Peptidomimetics

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αvβ3- or α5β1-Integrin-Selective Peptidomimetics for Surface Coating

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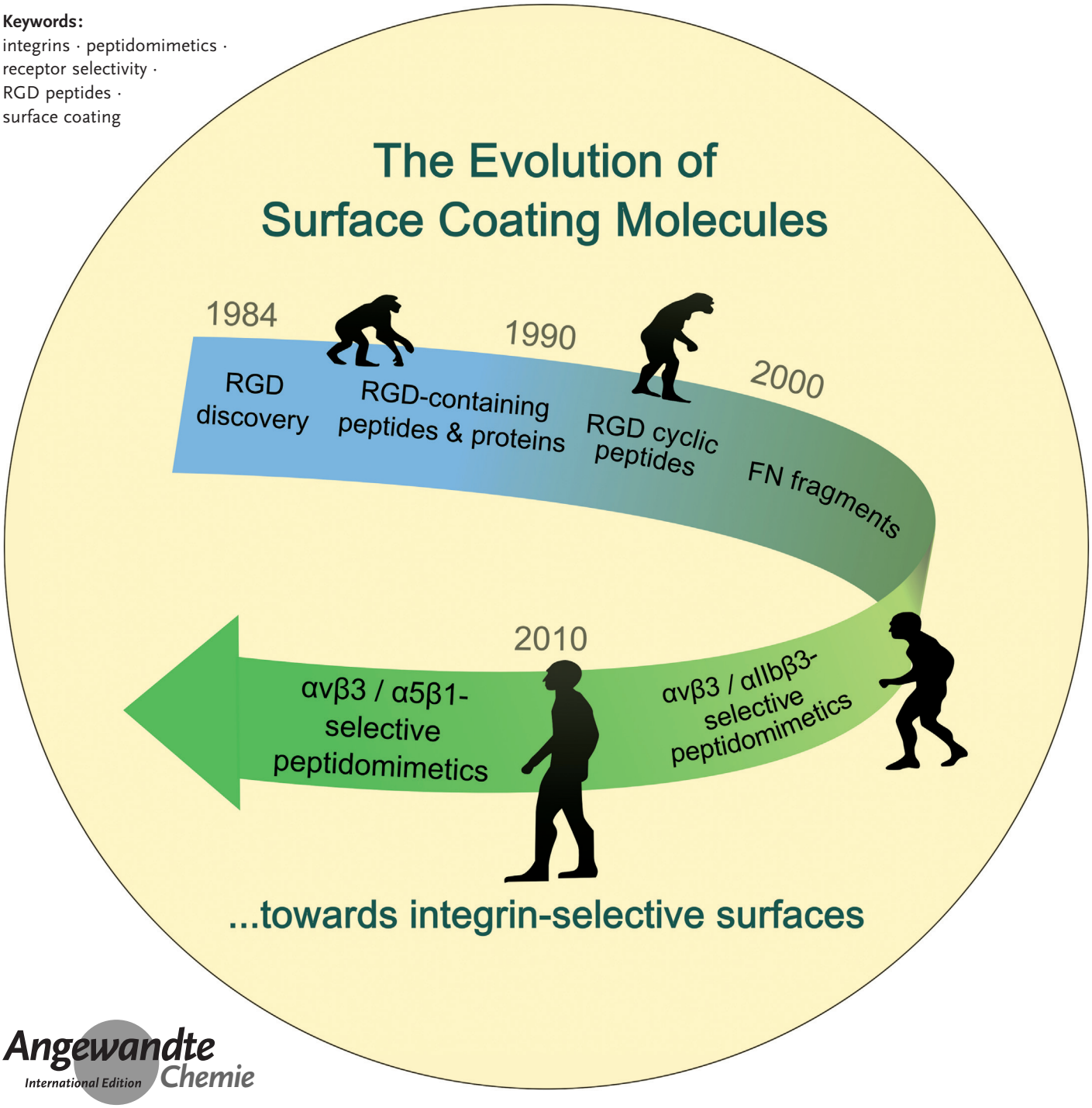
integrins · peptidomimetics · receptor selectivity · RGD peptides · surface coating



Engineering biomaterials with integrin-binding activity is a very powerful approach to promote cell adhesion, modulate cell behavior, and induce specific biological responses at the surface level. The aim of this Review is to illustrate the evolution of surface-coating molecules in this field: from peptides and proteins with relatively low integrin-binding activity and receptor selectivity to highly active and selective peptidomimetic ligands. In particular, we will bring into focus the difficult challenge of achieving selectivity between the two closely related integrin subtypes αvβ3 and α5β1. The functionalization of surfaces with such peptidomimetics opens the way for a new generation of highly specific cell-instructive surfaces to dissect the biological role of integrin subtypes and for application in tissue engineering and regenerative medicine.

From the Contents

1. Introduction

Integrins represent the most important family of cell adhesion receptors. These proteins are bidirectional, heterodimeric cell surface receptors, which are crucial for the interaction of cells with extracellular matrix (ECM) proteins.[1] By interacting with ECM ligands, integrins activate intracellular pathways of signal transduction and mediate cell migration and adhesion. Since the discovery and initial classification of integrins in the late 1980s,[2] extensive research has focused on the study of their structure, ligand recognition, and biological functions, thereby making this class of proteins the most studied adhesion receptors.

The integrin family consists of at least 24 subtypes, built by the noncovalent association of 18 α and 8 β subunits. These subunits are both type-I membrane proteins, each consisting of a large ectodomain and a typically short noncatalytic cytoplasmic domain, linked by a single transmembrane domain (Figure 1).[3] The affinity of integrins to their ligands is regulated by cellular signaling, which can lead to activation, so-called "inside-out" signaling.[4] For example, the formation of an intracellular salt bridge controls "inside-out" signaling, since abrogation of the salt bridge between the α and β subunit cytoplasmic domains (which stabilizes the resting state of the integrin) strengthens integrin adhesion to ECM ligands.[5] Conversely, the binding of ECM ligands induces conformational changes in the structure of integrins,[3c] provokes dissociation of the transmembrane helices, and contributes to clustering into oligomers, thereby leading to "outside-in" signal transduction.[4a,6] Interestingly, ligand binding can occur in the membrane-associated (resting) state of integrins; however signal transduction requires dissociation of the transmembrane helices of the integrins and subsequent oligomerization.[7] Thus, integrins are considered as bidirectional signaling machines that control cell polarity, adhesion, and survival. In the cell adhesion process, integrins mediate force transmission in focal adhesions (FAs) to ECM proteins, a process known as mechanotransduction.[8]

Embryogenesis, tissue development, angiogenesis, and immune system function are, therefore, highly dependent on integrin activity.[9] Moreover, integrins are also critically involved in pathological processes such as thrombosis, osteoporosis, tumor formation and progression, metastasis, and inflammation.[10] On the basis of these biological roles, it is not surprising that integrins have been targeted for the development of drugs to treat diverse pathologies.[10b,11] For example, antagonists of the platelet receptor αIIbβ3 (abciximab, eptifibatide, and tirofiban) have been marketed as inhibitors of platelet aggregation to reduce the risk of ischemia in acute coronary syndromes.[12] Natalizumab, a drug targeting integrin α4, was prescribed to patients suffering from multiple sclerosis[13] and Crohn's disease.[14] Efalizumab, an αLβ2 inhibitor, was approved for the treatment of psoriasis.[15] Cilengitide, a highly potent antagonist of integrins αvβ3, αvβ5, and α5β1, reached clinical phase III trials for the treatment of glioblastomas and is currently in phase II for other cancer types.[16] Although several limitations have been described for these drugs,[17] these examples illustrate the pharmacological potential of targeting integrin receptors.

Understanding the biological role of integrins is paramount to develop novel drugs with high potential and reduced side effects. Nonetheless, progress in this field has been hampered by the scarcity of integrin-specific ligands. The issue of ligand specificity is clearly illustrated by the canonical integrin-binding peptide RGD.[18] Although many integrins

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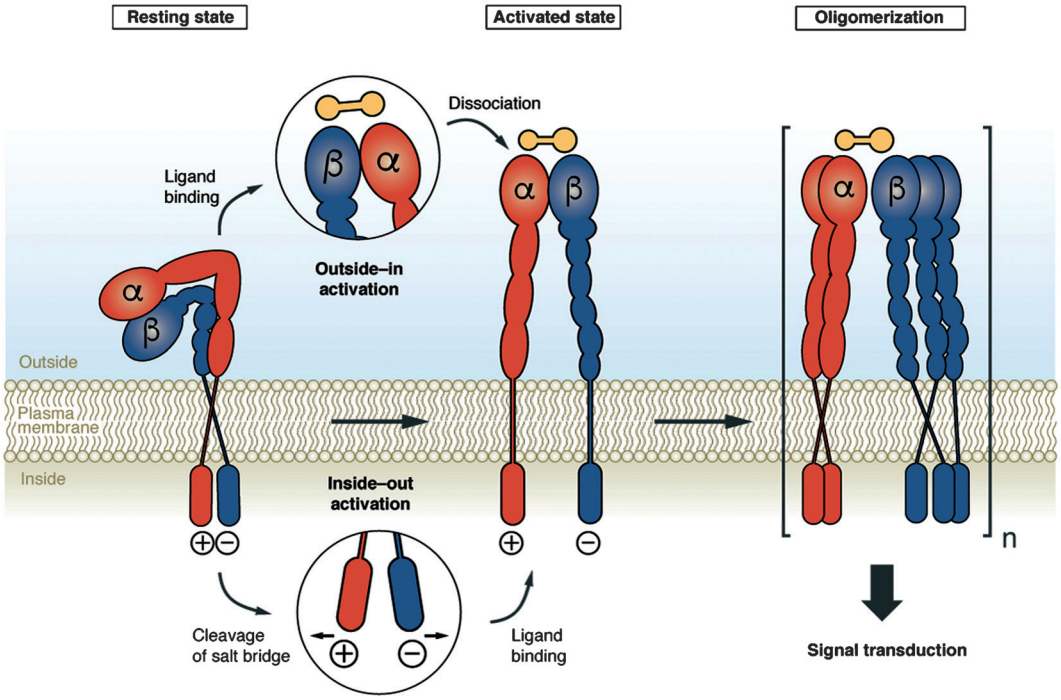
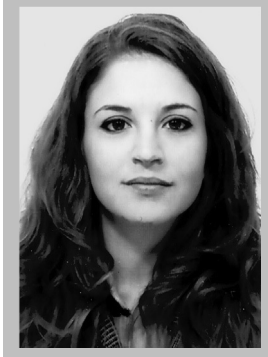


Figure 1. Integrin activation states as well as "inside-out" and "outside-in" signaling mechanisms. In the bent form, integrins have low affinity for their ECM ligands. Inside-out signaling includes cleavage of the intracellular salt bridge established between the cytoplasmic α and β subunits. This induces dissociation of the transmembrane helices and their reorganization and multimerization into a focal adhesion (FA), which binds ligands with high affinity. Conformational changes of the resting integrins and oligomerization are also induced by binding to ECM ligands. This causes stronger binding in the FA. Outside-in signaling requires integrin oligomerization.

Carles Mas-Moruno studied Chemistry at the University of Barcelona. He received his PhD in 2009 with Prof. F. Albericio at the Institute for Research in Biomedicine (IRB Barcelona). After postdoctoral research with Prof. H. Kessler at the Technical University of Munich (2009–2011) he earned a Marie Curie Career Integration Grant and joined the group of Biomaterials, Biomechanics, and Tissue Engineering of the Technical University of Catalonia. His research interests involve the development of novel chemical tools to functionalize biomaterials for application in regenerative medicine.

Florian Rechenmacher studied chemistry at the Technical University of Munich (TUM). After completing his MSc in 2009 in organic and biological chemistry, he joined the group of Prof. H. Kessler. The main research fields of his PhD (2013) were the functionalization of integrin α5β1 specific antagonists for various medicinal applications, and investigations of cyclic peptides for improvement of oral bioavailability.



Roberta Fraioli studied Materials Engineering at the Università degli Studi di Napoli Federico II. After obtaining her MSc in 2012, she joined the Biomaterials, Biomechanics, and Tissue Engineering Group at the Technical University of Catalonia (Barcelona, Spain) for her PhD studies. Recently, she visited the group of Prof. Dalby at the Centre for Cell Engineering at the University of Glasgow (UK). She is currently completing her PhD with Dr. Mas-Moruno on the functionalization of surfaces with integrin-selective peptide-based ligands to guide stem cell fate and foster implant osteointegration.

Stefanie Neubauer studied chemistry at the Technical University of Munich (TUM). In 2007 she joined the group of Prof. H. Kessler for her diploma and PhD in organic and biological chemistry, where she focused on the design, synthesis, and functionalization of peptidic and nonpeptidic integrin subtype specific antagonists for various medicinal applications, including surface coating and tumor imaging. As a postdoctoral fellow with Prof. J. P. Spatz at the Max Planck Institute in Stuttgart, she has been investigating functionalized integrin antagonists in biophysical and cellular studies.

bind the ECM through an RGD-specific recognition mechanism, these receptors are able to discriminate between distinct natural ligands containing the same RGD recognition motif.[19] The presence of complementary or synergistic domains, the nature of flanking residues, and the conformation and presentation of the RGD motif to integrins are key determinants of such integrin specificity.

In this regard, over the last three decades intensive efforts have been devoted to elucidate the structural features that govern integrin-specific interactions. Integrins αvβ3 and α5β1, key mediators of cell adhesion and differentiation, angiogenesis, and tumor growth, were considered very promising targets. By restricting the conformational space of RGD peptides through cyclization, introduction of D-amino acids, and through comprehensive structural studies, we developed in the early 1990s cyclic RGD peptides with very high affinities for αvβ3 and with selectivity against αIIbβ3.[20]

However, discrimination between the closely related αvβ3 and α5β1 integrins could not be achieved by cyclic peptides (excluding some remarkable exceptions, such as the recent development of *iso*DGR peptides).[21] Thus, selectivity between these two receptors was mostly achieved by synthetic RGD-based peptidomimetics. The development of such ligands was only possible after detailed structure–activity relationship studies and the determination of the crystal structures of αvβ3[22] and homology models for α5β1[23] (the crystal structure of this subtype was not reported until 2012).[24] These compounds have shown potential to be used as integrin antagonists for cancer treatment, tumor imaging, and for biophysical studies to elucidate the exact roles of these very important integrins.

Tobias Kapp studied chemistry at the Technical University of Munich (TUM). After completing his MSc in organic and bioorganic chemistry in 2011 he joined the group of Prof. H. Kessler. He is working on the design and synthesis of ligands for the chemokine receptor CXCR4 and several subtypes of the integrin receptor family. A major part of his work is also the development of functionalization strategies for these ligands to be used in biophysical and medical applications.

Horst Kessler studied chemistry in Leipzig and Tübingen and was appointed as full professor in 1971 at the J. W. Goethe University in Frankfurt. In 1989 he moved to the Technical University of Munich (TUM). In 2008, he became the Carl von Linde Professor at the Institute for Advanced Study at the TUM. His main interests are drug development from peptides and peptidomimetics as well as NMR spectroscopy.

In parallel to these studies, proteins from the ECM and short synthetic peptides have also been used to functionalize a wide range of materials, with the aim to improve their bioactivity by instructing cell-adhesive processes on the surfaces. An enormous body of research in this direction has shown that integrin activation and signaling on the surface of a bioinert material efficiently promotes cell attachment, proliferation, and differentiation, and thus this strategy has been used to develop a new generation of biomaterials for applications in tissue engineering and regenerative medicine. Surprisingly, the use of peptidomimetics to coat surfaces has been scarce and most of the strategies in this field have focused on using RGD-containing peptides and proteins with poor selectivity for integrin receptors.[25]

The aim of this Review is thus to discuss the use of RGD-based peptidomimetics with αvβ3/α5β1 integrin selectivity to install integrin-specific activity on the surface of biomaterials. We will present a historical perspective on the development of integrin-subtype peptidomimetics based on the RGD motif, illustrating some representative examples from our research group. The application of these types of molecules for surface coating, both for medical applications and biophysical studies, will be examined, and future prospects for this strategy will be outlined.

