

Supramolecular control of cell adhesion *via* ferrocene–cucurbit[7]uril host–guest binding on gold surfaces†

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Supramolecular control of adhesion of cells is demonstrated using synthetic integrin binding RGD peptide–ferrocene conjugates that were immobilized *via* host–guest chemistry onto cucurbit[7]uril coated gold surfaces.

Supramolecular construction processes provide innovative platforms for the study of fundamental aspects of cell biology, as a basis for the engineering of biomaterials with self-adaptive cell interface properties.¹ On surfaces, structured interfaces can be formed with molecular precision – on a scale most relevant to cellular function – through the spatial or temporal localization of ligands.^{2,3} With the appropriate combination of ligands, the surface functionality can be tuned to specifically manipulate and monitor cell adhesion and migration. This offers unique opportunities for the development of biomaterials for cell-growth engineering.⁴ The use of semi-synthetic proteins, growth factors or peptides in various patterns can direct adhesion and migration of *e.g.* endothelial cells. Supramolecular chemistry would allow for potentially reversible or stimuli-responsive surfaces with interesting properties,^{3,5} which are currently being integrated into biological systems^{6,7} and biomaterials with intended regenerative medicine applications.⁸ Here, important progress towards such supramolecular-based bio-interfaces is reported, based on the non-covalent immobilization of ferrocene-labelled cyclic RGD (Fc-cRGD) peptides onto gold surfaces coated with a CB[7] (cucurbit[7]uril) monolayer using host–guest interactions. Only surfaces featuring the

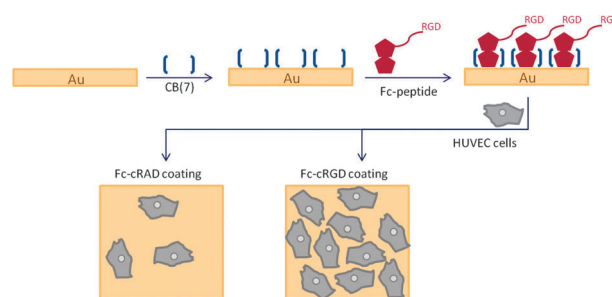


Fig. 1 Coating of gold surfaces with a CB[7] monolayer, pre-incubation with Fc-cRGD and reference compound Fc-cRAD, and subsequent endothelial cell adhesion to the supramolecularly functionalized surfaces.

correct supramolecular host–guest combination, displaying the RGD motif, enable enhanced molecular induced endothelial cell adhesion and wound recovery in a controlled manner.

To investigate supramolecular control of endothelial cell adhesion, the ferrocenylamine-CB[7] based host–guest system was selected (Fig. 1).^{9–11} The (ferrocenylmethyl)-trimethylammonium cation binds to CB[7] in solution with a K_d in the range of 10^{11} M^{-1} .¹² CB[7] spontaneously adsorbs onto gold surfaces and forms a stable self-assembled monolayer (SAM).^{13,14} Such supramolecular CB[7]-SAMs have been used for the site-selective immobilization of fluorescent proteins that were monovalently labelled with ferrocenylamine. Although the binding affinities to CB[7] SAMs might differ from solution, strong and reversible binding to CB[7]-coated gold surfaces was observed.¹⁵ This surface immobilization system bodes well for exploration of cellular applications such as cell adhesion studies, and is potentially attractive due to the ease of CB[7] SAM formation and the low toxicity of gold.

Crucial to the high affinity of this system is the amino functionality present in the ferrocene guest molecule. While the ferrocene ring system fills the lipophilic CB[7] cavity, the protonated amino functionality protrudes from the cavity, where it makes stabilizing electrostatic interactions with the polar carbonyl groups located at the CB[7] rim.¹² To enable studies of endothelial cell adhesion on such CB[7]-coated surfaces,

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RGD peptides^{16–19} were attached to the key ferrocenylamine moiety *via* an oligoethylene glycol (OEG)-based linker (**1**). The use of OEG was suitable for the envisioned purposes due to its biocompatibility and inertness to gold surfaces.²⁰ The choice of the linker length was based on previous investigations regarding the immobilization of ferrocenylamine-labelled fluorescent proteins onto CB[7]-SAMs.¹⁵

