



Introducing a combinatorial DNA-toolbox platform constituting defined protein-based biohybrid-materials

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ABSTRACT

The access to defined protein-based material systems is a major challenge in bionanotechnology and regenerative medicine. Exact control over sequence composition and modification is an important requirement for the intentional design of structure and function. Herein structural- and matrix proteins provide a great potential, but their large repetitive sequences pose a major challenge in their assembly. Here we introduce an integrative “one-vector-toolbox-platform” (OVTP) approach which is fast, efficient and reliable. The OVTP allows for the assembly, multimerization, intentional arrangement and direct translation of defined molecular DNA-tecton libraries, in combination with the selective functionalization of the yielded protein-tecton libraries. The diversity of the generated tectons ranges from elastin-, resilin, silk- to epitope sequence elements. OVTP comprises the expandability of modular biohybrid-materials via the assembly of defined multi-block domain genes and genetically encoded unnatural amino acids (UAA) for site-selective chemical modification. Thus, allowing for the modular combination of the protein-tecton library components and their functional expansion with chemical libraries via UAA functional groups with bioorthogonal reactivity. OVTP enables access to multitudes of defined protein-based biohybrid-materials for self-assembled superstructures such as nanoreactors and nanobiomaterials, e.g. for approaches in biotechnology and individualized regenerative medicine.

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1. Introduction

Challenging applications in material science, (bio)nanotechnology & regenerative medicine require molecular materials with

defined but adjustable properties ranging from the nano- to the macroscale. Controlling order, structure and function over this large dimensional space requires access to functionally & structurally programmable building blocks (tectons) [1–3].

Abbreviation: 2D, two-dimensional; 3D, three-dimensional; AA, amino acid; CL, cyclic-ligation; EbM, elastin based materials; ELP, elastin-like-protein; ECM, extracellular matrix; GdmCl, guanidinium chloride; hMSC, human mesenchymal stroma or stem cells; NCAM, neural cell adhesion molecule; NHDF, normal human dermal fibroblast; Ni-NTA, nickel - nitrilotriacetic acid; mEGFP, monomeric enhanced green fluorescent protein; pAzF, para-Azido-L-phenylalanine; PBO, protein-based vesicular organelle; PBS, phosphate buffered saline; OEPCR, overlap-extension polymerase chain reaction; OERCA, overlap-extension rolling circle amplification; ORI, origin of replication; OVTP, one-vector-toolbox platform; RCA, rolling circle amplification; SDL, sequential-directional ligation; SDS, sodium dodecylsulphate; SDS-PAGE, sodium dodecylsulphate polyacrylamide gel electrophoresis; SEM, standard error of the measurement; TEM, transmission electron microscopy; TCEP, tris(2-carboxyethyl)phosphine; THPTA, Tris-[1-(3-hydroxypropyl)-1H-[1,2,3]triazol-4-yl)methyl]amine; UAA, unnatural amino acid.

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Protein-based tectons constitute the most defined, efficient, reliable, flexible and functional molecular material toolbox in nature. Protein-based nanobiomaterials include the advantages of designability and precisely accessible molecules via blueprint-based biosynthesis. Standard synthetic methods in chemistry and biology are limited in accessing defined complex molecules, especially with large alternating repeating units and macromolecules with site-selective chemical modifications. Bio- and nanotechnology approaches have to overcome these restrictions [1,4], allowing to develop advanced hybrid-materials [5,6].

Structural proteins such as silk [7,8], resilin [9,10] and elastin [11–13] are suited for the engineering of materials with defined properties because of the strength, biointeractivity, resilience or elasticity of their native archetypes [8,14]. In order to fine tune and access new protein properties the sequence, the spatial position of hydrophobic and hydrophilic protein sequence blocks and the selective introduction of interaction sites, need to be encoded. Contemplatable sequences comprise homorepetitive and highly asymmetric sequence motives for the controlled formation of supramolecular structures. The allocation of a large variety of defined but tunable sequences as combinatorial libraries effectively provides adjustable biomaterials. Examples are protein-based vesicular organelles (PBOs) for drug formulation & synthetic biology, 3D-scaffolds with tunable elasticity and epitope presentation mimicking the extracellular matrix (ECM) for regenerative medicine approaches.

