JOURNAL OF MATERIALS SCIENCE: MATERIALS IN MEDICINE **12** (2001) 871–877

Biomimetic coatings functionalized with adhesion peptides for dental implants

S. ROESSLER, R. BORN, D. SCHARNWEBER, H. WORCH
Technische Universität Dresden, Institut für Werkstoffwissenschaft, D-01062 Dresden, Germany

A. SEWING, M. DARD
Merck Biomaterial GmbH, D-64271 Darmstadt, Germany

A complete biological integration into the surrounding tissues (bone, gingiva) is a critical step for clinical success of a dental implant. In this work biomimetic coatings consisting either of collagen type I (for the gingiva region) and hydroxyapatite (HAP) or mineralized collagen (for the bone interface) have been developed as suitable surfaces regarding the interfaces. Additionally, using these biomimetic coatings as a matrix, adhesion peptides were bound to further increase the specificity of titanium implant surfaces. To enhance cell attachment in the gingiva region, a linear adhesion peptide developed from a laminin sequence (TWYKIAFQRNRK) was bound to collagen, whereas for the bone interface, a cyclic RGD peptide was bound to HAP and mineralized collagen using adequate anchor systems. The biological potential of these coatings deduced from cell attachment experiments with HaCaT human keratinocytes and MC3T3-E1 mouse osteoblasts showed the best results for collagen and laminin sequence coating for the gingiva region and mineralized collagen and RGD peptide coatings for regions with bone contact. Our concept opens promising approaches to improve the biological integration of dental implants.

Introduction

Over the past years surface modification of dental implants focused mainly on micro and macro textures (e.g. titanium plasma spray coatings, etching) and to a lesser extent on plasma spray coatings of calcium phosphate phases (CPP) in the region of bone contact [1–3]. For the gingiva area polished surfaces are favored to minimize plaque adhesion, which may lead to periimplantitis [4–7]. However, metallic surfaces will not be integrated through specific biological mechanisms.

Immediately after contact with the biological fluids the implant surface is conditioned by proteins, glycoproteins etc., whereas the final composition is a result of a dynamic process determined by adsorption and desorption (Vroman effect [8]). The original surface characteristics influence the adsorption and conformation of proteins [9, 10]. As a consequence host cells interact with the conditioned metal surface. A practical application of these naturally occurring events is seen in the design of biological features on implant surfaces in order to induce a specific cellular response [10–12]. This objective may be addressed by different approaches. One concept is the immobilization of either adhesion proteins (collagens, fibronectin, laminin, vitronectin), bioactive peptides (RGD, KRSR) or growth factors (BMP, TGF-β) [12–20]. Another concept is seen in the formation of coatings by biomimetic processes that mimic the host tissue next to the implant (e.g. bone: mineralized collagen) to create a natural microenvironment (extracellular matrix) for cells [21–23].

We have developed a new strategy for dental implants which is based on both concepts. The basis for the biomimetic coatings is provided by the composition at the natural interface itself. Human gingival tissue comprises mainly of collagen type I. The main organic component of bone is also collagen type I with hydroxyapatite (HAP) as the main inorganic part. Therefore, biomimetic coatings consisting of collagen type I for the gingival region as well as mineralized collagen and HAP for the bone interface have been prepared on titanium surfaces for the latter both using a biomimetic approach. Biomimetic coatings can be functionalized to enhance cell attachment. For the gingival region a linear adhesion peptide using a laminin sequence (TWYKIAFQRNRK) [24] specific for $\alpha_6\beta_4$ and $\alpha_6\beta_1$ integrins was selected, whereas a cyclic α_v-selective RGD peptide [25] was taken for the bone interface.

Materials and methods

Ti6Al4V samples (ASTM 136) (∅ 10 mm, height 2–3 mm) were prepared by polishing to roughness values below 25 nm (RMS) on a 100 μm length scale. Prior to

use ultrasonic cleaning was done in 1% triton X-100, acetone and ethanol for 20 min each.

Collagen coating

Collagen type I coating of Ti6Al4V samples was performed according to Geißler *et al*. [26]. Briefly, for coating with fibrillar collagen (acid soluble calfskin collagen, Fluka, Deisenhofen, Germany) samples were incubated in a suspension of 65 mM phosphate buffer (pH 7.0, 25 °C) with a collagen concentration of about 1 mg ml^{-1} for 15 min and rinsed with distilled water.

