Biomaterials Science

COMMUNICATION

Supporting Information

Control of Stem Cell Response and Bone Growth on Biomaterials by Fully Non-Peptidic Integrin Selective Ligands

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1. RGD-based peptidomimetics

The αvβ3- or α5β1-selective peptidomimetics were designed, synthesized and characterized as described in previous studies.[1,2] In particular, for the *in vitro* studies the peptidomimetics were synthesized incorporating a mercaptopropionic acid as terminal group (compounds **1** and **2**), which allows functionalization of metallic samples via a Michael-type addition. The complete synthetic and characterization details are provided elsewhere[1] and their chemical structure shown in **Figure S1a**. Alternatively, the mimetics were also prepared with phosphonic acids as anchoring moieties to directly functionalize the titanium implants in the *in vivo* studies (compounds **3** and **4**). The synthesis and characterization of phosphonate derivatives was also previously reported.[2] However, in the particular case of the αvβ3-selective ligand (**3**), to improve its solubility, a short polyethylene glycol spacer and 4 phosphonic acids were used instead.

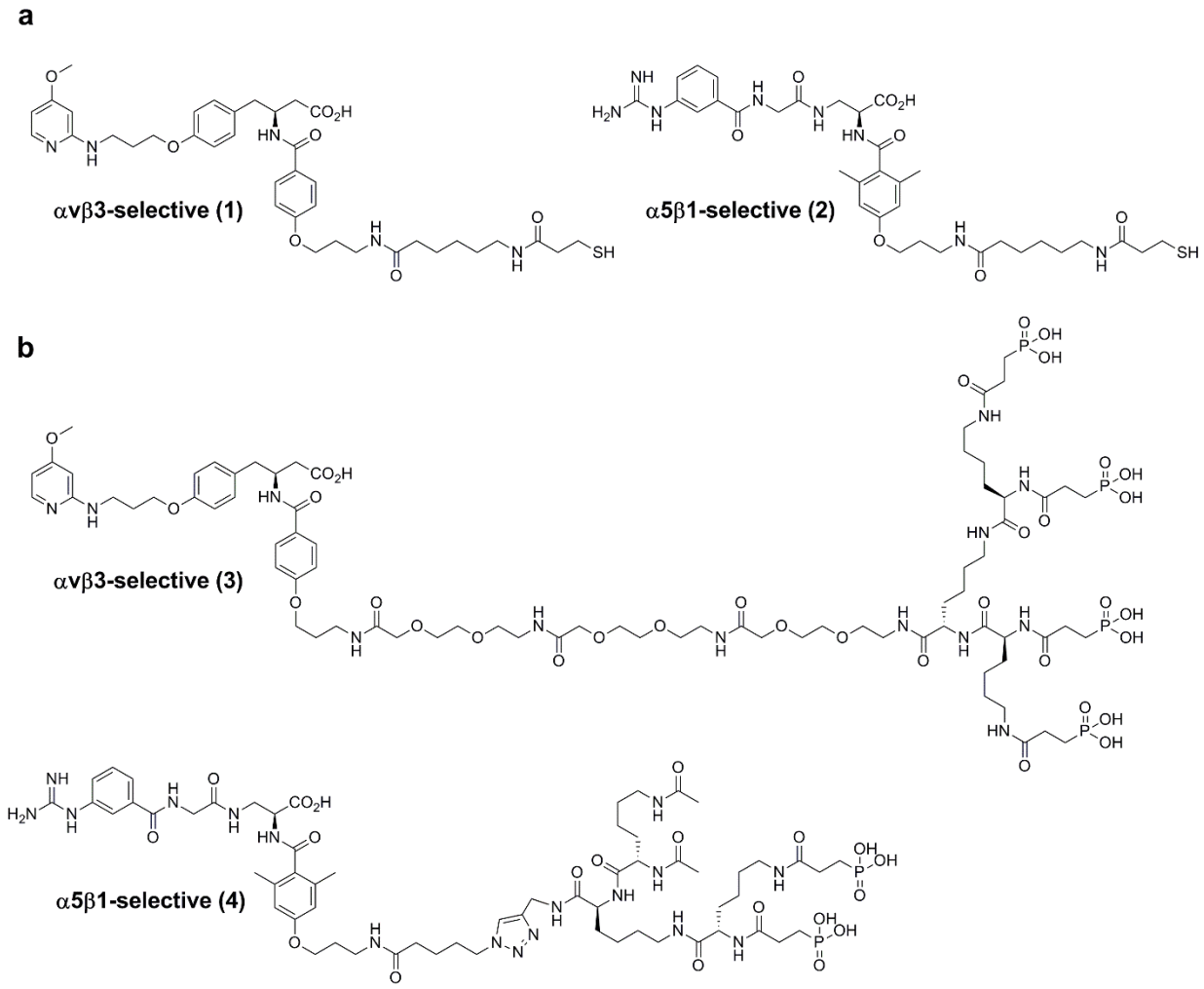


Figure S1. Chemical structure of the RGD-based peptidomimetics containing as anchoring groups either (a) a thiol group or (b) phosphonic acids.

2. Synthesis of peptidomimetic 3

General Procedures

GP1. *Loading of TCP-resin.* Peptide synthesis was carried out using TCP-resin (~80% maximum loading capacity) following standard Fmoc-strategy. Fmoc-Xaa-OH (1.2 eq.) were attached to the TCP-resin with *N*,*N*-diisopropylethylamine (DIEA; 2.5 eq.) in anhydrous DCM (8 mL/g resin) at room temperature (RT) for 1 h. Afterwards the resin is capped by addition of a solution of MeOH (0.2 mL/g resin) and DIEA, (5:1; v:v), for 15 min. The resin was filtered and washed with DCM (5x), NMP (3x), NMP/MeOH 1:1 (1x) and with MeOH (3x). The loading capacity was determined by gravimetric method after drying the resin under vacuum.

