assay (ELISA)

Supernatant concentrations of VEGF-A and PDGF-αβ were determined according the manufacturer's instructions using a commercially available sandwich ELISA technique kit (Quantikine; R&D Systems Inc., Minneapolis, MN, USA). Concentrations were given in pg/ml.

Statistics

Platelet activation on differently modified Ti surfaces was measured using platelet concentrate from three healthy volunteers. For each volunteer, the measurements were conducted in triplets. For analysis, descriptive statistical methods were found to be sufficient.

Results

Static water CA microscopy

The CA of examined samples is shown in Table 1.

PC

After 15 min of incubation, the control groups A–C showed a mean PC of 381664/µl. Simultaneous incubation with collagen resulted in relevant platelet consumption with visible large platelet aggregates (Table 2). Accordingly, the mean platelet consumption for collagen was 373,333/µl. In comparison with controls, for topographical-modified Ti samples, the decrease in PC was as follows: PT < A < SLA (Table 3). Platelet consumption for chemical-modified surfaces was: modA < modSLA (Table 4). For biomimetical coatings, the following platelet consumption was detected after 15 min of incubation: PT-RGD < A-RGD < SLA-RGD (Table 5; *total in* Fig. 2).

After 30 min of incubation, the control groups A–C showed a mean PC of 356,333/µl. The PC in the collagen group was marginal (Table 2). The mean platelet consumption for collagen was 348,000/µl. In comparison with controls, the decrease of PC for topographical modified samples was: PT < A < SLA (Table 3); for chemical modifications: modA < modSLA (Table 4) and for biomimetical modifications: PT-RGD < A-RGD < SLA-RGD (Table 5; *total in* Fig. 2).

***Table 2*. Mean and median values as well as standard deviation (SD) of platelet count, VEGF and PDGF concentration after 15 min (PC15, VEGF15 and PDGF15) and after 30 min (PC30, VEGF30 and PDGF30) of control and the collagen group**

	Control group	Collagen
PC15 (platelet/µl)		
Mean	381,667	9333
Median	401,000	10,000
SD	50,836	1155
PC30 (platelet/µl)		
Mean	356,333	8333
Median	368,000	8000
SD	51,500	577
VEGF15 (pg/ml)		
Mean	16	88
Median	14	87
SD	3	4
VEGF30 (pg/ml)		
Mean	20	127
Median	21	130
SD	4	10
PDGF15 (pg/ml)		
Mean	3134	6941
Median	3128	6894
SD	272	248
PDGF30 (pg/ml)		
Mean	3094	6845
Median	3109	6843
SD	212	138

VEGF, vascular endothelial growth factor; PDGF, platelet-derived growth factor; PC, platelet count.

***Table 3*. Mean and median values as well as standard deviation (SD) of platelet count, VEGF and PDGF concentration after 15 min (PC15, VEGF15 and PDGF15) and after 30 min (PC30, VEGF30 and PDGF30) of PT, A and SLA group**

	PT	A	SLA
PC15 (platelet/µl)			
Mean	341,500	285,333	155,000
Median	324,000	282,000	154,000
SD	41,608	16,258	17,521
PC30 (platelet/µl)			
Mean	324,000	181,666	124,000
Median	290,000	194,000	123,000
SD	10,066	41,884	9539
VEGF15 (pg/ml)			
Mean	14	27	32
Median	15	25	32
SD	2	8	5
VEGF30 (pg/ml)			
Mean	33	58	55
Median	32	59	55
SD	8	10	2
PDGF15 (pg/ml)			
Mean	2642	3093	4850
Median	2411	3210	5023
SD	424	267	402
PDGF30 (pg/ml)			
Mean	3510	4421	4377
Median	5319	4320	4443
SD	105	305	171

VEGF, vascular endothelial growth factor; PDGF, platelet-derived growth factor; PC, platelet count.