It was anticipated that a suitable strategy for attaching the ferrocenylamine guest molecule (**1**) to RGD peptides²¹ would require special consideration of the specific design of the guest molecule. Firstly, the ferrocenylamine group was initially developed for peptide synthesis as an amine protective group, and is typically cleaved under acidic conditions (TFA- β -thionaphthol-CH₂Cl₂, 2–4 h).²² Furthermore, compatibility with standard amide coupling chemistry²¹ would in all likelihood require further modification (at least one additional step) or laborious protection-deprotection steps of the ferrocenyl secondary amine functionality. Thus, an approach that introduced the ferrocenylamine guest into a fully unprotected peptide sequence was preferred to enable a divergent late stage attachment of the ferrocenylamine moiety.²³ Therefore a strategy based on copper-catalyzed azide-alkyne click chemistry²⁴ was chosen. The novel ferrocenylamine-PEG-azide **1** (Fig. 2) was readily prepared in one pot, *via in situ* imine formation followed by reduction using NaBH₄. Cyclic RGDFK, and RADFK²⁵ as reference, were synthesized using standard solid-phase peptide synthesis methods²⁶ and then coupled to pentynoic acid to form the alkyne derivatives **2** and **3**. Ligation of **1** to either **2** or **3** was achieved using CuSO₄ and sodium ascorbate in the presence of copper. This afforded the guest molecules Fc-cRAD (**4**) and Fc-cRGD (**5**), respectively, in useful yields after purification using reversed-phase-HPLC (ESI[†]).

CB[7] coated gold surfaces were prepared according to previous methods.^{11,13–15} Immobilization of the ferrocene-peptide conjugates onto the CB[7] coated surfaces was performed by immersion in aqueous solutions (50 μ M) of **4** or **5** for 3 h. After washing, the surfaces were characterized using

infrared reflection absorption spectroscopy (IR-RAS), water contact angle measurements (WCA) and X-ray photoelectron spectroscopy (XPS). The morphological properties of the CB[7]-coated gold surface monolayer have been previously described.¹⁴ Cyclic voltammetry (CV) measurements and dynamic force spectroscopy (DFS) suggested that CB[7] covers *ca.* 40–50% of the gold surface,^{13,14} and atomic force microscopy (AFM) showed that the surface adsorbed CB[7] molecules are accessible for the immobilization of ferrocene-labelled proteins *via* a specific monovalent CB[7]-ferrocene interaction.¹⁵ Here, the IR-RAS spectrum of the CB[7]-coated gold surface after incubation with Fc-cRGD **5** and subsequent blocking with (oligoethylene glycol)₆-thiol (OEG₆-SH) revealed additional peaks at 1670–1700 cm^{–1} (amide C=O bonds) and 1116 cm^{–1} (C–O–C), which are characteristic of the presence of the peptide-ferrocene conjugate and ethylene glycol, respectively (ESI[†]). The WCA of the CB[7]-SAMs increased from 32° to 58° upon incubation with Fc-cRGD **5**, which is indicative of the immobilization of the ferrocene-peptide conjugate and is in agreement with previously reported values for similar surfaces.¹⁵ XPS shows the expected C/N/O ratio of 3/2/1 for CB[7]-SAMs, and the appearance of an Fe signal upon incubation with Fc-cRGD **5** (ESI[†]). The surface characterization data thus demonstrate the successful immobilization of the ferrocene-peptide conjugates onto the CB[7]-SAMs *via* a ferrocene-CB[7] host-guest interaction.

Human umbilical vein endothelial cells (hUVECs) were used as a model system to study the cellular response to supramolecularly immobilized peptides **4** and **5**. Cells were seeded on CB[7]-SAMs using different chemical surface functionalizations, including blocking against unspecific interactions, and the cell response was monitored at different time points over a period of 48 h (Fig. 3a, 24 h, and Fig. S6–S9, ESI[†]). The blocking step using OEG₆-SH was evaluated at different steps in the surface preparation protocol. Addition of the short ethylene glycol chain directly after the incubation of the surface with the ferrocenylamine-peptides, *via* immersion in a 0.1 mM solution of OEG₆-SH for 2 min, was most effective (see ESI[†] for optimization of blocking conditions).

Supramolecular coating with Fc-cRGD **5** resulted in efficient adhesion and growth of hUVECs on the supramolecular surfaces as evidenced by the increase in the surface coverage and cell density after 24 h (Fig. 3a, (ii)). In contrast, surfaces pre-incubated with Fc-cRAD **4** showed less spreading of cells and a significantly reduced number (3-fold, ESI[†]) of adhered cells (Fig. 3a, (i)). Similarly, reference surfaces using bare gold (Fig. 3a, (iii)), CB[7]-only (Fig. 3a, (iv)), CB[7] with a ferrocenyl-PEG₃ linker or only **5** (ESI[†]) showed only minor cell adhesion and a more round cell morphology. After 48 h, a closed monolayer of cells was formed on the surfaces with CB[7] and Fc-cRGD **5**, whereas this state could not be reached in any of the control experiments including surfaces with CB[7] and Fc-cRAD **4**. A live-dead assay confirmed that the hUVECs remain viable on CB[7]-SAMs incubated with Fc-cRGD **5** compared to control tissue culture plates (TCP) and fibronectin coated TCP (ESI[†]). A wound assay on surfaces with CB[7] and Fc-cRGD **5** showed full recovery of the cell monolayer within 8 h, indicating effective