Hydroxyapatite coating

The HAP coating on Ti6Al4V was achieved by a two step process. First HAP was deposited on titanium by an electrochemically controlled method. The process of deposition is described in detail in Roessler *et al*. [27]. Briefly, cathodic polarization was carried out using galvanostatic mode (-100 A m^{-2}) in a $Ca^{2+}/H_xPO_4^{(3-x)-}$-containing electrolyte (0.03 M $CaCl_2$+ 0.02 M $NH_4H_2PO_4$) at pH 6.4 and 37 °C for 1 h. Second anodic polarization in phosphate buffer (pH 12, 36 °C) using galvanostatic mode (10 A m^{-2}, 40 V_{SCE}) was carried out to increase oxide thickness and hence partially incorporate the HAP layer into the anodic oxide layer. The coated samples were washed in distilled water and dried in air.

Mineralized collagen coating

Mineralization of collagen was accomplished by an electrochemically controlled process in a $Ca^{2+}/H_xPO_4^{(3-x)-}$-containing electrolyte at near physiological conditions for pH (6.4) and temperature (36 °C) under cathodic polarization of the sample. Prior to mineralization collagen fibrils were adsorbed onto a HAP layer previously deposited on the titanium surface by an electrochemically controlled procedure (see above) and subsequently mineralized by cathodic polarization in a $Ca^{2+}/H_xPO_4^{(3-x)-}$-containing solution (pH 6.4, 36 °C) at -10 A m^{-2} for 15 min.

Adhesion peptides

The linear laminin sequence was synthesized with a thiol anchor (NMI, Tübingen, Germany) and bound to amino groups of collagen using sulfosuccinimidyl 4-(p-maleimidophenyl)butyrat (SMPB). The peptide cyclo (-RGDfK) utilized with thiol or phosphonate anchor groups [28] was bound to collagen or CPP, respectively. The binding was performed with concentrations of 0.1 mmol for the RGD peptide and 1 mmol for the laminin sequence in the coating solution, which are in the range of saturation coverage for titanium samples given by ELISA measurements.

Scanning electron microscopy (SEM, DSM 982 Gemini, Carl Zeiss Oberkochen, Germany) at low acceleration voltage (1 kV) was utilized for morphology characterization. Fourier Transform Infrared Spectroscopy (FTS 2000, Perkin-Elmer) was used to characterize the chemical compositions of the deposited CPP and the mineralized collagen.

Cell attachment was measured in triplicate using hexosaminidase test 1 h after seeding [29]. ELISA measurements were related to cell number and enzyme activity by measuring absorbance in wells with known number of cells. Plating efficiency was calculated relative to the enzyme activity of the seeded cell number (50.000).

Results

Morphology and chemical composition

Morphology (SEM) and chemical composition (FTIR) of the biomimetic coatings comprising either collagen, HAP or mineralized collagen are described in the following. A polished titanium sample which was used

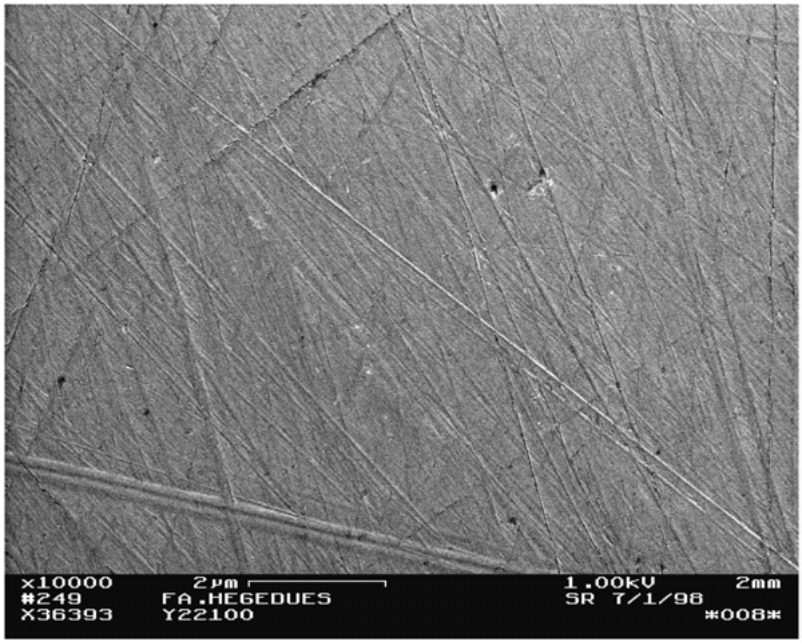

Figure 1 SEM-image of a Ti6Al4V surface (reference).