***Table 4*. Mean and median values as well as standard deviation (SD) of platelet count, VEGF and PDGF concentration after 15 min (PC15, VEGF15 and PDGF15) and after 30 min (PC30, VEGF30 and PDGF30) of modA and modSLA group**

	modA	modSLA
PC15 (platelet/µl)		
Mean	333,426	217,800
Median	324,000	233,000
SD	20,808	69,458
PC30 (platelet/µl)		
Mean	109,666	111,667
Median	112,000	120,000
SD	20,599	33,291
VEGF15 (pg/ml)		
Mean	19	16
Median	18	15
SD	5	2
VEGF30 (pg/ml)		
Mean	31	34
Median	36	33
SD	10	7
PDGF15 (pg/ml)		
Mean	2668	3286
Median	2654	3220
SD	89	117
PDGF30 (pg/ml)		
Mean	5111	4328
Median	5010	4328
SD	358	141

VEGF, vascular endothelial growth factor; PDGF, platelet-derived growth factor; PC, platelet count.

Table 5. **Mean and median values as well as standard deviation (SD) of platelet count, VEGF and PDGF concentration after 15 min (PC15, VEGF15 and PDGF15) and after 30 min (PC30, VEGF30 and PDGF30) of PT-RGD, A-RGD and SLA-RGD group**

	PT-RGD	A-RGD	SLA-RGD
PC15 (platelet/μl)			
Mean	294,333	158,000	167,000
Median	296,000	161,000	178,000
SD	10,599	6083	21,702
PC30 (platelet/μl)			
Mean	79,667	92,333	185,667
Median	86,000	80,000	216,000
SD	17,388	24,906	56,924
VEGF15 (pg/ml)			
Mean	31	25	20
Median	30	25	18
SD	3	9	5
VEGF30 (pg/ml)			
Mean	70	76	34
Median	67	76	34
SD	15	5	7
PDGF15 (pg/ml)			
Mean	2879	3460	3057
Median	2832	3427	3028
SD	206	227	219
PDGF30 (pg/ml)			
Mean	5255	5085	4205
Median	5252	5052	4032
SD	45	113	560

VEGF, vascular endothelial growth factor; PDGF, platelet-derived growth factor; PC, platelet count.

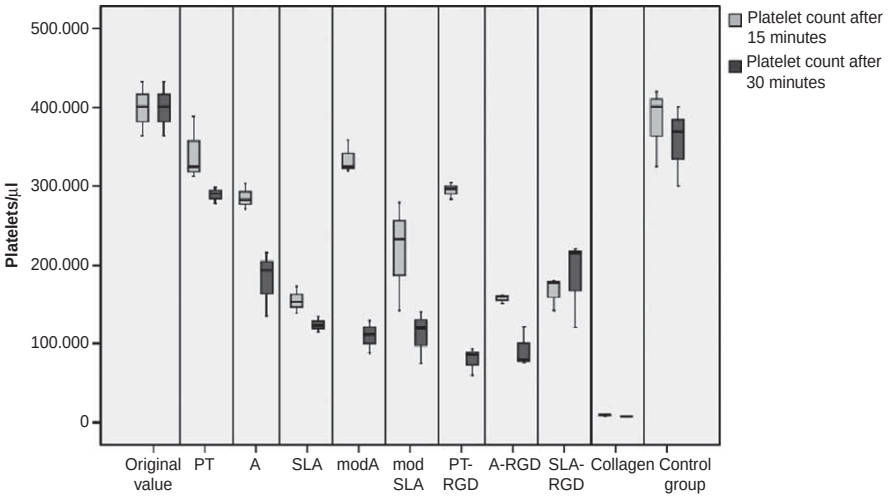

Fig. 2. Box plots of platelet count (PC) after 15 and 30 min. 15 min: the lowest PC is seen on SLA, A-RGD and SLA-RGD surfaces. 30 min: the lowest PC is seen on PT-RGD, A-RGD and modA-surfaces; the most significant decrease of PC in the defined time period was seen for modA, PT-RGD and modSLA. SLA, large grit- sandblasted, acid-etched; PT, pre-treated; modA, hydrophilically modified acid-etched; modSLA, modified large grit- sandblasted, acid-etched.