Current recombinant DNA-technology is optimized to access individual proteins for the intentional and rational design of new functional and structural materials [15,12,5]. Known strategies for the rational design of protein polymers via concatemerisation and recursive ligation techniques [16–21] providing defined sequences, but are limited if access to large numbers of diversified long sequences is required. Current combinatorial approaches using PCR based amplification strategies (e.g. OEPCR) or RCA (e.g. OERCA) [22,23] were developed with the focus on DNA-libraries with a statistical pool of structurally homogeneous sequences but different length. Thus, resulting repetitive genes can not achieve defined individual heterogeneous sequences required for the intentional design of new material libraries.

We present an approach creating complex but defined protein polymers in one unified system. Inspired from nature's principle of modularity [24] we developed the modular OVTP (Fig. 1A). This platform facilitates the generation of DNA-template entities (DNA-tectons) from the *de novo* synthesis, unimpaired by the grade of repetitive sequence motives. The facile implementation of structural and functional DNA-tectons derived from natural sequences and their free spatial arrangement allows to assemble defined composite sequences with the potential to create combinatorial libraries (Fig. 1B).

Several hundred DNA-tectons are generated so far. They are serving as DNA-templates for protein-tectons and subsequently as structural or functional building blocks for higher order structures *in vitro* or *in vivo* [1,3]. Furthermore, the OVTP permits the direct expression of all DNA-tectons and the integration of post-translational elements for subsequential combination or modification of the yielded tectons. Site-selective incorporation of unnatural amino acids (UAA) [25] enables the supplementation of protein-tectons specifically with synthetic molecules (chemical-tectons) (Fig. 1A & B). Thus, a protein-tecton-library can be further modified with chemical-tectons towards protein-hybrid tecton libraries (Fig. 1B, Supplementary Fig. 1).

Challenging applications for resulting complex protein-hybrid nanomaterials include individualized ECM-mimicry that is presented below. They could be used as environment for the differentiation of multipotent primary cells, a major challenge in

personalized medical approaches, or for the formation of complex vesicular assemblies with site-selective chemical functionalizations for biopharmaceutical and biotechnological applications. The OVTP could fill an apparent gap between the bioderived and the chemically synthesized biomaterial world. We believe that it will play an important role in the reliable and efficient rational production of defined, programmable composite 2D and 3D protein biomaterials for biomedical and pharmaceutical applications in future.

2. Methods and materials

2.1. Vectors and linker-regions for the modular one-vector-toolbox platform (OVTP)

Major components of the modular OVTP are vectors consisting of an adapted vector-backbone and a defined linker-region. Within the linker-regions the localization of the recognition sequences of the type IIS restriction enzymes Earl (NEB, New England Biolabs) and BspQI (NEB) and the restriction enzyme SacI (NEB) as well as the generated overhangs are precisely defined (compare Fig. 2A). Preserving these sequences for all platform vectors will permit their compatibility to each other. The type IIS restriction enzymes are cutting next to their recognition sequences and therefore allow for seamless cloning of constructs into the linker regions. The configuration of the flanking sequences between the NcoI site upstream and the MfeI site downstream is variable and characteristic for each of the different linker-regions (MCL, NMCL, NMCysL, NMBL, NMBXL, (TAG)NMBL, NMCysL (TAG); Supplementary Table 3). It is crucial for the OVTP that the restriction enzyme recognition sequences in the linker-module were absent in the vector backbone. Therefore, we removed all redundant restriction enzyme recognition sequences by site-directed mutagenesis [26] from the vector backbones of the OVTP vectors (Table 1A, Supplementary Table 1A).