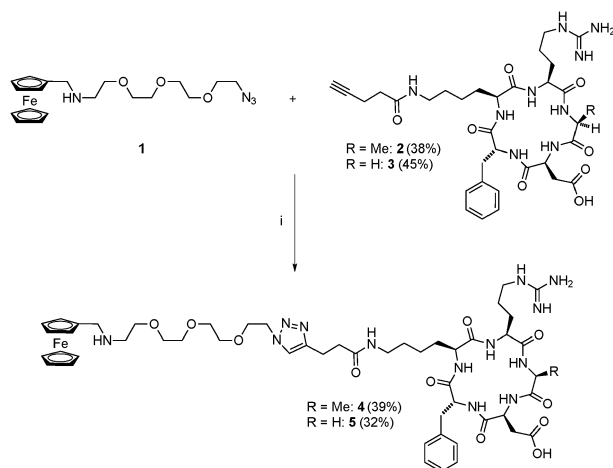


Fig. 2 Synthesis of ferrocene-peptide conjugates **4** and **5** *via* copper-catalyzed azide-alkyne cycloaddition of azide **1** to alkynes **2** or **3**. Reagents and conditions: (i) **1** (1.0 eq.), CuSO₄ (30 mol%), sodium ascorbate (50 mol%), copper ribbon, 40 °C, 24 h.

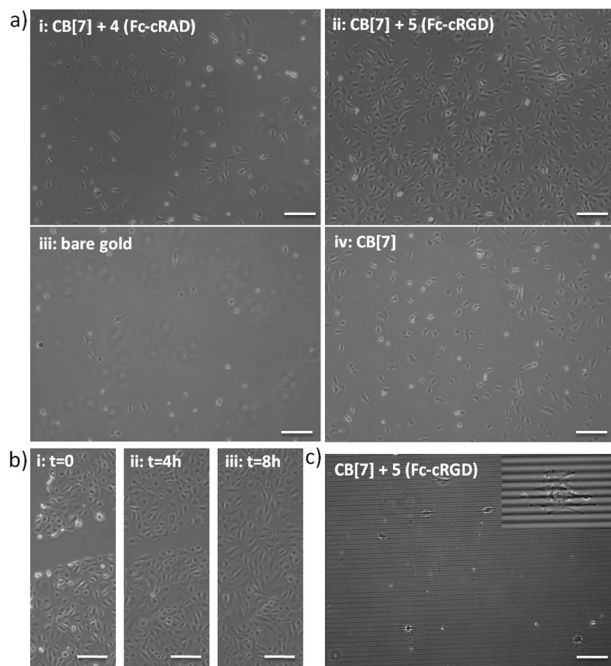


Fig. 3 Scale bar: 200 μm . (a) hUVECs seeded on gold surfaces pre-incubated with (i) CB[7] and 4, (ii) CB[7] and 5, (iii) only Au, (iv) CB[7], after 24 h proliferation. All surfaces were blocked with EG₆-SH as a last step. (b) Wound assay on a confluent monolayer of hUVECs on CB[7] and 5. (c) hUVECs seeded on a gold array functionalized with CB[7], Fc-RGD 5 and EG₆-SH, after 4 h proliferation, inset shows the magnified image.

direction of cell growth by the supramolecularly immobilized peptide epitopes (Fig. 3b).

To achieve patterned adhesion of hUVECs, glass substrates patterned with gold arrays were used.³ Prior to the functionalization of the gold arrays with CB[7], Fc-cRGD 5 and OEG₆SH as described above, the remaining glass areas were covalently blocked with polyethyleneglycol (ESI[†]). After seeding hUVECs for 4 h on these arrays the bright field images show that cells adhere specifically to the functionalized gold by having multiple contact points and mostly spreading over multiple gold stripes (Fig. 3c) whereas on the bare gold, the cells tended to align along single gold stripes (ESI[†]).

In conclusion, the ferrocene-CB[7] based host-guest system allows for supramolecular control of cell adhesion. The first example of spatial resolution of supramolecular cell adhesion using this system was demonstrated on a gold array. Combined with investigations into different surface types²⁷ and supramolecular approaches,^{3,28} such systems could lead to beneficial switching properties, building further on contemporary covalent methods^{29,30} by introducing reversibility and adaptability to surface immobilization through the chemical fine-tuning of the host-guest chemistry.