2. Biological Role of Integrin Subtypes αvβ3 and α5β1

Integrin subtypes αvβ3 and α5β1 were identified by Ruoslahti and co-workers in 1985 and originally named after their natural ECM ligands as the vitronectin (VN) and fibronectin (FN) receptors, respectively.[26] Both subtypes recognized the RGD sequence, which had been described as the minimal adhesive binding motif in 1984.[18] To date, one third of the 24 known integrin subtypes are reported to bind the RGD motif, including all αv integrins, the integrin α5β1, and the blood platelet integrin αIIbβ3.[27] Whereas αvβ3 can bind to several ECM proteins including VN, FN, osteopontin, and bone sialoprotein, the α5β1 integrin primarily recognizes FN as a consequence of the presence of the synergistic amino acid sequence PHSRN in the cell attachment site of the protein.[28] Nonetheless, both integrins have been described to bind to other ECM ligands with various degrees of affinity.[27b,c]

The formation and development of focal contacts is also integrin-dependent. The geometric localization, shape, and dimension of these points of anchorage to the ECM vary greatly with the integrin-expression profile, the ligands available in the microenvironment, and the culture time. In this context, Geiger and co-workers found that nascent focal complexes are rich in αvβ3, while α5β1 is present in mature fibrillar FAs.[29] Each subtype is associated with diverse organizations of the actin cytoskeleton and, therefore, of cell shape: cells overexpressing αvβ3 are characterized by broad lamellipodia and low RhoA activity (a small GTPase protein). In contrast, well-defined actin fibers and high RhoA activity are observed in α5β1-rich cells.[30] These observations on cell shape and actin organization are reflected well in the

force-sensing ability of each integrin subtype. Roca-Cusachs et al. showed that clusters of α5β1 support high matrix forces, while αvβ3 initiates mechanotransduction and is responsible for reinforcement in response to an applied force on FN-coated beads.[31] In agreement with this finding, it has also recently been shown that cells binding to substrates through α5β1 exert higher forces than if they bind through αvβ3.[32] In another study, Giannone and co-workers have reported distinct dynamic nanoscale organizations of β1 and β3 integrins, which can control local forces and signaling during cellular functions such as migration and ECM remodeling.[33] Recently, the group of Fässler has shown that α5β1 integrins accomplish force generation, whereas αv integrins mediate structural adaption to forces on FN-based microenvironments.[34] This study identified diverse functions of the integrins αvβ3 and α5β1, which cooperate to regulate cell contractility and rigidity sensing of cells.

Apart from FAs and the organization of actin fibers, the engagement of a specific integrin subtype has been shown to influence cell proliferation and differentiation. However, investigations on the role of the αvβ3 and α5β1 subunits on cell growth are often contradictory. The α5β1 receptor has been demonstrated to support cell adhesion and proliferation in several studies.[35] For example, blocking of α5β1 significantly reduced the expression of the transcription factor c-Fos, which is associated with cell proliferation.[35c] However, others authors observed no effect of this receptor on cell growth, neither in vitro nor in vivo.[36] A study by Martino et al.[37] on FN fragments presenting different affinities for α5β1 pointed out that blocking this receptor only affects proliferation on highly affine substrates (containing both RGD and PHSRN sequences), while full-length FN and fragments containing only the RGD motif are still capable of fostering cell growth because of the numerous unspecific signals mediated by other cell receptors. Fewer studies focused on the αvβ3 subtype. Murine cells overexpressing this integrin showed increased proliferation rates compared to nontransfected cells, and this effect was abolished by incubating transfected cells with an αvβ3-blocking antibody.[38] On the other hand, Garcia and co-workers observed no effect on proliferation after blocking this receptor.[35d]

The discrepancies observed in the literature may arise from different reasons. ECM ligands are often characterized for their affinity towards only one integrin subtype (e.g. αvβ3 or α5β1), but the determination of their binding affinity for other subtypes is often neglected. Thus, a biological effect may be associated to one specific integrin receptor, whilst underestimating the role of other integrins. Moreover, the pattern of integrin expression on each cell strongly varies depending on the cell type, culturing conditions, and substrate used, thus not allowing a direct comparison between different studies. On top of that, it should be taken into consideration that integrins have overlapping roles, and the suppressed function of one blocked integrin may be substituted by another.

The role of αvβ3 and α5β1 on the differentiation of mesenchymal stem cells (MSCs) is a very hot topic of research, with rising interest in stem cell therapies and in the development of cell instructive biomaterials. An increasing need to control the plasticity of stem cells is emerging, either for keeping them undifferentiated in culture[39] or for inducing a specific phenotype.[40] Apart from classical molecular mediators of differentiation, such as growth factors, the microenvironment has proved a promising tool for guiding the fate of stem cells.[41] In this regard, the role of integrins in the progression of the undifferentiated cell toward a specific lineage is not yet fully established. Several studies have detected a positive role of the α5β1 subtype in the induction of osteogenesis. This receptor has been shown to upregulate the expression of osteogenic markers and alkaline phosphatase (ALP) activity in vitro[36,37,42] and to induce osseointegration of implants[42b,43] and ectopic bone formation in vivo.[42a] Decreased levels of α5β1 were also associated with bone loss in an animal model of skeletal unloading.[44]

The role of the αvβ3 subtype remains controversial, with studies claiming a suppression of osteoblastic differentiation caused by this receptor,[38,42b] while others ascribe increased matrix mineralization to the binding of αvβ3.[45] In a recent study, Kilian and Mrksich[46] demonstrated that a cyclic RGD peptide with a high affinity for αvβ3 directed MSCs toward the osteoblastic lineage. They observed increased expression of several osteogenic markers, such as high ALP activity, high level of runt-related transcription factor-2 (Runx2), and greater cell spreading on surfaces coated with the cyclic peptide. Interestingly, a linear RGD peptide with a lower affinity for αvβ3 induced myogenic differentiation instead. However, few studies have focused on the osteogenic potential of αvβ3, since this receptor has been traditionally investigated for its role in bone resorption. In fact, osteoclasts are the cell type with highest in vivo expression of the αvβ3 integrin.[47] The binding of osteoclasts to the ECM is mediated by this integrin subtype, and interference with this receptor has been demonstrated to inhibit bone resorption.[47] This effect, which has been corroborated with blocking antibodies in vitro[48] and β3-lacking mice in vivo,[49] is attributed to the αvβ3-dependent migration of osteoclasts.[48] It is noteworthy that the role of αvβ3 in cell migration has been observed in many other cell types, from smooth muscle cells[50] to endothelial cells,[51] and various tumor cell lines.[9b]

Indeed, αvβ3 integrin is a critical regulator of physiological as well as pathological angiogenesis, which represents a critical step in tumor progression and metastasis.[9b,10b,52] At the very beginning of tumor progression, hypoxia can induce the so-called "angiogenic switch"[53] in dormant tumors, thus inducing secretion of growth factors, for example, vascular endothelial growth factor (VEGF), and as a consequence lead to upregulation of integrins. By interaction of αvβ3 with its natural ECM ligands, the migrating endothelial cells participate in the formation of new blood vessels, thus providing the tumor with oxygen and nutrients.[54] Since the first studies revealing that αvβ3 is involved in pathological angiogenesis,[55] many studies have shown upregulation of this subtype on tumor cells, thereby pointing towards a proangiogenic role of αvβ3. However, observations that mice lacking all αv integrins show extensive angiogenesis[56] and mice that lack β3 and β5 integrins show pathological angiogenesis and increased tumor growth[57] point to an important, but not essential, role of αvβ3 in the regulation of angiogenesis.[9a,58]

The biological function of α5β1 in angiogenesis is not fully established. Its ability to co-traffic with the epidermal growth factor receptor (EGFR)[59] as well as its upregulation during angiogenesis and on blood vessels in tumors[60] suggests a tumor-promoting role. Other reports point to a context-dependent function, with a promoting role in certain tumors and an inhibitory function in others. Recently, Hynes and co-workers have shown that αv and α5 may cooperate and even substitute each other during vascular remodeling.[61]

3. The Development of Subtype-Specific αvβ3 and α5β1 Ligands

3.1. RGD Peptides and Beyond

Seminal studies by Pierschbacher and Ruoslahti in 1984 described the tetrapeptide Arg-Gly-Asp-Ser (RGDS) as the minimal cell-binding motif in FN.[18] In these studies, synthetic peptides displaying this sequence inhibited the attachment of fibroblasts to surfaces coated with FN. Remarkably, coating agarose beads with this sequence also promoted the adhesion of fibroblasts. Further investigations on the role of each amino acid of the tetrapeptide revealed that Arg, Gly, and Asp were essential for the activity, but not Ser, which accepted a number of substitutions without loss of the biological activity.[18,62] Interestingly, the RGD motif was also found in fibrinogen and type I collagen, and short peptides derived from these proteins containing this sequence also supported cell attachment.[18] These remarkable findings suggested that cells expressed a common receptor to bind the ECM through the RGD recognition motif (i.e. integrins), and subsequent studies identified the RGD motif in many other ECM proteins, including VN,[63] von Willebrand factor,[64] osteopontin,[65] and laminin.[66]

Even if many integrins recognize ECM proteins through the RGD motif, the specificity governing this interaction is not trivial. This was illustrated by integrins α5β1 and αvβ3, which showed mutually exclusive specific interactions with the ECM. In detail, liposomes containing α5β1 were able to bind to FN-coated surfaces but not to VN-coated substrates. In contrast, the opposite behavior was observed when αvβ3 was inserted into liposomes.[26b] Nonetheless, the same RGD peptide inhibited protein binding in both cases. Nowadays, it is well-established that ligand specificity for integrins depends on multiple factors. Although there are notable examples of the influence of synergistic domains that confer integrin specificity (e.g. the PHSRN sequence, which synergizes the binding of RGD to α5β1 in FN),[28] the conformation and spatial presentation of the RGD motif within ECM proteins is one of the major determinants.[19,67]

The integration of an amino acid sequence into a cyclic peptide represents a feasible way to restrict its conformational space and increase its bioactivity and receptor selectivity.[68] Early studies with a disulfide-bridged RGD cyclopeptide showed an improved inhibition of the VN-mediated adhesion of fibroblasts, but no inhibitory activity of cell adhesion to FN, compared to the unselective linear control peptide.[69] In another study, the reduction of disulfide bonds in an RGD-containing venom peptide resulted in suppression of the inhibitory activity of the peptide, probably because of a reduced affinity for integrin through loss of its bioactive conformation.[70]

In this context, our group pioneered in the 1990s a series of studies to determine the effect of conformation on the selectivity of integrin subtypes.[20] The study of the effect of a single D-amino acid substitution in a cyclic peptide on its conformation and biological activity, a process named "spatial screening",[71] resulted in the development of the pentapeptide *c*(RGDfV) (Table 1), which showed a 100-fold increased inhibition of the adhesion of A375 cells to VN compared to the linear control peptide, as well as selectivity against the platelet integrin αIIbβ3.[20a] This peptide showed disruption of tumor-induced angiogenesis in a chick chorioallantoic membrane (CAM) model[55b] and served as the lead structure for the development of many other αvβ3-selective integrin ligands.[20c,72] As such, *c*(RGDfV) was subjected to a number of modifications such as the reduction of peptide bonds,[73] the incorporation of turn mimetics,[74] the use of sugar amino acids,[75] and the synthesis of retro-inverso analogues.[76] Although good selectivity was obtained between αvβ3 and αIIbβ3, these studies did not focus on achieving selectivity over α5β1.

N-Methylation of *c*(RGDfV) led to the drug candidate cilengitide *c*(RGDf(*N*Me)V) (Table 1),[16] which has antagonistic activity for αvβ3 in the subnanomolar range (IC_{50} = 0.58 nM) and for αvβ5 and α5β1 in the nanomolar range (IC_{50} = 11.7 nM and 13.2 nM, respectively).[77] Although peptidic compounds often show very poor enzymatic stability, cyclization and N-methylation led to high metabolic and enzymatic stability of this peptide.[78] As a consequence of its high activity against proangiogenic integrins, and also for its selectivity against integrin αIIbβ3, cilengitide has been developed as a drug candidate, and is currently undergoing clinical phase II trials for the treatment of several tumor types.[16b] Cilengitide reached a phase III trial for the treatment of glioblastomas;[16b,79] unfortunately patients treated with cilengitide and chemoradiotherapy did not live significantly longer and its use against these aggressive tumors was suspended.[80] Cross-talk between α5β1 and the tumor suppressor protein p53 was recently reported to mediate the induction of apoptosis in glioma cells. Such a biological effect has not been described for αvβ3. Thus, the reduced affinity of cilengitide towards α5β1 could explain the lack of efficacy of the peptide in the treatment of glioblastomas.[81] Cilengitide was administered intravenously twice a week or daily (2 g per patient) because of its short half-life in man (the drug is excreted after 4 h without being metabolized).[78b] Thus, its low concentration in the blood after a few hours may be enough to activate resting integrins but not to block binding and prevent signal transduction.[82] Up to now, no integrin ligand targeting αvβ3 or α5β1 has been able to get approval by the FDA.[11b]

Targeting the α5β1 subtype has been a hot topic of research as well, and for more than 20 years we and others have also focused on the development of peptides with affinity for this receptor. In this regard, cyclic peptides[72a] as well as linear peptides derived from phage display[83] have

Table 1: Structure of representative cyclic RGD peptides with affinity for αvβ3 and/or α5β1 integrins.