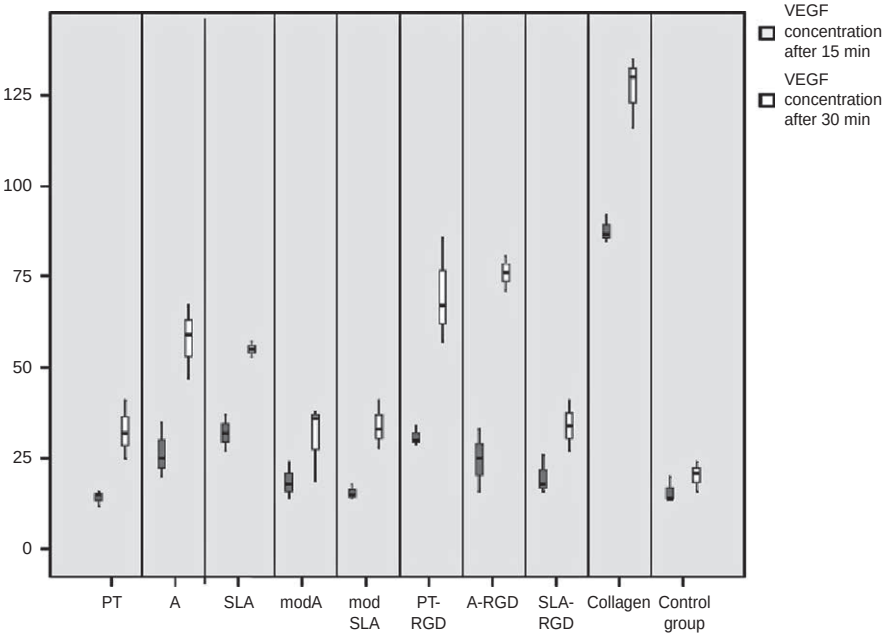

Fig. 3. Box plots of VEGF concentration after 15 and 30 min. 15 min: the highest VEGF concentration is seen on SLA, PT-RGD and A surfaces. 30 min: the highest VEGF concentration is seen on A-RGD, PT-RGD and A surfaces; the most significant increase of VEGF concentration in the defined time period was seen for modA, PT-RGD and modSLA. SLA, large grit- sandblasted, acid-etched; VEGF, vascular endothelial growth factor; PT, pre-treated; modA, hydrophilically modified acid-etched; modSLA, modified large grit- sandblasted, acid-etched.

VEGF-A and PDGF-αβ levels in the supernatant

After 15 min, mean VEGF concentrations for the control group and the collagen group were 16 and 88 pg/ml, respectively (Table 2). For the topographical-modified samples, concentrations were as follows: PT < A < SLA (Table 3); for chemical-modified samples: modA < modSLA (Table 4) and for biomimetical modifications: PT-RGD < A-RGD < SLA-RGD (Table 5; *total in* Fig. 3).

After 30 min, for the control group a mean VEGF concentration of 20 pg/ml, for the collagen group of 127 pg/ml was measured, respectively (Table 2). Topographical modifications showed the following VEGF concentrations: PT < A < ∩ SLA (Table 3). Chemical modifications resulted in the following levels: modA < modSLA (Table 4). After biomimetical coating, VEGF levels were as follows: PT-RGD < A-RGD < SLA-RGD (Table 5; *total in* Fig. 3).

After 15 min, and a mean PDGF concentration of 3134 pg/ml could be seen for the control group, a mean concentration of 6941 pg/ml for the collagen group (Table 2). For the topographical-modified samples, the following concentrations could be measured: PT < A < SLA (Table 3). For chemical-modified discs, results were: modA < modSLA (Table 4) and biomimetical modifications resulted in: PT-RGD < A-RGD < SLA-RGD (Table 5; *total in* Fig. 4).

PDGF concentrations after 30 min of incubation were 3094 pg/ml for the control and 6845 pg/ml for the collagen group (Table 2). The results of the topographical-altered titanium discs were as follows: PT < A < SLA (Table 3). Chemical modifications resulted in: modA < modSLA (Table 4); after biomimimetical modifications: PT-RGD < A-RGD < SLA-RGD (Table 5; *total in* Fig. 4).