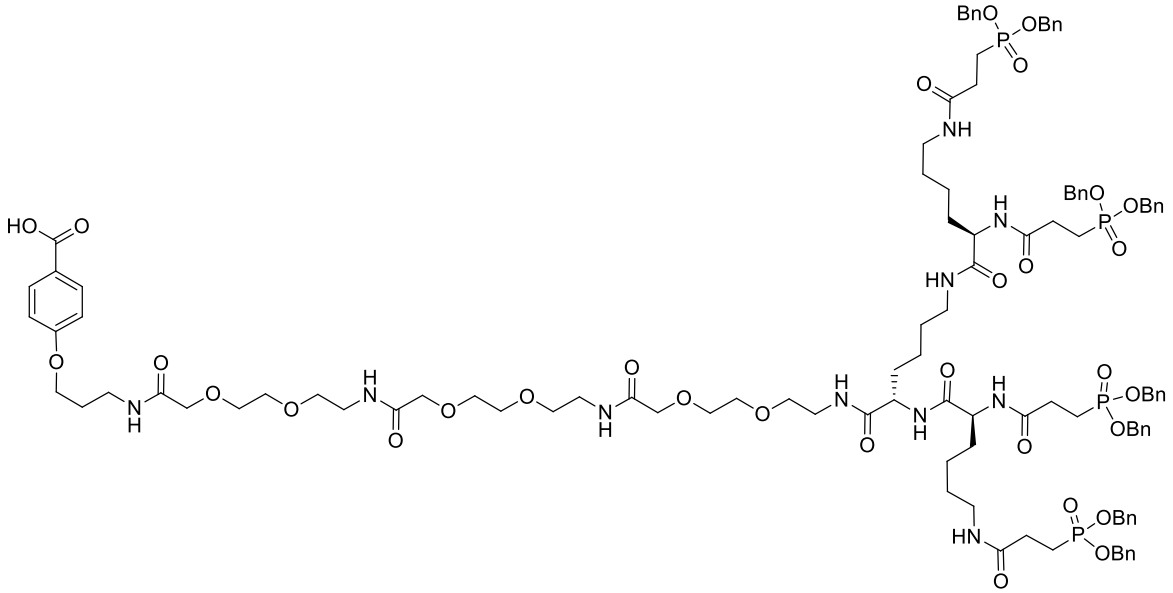
GP2. *Solid phase N-Alloc deprotection.* The resin was washed with DCM (3x) and then treated with a solution of *tetrakis*-triphenylphosphinepalladium (0.25 eq.) and phenylsilane (10 eq.) in DCM at ambient temperature. Care had to be taken due to gas evolution and the pressure had to be released from the reaction vessel from time to time. After 30 min of shaking, the mixture was filtered and the resin washed twice with a 0.5% solution of DDTC (sodium *N*,*N*-diethyldithiocarbamate) in DMF and a 0.5% solution of DIEA in DMF. The washing procedure was repeated (3x) and the resin washed with NMP (5x).

GP3. *Standard Amino Acid Coupling.* A solution of Fmoc-Xaa-OH (2 eq.), *O*-(7-azabenzotriazole-1yl)-*N*,*N*,*N'*,*N'*-tetramethyluronium-hexafluorophosphate (HATU) (2 eq.), 1-hydroxy-7-azabenzotriazole (HOAt; 2 eq.), and DIEA (5 eq.) in NMP (1 mL/g resin) was added to the free amino peptidyl-resin and shaken for 60 min at RT and washed with NMP (5x).

GP4. *On-resin Fmoc Deprotection.* The Fmoc peptidyl-resin was treated with 20% piperidine in NMP (v/v) for 15 min and a second time for 10 min. The resin was washed following with NMP (5x).

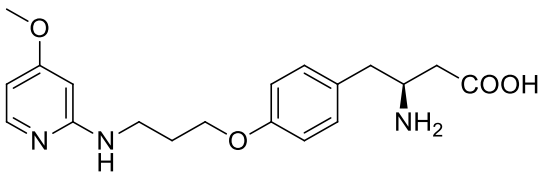
GP5. *Cleavage of Peptide from Resin.* For complete cleavage, the resin was washed with DCM (3x) and treated three times with a 20% hexafluoroisopropanol (HFIP) solution in DCM at RT for 10 min. Afterwadrs the resin was washed with DCM (3x) and the solvent of the combined solutions was evaporated under reduced pressure.

Protected tetraphosphonate spacer-anchoring unit (5)



500 mg of TCP resin was loaded with compound 4-[3-(((allyloxy)carbonyl)amino)propoxy]benzoic acid[1] according to **GP1**. After alloc deprotection (**GP2**), 8-(Fmoc-amino)-3,6-dioxaoctanoic acid was coupled to the resin as described in **GP3** and the Fmoc group subsequently removed according to **GP4**. The coupling and Fmoc-deprotection of the short PEG-amino acid was repeated successively two more times. Then, Fmoc-L-Lys(Fmoc)-OH was coupled (**GP3**) to the resin-bound spacer fragment and deprotected (**GP4**). These two steps were repeated a second time with twice the equivalents of Fmoc-L-Lys(Fmoc)-OH described in **GP3**. Next, the Fmoc group was removed and 3-(bis(benzyloxy)phosphoryl)propanoic acid (4 eq.) was coupled to the resin. Finally, compound **5** was cleaved from the resin according to **GP5**. **RP-HPLC** (10-90%, 5 min) R_t = 4.63 min. **MS** (ESI): m/z = 1141.09 $[(M+2H)/2]^+$.