As vector backbones we adapt a set of former expression vectors: the IPTG inducible pET28-vector backbone (Novagen), the constitutively expressing pIBHisC-vector backbone (Invitrogen/Life Technologies) and a vector backbone consisting of a completely reconstituted pEVOL vector [27] with an arabinose inducible promoter, a chloramphenicol resistance and the 15A ORI (Supplementary Materials section in Supplementary Data). The pET28-MCL vectors (or linker variants) are used for all cloning purposes (insertion, multimerization and rearrangement) and IPTG inducible protein expression in *Escherichia coli*. The pIB-NMCL vectors are used for cloning purposes in *E. coli* and for protein expression in insect cells (Sf9, HighFive; Invitrogen). The pBAD15a-NMBL vector is used for all cloning purposes and protein expression in *E. coli*. Because of its different ori it can be co-expressed with pET28-based OVTP vectors (Supplementary Table 1A).

2.2. DNA-tecton, protein-tecton, chemical-tecton

In synthetic biology basic units starting from natural amino acids up to synthetic molecules are linked together to build programmable tectons. These tectons can self-assemble to higher order units and beyond to form complex functional and structural assemblies [3]. Based on this specification we denominate the modular functional & structural programmable building blocks of the OVTP as DNA-tectons (DNA-template units of different length, complexity and determination), protein-tectons (protein units from the respective DNA-tectons, which could be further assembled to higher order structures via non-covalent or covalent interactions) and chemical-tectons (chemically synthesized (macro)molecules that could be conjugated to protein-tectons with UAA via bioorthogonal reactivities). The OVTP vectors are DNA-tecton harbouring vectors with a special integrated linker-region (Fig. 2A, Supplementary Table 3).

2.3. Cells and host organisms for the cloning of DNA-tectons and the expression of protein-tectons

All cloning steps such as monomer-integration, multimerization and arrangement of DNA-tectons were performed in *E. coli* DH5 α , XL1-blue, TOP10 strains (Invitrogen/Life Technologies). For the expression of DNA-tectons in pET28-vectors the OVTP vectors have to be transformed into *E. coli* BL21(DE3), BLR (from Novagen) or ER2566 (NEB) cells. DNA-tectons integrated in the pIB-vector could be expressed in lepidopteran insect cells (HiFive or Sf9; Invitrogen/Life Technologies). DNA-tectons in pMAV4rc-vector were expressed in *Physcomitrella patens* or *Arabidopsis thaliana* protoplasts. pBAD15a-vector constructs were expressed in *E. coli* DH5 α , BL21(DE3), BLR and ER2566 cells.

2.4. DNA- and amino acid sequences of selected DNA- and protein-tectons

The constructs used in this study are presented in Table 1. For some vectors with individual DNA-tectons the overall sequences are displayed in the Supplementary Materials section of Supplementary Data.

For the *de novo* synthesis and multimerisation of elastin-like-protein (ELP) variants we ordered chemically synthesized short single-stranded oligonucleotides from Invitrogen/Life Technology. The single-stranded oligonucleotides for monomers of DNA-tectons used for *de novo* generation of multimers via CL and SDL process are the following:

1. ELP-monomer (VPGVG, here: GVPGV) (ssOligonucleotides - upper and lower strand):
5'GGTGTTCGGGTGTT 3', 5'ACCAACACCCGAAC3'
ELP monomer (amino acid sequence, one letter code, from N→C):
GVPGV
2. Recombinant resilin monomer (ssOligonucleotides - upper and lower strand):
5'GGTGTTCGACCTTCTGATTCTTACGGTGCTCTGGTGGTGAAT3'
5'ACCATTACCACCAGGAGCACCCTAAGAATCAGAAGGTCGAC3'
Recombinant resilin monomer (amino acid sequence, one letter code, from N→C):
GGRPSDSYGAPGGN
3. Recombinant spider silk monomer (ssOligonucleotides - upper and lower strand):
5'GGTGTGGTGGTCTGGGTGGTCAAGGCTGCTGCTGCTGCTGCTA-
TGGGTGGTCTGGTCAAGGCTGGTACGGTGGTCTGGTCTGCTGCTGCTA-
5'ACCAGAGGTACCTGAGAACCCAGACCCGTAACCACTGACCAAGCAGCACC-
CCATAGCAGCAGCAGCAGCATACCAGCACCCTGACCAAGCAGCAGCAGC3'
Recombinant spider silk monomer (amino acid sequence, one letter code, from N→C):
GRGGLGGQAGMAAAAMGGAGQGQYGLGSQGS
4. FibronectinCS5-NG monomer (LifeTechnologies):
5'GTTTGGGTGGTGGTGAAGAAATCCAGATCGGTACATCCCGCTGAAGACG-
TTGACTACCACCTGTACCCGAAC3'

5'ACCGTTCGGGTACAGGTGGTAGTCAACGCTTCACGCGGGATGTGACCGATCT-
GGATTCTTCACCAACCCGAAC3'
FibronectinCS5-NG (amino acid sequence, one letter code, from N→C):
CGGGGEEIQGHIPREDVDYHLYPN

2.5. General recombinant DNA-technology, procedures and materials

General molecular biological procedures for growth and induction of bacterial cultures, DNA manipulation, transformation conditions of chemical competent *E. coli* cells and preparation of chemical competent cells were performed with standard methods as described in the literature [26]. Standard analytical and preparative DNA and protein techniques were performed as described in the literature [26] or described in the instructions provided by the manufacturers if not described otherwise. The required standard solutions and materials were prepared as described in literature [26].

2.6. Generation of DNA-tecton monomers from de novo

As an example for the *de novo* synthesis of DNA-tectons we used the elastin-like-proteins (ELP) monomers constituting the repetitive ELP pentameric peptide sequence (e.g. VPGVG). The DNA-monomers for subsequent multimerization are

Table 1

Vectors, linker-cassettes and DNA-tectons for the modular OVTP. A. Rebuilt and adapted vectors for the modular toolbox platform. I. Platform compatible and adapted vector backbones derived from pET28b vector (Novagen). II. Compatible “helper”-vectors used to remove redundant or objectionable 5' and 3' terminal base pairs from DNA-tectons to access *de novo* generated protein-tectons without any additional N- or C-terminal amino acids. III. Platform compatible vector with inserted TAG (amber) stop-codon for incorporation of an unnatural amino acid (UAA) into the resulting protein-tectons. B. Summary of DNA-tectons and resulting protein-tectons used in this study. I. Subset of different DNA-tecton multimers (*n* = repeat number) generated *de novo* presented in Fig. 3. A subset of these constructs are presented in Fig. 4 as expressed protein-tectons as well. II. C-terminal Cys-functionalised ELP constructs presented in Fig. 3 as DNA-tectons and in Fig. 4 as protein-tectons. III. Heteropolymeric constructs used as different structural protein-tectons for surface coatings shown in Fig. 5 performing cell adhesion and cellular growth assays. IV. Construct used for the incorporation of site-specific UAA on protein level. The chemically modified protein was used for *in vitro* studies labelling self-assembling 3D-structures as demonstrated in Fig. 4C. V. Three different variants of cellular recognition sequences (fibronectinCS5, RGD sequence) are presented. Spaced by either chemical or proteolytic AA recognition sequences (NG for chemical hydroxylamine cleavage or IEGR for Factor Xa recognition). Proteins are shown in Fig. 4A,II and results of cell adhesion experiments are demonstrated in Fig. 5 and Supplementary Table 1.