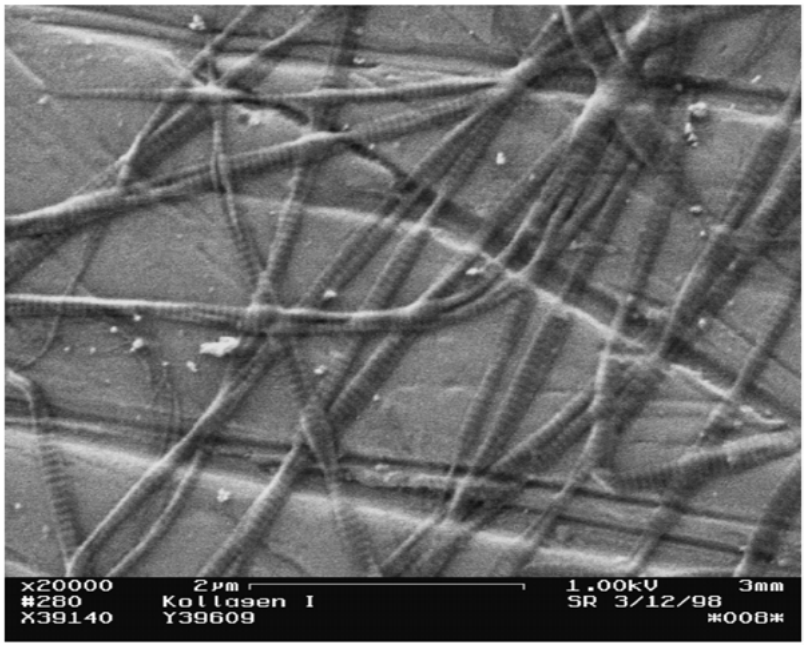

Figure 2 SEM-image of adsorbed collagen type I fibrils on Ti6Al4V.

as reference surface is shown in Fig. 1. Fig. 2 shows a titanium surface covered by a network of native collagen fibrils after adsorptive immobilization. The characteristic banding pattern of collagen type I fibrils (67 nm) is seen and a fibril width between 80–100 nm was measured with atomic force microscopy (AFM). Due to adsorption, fibrils deform and expose a ratio of width to height of about 10 which indicates strong interactions with the titanium surface [30]. Places in between the network of collagen fibrils appear free of collagen in SEM-images but are homogenously covered by collagen molecules and small aggregates as found with AFM [31]. It is important to note, that the titanium surface is homogenously covered by collagen either collagen fibrils or small aggregates. The coating consisting of electrochemically prepared HAP is shown in Fig. 3. The HAP crystals have a needle like appearance with < 500 nm length and < 60 nm width and height. The HAP needles form a porous structure with no preferential direction of crystallite orientation. The thickness of the coating is about 5 µm. Fig. 4 shows the mineralized collagen coating on a titanium surface. The mineralized collagen coatings consist basically of a two layer structure comprising a pure layer of HAP on the titanium surface with a mineralized collagen layer on top. Until complete mineralization was achieved the characteristic banding pattern of collagen fibrils (63–67 nm) remained visible. However, no periodic correlation of HAP crystals *c*-axes related to the banding pattern of collagen was found, instead collagen fibrils are covered by HAP. The electrochemical parameters used here result in partial mineralization of the collagen fibrils. The thickness of the coating is about 4 µm.

FTIR-spectroscopy has been used to analyze the

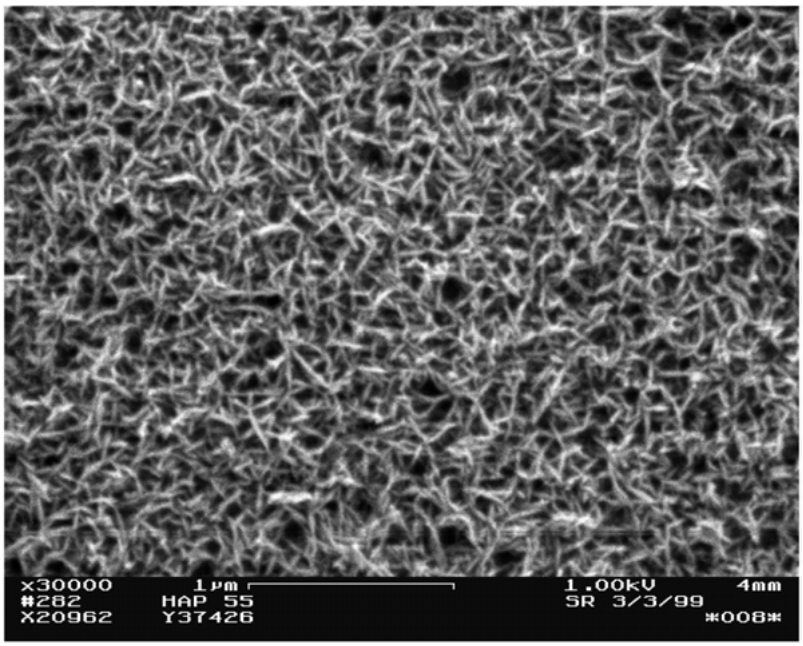

Figure 3 SEM-image of hydroxyapatite on Ti6Al4V.