Discussion

This study evaluated the impact of topographical, chemical and biomimetical modifications of titanium surfaces on platelet activation. After application of biomaterials and/or insertion of titanium implants, intensive and complex interactions between surface structures and acellular as well as cellular constituent parts of the extracellular environment can be observed (Davies 2003). Besides protein adsorption, platelet activation is essential for conditioning of the implant–bone interface for proper neovascularization and recruitment as well as differentiation of osteogenic cells (Hong et al. 1999; Park & Davies

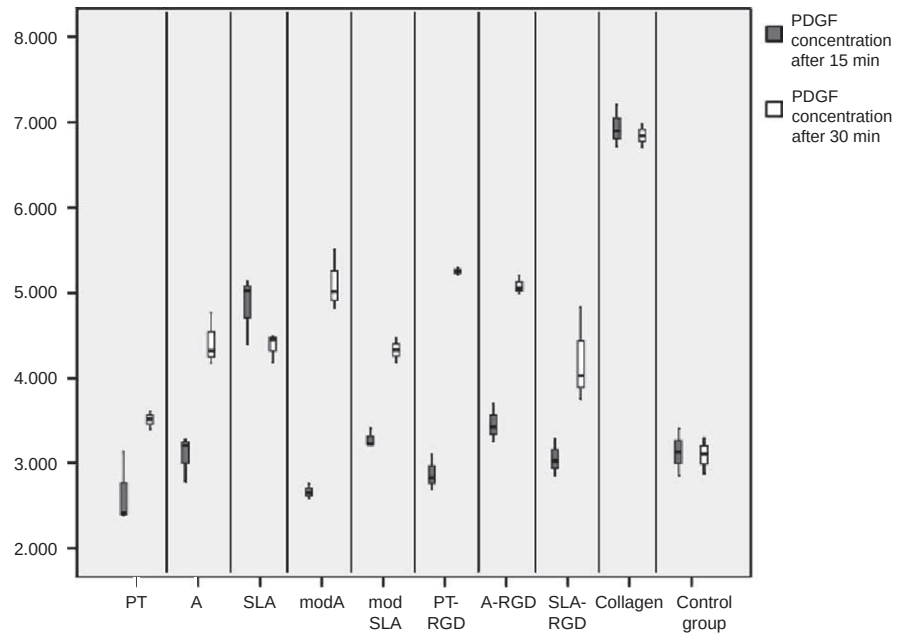

Fig. 4. Box plots of PDGF concentration after 15 and 30 min. 15 min: the highest PDGF concentration is seen on SLA, A-RGD and modSLA surfaces. 30 min: the highest PDGF concentration is seen on PT-RGD, modA and A-RGD; the most significant increase of PDGF concentration in the defined time period was seen for PT-RGD, modA and A. PDGF, platelet-derived growth factor; SLA, large grit- sandblasted, acid-etched; PT, pre-treated; modA, hydrophilically modified acid-etched; modSLA, modified large grit- sandblasted, acid-etched.

2000). Enhanced concentrations of activated platelets at wound sites are accompanied by increased local concentrations of released growth factors. Platelet-derived growth factors and cytokines have an important function in directing and potentiating of cellular responses in wound healing (Eppley et al. 2004; Kark et al. 2006) including aggravation of bony healing (Jakse et al. 2003; Plachokova, et al. 2008). Therefore, an increased concentration of those factors at local wound sites mediated by topographical, chemical and biomimetical modifications of biomaterials may be beneficial for early implant healing, especially in compromised situations. Accordingly, it could be shown that both VEGF and PDGF isolated as well as complex platelet concentrates are supporting cellular reactions *in vitro* (Weibrich et al. 2002; Han et al. 2007) as well as tissue regeneration (Howell et al. 1997; Cieslik-Bielecka et al. 2008). However, the beneficial clinical use of platelet-rich plasma (PRP) has been discussed controversially. One major drawback is the lacking of adequate mode of platelet activation directly at the wound site (Schilephake 2002; Boyapati & Wang 2006; Nikolidakis & Jansen 2008). Despite sophisticated time- and resource-consuming PRP preparation and activation procedures, the resulting platelet and growth factor concentrations have been shown to vary significantly from preparation kit to preparation kit as well as from patient to patient (Weibrich et al. 2003, 2005).