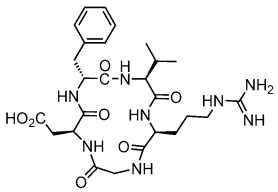
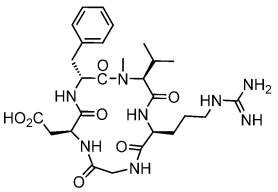
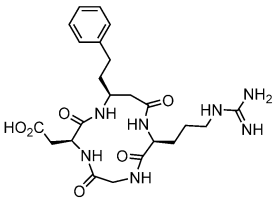
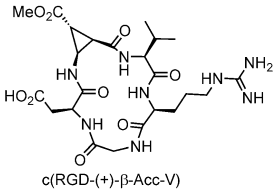
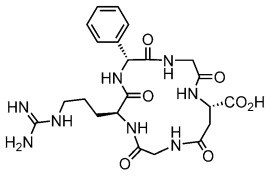
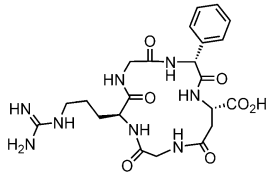
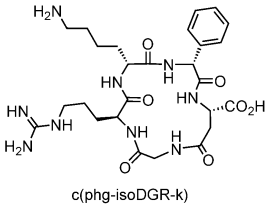
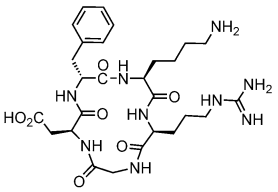
Structure	IC_{50} αvβ3 [nM]	IC_{50} α5β1 [nM]	Ref.
 c(RGDfV)	4.9[a]	480[b]	[20a, 72b]
 c(RGDf(NMe)V) (Cilengitide)	0.58[c]	13.2[c]	[16a, 77]
 c(RGD-β-HPhe)	63[d]	>1000[d]	[87, 88]
 c(RGD-(+)-β-Acc-V)	20	1500	[89]
 c(G-isoDGR-phg)	89	406	[21a]
 c(phg-isoDGR-G)	>1000	19	[21a]
 c(phg-isoDGR-k)	>1000	8.7	[21b]

Table 1: (Continued)

Structure	IC_{50} αvβ3 [nM]	IC_{50} α5β1 [nM]	Ref.
 c(RGDfK)	2.6[e]	133[e]	[21b, 72b]

[a] In the original publication (Ref. [20a]), the activity of this peptide was measured in terms of the inhibition of cell adhesion to VN and the laminin P1 fragment. The activity towards αvβ3 using isolated integrins was reported later in Ref. [72b]. [b] The activity towards α5β1 was not described in the original work (Ref. [20a]). The activity for this integrin was reported later in Ref. [72a]. [c] In the original publication (Ref. [16a]), only the activity for αvβ3 and αIIbβ3 was measured. IC_{50} values for the other integrins αvβ5 and α5β1 were reported later in Ref. [77]. [d] The activity towards αvβ3 was reported in Ref. [87]; for α5β1 in Ref. [88]. [e] In the original publication (Ref. [72b]), the activity of the peptide for αvβ3 was reported as the ratio $Q = IC_{50}[peptide]/IC_{50}[GRGDSPK]$. IC_{50} values for both αvβ3 and α5β1 have been published recently in Ref. [21b]. A comprehensive evaluation of the activity and selectivity profile of the peptides *c*(RGDfV), *c*(phg-*iso*DGR-k), and *c*(RGDfK) has recently been performed for RGD-binding integrins (T. G. Kapp, F. Rechenmacher, S. Neubauer, O. V. Maltsev, C. Mas-Moruno, A. E. Cavalcanti-Adam, J. Spatz, B. Geiger, H. Kessler, unpublished results).

been reported to be active for α5β1, but with no remarkable selectivity over the αvβ3 subtype. A few years later, a non-RGD linear peptide derived from FN that targets the synergistic domain of α5β1 was discovered, and its acetylated analogue, Ac-PHSCN-NH_2, later dubbed ATN-161, showed anti-invasive, anti-tumorigenic, and anti-metastatic activities in prostate cancer cell lines.[84] ATN-161 is currently undergoing clinical phase II trials for the treatment of cancer, however, activity for αvβ3[85] as well as for αvβ5[11b] is also reported.

The group of Sewald also synthesized several cyclic RGD peptides containing β-amino acids to investigate their influence on the peptide secondary structure.[86] In this way, the cyclic tetrapeptide *c*(RGD-β-HPhe) was identified as an αvβ3-active ligand (63 nM in an isolated integrin assay)[87] with very low affinity for α5β1 (>1000 μM in a cellular adhesion assay with K562 cells; Table 1).[88] In these studies, other highly active peptides were also reported, but with no remarkable selectivity between αvβ3 and α5β1. Later, the same group reported the synthesis, structural analysis, and biological evaluation of pentapeptides containing the constrained *cis*-β-aminocyclopropanecarboxylic acid (β-Acc).[89] The cyclic pentapeptide *c*(RGD-(+)-β-Acc-V) (Table 1) exhibited a very high activity for αvβ3 (20 nM) in a cellular adhesion assay of WM115 cells to FN and good selectivity over α5β1 (1.5 μM, 75-fold) from K562 cells on FN. In 2008, Pramanik et al. reported that a lipopeptide with the tetrapeptide sequence RGDK could selectively target genes to the α5β1 integrin receptor in vitro.[90]

These studies illustrate that the development of peptides with selectivity between integrins αvβ3 and α5β1 has been limited. In this regard, cyclic peptides containing the *iso*DGR sequence represent one of the few reported examples of peptides capable of achieving high binding affinities and

outstanding selectivity between these two receptors. The *iso*DGR motif, which results from the deamidation of asparagine at the fifth type repeat module I of FN, was identified by the Corti research group as an unexpected integrin binding motif in that protein.[91] Based on these findings, we designed head-to-tail cyclic peptides containing the *iso*DGR motif, and the spatial screening procedure was applied.[21a] In these peptides, the *iso*DGR sequence was flanked by one Gly and one aromatic amino acid (reported as crucial for binding with αvβ3).[72b] The aromatic residue was introduced in either the L- or D-configuration, thereby enabling the adoption of different peptide conformations. Interestingly, the relative position of the flanking residues determined the binding affinity towards αvβ3 or α5β1. This was illustrated by the *c*(phg-*iso*DGR-G) peptide, which exhibited an affinity for α5β1 in the nanomolar range ($IC_{50} = 19$ nM) but was inactive for αvβ3. In contrast, shifting the position of the flanking residues in *c*(G-*iso*DGR-phg) yielded the opposite biological behavior (Table 1). Such selectivity was corroborated with docking studies and cellular tests using α5β1- and αvβ3-expressing fibroblasts.[21a]

Follow-up studies based on the α5β1-selective peptide *c*(phg-*iso*DGR-G) were carried out after substituting Gly by other L- and D-amino acids.[21b] From the designed library, the best compound was *c*(phg-*iso*DGR-w), which displayed an increased affinity for α5β1 ($IC_{50} = 5.5$ nM) while maintaining selectivity over αvβ3. Interestingly, this peptide also exhibited a moderate affinity for the αvβ6 subtype ($IC_{50} = 92$ nM). The introduction of D-Lys instead of D-Trp in *c*(phg-*iso*DGR-k) further increased the activity for αvβ6 ($IC_{50} = 19$ nM), while retaining an excellent activity for α5β1 and selectivity over αvβ3 (Table 1). This peptide was functionalized with a thiol and anchored to a nanopatterned gold surface to study the adhesion and behavior of REF52 cells.[21b]

The recent determination of the X-ray structure of the head groups of α5β1 has shed further light onto the different binding modes of RGD ligands to αv and α5 subunits.[24] In particular, it was shown that the guanidine group of Arg binds to the αv subunit of αvβ3 only through side-on interactions with Asp218. In contrast, the binding of Arg to α5 in α5β1 is established through side-on and end-on interactions with Asp218 and Gln221, respectively. This has allowed the selectivity between the two receptors to be shifted through different N-methylation patterns on the guanidinium group.[92] For example, methylation at N_ω abrogated end-on interactions and totally prevented binding to α5, which in turn increased the selectivity for αvβ3 and αvβ6 receptors.

3.2. RGD-Based Peptidomimetics

The development of cyclic RGD peptides has been accompanied by the design and synthesis of totally non-peptidic antagonists that were aimed at improving the activity and selectivity profiles obtained by peptidic ligands.[20c,93] The first crystal structure of the extracellular segment of the αvβ3 integrin in 2001 was undoubtedly a major breakthrough in the design of selective integrin ligands.[22a] One year later, the crystal structure of the extracellular segment of integrin αvβ3 complexed with cilengitide was also elucidated.[22b] This study gave important insight into the binding modes of integrin ligands and served as a basis for docking studies of drug candidate molecules. As the crystal structure of α5β1 was unknown, in 2005 we published a three-dimensional model of this subtype based on homology modeling of the experimental three-dimensional structure of αvβ3 in its bound conformation.[23] Since the binding pockets of αvβ3 and α5β1 have strong similarities (αv:α5 53% identity; β3:β1 55% identity in the integrin's head group),[23] this model, together with the previously published crystal structures, paved the way for the rational design of selective ligands. In this section, we will only focus on the development of peptidomimetic ligands that have the capacity to differentiate between these two closely related subtypes. Integrin ligands which have been tested only for one of these subtypes as well as biselective integrin ligands, which are active for both αvβ3 and α5β1, are beyond the scope of this Review and have been described elsewhere.[94]

Apart from some peptidic ligands with low micromolar activities for α5β1 and isoDGR peptides, the first highly active α5β1 ligand was the small nonpeptidic molecule SJ749 (**M1**, Table 2). This molecule was developed by conformational restriction of an αvβ3 antagonist. It contains a spiro-oxazoline scaffold and exhibits an excellent activity for α5β1 ($IC_{50} = 0.18$ nM) and a selectivity of at least 200-fold over αvβ3.[95] Studies on the docking of **M1** into the α5β1 binding pocket revealed key specific interactions with the receptor, which were responsible for its high activity (Figure 2A).[23] **M1** was able to show inhibition of angiogenesis by affecting the adhesion and migration of endothelial cells,[96] inhibited the proliferation of tumor cells,[97] and facilitated cell apoptosis in a functional p53 background in the human glioblastoma cell line U87MG.[98]

The first rationally designed selective peptidomimetics were developed simultaneously by us[99] and Jerini AG.[100] The design of these compounds was based on previous docking studies into the crystal structure of αvβ3[101] and on the homology model of α5β1 in complex with **M1** (Figure 2A).[23] Useful insight for the design of antagonists was derived by comparing the two binding pockets. In particular, two regions seemed to be especially suitable for achieving selectivity between αvβ3 and α5β1: In the β-subunit, (β3)-Arg214 and (β3)-Arg216 are replaced by (β1)-Gly217 and (β1)-Leu219, respectively. The substitution of both Arg moieties by smaller residues expands the available space at this site of the α5β1 binding pocket, which, in comparison to the αvβ3 integrin, allows the introduction of bulky moieties into the core structure of the ligands. Secondly, the α5 subunit turned out to be less acidic as a result of the mutation of (αv)-Asp150 to (α5)-Ala159. Furthermore, the replacement of (αv)-Thr212 by (α5)-Gln221 results in a different geometry of this binding region, which offers the opportunity to gain selectivity by modification of the basic moieties.[99] As mentioned above, the modulation of selectivity between these two receptors by N-alkylation of the guanidinium group of Arg has recently been achieved.[24,92]

On the basis of these observations, we synthesized a series of peptidomimetics derived from a tyrosine scaffold, which

Table 2: Structure of representative RGD-based peptidomimetics with affinity for αvβ3 and/or α5β1 integrins.

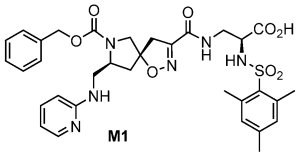
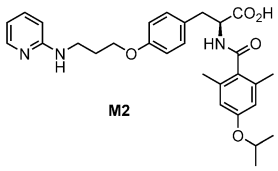
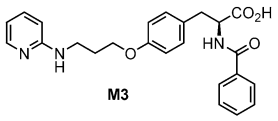
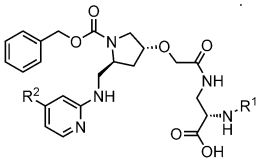
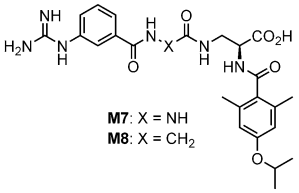
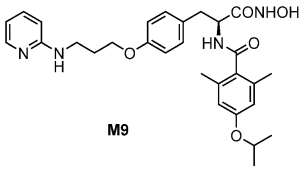
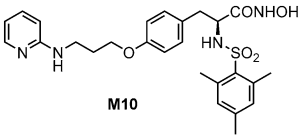
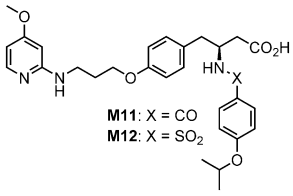
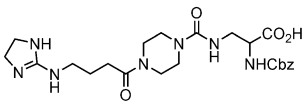
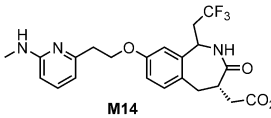
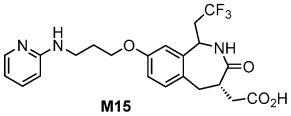
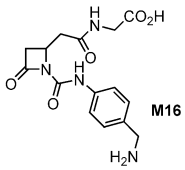
Compound	IC_{50} αvβ3 [nM]	IC_{50} α5β1 [nM]	Ref.
 **M1**	49	0.18	[95]
 **M2**	279	0.7	[99]
 **M3**	1.2	264	[99]
 **M4**: R^1 = SO_2-mes; R^2 = H **M5**: R^1 = CO-mes; R^2 = H **M6**: R^1 = CO-mes; R^2 = OMe	16 ca. 30000 3400	3.7 (**M4**) 3.5 (**M5**) 0.54 (**M6**)	[100]
 **M7**: X = NH **M8**: X = CH_2	>4750 9600	0.96 (**M7**) 0.86 (**M8**)	[107]
 **M9**	13.5	40	[109]
 **M10**	4.8	132	[109]
 **M11**: X = CO **M12**: X = SO_2	0.86 0.65	127 (**M11**) 108 (**M12**)	[110]
 **M13**	1.1	660	[111]
 **M14**	1.2[a]	110[a]	[112]

Table 2: (Continued)

Compound	IC_{50} αvβ3 [nM]	IC_{50} α5β1 [nM]	Ref.
 **M15**	0.9[a]	1000[a]	[112]
 **M16**	11[b]	763[b]	[113]

[a] Integrin binding activities in Ref. [112] are given as K_i values.
[b] Integrin binding activities in Ref. [113] are expressed as EC_{50} values using cells expressing either αvβ3 (SK-MEL-24) or α5β1 (K562).

had already been employed successfully in other integrin ligands.[102] The most potent α5β1-targeting ligand (**M2**, Table 2) was active for α5β1 in the subnanomolar range (IC_{50} = 0.7 nM) and displayed good selectivity over the αvβ3 integrin.[99] Docking studies revealed an optimal fitting of this compound into the α5β1 binding pocket (Figure 2B). Conversely, the removal of the two methyl groups from the aromatic moiety allowed it to fit into the αvβ3 binding pocket and led to the highly active αvβ3 integrin ligand (**M3**, Table 2; IC_{50} = 1.2 nM) with remarkable selectivity over α5β1.[99]

Stragies et al. from Jerini AG reported the development of highly active compounds derived from a virtual combinatorial library.[100] Since the starting compound (**M4**) of these series was active against α5β1 (IC_{50} = 3.7 nM), but showed little selectivity over αvβ3 (IC_{50} = 16 nM), variation of the hydrophobic side chain on R^1 (Table 2) was investigated. In this regard, replacement of the sulfonamide with an amide led to a remarkable drop in αvβ3-binding activity (IC_{50} ≈ 30000 nM), while completely maintaining the activity for α5β1 (**M5**, JSM6427). The fact that integrin α5β1 has a bulkier binding pocket seems to explain its capacity to accommodate both ligands, whereas the more sterically restricted region in αvβ3 only allows the binding of the conformation where the substituents on the sulfonamide group are twisted 90° about the SO_2–N bond relative to the planar amide.[99,100]

M5 inhibited choroidal neovascularization in a dose-dependent manner in monkey and rabbit models[103] and was investigated for the treatment of age-related macular degeneration (AMD) in clinical phase I studies. However, its therapeutic use appears to have been discontinued.[11b] The introduction of a 4-methoxy group on the 2-aminopyridine ring led to one compound (**M6**, Table 2) with even higher activities for α5β1 and still good selectivity over αvβ3. The very high α5β1-binding affinity of this compound was confirmed in a cellular adhesion assay with HEK293 cells.[100] Further investigations based on substituted 2-aminopyridine units linked to five- or six-membered heterocyclic ring systems, and a phenylalanine moiety yielded compounds with good selectivity for α5β1.[104]

Based on the observation that glycine can be replaced by azaglycine in RGD-containing linear peptides with preserva-