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Notes and references

- 1 M. P. Lutolf and J. A. Hubbell, *Nat. Biotechnol.*, 2005, **23**, 47.
- 2 R. N. Shah, N. A. Shah, M. M. Del Rosario Lim, C. Hsieh, G. Nuber and S. I. Stupp, *Proc. Natl. Acad. Sci. U. S. A.*, 2010, **107**, 3293.
- 3 Q. An, J. Brinkmann, S. Krabbenborg, J. de Boer and P. Jonkheijm, *Angew. Chem., Int. Ed.*, 2012, **51**, 12233.
- 4 M. J. Webber, J. A. Kessler and S. I. Stupp, *J. Intern. Med.*, 2010, **267**, 71.
- 5 L. Yang, A. Gomez-Casado, J. F. Young, H. D. Nguyen, J. Cabanas-Danés, J. Huskens, L. Brunsveld and P. Jonkheijm, *J. Am. Chem. Soc.*, 2012, **134**, 19199.
- 6 D. A. Uhlenheuer, K. Petkau and L. Brunsveld, *Chem. Soc. Rev.*, 2010, **39**, 2817.
- 7 Y.-H. Gong, C. Li, J. Yang, H.-Y. Wang, R.-X. Zhuo and X.-Z. Zhang, *Macromolecules*, 2011, **44**, 7499.
- 8 R. E. Kielyka, M. M. C. Bastings, G. C. van Almen, P. Besenius, E. W. L. Kemps and P. Y. W. Dankers, *Chem. Commun.*, 2012, **48**, 1452.
- 9 D. Sobransingh and A. E. Kaifer, *Chem. Commun.*, 2005, 5071.
- 10 E. Mason, X. Ling, R. Joseph, L. Kymereh-Mensah and X. Lu, *RSC Adv.*, 2012, **2**, 1213.
- 11 R. V. Pinjari and S. P. Gejji, *J. Phys. Chem.*, 2008, **112**, 12679.
- 12 S. Liu, C. Ruspici, P. Mukhopadhyay, S. Chakrabarti, P. Y. Zavalij and L. Isaacs, *J. Am. Chem. Soc.*, 2005, **127**, 15959.
- 13 Q. An, G. Li, C. Tao, Y. Li, Y. Wu and W. Zhang, *Chem. Commun.*, 2008, 1989.
- 14 A. Gomez Casado, P. Jonkheijm and J. Huskens, *Langmuir*, 2011, **27**, 11508.
- 15 J. F. Young, H. D. Nguyen, L. Yang, J. Huskens, P. Jonkheijm and L. Brunsveld, *ChemBioChem*, 2010, **11**, 180.
- 16 K.-E. Gottschalk and H. Kessler, *Angew. Chem., Int. Ed.*, 2002, **41**, 3767.
- 17 U. Hersel, C. Dahmen and H. Kessler, *Biomaterials*, 2003, **24**, 4385.
- 18 C. H. Yea, B. Lee, H. Kim, S. U. Kim, W. A. El-Said, J. Min, B. K. Oh and J. W. Choi, *Ultramicroscopy*, 2008, **108**, 1144.
- 19 S. P. Massia and J. A. Hubbell, *J. Cell Biol.*, 1991, **114**, 1089.
- 20 D. Coletti, F. A. Scaramuzzo, L. C. Montemiglio, A. Pristerà, L. Teodori, S. Admano and M. Berteri, *J. Biomed. Mater. Res., Part A*, 2008, **91**, 370.
- 21 S. D. Koster, J. Dittrich, G. Gasser, N. Husken, I. C. H. Castaneda, J. L. Jios, C. O. D. Vedova and N. Metzler-Nolte, *Organometallics*, 2008, **27**, 6326.
- 22 H. Eckert and C. Seidel, *Angew. Chem., Int. Ed. Engl.*, 1986, **98**, 168.
- 23 D. W. Lee, M. Park, M. Banerjee, S. H. Ha, T. Lee, K. Suh, S. Paul, H. Jung, J. Kim, N. Selvapalam, S. H. Ryu and K. Kim, *Nat. Chem.*, 2011, **3**, 154.
- 24 M. Meldal and C. W. Tornøe, *Chem. Rev.*, 2008, **108**, 2952.
- 25 G. J. Strijkers, E. Kluza, G. A. Van Tilborg, D. W. van der Schaft, A. W. Griffioen, W. J. Mulder and K. Nicolay, *Angiogenesis*, 2010, **13**, 161.
- 26 X. Dai, Z. Su and J. O. Liu, *Tetrahedron Lett.*, 2000, **41**, 6295.
- 27 I. Hwang, K. Baek, M. Jung, Y. Kim, K. M. Park, D.-W. Lee, N. Selvapalam and K. Kim, *J. Am. Chem. Soc.*, 2007, **129**, 4170.
- 28 A. Gonzalez-Campo, M. Brasch, D. A. Uhlenheuer, A. Gomez-Casado, L. Yang, L. Brunsveld, J. Huskens and P. Jonkheijm, *Langmuir*, 2012, **28**, 16364.
- 29 J. T. Koepsel and W. L. Murphy, *ChemBioChem*, 2012, **13**, 1717.
- 30 I. Choi and W.-S. Yeo, *ChemPhysChem*, 2013, **14**, 55.