(S)-3-amino-4-(4-(3-((4-methoxypyridin-2-yl)amino)propoxy)phenyl)butanoic acid (6)



Compound **6** was derived from the αvβ3 peptidomimetic fragment, (S)-methyl-3-(tert-butoxycarbonylamino)-4-(4-(3-(4-methoxypyridin-2-ylamino)-propoxy)phenyl)butanoate,[1] after deprotection of the Boc protecting group and hydrolsysi of the methyl ester. To this end, the αvβ3 peptidomimetic fragment (60 mg, 0.127 mmol, 1 eq.) was dissolved in a solution of MeOH/H_2O (3:1), and next LiOH (5 eq.) was added to the solution. The deprotection progress was monitored via mass spectrometry. After complete Boc deprotection, the crude product was dissolved in 5 mL DCM and 5 mL

TFA and is stirred for 6 h (control via mass spectrometry). After full deprotection the solvents were removed under reduced pressure and co-evaporated with toluene. **RP-HPLC** (10-90%, 8 min) R_t = 4.72 min. **MS** (ESI): m/z = 360.23 $[M+H]^+$.

Peptidomimetic 3

The protected tetraphosphonate spacer-anchoring unit **5** (78.6 mg, 34.5 µmol, 1.3 eq.) was dissolved in 1 mL DMF, and HATU (13.1 mg, 34.5 µmol, 1.3 eq.) and DIEA (13.1 µL, 0.13 mmol, 5 eq.) were added to the solution. After 1 h pre-activation, compound **6** (9.57 mg, 26.6 µmol, 1.0 eq.), dissolved in 1 mL DMF, was added to the solution and the reaction left under continuous stirring overnight. The benzyl protected product was purified via HPLC (yield 11.47 mg), followed by benzyl deprotection with a mixture of TFA/TIPS/H_2O (v/v/v, 95/2.5/2.5) for 2 h. Product **3** was finally precipitated with ether, washed and lyophilized. **RP-HPLC** (5-95%, 5 min) R_t = 2.02 min. **MS** (ESI): m/z = 634.60 $[(M+3H)/3]^+$ and m/z = 951.17 $[(M+2H)/2]^+$.

3. Ti surface functionalization and characterization

Ti disks were obtained by turning cylindrical Grade 2 commercially pure (CP) bars (10 mm in diameter, Technalloy S.A., Sant Cugat del Vallès, Spain). Polishing with silicon carbide grinding papers (Neuertek S.A., Eibar and Beortek S.A., Asua-Erandio, Spain) and with suspension of alumina particles (1 µm and 0.05 µm particle size) on cotton clothes was carried out to achieve a smooth mirror-like finish of the surface. The average roughness (Ra) of the samples was measured by white light interferometry and was below 20 nm. After ultrasonically rinsing with cyclohexane, isopropanol, distilled water, ethanol, and acetone, samples were passivated with 65% (v/v) HNO_3 for 1 h.

In vitro assays: Ti disks were silanized by immersion in 2% (v/v) APTES (3-(aminopropyl)-triethoxysilane, Sigma-Aldrich, St Louis, MO, USA) in anhydrous toluene (Sigma-Aldrich) under agitation at 70 ^{0}C for 1 h under nitrogen atmosphere. After rinsing samples with toluene, distilled water, ethanol, and acetone, a curing of the silane layer was performed at 120 ^{0}C for 5 min. To couple the crosslinking agent N-succinimidyl-3-maleimidopropionate (SMP) (Alfa Aesar, Karlsruhe, Germany), disks were immersed in a 7.5 M solution of SMP in DMF under agitation for 1 h at RT. Finally, the peptidomimetics with thiol anchor were immobilized on Ti surfaces by dissolving the biomolecules in phosphate buffered saline (PBS) at 100 µM and pH 6.5, and then depositing 100 µL of these solutions overnight on Ti disks at RT. VN and FN (both from Sigma-Aldrich) were used as positive controls and coated on silanized Ti at 50 µg/mL in PBS at pH 9.5. Uncoated polished Ti disks were selected as negative controls (Ti). This process has been widely used and optimized in previous reports [3-7] and is summarized in **Figure S2a**. The attachment of the two peptidomimetics on Ti via silanization was studied in another work by contact angle, X-ray photoelectron

spectroscopy (XPS) and fluorescence microscopy, showing comparable coating efficiency for both compounds (**Table S1**).[5]

To further characterize the peptidomimetic layer, the attachment of the two compounds was studied by quartz crystal microbalance with dissipation monitoring (QCM-D). To this end, Ti-sputtered gold sensors (QSX 310, Q-Sense, Sweden) were first silanized as described above with the only difference that the curing step was not done to avoid damaging of the disks. Peptidomimetic attachment was then measured using a D300 QCM-D equipment (Q-Sense) by monitoring changes in resonance frequency, Δf, and dissipation, ΔD, in real time (Qsoft software, Q-Sense). The sensors were first stabilized with PBS at pH 6.5 at 25 ºC, and the peptidomimetics subsequently injected at 100 μM (in PBS at pH 6.5) and kept in the sensor chamber for 60 min. Raw data (Δf) was analyzed using QTools software (Q-Sense) using the Sauerbrey model ($\Delta D < 1 \times 10^6$) as described elsewhere.[8] QCM data as layer thickness (nm) and density of molecule attached (pmol/cm^2) are shown in **Table S1**.