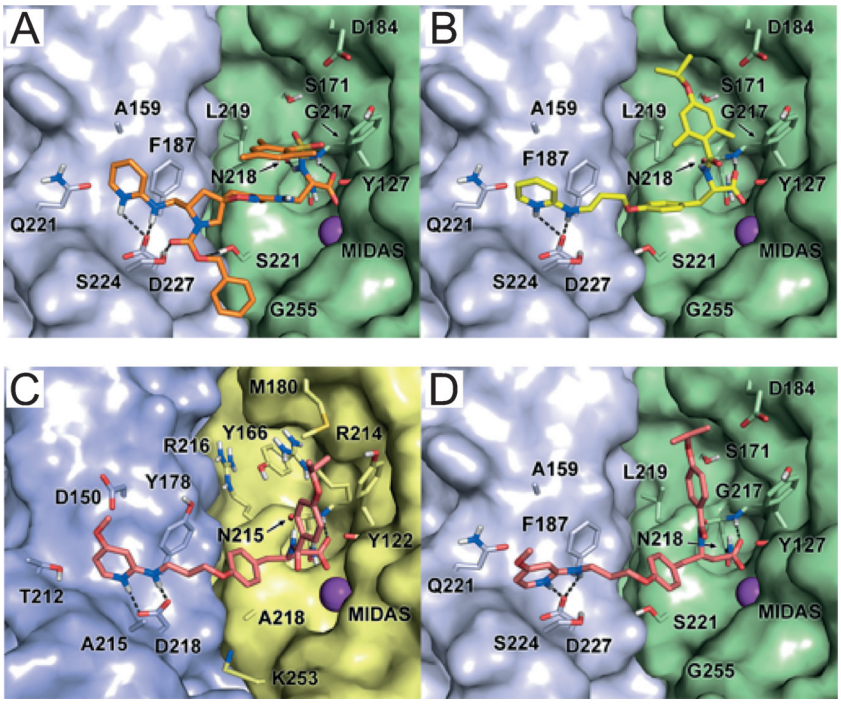


Figure 2. Binding modes in the integrin-binding pocket of exemplary peptidomimetics. Docking of A) SJ749 (**M1**; orange stick model) and B) **M2** (yellow stick model) into the α5β1 binding pocket. Both compounds show an optimal fit in the receptor. Binding modes of **M11** (pink sticks) into the αvβ3 (C) and α5β1 (D) binding pockets. The preferred fit of αvβ3 leads to a higher selectivity. The α5 and β1 subunits are represented as light blue and green surfaces, respectively, and the αv and β3 subunits are represented as blue and yellow surfaces, respectively. Amino acid side chains of the receptor that are important for the ligand binding are shown as sticks. The metal cation at the MIDAS is depicted as a magenta sphere. (Produced by F. Di Leva and L. Marinelli, Università degli Studi di Napoli Federico II, Italy.)

tion of biological activity and selectivity,[105] and its successful incorporation into αvβ3-active peptidomimetics,[106] this approach was also applied to develop α5β1 ligands. This strategy yielded compounds with very high affinity for α5β1 ($IC_{50} < 1$ nM) and outstanding selectivity over αvβ3 compared to the tyrosine scaffold (e.g. **M7**, Table 2).[107] The optimal selectivity profiles arise from the rigidity of the diacylhydrazone scaffold compared to the rather flexible tyrosine. Additionally, arylguanidyl and alkylguanidyl groups were used as basic moieties instead of the previously used 2-aminopyridine.[99] Nonetheless, we were able to show that only the C-terminal moiety of the molecule is responsible for selectivity. The substitution of azaglycine in **M7** by glycine (**M8**) had little effect on the biological activity and led to a highly active ($IC_{50} = 0.86$ nM) α5β1 ligand with remarkable selectivity over αvβ3 ($IC_{50} = 9600$ nM; Table 2).[107] As a consequence of this excellent integrin-subtype selectivity and its straightforward synthesis, this ligand was later used for a number of biological investigations and functionalized for different purposes.[32,81a,108] One year later, we reported for the first time the successful replacement of the carboxylic acid by an isosteric group, by using hydroxamic acids (**M9** and **M10**, Table 2).[109] Even though no superactive ligands were obtained, we were able to develop highly active αvβ3 ligands (IC_{50} values up to 5 nM) with good selectivity over α5β1 (1–2 orders of magnitude) and provided deep insight into the binding modes of these integrin antagonists. Compounds **M2** and **M9** only differ in the nature of the acid coordinating the metal at the metal-ion-dependent adhesion site (MIDAS), but show opposing selectivity for the two integrin subtypes (Table 2). The ligand containing the carboxylic acid is a highly active α5β1 ligand, whereas the selectivity in the case of the compound with the hydroxamic acid ligand is shifted to αvβ3 with strongly reduced α5β1 activity. Docking models revealed that the reason for the difference is the increased distance between the acidic and basic groups, which favors αvβ3 affinity.[109] This principle could also be proven for other analogues based on the same scaffold, such as **M10** (Table 2), which showed higher affinity for αvβ3 and improved selectivity over α5β1.

There was a high demand for αvβ3-specific ligands to investigate the precise roles of the two integrin receptors α5β1 and αvβ3 in biological processes. Extensive research was thus stimulated to develop novel selective ligands, as cyclic RGD peptides, in general, did not show a satisfactory selectivity over α5β1. Based on the three-dimensional structure of the αvβ3 binding pocket and the pharmacophoric requirements of already published peptidomimetics, we were able to develop novel αvβ3-subtype-specific compounds. The backbone of these RGD ligands is presented as β-homotyrosine, which was shown to be essential for selectivity against α5β1 and αIIbβ3.[99] As a result of the steric and electrostatic demands of the amino acid residues presented in the binding region of the α-subunits, a 4-methoxypyridine residue was incorporated into the backbone as a basic moiety and mimic for Arg (**M11** and **M12**, Table 2, Figure 2C,D).[110]

Previous studies revealed an enhanced affinity profile towards αvβ3 on introducing the aromatic moiety through a sulfonamide group instead of a carboxamide bond.[100] This effect was based on the relative structural orientation of these chemical groups and differential fitting in the integrin-binding pocket. However, both **M11** and **M12** showed subnanomolar activities for αvβ3 (**M11**, $IC_{50} = 0.86$ nM; **M12**, $IC_{50} = 0.65$ nM) with almost no difference in the affinity profile because of an optimized fitting of the ligands into the αvβ3 integrin-binding pocket (Figure 2C).[110] These compounds or their functionalized derivatives were potent enough to be used for in vitro and in vivo applications.[32,81a,108] Furthermore, the ligands showed dose-dependent anti-angiogenetic effects on spontaneous, basic fibroblast growth factor (bFGF) and VEGF induced capillary sprouting in a rat aorta ring system, and also induced an antitumor effect in mice bearing WEHI-164 fibrosarcomas.[108b]

Other research groups have also described ligands with αvβ3/α5β1 selectivity. The group of DeGrado, for example,

reported the design and synthesis of a library of αvβ3-selective antagonists based on a diaminopropionic acid scaffold.[111] The most active compound from these series, **M13**, showed high αvβ3 affinity ($IC_{50} = 1.1$ nM) and high selectivity over α5β1 ($IC_{50} = 660$ nM; Table 2). Two highly active and selective αvβ3 inhibitors have also been reported by SmithKline.[112] The compounds are derivatives of benzodiazepine and showed excellent pharmacokinetic profiles in rats. Biological evaluation in an isolated receptor assay showed αvβ3 affinities (IC_{50} values) of 1.2 and 0.9 nM for **M14** and **M15**, respectively (Table 2). Their high potential was confirmed in a cell-adhesion assay of αvβ3-expressing HEK cells. The α5β1 activity was reported to be $IC_{50} = 110$ nM and 1000 nM, respectively. Very recently, Galletti et al. reported the development of α5β1/αvβ3-active peptidomimetics based on a β-lactam scaffold. The most active and selective compound **M16** exhibited $EC_{50} = 11$ nM in an assay using αvβ3-expressing SK-MEL-24 cells. In contrast, when α5β1-expressing K562 cells were used, the EC_{50} value dropped to 763 nM, thus making this compound a relatively selective αvβ3 inhibitor.[113]

4. Coating of Surfaces with αvβ3- or α5β1-Selective Ligands

4.1. General Considerations for Surface Coating

Immobilizing bioactive molecules onto a biomaterial substrate represents a critical step in the process of surface biofunctionalization that needs to be carefully designed. Even ligands with a high affinity for integrins may fail to support cell adhesion if their binding to the surface and/or presentation and accessibility to integrin receptors are not optimal. The following considerations should be taken into account:

1) Method of immobilization: Integrin ligands can be coated on the surface of materials by simple physical adsorption.[25b,114] This method, also known as physisorption, relies on the establishment of noncovalent interactions (e.g. electrostatic interactions, van der Waal forces, and hydrogen bonds) between the ligand and the substrate. Although this procedure is commonly used to immobilize proteins and large molecules, it is based on weak interactions and does not ensure a stable binding of small molecules. Moreover, the adsorption of the molecules takes place in a nonspecific manner, which may affect their conformation or hinder motifs required for the activity. For these reasons, a chemical anchoring to the biomaterial is preferable.[25b,114] Covalent immobilization offers much higher stability, which is important for clinical applications. Moreover, coating molecules can be functionalized with anchoring groups, which may provide chemoselective binding to the surface without affecting the pharmacophoric properties of the molecule.
2) Anchoring unit: The anchor moiety should allow a strong binding to the surface. For this purpose, the surface can be modified (e.g. by silanization) to expose a wide range of functional groups that can be used to anchor the integrin ligands in a highly chemoselective manner. Moreover, the chemistry of the surface can be exploited to select substrate-specific anchors. For example, thiols bind to gold and phosphonates to titanium oxide (and other metal oxides) with high affinity. This topic has been covered in great detail in the literature.[25a,115]
3) Spacer units: Though often underestimated, the use of a chemical spacer is a crucial element in a the coating system. In particular, the importance in keeping a minimum distance (i.e. > 3.5 nm) between the RGD motif and the surface has been highlighted to engage integrin-mediated adhesion.[116] This distance can be achieved by using chemical spacers, which ensure a correct accessibility of the peptide and an adequate interaction with integrin receptors.[116,117] Chemical groups typically used as spacer units include polyglycine, aminohexanoic acid, and polyethylene glycol (PEG). Choosing the right spacer might be a difficult task, since not only an optimal length but also other physicochemical properties need to be carefully considered. For example, the hydrophilic or hydrophobic nature of the spacers and their conformation in solution also play important roles. In a recent study we showed that polyproline helices can also be used as spacers.[118] Polyproline chains prefer an extended conformation in solution, whereas PEG chains adopt coiled conformations, which make it difficult to assess the exact distance between the biomaterial and the integrin-binding peptide.

4.2. Coating with RGD Peptides and Proteins

The discovery of the RGD motif as a cell-recognition sequence was accompanied by a series of early studies in which synthetic peptides containing this sequence were used to promote cell attachment on different surfaces.[18] Since then, the RGD motif has been widely used to coat the surface of biomaterials with the aim of improving their bioactivity and conferring cell-instructing properties. Hence, RGD-biofunctionalized materials have been investigated for a myriad of biomedical purposes, including bone, neural, and cardiovascular applications.[25a,119] In addition to RGD peptides, many other cell-binding motifs (integrin-dependent or not) have been described over the last few years. These findings have notoriously increased the molecular tools available for surface functionalization and expanded the initial potential of RGD peptides. For a comprehensive review of the state of the art in this field, the reader is referred to the current literature.[25b,115,120]

Despite the versatility of RGD-based synthetic peptides, their use in biomaterials has found three major limitations: 1) Peptides often display a lower potential for cell adhesion than full-length ECM proteins, mainly because they lack synergistic or complimentary domains present in native proteins and required for optimal cell signaling. 2) Linear peptides possess high conformational freedom and, therefore, fail to exhibit receptor selectivity. 3) Linear peptides and large cyclic peptides are susceptible to enzymatic cleavage and are consequently easily degraded in vivo. As a result of these drawbacks, the translation of promising in vitro data to

successful in vivo outcomes has not been possible.[25b,120a] Alternatively, the use of ECM proteins appears to be an intuitive way to mimic the complexity of cell–matrix interactions. However, their use remains controversial and several disadvantages have also been reported, including unwanted inflammatory responses, risk of infections, short biological half-life, and rapid clearance.[25b,120a] These shortcomings are a matter of extensive debate in the field and illustrates the necessity of finding new strategies for surface functionalization.[121] Moreover, none of these classical strategies has achieved integrin-subtype selectivity. In the following, three representative approaches to improve the activity and selectivity of ECM-based molecules for surface coating are presented.

4.2.1. Coating with Cyclic RGD Peptides

As described above, the limited biological profiles of linear RGD peptides have been significantly improved by the use of cyclic counterparts.[68] Moreover, small cyclic peptides are much more stable against enzymatic cleavage, especially when they contain D-amino acids and/or N-methylated amide bonds. Structure–activity relationship studies of the αvβ3-binding *c*(RGDfV) (Table 1) stem peptide revealed that the amino acid at the 5th position (i.e. Val) was not essential for its integrin-binding activity.[72b] Such a finding was of great value for applications in surface coating. For example, replacement of valine by lysine in *c*(RGDfK) leads to retention of the integrin-binding activity of the peptide, but provides a new functional group that can be further functionalized (Table 1).[72b] This has allowed the production of cyclic peptides containing different spacer–anchor systems for coating a variety of surfaces, thereby resulting in substrates with high αvβ3-binding activity. Given the importance of this integrin in bone biology, this peptide has been widely used to coat implant materials, with improved levels of osteoblast adhesion in vitro[116,117a,122] and bone formation in vivo.[116b,123] As previously mentioned, a cyclic RGD has also recently been described to promote osteogenic differentiation on MSCs.[46] However, since cyclic RGD peptides also display some affinity for α5β1 the aforementioned biological effects can not be ascribed univocally to the αvβ3 subtype.

4.2.2. Coating with Engineered Protein Fragments

The production of protein fragments of the ECM by recombinant methods has also been exploited to achieve integrin selectivity, but mainly towards α5β1. The Garcia research group engineered a recombinant fragment of FN spanning the 7th to the 10th type III repeats of the protein (FN-$III_{7–10}$). This fragment, which contains the RGD sequence and the synergy motif PHSRN, directed the α5β1-dependent adhesion of osteoblast-like cells, their spreading, and assembly of FAs on functionalized surfaces.[35a] In a subsequent study, this fragment also showed enhanced values of osteoblast adhesion compared with a linear RGD peptide and the oligopeptide RGD-G_{13}-PHSRN. Interestingly, whereas cell adhesion on surfaces functionalized with FN-$III_{7–10}$ was shown to be mediated by α5β1, the binding of cells on the surfaces coated with the peptides was αvβ3-dependent.[35b] The lack of α5β1-binding activity for the RGD-G_{13}-PHSRN construct could be explained by the great flexibility of the polyglycine spacer, which may not match the optimal distance between the RGD and PHSRN motifs adopted in the context of the protein fragment and required for integrin binding.[124] The immobilization of FN-$III_{7–10}$ on titanium surfaces also promoted differentiation of bone marrow stromal cells into osteoblasts and improved implant osseointegration in vivo compared to surfaces coated with linear RGD peptides[42b] or full-length FN.[43a] In a recent study, this fragment also improved osseointegration of stainless-steel screws in healthy and osteoporotic rats.[43b] Other authors have also shown an enhanced osteogenic differentiation of human MSCs on surfaces functionalized with a recombinant fragment derived from the 9th and 10th type III domains (FN-$III_{9–10}$), compared to surfaces modified with the 10th type III domain of FN (FN-III_{10}), which does not contain the synergy binding site for α5β1.[37] It is noteworthy that the extent of osteoblastic differentiation for each fragment correlated with their selectivity towards α5β1. As previously discussed, these studies highlight a crucial role of α5β1 in cell adhesion, proliferation, and differentiation, compared to a somewhat more modest role for αvβ3 in these processes. However, these studies used linear RGD peptides as a comparison, which show low affinity for αvβ3. The positive biological outcomes obtained with highly αvβ3-active cyclic RGD peptides suggest a more important function for this integrin. Comprehensive studies in this regard are lacking.