A. Rebuilt and adapted vectors for the modular toolbox platform				
<i>I. Platform compatible cloning & expression vectors</i>				
pET28-MCL	Generation, arrangement and expression of DNA-tectons in <i>E. coli</i> ; linker-region with special equipment of unique restriction enzymes			
pET28-NMCL	Generation, arrangement and expression of DNA-tectons in <i>E. coli</i> ; linker-region with special equipment of unique restriction enzymes			
pET28-NMCysL	Generation, arrangement and expression of DNA-tectons in <i>E. coli</i> ; linker-region with special equipment of unique restriction enzymes			
<i>II. platform compatible cloning or expression vectors (special manipulation of tectons sequences)</i>				
pIB-ELPL	Removal of nucleotides encoding for abditable or disadvantageous N-terminal amino acids from DNA-tectons in <i>E. coli</i>			
pET28-NMBXL	Removal of nucleotides encoding for abditable or disadvantageous C-terminal amino acids from DNA-tectons in <i>E. coli</i>			
<i>III. platform compatible cloning and expression vector for the insertion of unnatural amino acids (UAA) into expressed protein-tectons</i>				
pET28-(TAG)NMBL	Properties as described for A. I. DNA-tecton vectors applicable for the N-terminal insertion of UAA into the expressed protein-tectons in <i>E. coli</i>			
B. Summary of DNA-tectons and resulting protein-tectons used in this study				
Vector - linker - DNA-tecton	Repeating unit	DNA-tecton	Protein-tecton	Structure and property of protein-tecton
<i>I. De novo generated DNA-tectons for structural homopolymeric protein-tectons</i>				
pET28-MCL-(VPGVG)n	n = 20, 40, 60, 80, 100	[V]n	Vn-His	ELP with weak hydrophobic AA V at the fourth pentamer position
pET28-MCL-(res)n	n = 12, 18, 24, 30	[res]n	(resilin)n	Multimeric recombinant resilin
pET28-MCL-(spisi)n	n = 10, 20, 40, 60, 80	[spisi]n	(spisi)n	Multimeric recombinant spider silk
<i>II. De novo generated DNA-tectons for homopolymeric protein-tectons with unique amino acid Cys provide a reactive sulfhydryl-side group</i>				
pET28-(VPGVG)n(VPGCG)1	n = 20	[V]n[C]1	His-V20C1	[V]20 ELP with AA Cys at 4. posit. in the C-terminal ELP-pentamer
pET28-(VPGRG)n(VPGCG)1	n = 20	[R]n[C]1	His-R20C1	Positively charged [R]20 with Cys in the C-terminal ELP-pentamer
pET28-(VPGFG)n(VPGCG)1	n = 20, 40	[F]n[C]1	FnC1	Hydrophobic [F]n ELP with Cys in the C-terminal ELP-pentamer
<i>III. De novo generated DNA-tectons for heteropolymeric and block-copolymeric protein-tectons</i>				
pET28-NMCL-([V]2[Y]1)n	n = 15	[V2Y]15	His-(V2Y)15	[V]20 ELP with AA Cys at 4. position in the C-terminal ELP-pentamer
pET28-NMCL-[V2Y]5[F]n	n = 20	[V2Y]5[F]20	(V2Y)F20-His	Diblock-copolymer (db-cp) with [V2Y]5 and [F]20 block
pET28-NMCL-[R]n[F]n	n = 20	R20F20	R20F20	db-cp with positively charged [R]20 and hydrophobic [F]20 block
pET28-NMCL-[E]n-mEGFP	n = 40	E40-mEGFP	E40-mEGFP-His	db-cp with negatively charged [E]40 block and mEGFP domain
pET28-NMCL-mEGFP-[E]n[F]n	n = 20	mEGFP-E20F20	mEGFP-E20F20-His	Triblock-cp with mEGFP domain and [E]20 and [F]20 block
<i>IV. De novo generated DNA-tectons for block-copolymeric protein-tecton with incorporated unnatural amino acid (UAA)</i>				
pET28-(TAG)NMBL-mEGFP-[E]n[F]n	n = 20	(TAG)mEGFP-E20F20	pAzF-mEGFP-E20F20-His	mEGFP-E20F20 triblock-copol. with N-terminal UAA (azide-group)
<i>V. De novo generated DNA-tectons for multimeric protein-tectons with cellular adhesion peptide sequences</i>				
pET28-NMCysL-(RGDser-NG)n	n = 80	[RGDser-NG]80	RGD-NG80-His	Multimeric RGD sequence separated by HA cleavage sequence
pET28-NMCysL-(RGD-IEGR)n	n = 40	[RGD-IEGR]40	RGD-IEGR40-His	Multimeric RGD sequence separated by Factor XA cleavage seq.
pET28-NMCysL-(fibroCS5-NG)n	n = 80	[fibroCS5-NG]40	fibroCS5-NG40-His	fibronectins5 sequence separated by Factor XA cleavage seq.