In vivo assays: In this case, peptidomimetics with phosphonic acid anchors were directly deposited on Ti samples (100 μL solutions at 100 μM in PBS, overnight, RT) (**Figure S2b**). The use of phosphonic acids to attach biomolecules on Ti has been reported and optimized by us in previous studies.[2,9,10] Binding to Ti was analyzed using an XPS equipment (SPECS Surface Nano Analysis GmbH, Berlin, Germany) with a Mg anode XR50 source operating at 150 W and a Phoibos 150 MCD-9 detector. XPS characterization is shown in **Figure S5**. The attachment of the molecules was also analyzed by QCM-D using non-silanized disks and fitting the data to a Voigt viscoelastic model ($\Delta D > 1 \times 10^6$) [8] (**Table S2**).

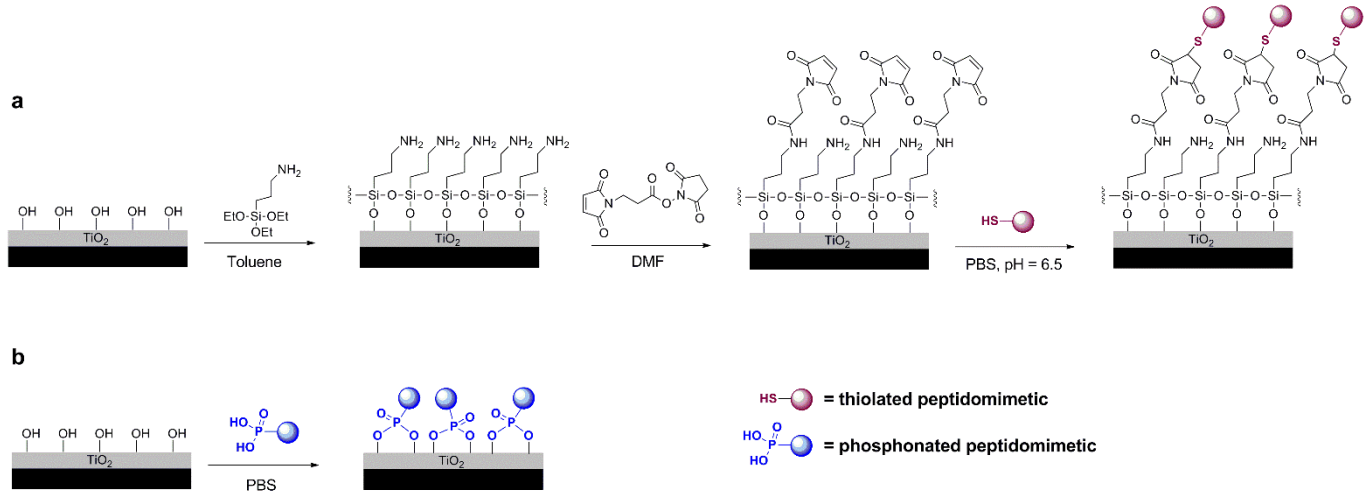


Figure S2. Strategies of surface functionalization. (a) Covalent attachment via silanization and addition of a maleimide containing crosslinker. The mimetic is bound via the thiol group (Michael addition). (b) Direct chemisorption to Ti dioxide through phosphonic acids.

4. Cell culture

All reagents were purchased from Sigma-Aldrich, unless otherwise noted. Human MSCs (hMSCs) (SCR 108, Merck Millipore) were cultured in Advanced Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% (v/v) fetal bovine serum (FBS), 50 U/mL penicillin, 50 µg/mL streptomycin and 1% (w/v) L-glutamine. Cells were maintained at 37 °C, in a humidified atmosphere containing 5% (v/v) CO_2 and culture medium was changed twice a week. Upon reaching 70 % confluence, cells were detached by trypsin-EDTA and subcultured into a new flask. Cells at passages between 1 and 4 were used to carry out all the experiments. Prior to seeding, samples were blocked in 1% (w/v) bovine serum albumin (BSA) for 30 min at RT to avoid non-specific protein adsorption and rinsed with PBS.

5. Cell adhesion and proliferation

Cell immunostaining: To evaluate cell adhesion, hMSCs were plated at 10^4 cells/mL and incubated for 6 h in serum-free medium at 37 °C and 5% (v/v) CO_2 containing atmosphere. Immunofluorescent staining of cell nuclei and actin fibers was performed to study the number, area and morphology of attached cells. After the incubation period, non-adherent cells were removed by gently washing samples with PBS, and adherent hMSCs were fixed with paraformaldehyde (PFA, 4% w/v in PBS) for 20 min, and permeabilized with 500 µL/disk of 0.05% (w/v) Triton X-100 in PBS for 20 min. The surface was then blocked with 1% BSA (w/v) in PBS for 30 min, and actin fibers and nuclei were stained by incubating with rhodamine-conjugated phalloidin (1:300, in Triton 0.05% (w/v) in PBS) for 1 h and with 4',6-diamidino-2-phenylindole (DAPI) (1:1000, in PBS-Glycine 20 mM) for 2 min at RT in the dark, respectively. Samples were rinsed three times with PBS-Glycine for 5 min between each step of the staining procedure. Ti disks were examined under a fluorescence inverted microscope (AF7000, Leica, Germany), and quantification of nuclei and cell projected area was done with the Fiji/Image-J package.

Cell proliferation: To study proliferation of cells on the substrates, 6×10^3 cells/mL were plated on samples in serum-free medium and incubated as previously explained. 6-hours post seeding, medium was aspired and replaced with complete medium. After 3, 6, and 8 days of incubation, cell number was evaluated with the Alamar Blue assay (Invitrogen Life Technologies, Merelbeke, Belgium): briefly, Alamar Blue-containing medium (10% (v/v)) was added for 1 h, and fluorescence of the dye was quantified according to the manufacturer instructions with a multimode microplate reader (Infinite M200 PRO, Tecan GroupLtd., Männedorf, Switzerland).