In this study, we used non-pre-activated platelet concentrate with a comparable low PC of a mean of 399,000/µl to examine early platelet interactions with different titanium surfaces. In prior studies, it could be shown that pure titanium surface microtextures can modulate platelet activity (Park et al. 2001), even more than calcium-phosphate coatings (Kikuchi et al. 2005). Therefore we evaluated five different, topographical- and chemical-modified titanium surfaces regarding their role in platelet activation. The RGD motive has an outstanding impact on specific platelet activation (Sanchez-Cortes & Mrksich 2009). Besides, it is heat resistant and can be sterilized without damaging (Hersel, et al. 2003). As it can be manufactured synthetically, there is no potential for the transmission of diseases and its use might be beneficial in later clinical settings (Avila et al. 2009). Therefore, on three differently modified Ti surfaces (PT, A, SLA), RDG peptides were additionally immobilized.

For the pure topographical surface modifications, platelet consumption was increased both with time (30 min > 15 min) as well as with increasing surface roughness (SLA > A > PT). The hydrophilically modified surfaces (modA, modSLA) showed only moderate platelet consumption after 15 min; after 30 min, a strong drop of PC was observed. For the PT-RGD and A-RGD surfaces, platelet consumption was, compared with the PT and A surfaces, higher both after 15 and 30 min. Compared with the SLA surfaces, platelet consumption was slightly lower on the SLA-RGD surface.

The quantification of VEGF and PDGF serves as a direct measurement of platelet degranulation (Jurk & Kehrel 2005). Two comparable early incubation times (15 and 30 min) were chosen for investigation. The active secretion of growth factors into the environment is initiated 10 min after the begin of the blood clotting process (Marx 2004). Approximatley 95% of all pre-synthesized growth factors are secreted within one hour (Kevy & Jacobson 2001).

Analogous to the PC results, for the pure topographical surface modifications, VEGF and PDGF levels increased with time (30 > 15 min) and – after 15 min – with increasing surface roughness (SLA > A > PT). After 30 min of incubation, the highest VEGF and PDGF levels were measured on the A surface. Again, the hydrophilically modified surfaces (modA, modSLA) showed only moderate VEGF and PDGF levels after 15 min; however, after 30 min, an increase in cytokines was observed. For the PT-RGD and A-RGD surfaces, VEGF and PDGF levels were, compared with the PT and A surfaces, higher both after 15 and 30 min with the strongest differences after 30 min. Compared with the SLA surfaces, VEGF and PDGF expression was lower on the SLA-RGD surface.

Our results indicate that rough surface topographies (SLA $R_a = 3.2$) promote a fast and profound platelet activation. This is in concordance to former studies (Park & Davies 2000; Park et al. 2001; Kikuchi et al. 2005). On highly hydrophilic modA and modSLA surfaces, after 15 min, platelet activation and degranulation kinetics were lower compared with rather hydrophobic (A, SLA) surfaces. Nevertheless, after 30 min, a more profound decrease in PC and an increase in cytokine concentration could be evaluated. Fibrinogen that has been absorbed on titanium surfaces has shown to increase – via $\alpha_{IIb}\beta_3$ integrin receptor and RGD sequence – platelet adhesion and activation (Savage & Ruggeri 1991; Broberg et al. 2002). As RDG modifications of different surfaces also showed a more profound platelet activation after 30 min, an influence of augmented protein absorption on hydrophilic surfaces (Scheideler et al. 2005) related to early platelet activation is possible.

Altogether, RGD modifications of differently treated surfaces resulted in additional platelet activation, especially after 30 min and for plainer surfaces. RGD modifications of SLA surfaces were showing less platelet activation. This may be due to the binding of RGD on different elevation levels. Accordingly, one platelet may be multiple bound and therefore inhibited (Zhang et al. 2008). This effect may also explain the slightly better results obtained with PT-RGD ($R_a = 0.04$) compared with A-RGD ($R_a = 0.83$).

Conclusion

In this *in vitro* study, the influence of topographical, chemical and biomimetical modifications on platelet activation has been shown. It seems that rougher surfaces are promoting a faster activation of platelets. Chemical and biomimetical modifications can initiate later but even more distinct platelet degranulation on surfaces. A correlation between chemical/biomimetical modifications with a low CA/high hydrophilic potential together with later platelet activation can be assumed. RGD modifications have shown to increase fast platelet activation on plainer surfaces as well. The use of combined topographical and biomimetical modifications seems to be suitable in critical clinical situations.

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