4.2.3. Coating with Multiple Peptide Motifs

The combination of distinct peptide motifs to exert integrin-selective effects is another interesting strategy. It takes advantage of the favorable properties of synthetic peptides compared to proteins, while improving their activity and specificity. In this regard, we have recently introduced a novel peptide-based platform with the capacity to simultaneously present two distinct bioactive sequences on the surface of biomaterials.[125] In a proof of concept study, the combination of the RGD and PHSRN in this platform supported a very homogeneous spreading of osteoblasts over the entire surface. In contrast, the spreading of cells on surfaces coated with a mixture of the two motifs was not homogeneous, probably because of the random orientation and spacing of the RGD and PHSRN sequences, which did not match the spatial conformation required for binding to α5β1.[124]

4.3. Coating with RGD-Based Peptidomimetics

The previous examples illustrate the extensive effort devoted to install integrin-selective activity on the surface of biomaterials. However, the majority of approaches in the literature are focused on RGD-containing peptides and proteins with relatively poor integrin-receptor selectivity. This has hampered the dissection of the roles of integrins in

cell behavior and has also resulted in frustrating preclinical outcomes. It is thus surprising that integrin-selective peptidomimetics are rarely applied as surface-coating molecules: in addition to their capacity to exhibit excellent integrin-binding activity and subtype-selectivity profiles, nonpeptidic ligands are devoid of the intrinsic pharmacokinetic limitations of peptides and proteins.

Probably one of the first examples reporting the use of an RGD peptidomimetic for surface coating was described by Marchand-Brynaert and co-workers at the end of the last century.[126] In these studies, an RGD peptidomimetic based on a tyrosine scaffold (**C1**, Table 3) grafted on a poly(ethylene terephthalate) (PET) membrane supported the adhesion of adenocarcinoma epithelial cells (Caco-2) to similar levels as PET surfaces grafted with an RGDS peptide, but lower than surfaces coated with FN. Unfortunately, the activity of this compound was only evaluated for αIIbβ3 and thus the affinity for other subtypes is unknown. Although the authors showed that this compound could adopt a conformation similar to that of *c*(RGDfV), thus indicating a potential activity for αvβ3,[126a] the fact that it failed to inhibit the binding of αvβ3-expressing cells to VN[126b] suggested a low affinity for this receptor. Furthermore, the flexibility of the compound could be associated with poor receptor selectivity. Nonetheless, this work demonstrated for the first time that the cell-binding properties of synthetic peptidomimetics could be recapitulated on the surface of biologically relevant materials. To increase the affinity for αvβ3, the guanidine function was rigidified with an isonipecotic group and the α-amino substituent replaced by a bulkier hydrophobic moiety (**C2** and **C3**, Table 3). Such modifications resulted in affinities for this integrin in the nanomolar range; however, selectivity against αIIbβ3 could not be attained.[127] In accordance with the improvement in αvβ3 activity, these compounds efficiently improved the adhesion of Caco-2 cells on PET surfaces, and inhibited integrin-mediated binding of these cells onto VN-coated materials. It is noteworthy that the cell adhesion capacity was higher than to an RGDS control peptide.[128] In follow-up studies, exchanging the relative positions of the Arg surrogate and the spacer yielded highly active αvβ3 antagonists, which showed selectivity against αIIbβ3 (**C4**, Table 3).[129] Grafting of these compounds on PET surfaces improved the adhesion of human endothelial cells.[129,130]

In another example, an Arg-Lys dipeptide, in which the C-terminus of Arg was bound to the ε-amino group of Lys, was produced as an RGD mimic (**C5**, Table 3).[131] This compound was very stable to enzymatic degradation compared to the linear RGD peptide. When immobilized on dextran-coated surfaces it promoted the extensive adhesion and spreading of BALB/c-3T3 cells in comparable amounts as substrates grafted with GRGDSP. However, on the basis of the flexibility of this construct, modest integrin affinity and lack of receptor specificity are expected.

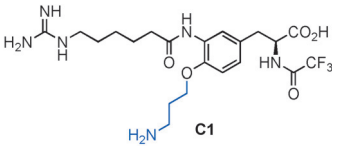
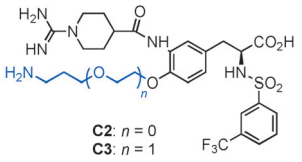
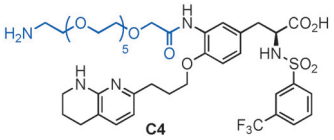
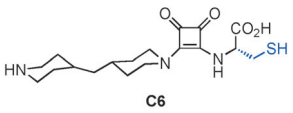
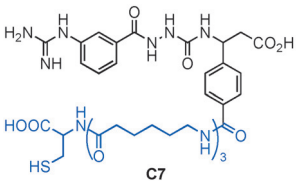
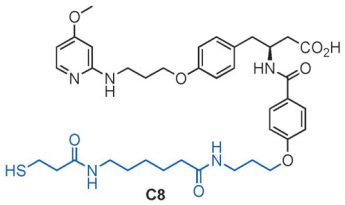
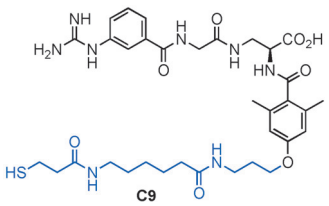
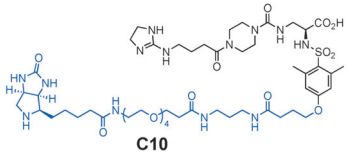
Squaramide-based RGD mimics have also been described. The immobilization of **C6** on self-assembled monolayers on gold substrates mediated stronger rates of cell adhesion, more mature stress fibers, and higher numbers of FAs compared to a linear RGD control. These biological effects were attributed to the increased rigidity of the squaramide moiety, which in turn would increase the affinity of the ligand for αvβ3. However, the authors did not check the integrin-binding affinity of their ligands.[132]

In parallel to these studies, we reported in 2004 the first example of a highly αvβ3-binding nonpeptidic ligand that was selective against αIIbβ3 for surface coating (**C7**, Table 3).[133] This compound exhibited stimulated osteoblast adhesion on titanium to similar levels as the cyclic *c*(RGDfK) peptide. This was also the first report to describe the functionalization of a metallic implant material with a nonpeptidic ligand. In this study, the importance of a suitable spacer was again illustrated, since the use of shorter linkers significantly reduced the adhesion of osteoblasts to the surfaces. Likewise, the position chosen to functionalize the ligand was found to be crucial to retain the biological activity. At the time of this study there were still no reported α5β1-selective ligands and the affinity for this integrin was not studied.

The first example of surface functionalization with nonpeptidic ligands capable of discriminating between αvβ3 and α5β1 was also reported by us a decade later,[108a] and was the result of our extensive work on the development of integrin-selective peptidomimetics (see Section 3). In this study, αvβ3- (**C8**) or α5β1-specific (**C9**) ligands (Table 3; derived from the stem compounds **M11** and **M8**, respectively, Table 2) were immobilized on nanostructured gold surfaces and their capacity to mediate integrin-dependent cell adhesion was analyzed with genetically modified fibroblasts expressing either αvβ3 or α5β1. It was found that αvβ3-expressing fibroblasts adhered exclusively to surfaces coated with **C8**. In contrast, surfaces functionalized with compound **C9** only supported the adhesion and spreading of fibroblasts expressing α5β1 (Figures 3 and 4A), thereby providing striking evidence of the integrin selectivity displayed by the surfaces. In this study, integrin-mediated cell adhesion could be triggered through one specific integrin, thus opening up new prospects to elucidate the role of these two subtypes in cell adhesion and other biological processes (Figure 4).

In a subsequent study, these compounds were bound to PEG-based micropillars, covered at the top with gold nanoparticles, to investigate the contribution of αvβ3 or α5β1 integrins to cell traction forces (Figure 4B).[32] Force measurements after seeding rat embryonic fibroblasts on these pillars revealed that cells binding to the pillars through α5β1 exerted higher forces on the pillars than cells binding through αvβ3, in good correlation with previous findings.[31] Integrin-mediated cell adhesion could also be achieved on titanium surfaces coated with the same compounds but functionalized with phosphonates as anchor molecules.[117b] To this end, click chemistry was used to enable the modification of a large variety of surfaces in a straightforward manner. The possibility of using different anchors to coat surfaces of distinct chemistry has recently been exploited to construct binary nano- and micropatterned arrays in an orthogonal fashion (Figure 4C).[134] For example, surfaces containing alternating stripes of gold and metal oxide (e.g. Fe_2O_3 or TiO_2) were functionalized with the αvβ3- and α5β1-selective mimetics, respectively.[134a] To achieve an orthogonal attachment, the αvβ3 ligand contained a thiol group, while the α5β1 ligand

Table 3: Structure of representative functionalized RGD-based peptidomimetics with affinity for αvβ3 and/or α5β1 integrins.

Compound[a]	IC_{50} [nM]	Surface	Coating chemistry	Biological results	Ref.
 **C1**	αvβ3: n.r. αIIbβ3: 320000	poly(ethylene terephthalate) (PET)	i) oxidation of PET hydroxy groups to carboxylic acids ii) amide bond formation by carbodiimide chemistry	increased values of surface occupancy by Caco-2 cells ≈RGD < FN	[126]
 **C2**: $n = 0$ **C3**: $n = 1$	C2; αvβ3: 63 αIIbβ3: 11 C3; αvβ3: 765 αIIbβ3: 5	PET	i) tosylation of PET hydroxy groups ii) nucleophilic substitution	increased values of surface occupancy by Caco-2 cells > RGD < VN	[127,128]
 **C4**	αvβ3: 0.7 αIIbβ3: 51	PET	i) activation of PET hydroxy and carboxy groups with trifluorotriazine ii) aromatic nucleophilic substitution	improved adhesion of human endothelial cells > RGD	[129,130]
 **C5**	αvβ3: n.m. αIIbβ3: n.m.	dextran-coated TCPS	i) oxidation of dextran hydroxy groups to aldehydes ii) nucleophilic addition iii) Schiff base reduction	enhanced adhesion and spreading of BALB/c-3T3 ≈RGD	[131]
 **C6**	αvβ3: n.m. αIIbβ3: n.m.	SAMs of PEGylated alkanethiols on gold	thiol addition to squaramate reactive moiety	faster and stronger cell attachment, mature stress fibers, and more focal adhesions > RGD ≈c(RGDfK)	[132]
 **C7**	αvβ3: 0.72 αIIbβ3: 3150	Ti6Al4V	thiol binding to metal oxide	enhanced adhesion of MC3T3 mouse osteoblasts	[133]
 **C8**	αvβ3: 1.8 α5β1: 130	a) gold b) Ti6Al4V /metal oxide c) TiO_2	a) thiol binding to gold b) the thiol group is replaced by a phosphonate anchor c) binding through Michael addition to a maleimide-containing silane layer	multiple effects (see Figure 4 for details)	[32,108a, 117b,137]
 **C9**	αvβ3: 229 α5β1: 1.5	as above, but with **C8**	as above, but with **C8**	as above, but with **C8**	[32,108a, 117b,137]
 **C10**	αvβ3: 0.47[b]	streptavidin-coated polystyrene	biotin-streptavidin binding	enhanced adhesion of M21 melanoma cells, activation of integrin αvβ3 ≈cRGD > VN, FN (integrin specificity)	[135]

[a] The bioactive moiety of the molecules is depicted in black; the spacer-anchor units in blue. [b] Integrin binding activity in Ref. [135] is given as the K_d value. The binding of αvβ5 or α5β1 was not detected. n.m. = not measured.

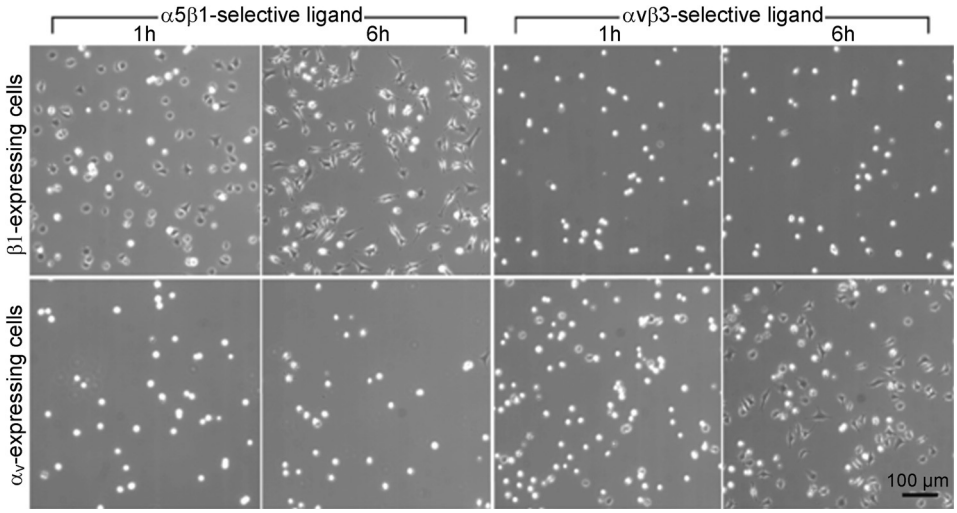


Figure 3. Cell-adhesion assay. αvβ3-expressing fibroblasts adhere and spread on surfaces coated with compound **C8** (αvβ3-selective) but not on surfaces coated with **C9** (α5β1-selective). The opposite behavior is observed when cells expressing α5β1 are used. From Ref. [108a].

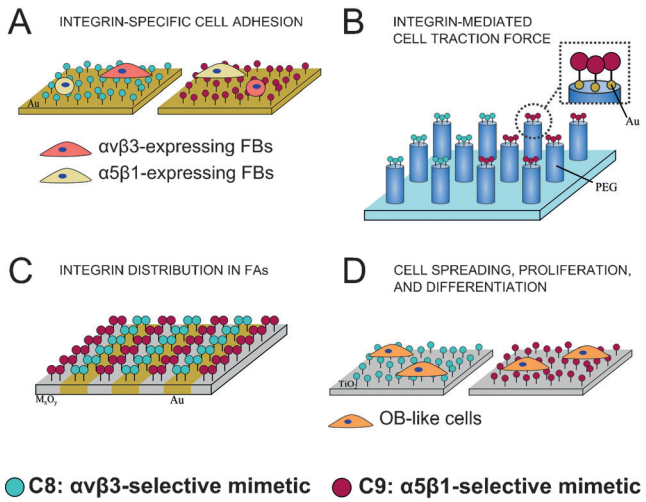


Figure 4. Application of αvβ3-selective (peptidomimetic **C8**) and α5β1-selective (peptidomimetic **C9**) ligands. These molecules can be used for biophysical studies of integrin-mediated cell adhesion (A) and cell traction forces (B), as well as to investigate integrin distribution (segregation versus colocalization) in FAs (C). Moreover, integrin-selective peptidomimetics can also be used to install osteointegrative properties on the surface of implant materials (D).

contained a phosphonate anchor. This strategy further allowed the incubation and segregation of integrins on the surface, thereby obtaining arrays of αvβ3 integrins on the gold stripes and of α5β1 on the Fe_2O_3/TiO_2 stripes. Such binary systems allowed the study of integrin distribution during FAs. Interestingly, osteosarcoma U2OS cells showed clusters of αvβ3 on gold surfaces (αvβ3-selective), whereas colocalization of both αvβ3 and α5β1 in clusters was observed on metal oxide surfaces (α5β1-selective).[134a] These findings suggest that α5β1 activation may promote diffusion and recruitment of αvβ3 integrins through an inside-out signaling. Such intimate cross-talk between these two integrin subtypes warrants further investigations.