6. Cell differentiation

RT-PCR: Gene expression was evaluated by RT-PCR analysis. 10^4 cells/well were plated on metallic disks and cultured for 7 days in basal medium. At harvest, cells were lysed and total RNA was extracted using RNeasy® Mini Kit (Qiagen, Hilden, Germany), according to manufacturer instructions. Total RNA was quantified with NanoDrop ND-1000 spectrophotometer (Thermo Scientific, Waltham, MA, USA). 200 ng of RNA were reverse transcripted to cDNA with QuantiTect Reverse Transcription Kit (Qiagen). A StepOnePlus Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) with QuantiTect SYBR Green RT-PCR Kit (Qiagen) and gene-specific primers for runt-related transcription factor 2 (RUNX2 – primer sequences: FW: CGGAATGCCTCTGCTGTTAT, RV: TGGGGAGGATTTGTGAAGAC) and osteocalcin (OCN - primer sequences: FW: ATGAGAGCCCTCACACTCCT, RV: CTTGGACACAAAGGCTGCAC) were used, doing a 5 min incubation at 95 °C and 40 amplification cycles (10 sec at 95 °C and 30 sec at 60 °C), followed by a melt curve. Melting curve analysis was done to prove specificity and gene expression was normalized to β-ACTIN (primer sequences: FW: AGAGCTACGAGCTGCCTGAC, RV: CGTGGATGCCACAGGACT).

Western blot: hMSCs were plated at a concentration of 3×10^5 cells/mL in basal serum-free medium and incubated for 6 hours. Medium was aspired 6 h post-seeding and FBS-supplemented medium was added. Medium was changed every 2-3 days. On days 7 and 18, cells were washed with PBS and detached by trypsin-EDTA for protein isolation. The pellet was then resuspended with lysis buffer, containing 20 mM TRIS, 5 mM EDTA, 1% NP40 (IGEPAL CA-630), 150 mM NaCl, pH 7.4, and supplemented with Pierce Protease Inhibitor tablets (#88266, Thermo Fisher Scientific) and with Pierce Phosphatase Inhibitor Mini tablets (#88667, Thermo Fisher Scientific). Extracts were then sonicated and centrifuged at 14,000 rpm for 15 min at 4 °C. The supernatants were collected and the protein concentration was quantified by Bradford assay (BioRad). Next, for Western blot analysis protein samples were denatured at 90 °C for 5 min and 30 μg of total protein was separated by 4–15% precast polyacrylamide gel (BioRad) and transferred to PVDF membranes (Immobilon-P, Millipore). After blocking with 5% non-fat dry milk, the membranes were incubated overnight at 4ºC with the following primary antibodies: RUNX2 (Santa Cruz Biotechnologies), OCN (Santa Cruz Biotechnologies) and β-ACTIN (Sigma-Aldrich). After extensive washing, membranes were incubated with horseradish peroxidase (HRP) conjugated anti-mouse and anti-rabbit IgG secondary antibodies (Jackson Laboratories), 1:20000 dilutions, for 1 h at RT. After additional washes, the membranes were examined using Luminata Forte Western HRP Substrate (Millipore) following manufacturer's instructions, and images were taken with GeneSnap (Syngene). The density of the bands was analyzed using the Image Studio Lite (Li-Cor) software and normalized to the β-ACTIN signal.

7. *In vivo* implantation

To test the ability of the biomolecule coating to induce bone growth *in vivo*, a rat partial thickness calvarial defect was used. All animal procedures (housing, care and experimentation) were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of Dankook University and approved by the Animal Ethics Committee of Dankook University Institutional Animal Care & Use Committee, Republic of Korea. Eighteen 11 week-old, 250-300 g healthy male Sprague-Dawley rats were used. Animals were acclimatized for 7 days before implantation, and each rat was housed in a separate cage under temperature- and humidity-controlled environment, exposed to a 12 h light-dark cycle, and had free access to water and food. Custom-made rod-type Ti implants (5.5 mm diameter, 5 mm long) were prepared and functionalized by immersion in the mimetics solution. After coating, samples were washed three times in sterile water and sterilized by incubating in 70% ethanol for 30 min. Implant placement was performed under general anesthesia using an intramuscular injection of a mixture of ketamine (80 mg/kg) and xylazine (10 mg/kg). The animals were randomly allocated to one of three groups before implantation (n=6): Ti as the control group, αvβ3-selective mimetic-coated Ti, and α5β1-selective mimetic-coated Ti as study groups. After shaving over the cranial lesion, the surgical site was scrubbed with iodine and 70% ethanol, and a linear skin incision was made. A full thickness flap was retracted and the calvarial bone was exposed. Two 5.5 mm diameter partial thickness calvarial bone defect were prepared in each rat on each side of the parietal bone under cooling conditions with sterile saline buffer, using a dental handpiece and a 5.5 mm diameter LS-Reamer (Neobiotech, Seoul, South Korea). The implants were covered by 3D printed rigid polymer caps and secured to the calvarial bone using fixation screws via its anchoring rings. Rigid polymer caps (5.5 mm inner diameter and 5 mm height) were custom-made (Taulman 618 Nylon, Taulman 3D, Missouri, US) by 3D printing (NP-Mendel, Opencreators, South Korea). The subcutaneous tissues and periosteum was sutured with absorbable sutures (4-0 Vicryl®, Ethicon, Germany), and the skin was closed with non-absorbable suture material (4-0 Prolene, Ethicon, Germany). The animals were monitored daily for possible clinical signs of infection, inflammation, and any adverse reaction. After two and four weeks, the animals were euthanized by CO_2 inhalation and the tissue part of the calvarium surrounding the cap was harvested and fixed in 10% neutral buffered formalin for 24 hours at RT. The samples were then decalcified with RapidCalTM solution (BBC Chemical Co., Stanwood, WA), dehydrated and embedded in paraffin using standard procedures. Five serial sections (5 μm) were cut at the central of the defects, and the deparaffinized sections were subjected to hematoxylin & eosin (H&E) stain, and then imaged using a light microscope.