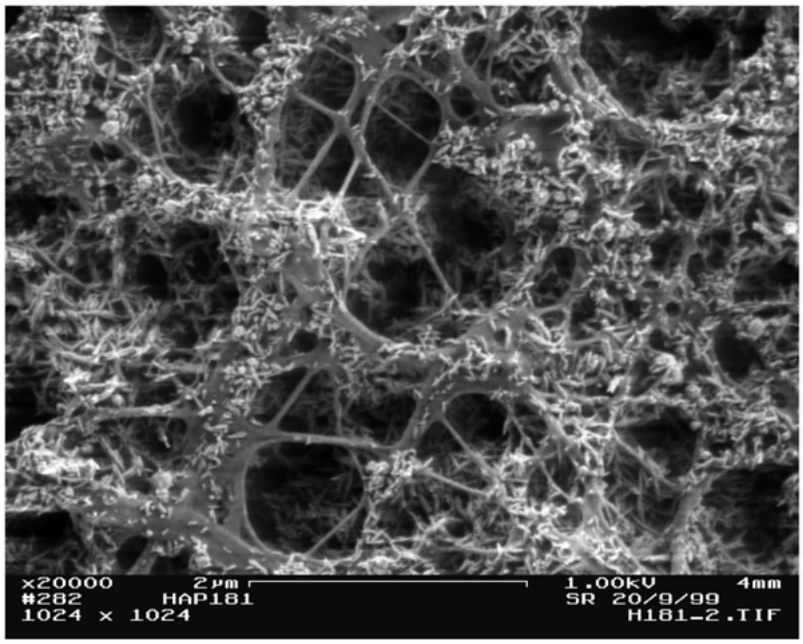

Figure 4 SEM-image of mineralized collagen type I coating on Ti6Al4V.

chemical composition of the coatings and characteristic spectra are shown in Fig. 5. As intensities resulting from adsorbed collagen films were too low, thick collagen films were prepared by drying high concentrated collagen suspension on titanium. The spectrum of collagen I fibrils shows maxima at 1660, 1555 and 1280 cm^{-1} which can be assigned to protein amide-I-, -II-, and -III-bands respectively. The FTIR-spectrum of HAP clearly shows splitting into the antisymmetric v_3 and v_4 vibration modes (1050, 1090 cm^{-1} and 570,600 cm^{-1}). The previously inactive v_1 vibration of the free phosphate ion can also be seen in the HAP spectrum. Additional bands at 874, 1587 and 1419 cm^{-1} can be associated to carbonate ions (CO_3^{2-}) resulting from the reaction of OH ions with carbon dioxide from air. The characteristic OH libration at 630 cm^{-1} is also evident. The OH stretching vibration at 3570 cm^{-1} is either very weak or, due to the reaction of CO_2 with the hydroxyl groups of the electrochemically controlled deposited HAP, not detectable. It is further conceivable that the OH stretching vibration is overlapped by a broad band resulting from adsorbed water. FTIR- and XRD-spectra of CPP using a electrochemically assisted procedure have been published in detail previously [27]. The spectrum of mineralized collagen shows the characteristic phosphate bands between 1000 and 1100 cm^{-1} as well as the amide-bands and can be interpreted as a superposition of the two components HAP and collagen I. For the amide-I-band a broadening and shift to lower wavenumbers was found. FTIR-spectra of bone (cranial bone) are almost similar to our biomimetic coatings (mineralized collagen). The shift of the amide-I-band of mineralized collagen corresponds to the broad amide-I-band of bone.

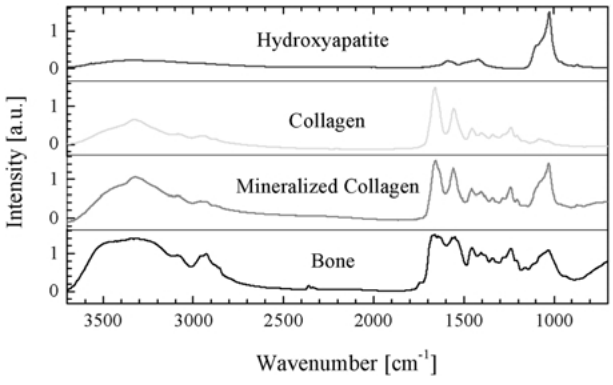

Figure 5 FTIR-spectra for hydroxyapatite, collagen type I, mineralized collagen type I and bone (cranial bone).