The Kiessling research group has also recently reported the use of peptidomimetics to study integrin-specific cell behavior on surfaces.[135] In this study, an αvβ3 inhibitor[111] was biotinylated (**C10**, Table 3) and immobilized on streptavidin-coated surfaces. The resulting surfaces had a high affinity for αvβ3, (i.e. no binding of integrins αvβ5 or α5β1 was detected) as well as enhanced adhesion of M21 melanoma cells and activated αvβ3 signaling. Taken together, these recent findings demonstrate that integrin-tailored surfaces can be used to unravel the specific role of the integrin subtypes in cell-adhesion processes.[136]

Moreover, this strategy can also be used to dictate cell behavior on the surface of biomaterials. In this regard, we have recently coated titanium surfaces with compounds **C8** or **C9** and studied the behavior of osteoblast-like cells on the surfaces.[137] For the first time, it was shown that integrin-binding peptidomimetics are able to support and promote all the biological processes required to ensure a reliable osseointegration of an implant material: the immobilization of these molecules on titanium significantly enhanced the attachment, spreading, proliferation, ALP production, and mineralization of osteoblast-like cells (Figure 4D). Remarkably, the biological activity exhibited by these molecules was comparable to that observed on surfaces coated with native proteins of the ECM. These results are of relevance because they show an unprecedented biological activity for low-molecular-weight ligands, and demonstrate that the activity of complex ECM proteins can be recapitulated by synthetic integrin-binding ligands.

5. Summary and Outlook

The development of integrin-selective ligands for surface coating has been a long and challenging journey. In this regard, the use of integrin-binding molecules as surface-coating agents has evolved over the last three decades (Figure 5). Early studies focused on the use of RGD-containing peptides and proteins with, in general, relatively low integrin-binding activity and receptor selectivity. The biological profile of these ligands has been improved by different approaches, including the development of cyclic RGD peptides and fragments derived from FN. In 2004 we published the first coating of a surface with a highly active peptidomimetic for αvβ3 and selectivity over αIIbβ3.[133]

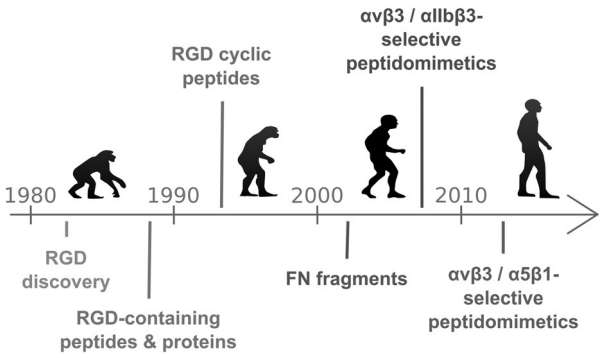


Figure 5. Evolution of surface-coating strategies over the last decades. The principal milestones in the field are highlighted.

Selectivity between αvβ3 and α5β1 on a surface was not achieved until 2013.[108a]

Peptidomimetics overcome the majority of limitations displayed by peptides and proteins. They exhibit very potent integrin-binding affinities (with IC_{50} values in the sub- to nanomolar range) and excellent selectivity. In addition to that, they are highly stable to enzymatic degradation, changes of pH value and temperature, and are devoid of immunogenic responses. They can, furthermore, be immobilized on surfaces at high densities and produced on a large scale. It is, thus, surprising that their use as coating molecules has rarely been documented in the literature. This can be attributed, in part, to the fact that their production requires expertise in synthetic organic chemistry. Moreover, their development often requires comprehensive structure–activity relationship studies, and achieving receptor selectivity is not straightforward. Last, but not least, the functionalization of the peptidomimetic ligands for surface coating without loss of biological activity is another crucial issue.

This Review illustrates all these aspects and provides examples of the design of peptidomimetics and their application in surface coating. Insights from these studies may help to produce novel types of mimetics with improved selectivity profiles and increased affinity for other integrins. The studies described in this Review have demonstrated the following:

1. Surfaces functionalized with integrin-selective peptidomimetics (i.e. capable of discriminating between αvβ3 and α5β1) are very useful tools to elucidate the role of integrins in cell biology. The possibility to selectively engage one integrin subtype opens new prospects in the study of integrin-mediated signaling, for example in the differentiation of stem cells, where the specific role of integrin subtypes remains unknown.
2. The capacity to elicit integrin activation can be used to tailor cell-specific responses and modulate cell behavior on biomaterials for regenerative purposes. For example, peptidomimetics have been proposed as promising molecules to improve the osseointegration of implant materials. Their application in the regeneration of other organs and tissues has yet to be explored.

Further studies with these types of molecules are thus warranted. Given the importance of αvβ3 and α5β1 in the osteogenic differentiation of MSCs, integrin-selective surfaces could be used to tune the differentiation of these cells into the osteoblastic lineage. Moreover, the ability of these peptidomimetic-coated biomaterials to promote bone growth has not been proven in vivo. Both strategies are currently being investigated in our laboratories.

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[1] R. O. Hynes, *Cell* **2002**, *110*, 673–687.
[2] R. O. Hynes, *Cell* **1987**, *48*, 549–550.
[3] a) M. J. Humphries, *Biochem. Soc. Trans.* **2000**, *28*, 311–339; b) X. C. Dong, L. Z. Mi, J. H. Zhu, W. Wang, P. Hu, B. H. Luo, T. A. Springer, *Biochemistry* **2012**, *51*, 8814–8828; c) J.-P. Xiong, B. Mahalingham, J. L. Alonso, L. A. Borrelli, X. Rui, S. Anand, B. T. Hyman, T. Rysiok, D. Muller-Pompalla, S. L. Goodman, M. A. Arnaout, *J. Cell Biol.* **2009**, *186*, 589–600.
[4] a) S. J. Shattil, C. Kim, M. H. Ginsberg, *Nat. Rev. Mol. Cell Biol.* **2010**, *11*, 288–300; b) T. A. Springer, M. L. Dustin, *Curr. Opin. Cell Biol.* **2012**, *24*, 107–115.
[5] a) P. E. Hughes, F. Diaz-Gonzalez, L. Leong, C. Wu, J. A. McDonald, S. J. Shattil, M. H. Ginsberg, *J. Biol. Chem.* **1996**, *271*, 6571–6574; b) M. A. Müller, L. Brunie, A.-S. Bächer, H. Kessler, K.-E. Gottschalk, U. Reuning, *Cell. Signalling* **2014**, *26*, 2493–2503.
[6] a) See Ref. [4a]; b) K. R. Legate, S. A. Wickstrom, R. Fässler, *Genes Dev.* **2009**, *23*, 397–418.
[7] a) K.-E. Gottschalk, H. Kessler, *Angew. Chem. Int. Ed.* **2002**, *41*, 3767–3774; *Angew. Chem.* **2002**, *114*, 3919–3927; b) K.-E. Gottschalk, H. Kessler, *Structure* **2004**, *12*, 1109–1116; c) M. A. Müller, J. Opfer, L. A. Volkhardt, L. Brunie, E.-K. Sinner, D. Boettiger, A. Bochen, H. Kessler, K.-E. Gottschalk, U. Reuning, *J. Mol. Biol.* **2013**, *425*, 2988–3006.
[8] B. Geiger, J. P. Spatz, A. D. Bershadsky, *Nat. Rev. Mol. Cell Biol.* **2009**, *10*, 21–33.
[9] a) R. Silva, G. D'Amico, K. M. Hodivala-Dilke, L. E. Reynolds, *Arterioscler. Thromb. Vasc. Biol.* **2008**, *28*, 1703–1713; b) C. J. Avraamides, B. Garmy-Susini, J. Varner, *Nat. Rev. Cancer* **2008**, *8*, 604–617; c) J. E. Smith-Garvin, G. A. Koretzky, M. S. Jordan, *Ann. Rev. Immunol.* **2009**, *27*, 591–619.
[10] a) S. E. Winograd-Katz, R. Fässler, B. Geiger, K. R. Legate, *Nat. Rev. Mol. Cell Biol.* **2014**, *15*, 273–288; b) J. S. Desgrosellier, D. A. Cheresh, *Nat. Rev. Cancer* **2010**, *10*, 9–22.
[11] a) D. Cox, M. Brennan, N. Moran, *Nat. Rev. Drug Discovery* **2010**, *9*, 804–820; b) S. L. Goodman, M. Picard, *Trends Pharmacol. Sci.* **2012**, *33*, 405–412; c) K. Ley, J. Rivera-Nieves, W. J. Sandborn, S. Shattil, *Nat. Rev. Drug Disc.* **2016**, *15*, 173–183.

[12] M. P. Bonaca, P. G. Steg, L. J. Feldman, J. F. Canales, J. J. Ferguson, L. Wallentin, R. M. Califf, R. A. Harrington, R. P. Giugliano, *J. Am. Coll. Cardiol.* **2009**, *54*, 969 – 984.

[13] G. P. A. Rice, H. P. Hartung, P. A. Calabresi, *Neurology* **2005**, *64*, 1336 – 1342.

[14] S. R. Targan, B. G. Feagan, R. N. Fedorak, B. A. Lashner, R. Panaccione, D. H. Present, M. E. Spehlmann, P. J. Rutgeerts, Z. Tulassay, M. Volfova, D. C. Wolf, C. Hernandez, J. Bornstein, W. J. Sandborn, *Gastroenterology* **2007**, *132*, 1672 – 1683.

[15] J. E. Frampton, G. L. Plosker, *Am. J. Clin. Dermatol.* **2009**, *10*, 51 – 72.

[16] a) M. A. Dechantsreiter, E. Planker, B. Mathä, E. Lohof, G. Hölzemann, A. Jonczyk, S. L. Goodman, H. Kessler, *J. Med. Chem.* **1999**, *42*, 3033 – 3040; b) C. Mas-Moruno, F. Rechenmacher, H. Kessler, *Anti-Cancer Agents Med. Chem.* **2010**, *10*, 753 – 768.

[17] a) D. Cox, *Curr. Pharm. Des.* **2004**, *10*, 1587 – 1596; b) C. Warnke, T. Menge, H. P. Hartung, M. K. Racke, P. D. Cravens, J. L. Bennett, E. M. Frohman, B. M. Greenberg, S. S. Zamvil, R. Gold, B. Hemmer, B. C. Kieseier, O. Stüve, *Arch. Neurol.* **2010**, *67*, 923 – 930; c) L. Kappos, D. Bates, G. Edan, M. Eraksoy, A. Garcia-Merino, N. Grigoriadis, H. P. Hartung, E. Havrdová, J. Hillert, R. Hohlfeld, M. Kremenchutzky, O. Lyon-Caen, A. Miller, C. Pozzilli, M. Ravnborg, T. Saida, C. Sindic, K. Vass, D. B. Clifford, S. Hauser, E. O. Major, P. W. O'Connor, H. L. Weiner, M. Clanet, R. Gold, H. H. Hirsch, E. W. Radü, P. S. Sørensen, J. King, *Lancet Neurol.* **2011**, *10*, 745 – 758; d) E. O. Major, *Annu. Rev. Med.* **2010**, *61*, 35 – 47.

[18] M. D. Pierschbacher, E. Ruoslahti, *Nature* **1984**, *309*, 30 – 33.

[19] M. D. Pierschbacher, E. Ruoslahti, *Cell* **1986**, *44*, 517 – 518.

[20] a) M. Aumailley, M. Gurrath, G. Müller, J. Calvete, R. Timpl, H. Kessler, *FEBS Lett.* **1991**, *291*, 50 – 54; b) M. Gurrath, G. Müller, H. Kessler, M. Aumailley, R. Timpl, *Eur. J. Biochem.* **1992**, *210*, 911 – 921; c) R. Haubner, D. Finsinger, H. Kessler, *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 1374 – 1389; *Angew. Chem.* **1997**, *109*, 1440 – 1456.

[21] a) A. O. Frank, E. Otto, C. Mas-Moruno, H. B. Schiller, L. Marinelli, S. Cosconati, A. Bochen, D. Vossmeyer, G. Zahn, R. Stragies, E. Novellino, H. Kessler, *Angew. Chem. Int. Ed.* **2010**, *49*, 9278 – 9281; *Angew. Chem.* **2010**, *122*, 9465 – 9468; b) A. Bochen, U. K. Marelli, E. Otto, D. Pallarola, C. Mas-Moruno, F. S. Di Leva, H. Boehm, J. P. Spatz, E. Novellino, H. Kessler, L. Marinelli, *J. Med. Chem.* **2013**, *56*, 1509 – 1519.

[22] a) J. P. Xiong, T. Stehle, B. Diefenbach, R. Zhang, R. Dunker, D. L. Scott, A. Joachimiak, S. L. Goodman, M. A. Arnaout, *Science* **2001**, *294*, 339 – 345; b) J. P. Xiong, T. Stehle, R. Zhang, A. Joachimiak, M. Frech, S. L. Goodman, M. A. Arnaout, *Science* **2002**, *296*, 151 – 155.

[23] L. Marinelli, A. Meyer, D. Heckmann, A. Lavecchia, E. Novellino, H. Kessler, *J. Med. Chem.* **2005**, *48*, 4204 – 4207.

[24] M. Nagae, S. Re, E. Mihara, T. Nogi, Y. Sugita, J. Takagi, *J. Cell Biol.* **2012**, *197*, 131 – 140.

[25] a) U. Hersel, C. Dahmen, H. Kessler, *Biomaterials* **2003**, *24*, 4385 – 4415; b) C. Mas-Moruno, M. Espanol, E. B. Montufar, G. Mestres, C. Aparicio, F. J. Gil, M. P. Ginebra in *Biomaterials Surface Science* (Eds.: A. Taubert, J. F. Mano, J. C. Rodríguez-Cabello), Wiley-VCH, Weinheim, **2013**, pp. 337 – 374.

[26] a) R. Pytela, M. D. Pierschbacher, E. Ruoslahti, *Cell* **1985**, *40*, 191 – 198; b) R. Pytela, M. D. Pierschbacher, E. Ruoslahti, *Proc. Natl. Acad. Sci. USA* **1985**, *82*, 5766 – 5770.

[27] a) J. D. Humphries, A. Byron, M. J. Humphries, *J. Cell Sci.* **2006**, *119*, 3901 – 3903; b) E. F. Plow, T. A. Haas, L. Zhang, J. Loftus, J. W. Smith, *J. Biol. Chem.* **2000**, *275*, 21785 – 21788; c) S. Gronthos, P. J. Simmons, S. E. Graves, P. G. Robey, *Bone* **2001**, *28*, 174 – 181.

[28] S. Aota, M. Nomizu, K. M. Yamada, *J. Biol. Chem.* **1994**, *269*, 24756 – 24761.

[29] a) E. Zamir, M. Katz, Y. Posen, N. Erez, K. M. Yamada, B. Z. Katz, S. Lin, D. C. Lin, A. Bershadsky, Z. Kam, B. Geiger, *Nat. Cell Biol.* **2000**, *2*, 191 – 196; b) R. Zaidel-Bar, C. Ballestrem, Z. Kam, B. Geiger, *J. Cell Sci.* **2003**, *116*, 4605 – 4613.

[30] M. R. Morgan, A. Byron, M. J. Humphries, M. D. Bass, *IUBMB Life* **2009**, *61*, 731 – 738.

[31] P. Roca-Cusachs, N. C. Gauthier, A. Del Rio, M. P. Sheetz, *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 16245 – 16250.