8. Statistical analysis

Statistical comparisons were based on analysis of variance (ANOVA) with Tukey post hoc test for pairwise comparisons. Results are presented as mean + SEM. All *in vitro* cellular assays were done using 3 replicates per condition, and repeated at least in 2 independent experiments.

9. Characterization of the functionalization via silanization (Table S1)

The attachment of the peptidomimetics via silanization (αvβ3-s and α5β1-s) was characterized in a previous study by means of contact angle measurements, fluorescent labeling and XPS.[5] The thickness and density of the peptidomimetic layer was further characterized using QCM-D, as described above (section 3). The physicochemical characterization of the surfaces is summarized in Table S1.

Table S1

Condition	CA (º)	SE (mN/m)	Ti 2p (%)	Si 2p (%)	N 1s (%)	N/Si	FI (a.u.)	Thickness (nm)	Density (pmol/cm^2)
Ti	66.8 ± 3.5	47.5 ± 2.2	16.0 ± 0.8	0.1 ± 0.1	0.5 ± 0.1	-	0.01	-	-
APTES	65.2 ± 0.5	49.6 ± 0.1	7.2 ± 1.4	7.3 ± 1.2	6.1 ± 0.6	0.83	-	-	-
αvβ3-s	46.2 ± 4.5	60.8 ± 1.8	5.3 ± 1.2	4.5 ± 0.6	6.5 ± 0.7	1.44	0.11	0.43	70 ± 4
α5β1-s	44.0 ± 6.2	61.1 ± 3.3	6.1 ± 0.8	4.5 ± 0.7	6.5 ± 0.7	1.43	0.10	0.41	68 ± 5

CA, contact angle; SE, surface Energy; FI, fluorescence intensity; a.u., arbitrary units

10. Cell proliferation and gene expression (Figure S3)

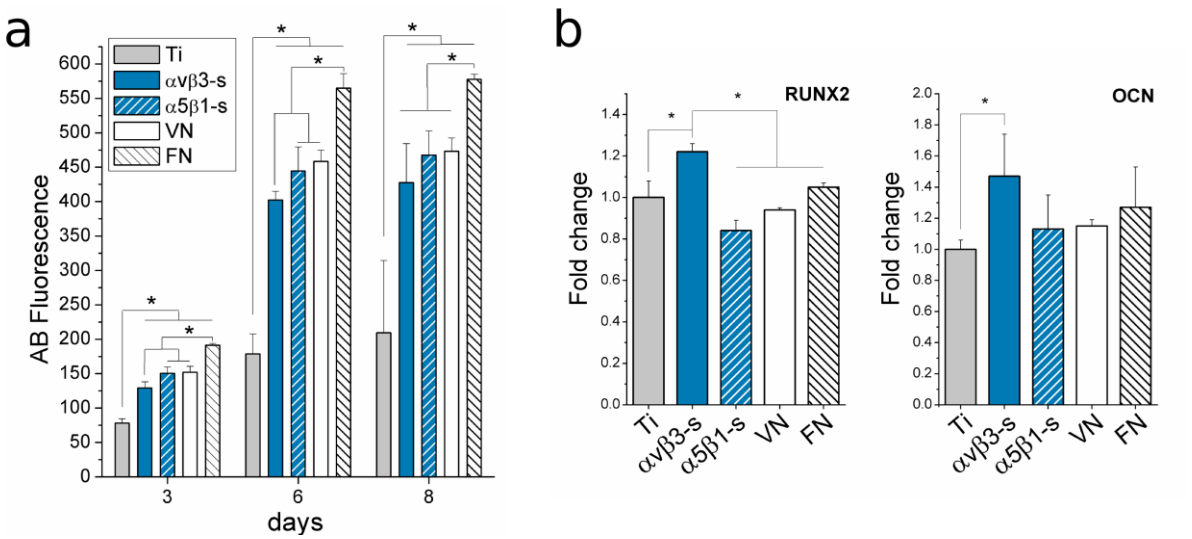


Figure S3. (a) Alamar blue assay of cell proliferation indicates that cell growth follows this trend: FN>VN~α5β1-s>αvβ3-s>Ti. * means $p<0.01$. (b) Expression of osteogenic markers RUNX2 and OCN on the functionalized substrates. Fold change is relative to Ti. Increased expression is observed on the αvβ3-selective mimetic for both genes, compared to Ti. * means $p<0.05$.