Cell adhesion

Cell adhesion experiments were carried out with HaCaT human keratinocytes and MC3T3-E1 mouse osteoblasts representing two different cell types in contact with a dental implant. Fig. 6 shows the plating efficiency for the basic coatings of collagen, mineralized collagen and HAP and for the corresponding states with bound adhesion peptides. MC3T3-E1 cells were not tested on surfaces coated with the laminin sequence. According to their differentiation stage there is a risk that they express the corresponding integrin receptors leading to non-specific results. For HaCaT cells the strongest influence on cell adhesion is coming from collagen, leading to a plating efficiency of 37% for the collagen coating in contrast to 2% for the uncoated control. This is confirmed by the mineralized collagen surfaces where values in the range of 40% are reached, while hydroxyapatite is less favored with 11%. Binding of the laminin sequence to the collagen layer leads to a further increase of cell adhesion (57%). For the mineralized collagen no influence of the laminin sequence could be observed. The RGD peptide shows no significant influence on HaCaT cells except on the hydroxyapatite surface. Cell adhesion of MC3T3-E1 mouse osteoblasts is improved to values around 32% for mineralized collagen and HAP coatings, while the control reaches 5% and the collagen coating 10%. The binding of the RGD adhesion peptide in this case also improves the plating efficiency leading to values above 50% independent on anchor and coating type.

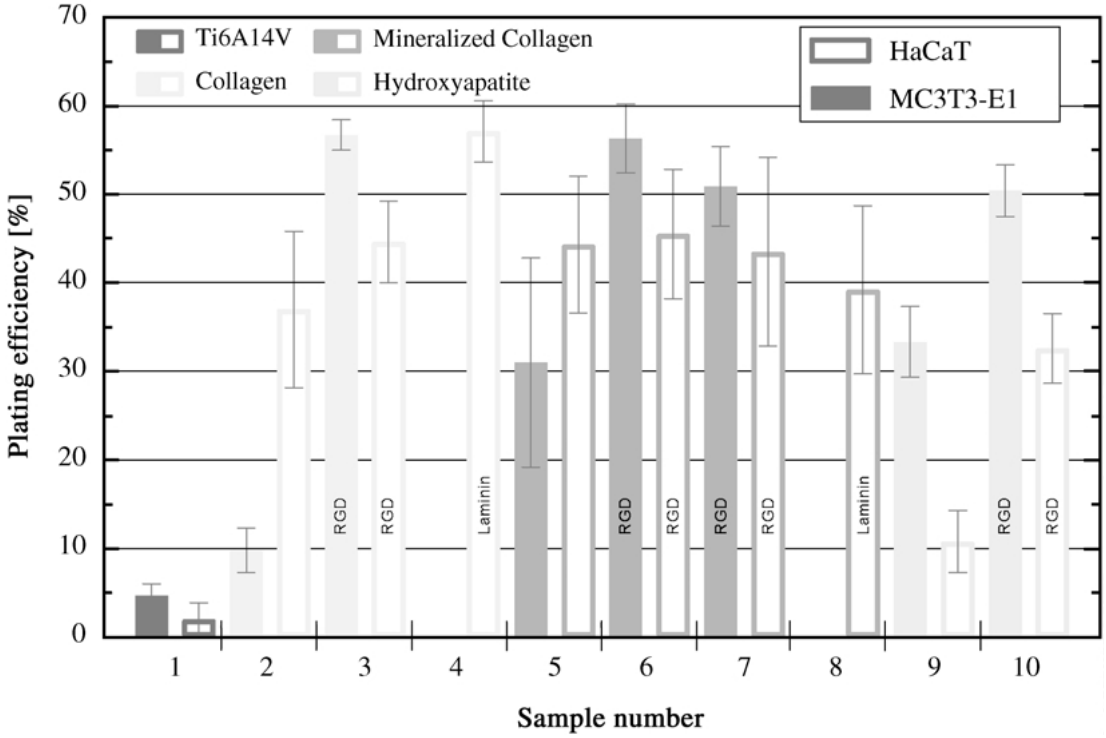

Figure 6 Cell adhesion of MC3T3-E1 and HaCaT cells on different biomimetic coatings and their functionalized surface states: 1 Ti6Al4V reference, 2–4 collagen, 5–8 mineralized collagen, 9–10 hydroxyapatite. Adhesion peptides were bound to collagen (3, 4, 6, 8) or to hydroxyapatite (7, 10).