[32] S. Rahmouni, A. Lindner, F. Rechenmacher, S. Neubauer, T. R. A. Sobahi, H. Kessler, E. A. Cavalcanti-Adam, J. P. Spatz, *Adv. Mater.* **2013**, *25*, 5869 – 5874.

[33] O. Rossier, V. Octeau, J. B. Sibarita, C. Leduc, B. Tessier, D. Nair, V. Gatterdam, O. Destaing, C. Albiges-Rizo, R. Tampe, L. Cognet, D. Choquet, B. Lounis, G. Giannone, *Nat. Cell Biol.* **2012**, *14*, 1057 – 1067.

[34] H. B. Schiller, M. R. Hermann, J. Polleux, T. Vignaud, S. Zanivan, C. C. Friedel, Z. Q. Sun, A. Raducanu, K. E. Gottschalk, M. Thery, M. Mann, R. Fässler, *Nat. Cell Biol.* **2013**, *15*, 625 – 636.

[35] a) S. M. Cutler, A. J. Garcia, *Biomaterials* **2003**, *24*, 1759 – 1770; b) T. A. Petrie, J. R. Capadona, C. D. Reyes, A. J. Garcia, *Biomaterials* **2006**, *27*, 5459 – 5470; c) E. A. Cowles, L. L. Brailey, G. A. Gronowicz, *J. Biomed. Mater. Res.* **2000**, *52*, 725 – 737; d) B. Keselowsky, L. Wang, Z. Schwarts, A. J. Garcia, B. D. Boyan, *J. Biomed. Mater. Res. Part A* **2007**, *80*, 700 – 710.

[36] O. Fromigué, J. Brun, C. Marty, S. Da Nascimento, P. Sonnet, P. J. Marie, *J. Cell. Biochem.* **2012**, *113*, 3029 – 3038.

[37] M. M. Martino, M. Mochizuki, D. A. Rothenfluh, S. A. Rempel, J. A. Hubbell, T. H. Barker, *Biomaterials* **2009**, *30*, 1089 – 1097.

[38] S. L. Cheng, C. F. Lai, S. D. Blystone, L. V. Avioli, *J. Bone Miner. Res.* **2001**, *16*, 277 – 288.

[39] R. J. McMurray, N. Gadegaard, P. M. Tsimbouri, K. V. Burgess, L. E. McNamara, R. Tare, K. Murawski, E. Kingham, R. O. C. Oreffo, M. J. Dalby, *Nat. Mater.* **2011**, *10*, 637 – 644.

[40] J. E. Frith, R. J. Mills, J. E. Hudson, J. J. Cooper-White, *Stem Cells Dev.* **2012**, *21*, 2442 – 2456.

[41] a) F. Guilak, D. M. Cohen, B. T. Estes, J. M. Gimble, W. Liedtke, C. S. Chen, *Cell Stem Cell* **2009**, *5*, 17 – 26; b) W. P. Daley, S. B. Peters, M. Larsen, *J. Cell Sci.* **2008**, *121*, 255 – 264; c) S. Cosson, E. A. Otte, H. Hezaveh, J. J. Cooper-White, *Stem Cells Transl. Med.* **2015**, *4*, 156 – 164.

[42] a) Z. Hamidouche, O. Fromigué, J. Ringe, T. Häupl, P. Vaudin, J.-C. Pagès, S. Srouji, E. Livne, P. J. Marie, *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 18587 – 18591; b) T. A. Petrie, J. E. Raynor, C. D. Reyes, K. L. Burns, D. M. Collard, A. J. Garcia, *Biomaterials* **2008**, *29*, 2849 – 2857.

[43] a) T. A. Petrie, C. D. Reyes, K. L. Burns, A. J. Garcia, *J. Cell. Mol. Med.* **2009**, *13*, 2602 – 2612; b) R. Agarwal, C. Gonzalez-Garcia, B. Torstrick, R. E. Guldberg, M. Salmeron-Sanchez, A. J. Garcia, *Biomaterials* **2015**, *63*, 137 – 145.

[44] C. Dufour, X. Holy, P. J. Marie, *Exp. Cell Res.* **2007**, *313*, 394 – 403.

[45] a) G. B. Schneider, R. Zaharias, C. Stanford, *J. Dent. Res.* **2001**, *80*, 1540 – 1544; b) C. F. Lai, S. L. Cheng, *J. Bone Miner. Res.* **2005**, *20*, 330 – 340.

[46] K. A. Kilian, M. Mrksich, *Angew. Chem. Int. Ed.* **2012**, *51*, 4891 – 4895; *Angew. Chem.* **2012**, *124*, 4975 – 4979.

[47] L. T. Duong, G. A. Rodan, *Front. Biosci.* **1998**, *3*, 757 – 768.

[48] C. P. Carron, D. M. Meyer, V. W. Engleman, J. G. Rico, P. G. Ruminski, R. L. Ornberg, W. F. Westlin, G. A. Nickols, *J. Endocrinol.* **2000**, *165*, 587 – 598.

[49] K. P. McHugh, K. Hodivala-Dilke, M. H. Zheng, N. Namba, L. Jonathan, D. Novack, X. Feng, F. P. Ross, R. O. Hynes, S. L. Teitelbaum, *J. Clin. Invest.* **2000**, *105*, 433 – 440.

[50] S. Stefansson, D. A. Lawrence, *Nature* **1996**, *383*, 441 – 443.

[51] D. I. Leavesley, M. A. Schwartz, M. Rosenfeld, D. A. Cheresh, *J. Cell Biol.* **1993**, *121*, 163 – 170.

[52] S. D. Robinson, K. M. Hodivala-Dilke, *Curr. Opin. Cell Biol.* **2011**, *23*, 630 – 637.

[53] G. Bergers, L. E. Benjamin, *Nat. Rev. Cancer* **2003**, *3*, 401 – 410.

[54] S. M. Weis, D. A. Cheresh, *Nat. Med.* **2011**, *17*, 1359 – 1370.

[55] a) P. C. Brooks, R. A. F. Clark, D. A. Cheresh, *Science* **1994**, *264*, 569 – 571; b) P. C. Brooks, A. M. P. Montgomery, M. Rosenfeld, R. A. Reisfeld, T. H. Hu, G. Klier, D. A. Cheresh, *Cell* **1994**, *79*, 1157 – 1164.

[56] B. L. Bader, H. Rayburn, D. Crowley, R. O. Hynes, *Cell* **1998**, *95*, 507 – 519.

[57] L. E. Reynolds, L. Wyder, J. C. Lively, D. Taverna, S. D. Robinson, X. Z. Huang, D. Sheppard, R. O. Hynes, K. M. Hodivala-Dilke, *Nat. Med.* **2002**, *8*, 27 – 34.

[58] A. R. Ramjaun, K. Hodivala-Dilke, *Int. J. Biochem. Cell Biol.* **2009**, *41*, 521 – 530.

[59] P. T. Caswell, M. Chan, A. J. Lindsay, M. W. McCaffrey, D. Boettiger, J. C. Norman, *J. Cell Biol.* **2008**, *183*, 143 – 155.

[60] a) S. Kim, K. Bell, S. A. Mousa, J. A. Varner, *Am. J. Pathol.* **2000**, *156*, 1345 – 1362; b) P. Parsons-Wingerter, I. M. Kasman, S. Norberg, A. Magnussen, S. Zanivan, A. Rissone, P. Baluk, C. J. Favre, U. Jeffry, R. Murray, D. M. McDonald, *Am. J. Pathol.* **2005**, *167*, 193 – 211.

[61] A. van der Flier, K. Badu-Nkansah, C. A. Whittaker, D. Crowley, R. T. Bronson, A. Lacy-Hulbert, R. O. Hynes, *Development* **2010**, *137*, 2439 – 2449.

[62] M. D. Pierschbacher, E. Ruoslahti, *Proc. Natl. Acad. Sci. USA* **1984**, *81*, 5985 – 5988.

[63] S. Suzuki, A. Oldberg, E. G. Hayman, M. D. Pierschbacher, E. Ruoslahti, *EMBO J.* **1985**, *4*, 2519 – 2524.

[64] E. F. Plow, M. D. Pierschbacher, E. Ruoslahti, G. A. Marguerie, M. H. Ginsberg, *Proc. Natl. Acad. Sci. USA* **1985**, *82*, 8057 – 8061.

[65] A. Oldberg, A. Franzen, D. Heinegard, *Proc. Natl. Acad. Sci. USA* **1986**, *83*, 8819 – 8823.

[66] D. S. Grant, K. I. Tashiro, B. Segui-Real, Y. Yamada, G. R. Martin, H. K. Kleinman, *Cell* **1989**, *58*, 933 – 943.

[67] E. Ruoslahti, M. D. Pierschbacher, *Science* **1987**, *238*, 491 – 497.

[68] a) H. Kessler, *Angew. Chem. Int. Ed. Engl.* **1982**, *21*, 512 – 523; *Angew. Chem.* **1982**, *94*, 509 – 520; b) V. J. Hruby, *Life Sci.* **1982**, *31*, 189 – 199.

[69] M. D. Pierschbacher, E. Ruoslahti, *J. Biol. Chem.* **1987**, *262*, 17924 – 17928.

[70] J. J. Calvete, W. Schafer, T. Soszka, W. Lu, J. J. Cook, B. A. Jameson, S. Niewiarowski, *Biochemistry* **1991**, *30*, 5225 – 5229.

[71] H. Kessler, R. Gratias, G. Hessler, M. Gurrath, G. Müller, *Pure Appl. Chem.* **1996**, *68*, 1201 – 1205.

[72] a) M. Pfaff, K. Tangemann, B. Müller, M. Gurrath, G. Müller, H. Kessler, R. Timpl, J. Engel, *J. Biol. Chem.* **1994**, *269*, 20233 – 20238; b) R. Haubner, R. Gratias, B. Diefenbach, S. L. Goodman, A. Jonczyk, H. Kessler, *J. Am. Chem. Soc.* **1996**, *118*, 7461 – 7472.

[73] A. Geyer, G. Müller, H. Kessler, *J. Am. Chem. Soc.* **1994**, *116*, 7735 – 7743.

[74] R. Haubner, W. Schmitt, G. Hölzemann, S. L. Goodman, A. Jonczyk, H. Kessler, *J. Am. Chem. Soc.* **1996**, *118*, 7881 – 7891.

[75] E. Lohof, E. Planker, C. Mang, F. Burkhart, M. A. Dechantsreiter, R. Haubner, H. J. Wester, M. Schwaiger, G. Hölzemann, S. L. Goodman, H. Kessler, *Angew. Chem. Int. Ed.* **2000**, *39*, 2761 – 2764; *Angew. Chem.* **2000**, *112*, 2868 – 2871.

[76] J. Wermuth, S. L. Goodman, A. Jonczyk, H. Kessler, *J. Am. Chem. Soc.* **1997**, *119*, 1328 – 1335.

[77] C. Mas-Moruno, J. G. Beck, L. Doedens, A. O. Frank, L. Marinelli, S. Cosconati, E. Novellino, H. Kessler, *Angew. Chem. Int. Ed.* **2011**, *50*, 9496 – 9500; *Angew. Chem.* **2011**, *123*, 9668 – 9672.

[78] a) J. Chatterjee, F. Rechenmacher, H. Kessler, *Angew. Chem. Int. Ed.* **2013**, *52*, 254 – 269; *Angew. Chem.* **2013**, *125*, 268 – 283; b) A. Becker, O. von Richter, A. Kovar, H. Scheible, J. J. van Lier, A. Johne, *J. Clin. Pharm.* **2015**, *55*, 815 – 824.

[79] R. Stupp, M. E. Hegi, T. Gorlia, S. C. Erridge, J. Perry, Y. K. Hong, K. D. Aldape, B. Lhermitte, T. Pietsch, D. Grujicic, J. P. Steinbach, W. Wick, R. Tarnawski, D. H. Nam, P. Hau, A. Weyerbrock, M. J. Taphoorn, C. C. Shen, N. Rao, L. Thurzo, U. Herrlinger, T. Gupta, R. D. Kortmann, K. Adamska, C. McBain, A. A. Brandes, J. C. Tonn, O. Schnell, T. Wiegel, C. Y. Kim, L. B. Nabors, D. A. Reardon, M. J. van den Bent, C. Hicking, A. Markivskyy, M. Picard, M. Weller, *Lancet Oncol.* **2014**, *15*, 1100 – 1108.

[80] a) L. B. Nabors, K. L. Fink, T. Mikkelsen, D. Grujicic, R. Tarnawski, D. H. Nam, M. Mazurkiewicz, M. Salacz, L. Ashby, V. Zagonel, R. Depenni, J. R. Perry, C. Hicking, M. Picard, M. E. Hegi, B. Lhermitte, D. A. Reardon, *Neuro-Oncology* **2015**, *17*, 708 – 717; b) W. P. Mason, *Neuro-Oncology* **2015**, *17*, 634 – 635.

[81] a) A. M. Ray, F. Schaffner, H. Janouskova, F. Noulet, D. Rognan, I. Lelong-Rebel, L. Choulier, A. F. Blandin, M. Lehmann, S. Martin, T. Kapp, S. Neubauer, F. Rechenmacher, H. Kessler, M. Dontenwill, *Biochim. Biophys. Acta Gen. Subj.* **2014**, *1840*, 2978 – 2987; b) G. Renner, H. Janouskova, F. Noulet, E. Guerin, S. Bär, J. Nuesch, F. Rechenmacher, S. Neubauer, H. Kessler, L. Choulier, N. Etienne-Selloum, M. Lehmann, I. Lelong-Rebel, S. Martin, M. Dontenwill, *Cell Death Differ.*, DOI:10.1038/cdd.2015.131.

[82] A. R. Reynolds, I. R. Hart, A. R. Watson, J. C. Welti, R. G. Silva, S. D. Robinson, G. Da Violante, M. Gourlaouen, M. Salih, M. C. Jones, D. T. Jones, G. Saunders, V. Kostourou, F. Perron-Sierra, J. C. Norman, G. C. Tucker, K. M. Hodivala-Dilke, *Nat. Med.* **2009**, *15*, 392 – 400.

[83] E. Koivunen, D. A. Gay, E. Ruoslahti, *J. Biol. Chem.* **1993**, *268*, 20205 – 20210.

[84] D. L. Livant, R. K. Brabec, K. J. Pienta, D. L. Allen, K. Kurachi, S. Markwart, A. Upadhyaya, *Cancer Res.* **2000**, *60*, 309 – 320.

[85] P. Khalili, A. Arakelian, G. Chen, M. L. Plunkett, I. Beck, G. C. Parry, F. Doñate, D. E. Shaw, A. P. Mazar, S. A. Rabbani, *Mol. Cancer Ther.* **2006**, *5*, 2271 – 2280.

[86] A. Müller, F. Schumann, M. Koksch, N. Sewald, *Lett. Pept. Sci.* **1997**, *4*, 275 – 281.

[87] F. Schumann, A. Müller, M. Koksch, G. Müller, N. Sewald, *J. Am. Chem. Soc.* **2000**, *122*, 12009 – 12010.

[88] D. Zimmermann, E. W. Guthohrlein, M. Malesevic, K. Sewald, L. Wobbe, C. Heggemann, N. Sewald, *ChemBioChem* **2005**, *6*, 272 – 276.

[89] S. Urman, K. Gaus, Y. Yang, U. Strijowski, N. Sewald, S. De Pol, O. Reiser, *Angew. Chem. Int. Ed.* **2007**, *46*, 3976 – 3978; *Angew. Chem.* **2007**, *119*, 4050 – 4053.

[90] D. Pramanik, B. K. Majeti, G. Mondal, P. P. Karmali, R. Sistla, O. G. Ramprasad, G. Srinivas, G. Pande, A. Chaudhuri, *J. Med. Chem.* **2008**, *51*, 7298 – 7302.