11. Western blot analysis (Figure S4)

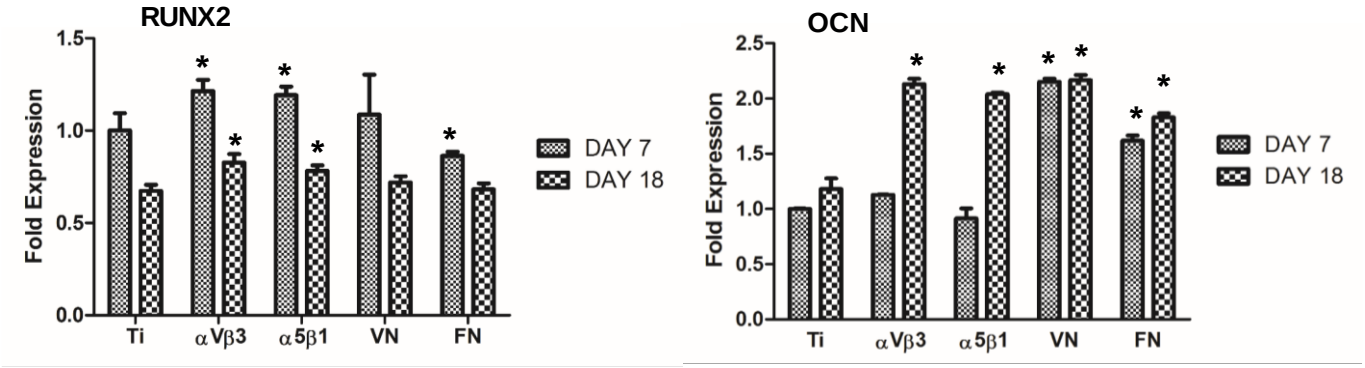


Figure S4. Protein fold expression of the osteogenic markers RUNX2 and OCN on hMSCs seeded on functionalized samples, analyzed by western blot analysis after 7 and 18 days of incubation. Fold change is relative to Ti at day 7. The expression of RUNX2 was significantly increased by the peptidomimetics (at days 7 and 18) and FN (at day 7), but not by VN. OCN expression was also significantly increased by the peptidomimetics, but only at day 18, and by the ECM proteins at both time points. * means $p<0.05$.

12. Characterization of the functionalization via chemisorption

XPS (Figure S5)

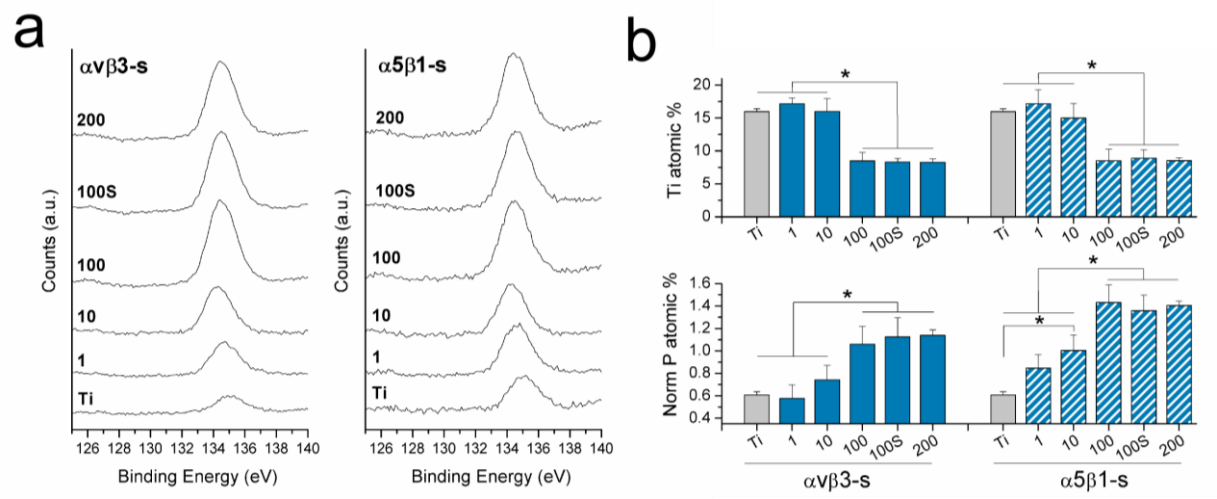


Figure S5. P 2p spectra (a) and Ti and P atomic percentage (b) of uncoated Ti (Ti) and mimetic-functionalized Ti at different coating concentrations (1 μM, 10 μM, 100 μM, 200 μM) and after an ultrasonication treatment to verify stability (100S). * means $p<0.05$. Atomic percentages of Ti and P decrease and increase, respectively, at increasing concentrations, reaching a plateau at 100 μM. Neither atomic percentages nor P spectra are modified by the ultrasonication treatment, confirming stability of the coating.

QCM-D analysis

QCM-D analysis was performed to measure the amount of peptidomimetic bound by chemisorption on the Ti substrates, yielding a comparable density of molecule attachment for both compounds after a thorough wash to remove non adherent molecules (Table S2). The higher density of peptidomimetic obtained with this method correlates well with the superior potential described for phosphonate anchors to bind Ti substrates, as previously described by us.[10]

Table S2

Condition	Density (pmol/cm^2)
αvβ3-s	561 ± 20
α5β1-s	502 ± 82

13. Summary of the histological observations (Table S3)

For each experimental condition tested *in vivo* (Ti, αvβ3-s and α5β1-s), replicates (n=6) were stratified according to the amount of new bone growth (increasing from grade I to V), as described in the classification.