Discussion

The initial biological reactions occurring at the host tissue implant interface are essential for the success of implantation [10]. These events may be tailored towards a more physiological process by surface modifications which minimize nonspecific adsorption of proteins [17, 32], promote adhesion of cells [11, 20, 33] and present a microenvironment closely related to the host tissue [10, 21]. In order to achieve such surface properties the main components of the extracellular matrix (collagen, HAP, mineralized collagen) have been immobilized on Ti6Al4V using adsorption or biomimetic processes. It was shown that collagen I coatings could be achieved by a simple adsorption process which proved to be stable against concurrence adsorption [26]. On the other hand CPP are considered the material of choice for the bone interface. In contrast to ref. [34–37] who also used electrochemistry for calcium phosphate deposition, we found CPP presenting a high similarity to natural CPP. In analogy to the natural process an amorphous calcium phosphate precursor is first formed [27] which then transforms to nanocrystalline HAP needles. The dimensions of the HAP needles compared to HAP crystals in bone were higher by about one order of magnitude [38] which was also reported by Schirkanzadeh [37].

Aiming to achieve a more precise resemblance to the complex structure of bone a biomimetic process for mineralization of collagen was developed. Although there are a number of studies on the development of bone-like substitutes containing collagen and CPP [39–42] only a few studies address the formation of bone-like structures on metallic implant surfaces. Self-assembled bilayers of positively and negatively charged poly(amino acids) on titanium are used by Hwang *et al.* [43] to induce the formation of organoapatite. Serro *et al.* studied the deposition of CPP on titanium in the presence of albumin and fibronectin [44, 45]. However, these processes do not resemble the natural composition of bone. Using collagen as the organic component of the bone matrix our mineralization method leads to a structure, which mimics, to some extent, the main composition and microstructure of bone. Further, the FTIR-spectra of mineralized collagen display phosphate vibrations and amide-bands that match those of bone. Also the shift of the amide-I-band of mineralized collagen as compared to nonmineralized collagen corresponds well with the broad amide-I-band of bone.

The following methods are used for surface modification of biomaterials with proteins or molecules: adsorption, covalent coupling and self-assembly techniques [17, 32, 33, 46]. The latter are rather difficult on metal oxides, whereas covalent coupling requires intermediate layers. Biomimetic coatings which additionally have a biological function could offer an alternative to the covalent coupling process via classical chemistry. The main biological function is seen in the microenvironment in which peptide ligands are presented, which is an important factor for cell adhesion [33].

There are numerous studies suggesting that cell attachment plays a dominant role for further cellular proliferation, differentiation and biochemical activity [47, 48]. Cell attachment of human gingival epithelial cells on coated titanium surfaces have led to different results. Dean *et al.* [13] report the greater affinity to laminin coated surfaces, while Park *et al.* [12] only found minor differences between collagen I, collagen IV and laminin coatings in comparison to titanium. For HaCaT human keratinocytes we have shown the positive influence of collagen I on cell attachment which can still be increased by functionalization with the laminin

sequence. The finding that more HaCaT cells adhere on collagen I coated titanium surfaces is in agreement with a recent publication of Klein *et al*. [49], who measured cell adhesion on sand-blasted surfaces coated with collagen I and IV. The results for the $\alpha_6\beta_4$ and $\alpha_6\beta_{41}$ selective laminin sequence is supported by Räisänen *et al*. [50] who demonstrated the important role of the $\alpha_6\beta_4$ integrin in cell attachment of HaCaT cells. Our cyclic RGD adhesion peptide has no influence on HaCaT cell attachment except for the hydroxyapatite surface. In this case as well as for the laminin sequence on mineralized collagen, which shows lower results then on collagen itself, the integrin receptors may be influenced by presence of divalent Ca^{2+} ions [51].

The hydroxyapatite and mineralized collagen coatings act positively in cell attachment of MC3T3-E1 mouse osteoblasts in contrast to collagen and titanium surfaces. The binding of the RGD adhesion peptide greatly increases cell adhesion independently of coating type. For collagen, values as high as for the calcium phosphate coatings are reached demonstrating the high efficiency of the chosen cyclic RGD peptide. A high selectivity can also be deduced from the negligible influence of this peptide on the HaCaT cells. Similar property has also been demonstrated by Kantlehner *et al*. [52] for other cell types.

Conclusion

Biomimetic coatings consisting of the main components of bone can be produced on titanium implant surfaces using electrochemical controlled methods. SEM and FTIR-spectroscopy of biomimetic coatings show structural and chemical properties comparable to bone. Further, adhesion promoting peptides could be immobilized on collagen, HAP or mineralized collagen without losing their functionality. Biomimetic coatings can be regarded as a good alternative to the conventional covalent coupling to metallic implant surfaces. Cell adhesion experiments provide evidence to our concepts to coat metal implants with the main components of the extracellular matrix presenting additionally an adhesion promoting peptide. Coating and adhesion peptide have to be chosen regarding to the host tissue at the interface.