[91] a) F. Curnis, R. Longhi, L. Crippa, A. Cattaneo, E. Donossola, A. Bachi, A. Corti, *J. Biol. Chem.* **2006**, *281*, 36466 – 36476; b) S. Takahashi, M. Leiss, M. Moser, T. Ohashi, T. Kitao, D. Heckmann, A. Pfeifer, H. Kessler, J. Takagi, H. P. Erickson, R. Fässler, *J. Cell Biol.* **2007**, *178*, 167 – 178.

[92] T. Kapp, M. Fottner, O. V. Maltsev, H. Kessler, *Angew. Chem. Int. Ed.* **2016**, *55*, 1540 – 1543; *Angew. Chem.* **2016**, *128*, 1559 – 1563.

[93] R. M. Keenan, W. H. Miller, C. Kwon, F. E. Ali, J. F. Callahan, R. R. Calvo, S. M. Hwang, K. D. Kopple, C. E. Peishoff, J. M. Samanen, A. S. Wong, C. K. Yuan, W. F. Huffman, *J. Med. Chem.* **1997**, *40*, 2289 – 2292.

[94] a) M. Paolillo, M. A. Russo, M. Serra, L. Colombo, S. Schinelli, *Mini-Rev. Med. Chem.* **2009**, *9*, 1439 – 1446; b) A. Perdih, M. S. Dolenc, *Curr. Med. Chem.* **2010**, *17*, 2371 – 2392; c) T. G. Kapp, F. Rechenmacher, T. R. Sobahi, H. Kessler, *Expert Opin. Ther. Pat.* **2013**, *23*, 1273 – 1295; d) U. K. Marelli, F. Rechenmacher, T. R. A. Sobahi, C. Mas-Moruno, H. Kessler, *Front. Oncol.* **2013**, *3*, 222; e) H. M. Sheldrake, L. H. Patterson, *J. Med. Chem.* **2014**, *57*, 6301 – 6315.

[95] J. M. Smallheer, C. A. Weigelt, F. J. Woerner, J. S. Wells, W. F. Daneker, S. A. Mousa, R. R. Wexler, P. K. Jadhav, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 383 – 387.

[96] S. A. Mousa, S. Mohamed, J. M. Smallheer, P. K. Jadhav, J. A. Varner, *Blood* **1999**, *94*, 620A-620A.

[97] A. Maglott, P. Bartik, S. Cosgun, P. Klotz, P. Ronde, G. Fuhrmann, K. Takeda, S. Martin, M. Dontenwill, *Cancer Res.* **2006**, *66*, 6002 – 6007.

[98] E. Martinkova, A. Maglott, D. Y. Leger, D. Bonnet, M. Stiborova, K. Takeda, S. Martin, M. Dontenwill, *Int. J. Cancer* **2010**, *127*, 1240 – 1248.

[99] D. Heckmann, A. Meyer, L. Marinelli, G. Zahn, R. Stragies, H. Kessler, *Angew. Chem. Int. Ed.* **2007**, *46*, 3571 – 3574; *Angew. Chem.* **2007**, *119*, 3641 – 3644.

[100] R. Stragies, F. Osterkamp, G. Zischinsky, D. Vossmeyer, H. Kalkhof, U. Reimer, G. Zahn, *J. Med. Chem.* **2007**, *50*, 3786 – 3794.

[101] L. Marinelli, A. Lavecchia, K.-E. Gottschalk, E. Novellino, H. Kessler, *J. Med. Chem.* **2003**, *46*, 4393 – 4404.

[102] G. P. Curley, H. Blum, M. J. Humphries, *Cell. Mol. Life Sci.* **1999**, *56*, 427 – 441.

[103] a) G. Zahn, D. Vossmeyer, R. Stragies, M. Wills, C. G. Wong, K. U. Loffler, A. P. Adamis, J. Knolle, *Arch. Ophthalmol.* **2009**, *127*, 1329 – 1335; b) G. Zahn, K. Volk, G. P. Lewis, D. Vossmeyer, D. Stragies, J. S. Heier, P. E. Daniel, Jr., A. P. Adamis, E. A. Chapin, S. K. Fisher, F. G. Holz, K. U. Löffler, J. Knolle, *Invest. Ophthalmol. Vis. Sci.* **2010**, *51*, 1028 – 1035.

[104] a) G. Zischinsky, F. Osterkamp, D. Vossmeyer, G. Zahn, D. Scharn, A. Zwintscher, R. Stragies, *Bioorg. Med. Chem. Lett.* **2010**, *20*, 65 – 68; b) G. Zischinsky, F. Osterkamp, D. Vossmeyer, G. Zahn, D. Scharn, A. Zwintscher, R. Stragies, *Bioorg. Med. Chem. Lett.* **2010**, *20*, 380 – 382.

[105] C. Gibson, S. L. Goodman, D. Hahn, G. Hölzemann, H. Kessler, *J. Org. Chem.* **1999**, *64*, 7388 – 7394.

[106] a) C. Gibson, G. A. G. Sulyok, D. Hahn, S. L. Goodman, G. Hölzemann, H. Kessler, *Angew. Chem. Int. Ed.* **2001**, *40*, 165 – 169; *Angew. Chem.* **2001**, *113*, 169 – 173; b) G. A. G. Sulyok, C. Gibson, S. L. Goodman, G. Hölzemann, M. Wiesner, H. Kessler, *J. Med. Chem.* **2001**, *44*, 1938 – 1950.

[107] D. Heckmann, A. Meyer, B. Laufer, G. Zahn, R. Stragies, H. Kessler, *ChemBioChem* **2008**, *9*, 1397 – 1407.

[108] a) F. Rechenmacher, S. Neubauer, J. Polleux, C. Mas-Moruno, M. De Simone, E. A. Cavalcanti-Adam, J. P. Spatz, R. Fässler, H. Kessler, *Angew. Chem. Int. Ed.* **2013**, *52*, 1572 – 1575; *Angew. Chem.* **2013**, *125*, 1612 – 1616; b) S. Neubauer, F. Rechenmacher, A. J. Beer, F. Curnis, K. Pohle, C. D'Alessandria, H. J. Wester, U. Reuning, A. Corti, M. Schwaiger, H. Kessler, *Angew. Chem. Int. Ed.* **2013**, *52*, 11656 – 11659; *Angew. Chem.* **2013**, *125*, 11870 – 11873.

[109] D. Heckmann, B. Laufer, L. Marinelli, V. Limongelli, E. Novellino, G. Zahn, R. Stragies, H. Kessler, *Angew. Chem. Int. Ed.* **2009**, *48*, 4436 – 4440; *Angew. Chem.* **2009**, *121*, 4501 – 4506.

[110] S. Neubauer, F. Rechenmacher, R. Brimioulle, F. S. Di Leva, A. Bochen, T. R. Sobahi, M. Schottelius, E. Novellino, C. Mas-Moruno, L. Marinelli, H. Kessler, *J. Med. Chem.* **2014**, *57*, 3410 – 3417.

[111] J. W. Corbett, N. R. Graciani, S. A. Mousa, W. F. DeGrado, *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1371 – 1376.

[112] W. H. Miller, D. P. Alberts, P. K. Bhatnagar, W. E. Bondinell, J. F. Callahan, R. R. Calvo, R. D. Cousins, K. F. Erhard, D. A. Heerding, R. M. Keenan, C. Kwon, P. J. Manley, K. A. Newlander, S. T. Ross, J. M. Samanen, I. N. Uzinskas, J. W. Venslavsky, C. C.-K. Yuan, R. C. Haltiwanger, M. Gowen, S.-M. Hwang, I. E. James, M. W. Lark, D. J. Rieman, G. B. Stroup, L. M. Azzarano, K. L. Salyers, B. R. Smith, K. W. Ward, K. O. Johanson, W. F. Huffman, *J. Med. Chem.* **2000**, *43*, 22 – 26.

[113] P. Galletti, R. Soldati, M. Pori, M. Durso, A. Tolomelli, L. Gentilucci, S. D. Dattoli, M. Baiula, S. Spampinato, D. Giacomini, *Eur. J. Med. Chem.* **2014**, *83*, 284 – 293.

[114] Y. Hirano, D. J. Mooney, *Adv. Mater.* **2004**, *16*, 17 – 25.

[115] a) S. Bauer, P. Schmuki, K. von der Mark, J. Park, *Prog. Mater. Sci.* **2013**, *58*, 261 – 326; b) A. E. Rodda, L. Meagher, D. R. Nisbet, J. S. Forsythe, *Prog. Polym. Sci.* **2014**, *39*, 1312 – 1347.

[116] a) M. Kantlehner, D. Finsinger, J. Meyer, P. Schaffner, A. Jonczyk, B. Diefenbach, B. Nies, H. Kessler, *Angew. Chem. Int. Ed.* **1999**, *38*, 560 – 562; *Angew. Chem.* **1999**, *111*, 587 – 590; b) M. Kantlehner, P. Schaffner, D. Finsinger, J. Meyer, A. Jonczyk, B. Diefenbach, B. Nies, G. Hölzemann, S. L. Goodman, H. Kessler, *ChemBioChem* **2000**, *1*, 107 – 114.

[117] a) C. Mas-Moruno, P. M. Dorfner, F. Manzenrieder, S. Neubauer, U. Reuning, R. Burgkart, H. Kessler, *J. Biomed. Mater. Res. Part A* **2013**, *101*, 87 – 97; b) F. Rechenmacher, S. Neubauer, C. Mas-Moruno, P. M. Dorfner, J. Polleux, J. Guasch, B. Conings, H. G. Boyen, A. Bochen, T. R. Sobahi, R. Burgkart, J. P. Spatz, R. Fässler, H. Kessler, *Chem. Eur. J.* **2013**, *19*, 9218 – 9223; c) J. Auernheimer, C. Dahmen, U. Hersel, A. Bausch, H. Kessler, *J. Am. Chem. Soc.* **2005**, *127*, 16107 – 16110.

[118] D. Pallarola, A. Bochen, H. Boehm, F. Rechenmacher, T. R. Sobahi, J. P. Spatz, H. Kessler, *Adv. Funct. Mater.* **2014**, *24*, 943 – 956.

[119] H. Shin, S. Jo, A. G. Mikos, *Biomaterials* **2003**, *24*, 4353 – 4364.

[120] a) A. Shekaran, A. J. Garcia, *J. Biomed. Mater. Res. Part A* **2011**, *96*, 261 – 272; b) K. G. Sreejalekshmi, P. D. Nair, *J. Biomed. Mater. Res. Part A* **2011**, *96*, 477 – 491.

[121] D. F. Williams, *Biomaterials* **2011**, *32*, 4195 – 4197.

[122] a) J. Auernheimer, D. Zukowski, C. Dahmen, M. Kantlehner, A. Enderle, S. L. Goodman, H. Kessler, *ChemBioChem* **2005**, *6*, 2034 – 2040; b) U. Magdolen, J. Auernheimer, C. Dahmen, J. Schauwecker, H. Gollwitzer, J. Tübel, R. Gradinger, H. Kessler, M. Schmitt, P. Diehl, *Int. J. Mol. Med.* **2006**, *17*, 1017 – 1021.

[123] a) B. Elmengaard, J. E. Bechtold, K. Soballe, *Biomaterials* **2005**, *26*, 3521 – 3526; b) B. Elmengaard, J. E. Bechtold, K. Søballe, *J. Biomed. Mater. Res. Part A* **2005**, *75*, 249 – 255; c) H. C. Kroese-Deutman, J. Van den Dolder, P. H. M. Spauwen, J. A. Jansen, *Tissue Eng.* **2005**, *11*, 1867 – 1875; d) S. Rammelt, T. Illert, S. Bierbaum, D. Scharnweber, H. Zwipp, W. Schneiders, *Biomaterials* **2006**, *27*, 5561 – 5571.

[124] a) D. J. Leahy, I. Aukhil, H. P. Erickson, *Cell* **1996**, *84*, 155 – 164; b) S. Johansson, G. Svineng, K. Wennerberg, A. Armulik, L. Lohikangas, *Front. Sci. Ser.* **1997**, *2*, d126 – 146.

[125] C. Mas-Moruno, R. Fraioli, F. Albericio, J. M. Manero, F. J. Gil, *ACS Appl. Mater. Interfaces* **2014**, *6*, 6525 – 6536.

[126] a) T. Boxus, R. Touillaux, G. Dive, J. Marchand-Brynaert, *Bioorg. Med. Chem.* **1998**, *6*, 1577 – 1595; b) J. Marchand-Brynaert, E. Detrait, O. Noiset, T. Boxus, Y.-J. Schneider, C. Remacle, *Biomaterials* **1999**, *20*, 1773 – 1782.

[127] S. Biltresse, M. Attolini, G. Dive, A. Cordi, G. C. Tucker, J. Marchand-Brynaert, *Bioorg. Med. Chem.* **2004**, *12*, 5379 – 5393.

[128] S. Biltresse, M. Attolini, J. Marchand-Brynaert, *Biomaterials* **2005**, *26*, 4576 – 4587.

[129] V. Rerat, G. Dive, A. A. Cordi, G. C. Tucker, R. Bareille, J. Amedee, L. Bordenave, J. Marchand-Brynaert, *J. Med. Chem.* **2009**, *52*, 7029 – 7043.

[130] M. Rémy, R. Bareille, V. Rerat, C. Bourget, J. Marchand-Brynaert, L. Bordenave, *J. Biomater. Sci. Polym. Ed.* **2013**, *24*, 269 – 286.
[131] G. Ehteshami, D. C. Brune, J. C. Lopez, S. P. Massia, *Acta Biomater.* **2005**, *1*, 85 – 91.
[132] S. K. Narasimhan, P. Sejwal, S. Zhu, Y.-Y. Luk, *Bioorg. Med. Chem.* **2013**, *21*, 2210 – 2216.
[133] C. Dahmen, J. Auernheimer, A. Meyer, A. Enderle, S. L. Goodman, H. Kessler, *Angew. Chem. Int. Ed.* **2004**, *43*, 6649 – 6652; *Angew. Chem.* **2004**, *116*, 6818 – 6821.
[134] a) J. Guasch, B. Conings, S. Neubauer, F. Rechenmacher, K. Ende, C. G. Rolli, C. Kappel, V. Schaufler, A. Micoulet, H. Kessler, H.-G. Boyen, E. A. Cavalcanti-Adam, J. P. Spatz, *Adv. Mater.* **2015**, *27*, 3737 – 3747; b) J. Guasch, J. Diemer, H. Riahinezhad, S. Neubauer, H. Kessler, J. P. Spatz, *Chem. Mater.* **2016**, *28*, 1806 – 1815.
[135] J. R. Klim, A. J. Fowler, A. H. Courtney, P. J. Wrighton, R. T. C. Sheridan, M. L. Wong, L. L. Kiessling, *ACS Chem. Biol.* **2012**, *7*, 518 – 525.
[136] a) V. Schaufler, H. Czichos-Medda, V. Hirschfeld-Warnecken, S. Neubauer, F. Rechenmacher, R. Medda, H. Kessler, B. Geiger, J. P. Spatz, E. A. Cavalcanti-Adam, *Cell Adh. Migr.* DOI: 10.1080/19336918.2016.1163453; b) D. Missirlis, T. Haraszti, C. v. C. Scheele, T. Wiegand, C. Diaz, S. Neubauer, F. Rechenmacher, H. Kessler, J. P. Spatz, *Nat. Sci. Rep.* **2016**, *6*, 23258 – 23258.
[137] R. Fraioli, F. Rechenmacher, S. Neubauer, J. M. Manero, J. Gil, H. Kessler, C. Mas-Moruno, *Colloids Surf. B* **2015**, *128*, 191 – 200.

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