Table S3

Grade	Classification	Number of samples (2 weeks)			Number of samples (4 weeks)		
		Ti	αvβ3-s	α5β1-s	Ti	αvβ3-s	α5β1-s
I	No bone	4	1	2	2	1	1
II	Minimal bone in only one side	1	1	1	2	0	1
III	Moderate bone in only one side	1	2	2	1	1	4
IV	Moderate bone in both sides	0	1	1	1	2	0
V	Substantial bone in both sides	0	1	0	0	2	0

14. Representative histological examples (Figure S6)

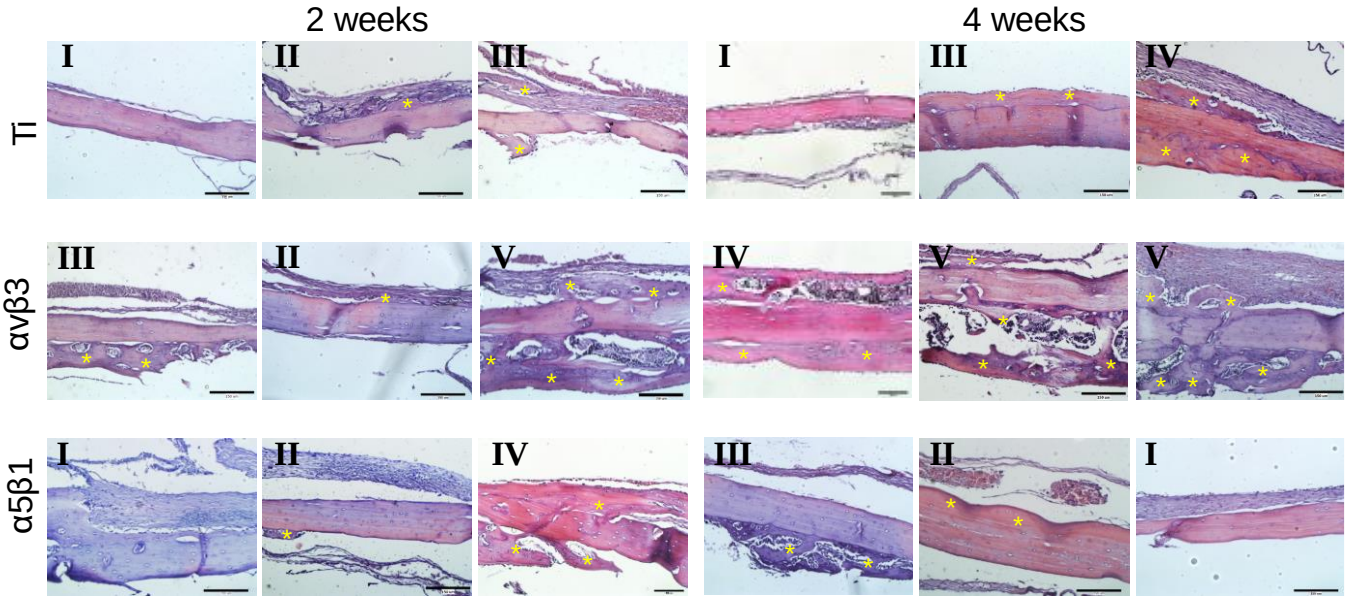


Figure S6. Representative histological examples for each condition at the two implantation periods. Each image indicates the extent of bone formation according to the grading (I to V) described in Table S3. Yellow stars indicate new bone. Scale bar = 150 µm.

15. Supplementary larger size histological image (Figure S7)

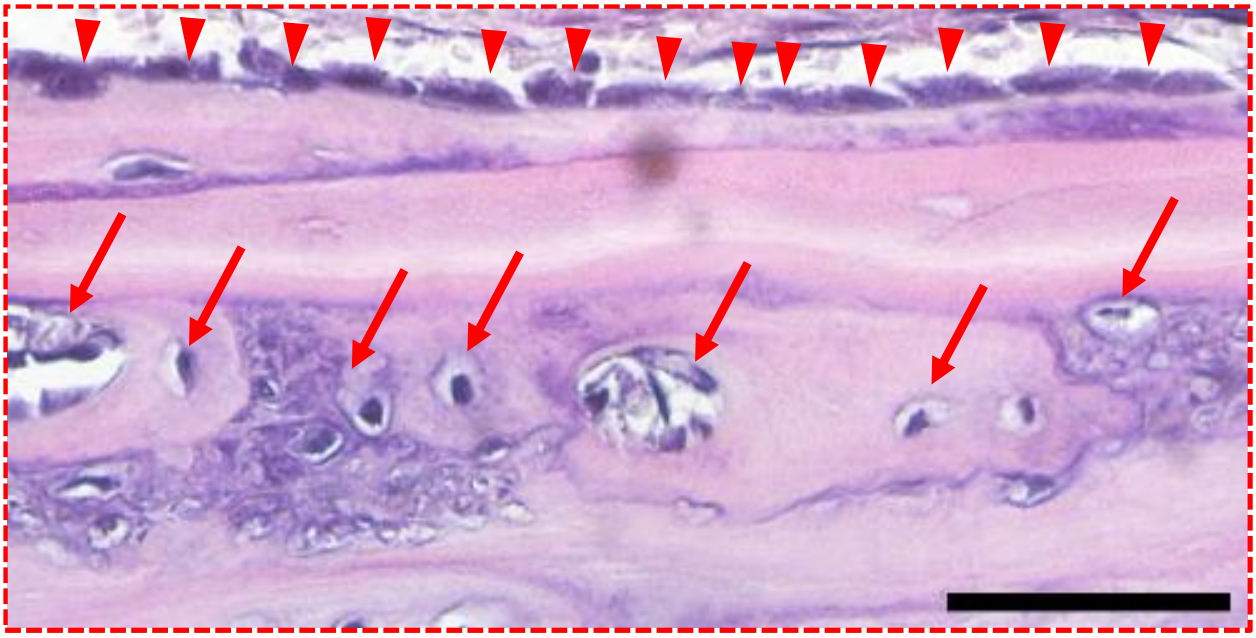


Figure S7. Larger size image of H&E staining of new bone formed on αvβ3-coated implant (inset in figure 3b). Red arrow heads indicate lining osteoblasts, while red arrows indicate lacunae with osteocyte(s) inside. This image represents a typical histological morphology of a woven bone structure (not lamellar bone).

16. References

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