Acknowledgment

The financial support by BMBF (03N4021) is gratefully acknowledged. The authors like to thank B. Blessing and S. Perschbacher for performing the cell experiments and BoneMaster GmbH, Dresden, for providing the hydroxyapatite coatings.

References

1. M. BRIEN, H. SCHLIEPHAKE, J. BECKER and P. A. REICHART, *Z. Zahnärtzl. Implantol.* **14** (1998) 208.
2. D. BUSER, R. K. SCHENK, S. STEINEMANN, J. P. FIORELLINI, C. H. FOX and H. STICH, *J. Biomed. Mater. Res.* **25** (1991) 889.
3. G. L. DELANGE and K. DONATH, *Biomaterials* **9** (1988) 302.
4. L. W. LINDQUIST, B. ROCKLER and G. E. CARLSSON, *J. Prosthet. Dent.* **59** (1988) 59.
5. M. QUIRYNEN, H. C. VAN DER MEI, C. M. BOLLEN, A. SCHOTTE, M. MARECHAL and G. I. DOORNBUSCH, *J. Dent. Res.* **72** (1993) 1304.
6. M. QUIRYNEN, C. M. BOLLEN, W. PAPAIOANNOU, J. VAN ELDERE and D. VAN STEONBERGHE, *J. Oral Maxillofac. Impl.* **11** (1996) 169.
7. C. D. WU-YUAN, K. J. EGANHOUSE, J. C. KELLER and K. S. WALTERS, *J. Oral Implantol.* **21** (1995) 207.
8. L. VROMAN and A. L. ADAMS, *J. Colloid Interface Sci.* **111** (1986) 391.
9. D. M. BRUNETTE, *Int. J. Oral Maxillofac. Impl.* **3** (1988) 231.
10. C. J. KIRKPATRICK, M. WAGNER, H. KÖHLER, F. BITTINGER, M. OTTO and C. L. KLEIN, *J. Mater. Sci.* **8** (1997) 131.
11. K. ANSELME, *Biomaterials* **21** (2000) 667.
12. J. C. PARKS, H. M. KIM and J. S. KO, *J. Oral. Maxillofac. Impl.* **13** (1998) 826.
13. J. W. DEAN, K. C. CULBERTSON and A. M. DANGELO, *J. Oral. Maxillofac. Impl.* **10** (1995) 721.
14. K. C. DEE, T. T. ANDERSEN and R.BIZIOS, *J. Biomed. Mater. Res.* **40** (1998) 371.
15. D. M. FERRIS, G. D. MOODIE, P. M. DIOMOND, C. W. D. GIORANNI, M. G. EHRLICH and R. F. VALENTINI, *Biomaterials* **20** (1999) 2323.
16. B. F. LOWENBERG, R. M. PILLAR, J. E. AUBIN, J. SODEK and A. H. MELCHER, *ibid.* **9** (1988) 302.
17. D. A. PULEO and A. NANCI, *ibid.* **20** (1999) 2311.
18. S. ROESSLER, C. WOLF, D. SCHARNWEBER and H. WORCH, *J. Adhes. Sci. Technol.* **14** (2000) 453.
19. H. WORCH in ''Metals as Biomaterials'', edited by J. A. Helsen and H. J.Breme (John Wiley & Sons Ltd, 1998) p. 177.
20. P. SCHAFFNER, J. MEYER, M. DARD, B. NIES, S. VERRIER, H. KESSLER and M. KANTLEHNER, *J. Mater. Sci.: Mater. Med.* **10** (1999) 837.
21. J. P. MAZZUCOTELLI, C. KLEIN-SOYER, A. BERETZ, C. BRISSON, G. ARCHIPOFF and J. P. CAZENAVE, *Int. J. Artif. Organs* **14** (1991) 482.
22. S. ROESSLER, D. SCHARNWEBER, R. BORN, H. WORCH, A. SEWING and M. DARD, in ''Proceedings of the 6th World Biomaterials Congress'' (Kamuela, Hawaii, May 2000, Transactions) p. 1040.
23. H. B. WEN, J. R. DE WIJN, F. Z. CUI and K. DE GROOT, *J. Biomed. Mater. Res.* **41** (1998) 227.
24. M. NOMIZU, W. H. KIM, K. YAMAMURA, A. UTANI and SANG-YO, *J. Biol. Chem.* **270** (1995) 20583.
25. M. KANTLEHNER, D. FINSINGER, J. MEYER, P. SCHAFFNER, A. JONCZYK, B. DIEFENBACHER, B. NIES and H. KESSLER, *Angew. Chem.* **111** (1999) 587.
26. U. GEISSLER, U. HEMPEL, C. WOLF, D. SCHARNWEBER, H. WORCH and K. W. WENZEL, *J. Biomed. Mater. Res.* **51** (2000) 752.
27. S. ROESSLER, M. STOELZEL, R. BORN, D. SCHARNWEBER, H. WORCH, A. SEWING and M. DARD, *ibid.* Submitted May, 2001.
28. A. JONCZYK, S. GOODMAN, B. DIEFENBACH, A. SUTTER and H. KESSLER, WO-A97/14716.
29. U. LANDEGREN, *J. Immunol. Meth.* **67** (1984) 379.
30. S. ROESSLER, D. SCHARNWEBER and H. WORCH, *J. Mater. Sci. Lett.* **18** (1999) 577.
31. S. ROESSLER, C. WOLF, D. SCHARNWEBER, H. WORCH, A. SEWING and M. DARD, in ''Proceedings of the 6th World Biomaterials Congress'' (Kamuela, Hawaii, May 2000, Transactions) p. 1045.
32. A. NANCI, J. D. WUEST, L. PERU, P. BRUNET, V. SHARMA, S. ZALZAL and M. D. MCKEE, *J. Biomed. Mater. Res.* **40** (1998) 342.
33. B. T. HOUSEMAN and M. MRKSICH, *Biomaterials* **22** (2001) 943.
34. S. BAN and S. MARUNO, *J. Biomed. Mater. Res.* **42** (1998) 387.
35. H. ISHIZAWA and M. OGINO, *ibid.* **31** (1996) 6279.
36. J. REDEPENNING, T. SCHLESSINGER, S. BURNHAM, L. LIPPIELLO and J. MIYANO, *ibid.* **30** (1996) 287.
37. M. SHIRKHANZADEH, *J. Mater. Sci.: Mater. Med.* **9** (1998) 67.

38. R. Z. LEGEROS, in ''Calcium Phosphates in Oral Biology and Medicine'' (Basel, Kräger, 1991) p. 110.
39. J. H. BRADT, M. MERTIG, A. TERESIAK and W. POMPE, *Chem. Mater.* **11** (1999) 2694.
40. Y. DOI, T. HORIGUCHI, Y. MORIWAKI, H. KIITAGO, T. KAJIMOTO and Y. J. I. IWAYAMA, *J. Biomed. Mater. Res.* **31** (1996) 43.
41. C. DU, Z. F. CUI, W. ZHANG, Q. L. FENG and K. DEGROOT, *ibid.* **50** (1999) 518.
42. K. S. TEN HIUSEN, R. MARTIN, M. KLIMKIEWICZ and P. W. BROWN, *ibid.* **29** (1995) 803.
43. J. J. HWANG, K. JAEGER, J. HANCOCK and S. I. STUPP, *ibid.* **47** (1999) 504.
44. A. P. V. A. DE SERRO, A. C. FERNANDES, B. J. V. SARAMAGO, J. LIMA and M. A. BARBOSA, *Biomaterials* **18** (1997) 963.
45. A. P. V. A. DE SERRO, A. C. FERNANDES and B. J. V. SARAMAGO, *J. Biomed. Mater. Res.* **49** (2000) 345.
46. M. MRKSICH and G. M. WHITESIDES, *Ann. Rev. Biophys. Biomol. Struct.* **25** (1996) 55.
47. E. D. HAY, in ''Cell biology of extracellular matrix'' (Plenum Press, New York, 1991) p. 343.
48. A. BRUCE, B. DENNIS, L. JULIAN, R. MARTIN, R. KEITH and D. W. JAMES, in ''Molecular biology of the cell'' (Garland, New York, 1994) p. 963.
49. M. KLEIN, T. HOHLFELD, P. MOORMANN and H. MENNEKING, *Int. J. Oral. Maxillofac. Impl.* **15** (2000) 247.
50. L. RÄISÄNEN, M. KÖNÖNEN, J. JUHANOJA, P. VARPAVAARA, J. HAUTANIEMI and M. HORMIA, *J. Biomed. Mater. Res.* **49** (2000) 79.
51. B. DIEFENBACH, Ph.D. Thesis, TH Darmstadt, Germany (1995).
52. M. KANTLEHNER, P. SCHAFFNER, D. FINSINGER, J. MEYER, A. JONCZYK, B. DIEFENBACHER, B. NIES, G. HÖLZEMANN, S. L. GOODMAN and H. KESSLER, *Chembiochem.* **1** (2000) 107.

Received 14 May
and accepted 21 May 2001