generated by annealing of chemically synthesized complementary 15 bp ssDNA oligonucleotides. The detailed procedure is described in the Extended Experimental Procedures section of the [Supplementary Data](#).

2.7. Cyclic-ligation (CL) and sequential-directional ligation (SDL) of DNA-tectons

The insertion and multimerization of DNA-tectons is a recursive process of subsequent digestion of a vector next to an integrated DNA-tecton and ligation of another copy of a DNA-tecton into the vector. To allow more than one of these integration events in one reaction we develop the CL reaction to multimerize and integrate oligomeric DNA-template blocks into the platform vectors. The limitations which arose from the circularization of inserts used in CL were circumvented by SDL. For details compare [Fig. 3A](#) and [C](#) and the Extended Experimental Procedures section of the [Supplementary Data](#).

2.8. Protein-expression and purification

Positively analysed clones containing integrated multimers with correct DNA-sequence were transformed directly into competent *E. coli* BL21 (DE3), BLR or ER2566 cells for protein expression. The protein expression was performed with standard methods in LB-, TB-medium as described elsewhere [26] and in the Extended Experimental Procedures section of the [Supplementary Data](#).

2.9. Bioorthogonal incorporation of UAA

Plasmids pET28-(TAG)NMBL-mEGFP-E20F20-His and the pEVOLpAzF containing the orthogonal pair of t-RNA/t-RNA-synthetase (t-RNA-RS/AzPheRS for azido-phenylalanine incorporation) were cotransformed into *E. coli* strain ER2566. For the expression of the desired protein construct pAzF-mEGFP-E20F20-His bacteria were grown while shaking at 250 rpm in 20 ml LB medium containing 34 µg/ml chloramphenicol and 40 µg/ml kanamycin at 37 °C until they reached an OD₆₀₀ of 0.7. At this point 2 mM (f.c.) azidophenylalanine was added to the media. After 10 min incubation the tRNA synthetase expression under control of an arabinose promoter was induced with 0.2% arabinose and target protein expression by adding 1 mM IPTG. After shaking for 48 h at 20 °C and 180 rpm cells were harvested by centrifugation at 10,000 g for 10 min. The supernatant (20 ml) including the green fluorescent pAzF-mEGFP-E20F20-His was concentrated and 3 times buffer exchanged with vivaspin 20 (10 kDa Mw cut off) against PB buffer (50 mM PB pH 7.5/250 mM NaCl).

2.10. Protein modification and chemical conjugation

The 1,3 dipolar copper catalysed azid-alkyne [3 + 2] cycloaddition (CuAAC), also called “click reaction”, was done according to established protocols. To 150 µl pAzF-mEGFP-E20F20-His (f.c. 20 µmol/l = 1 eq) in 50 mM PB pH 7.5/250 mM NaCl and 4 µl chromeo dye 642 (f.c. 250 µmol/l = 12.5 eq). After addition of 5 µl click solution the reaction was shaken over night at RT and 500 rpm. The “click solution”

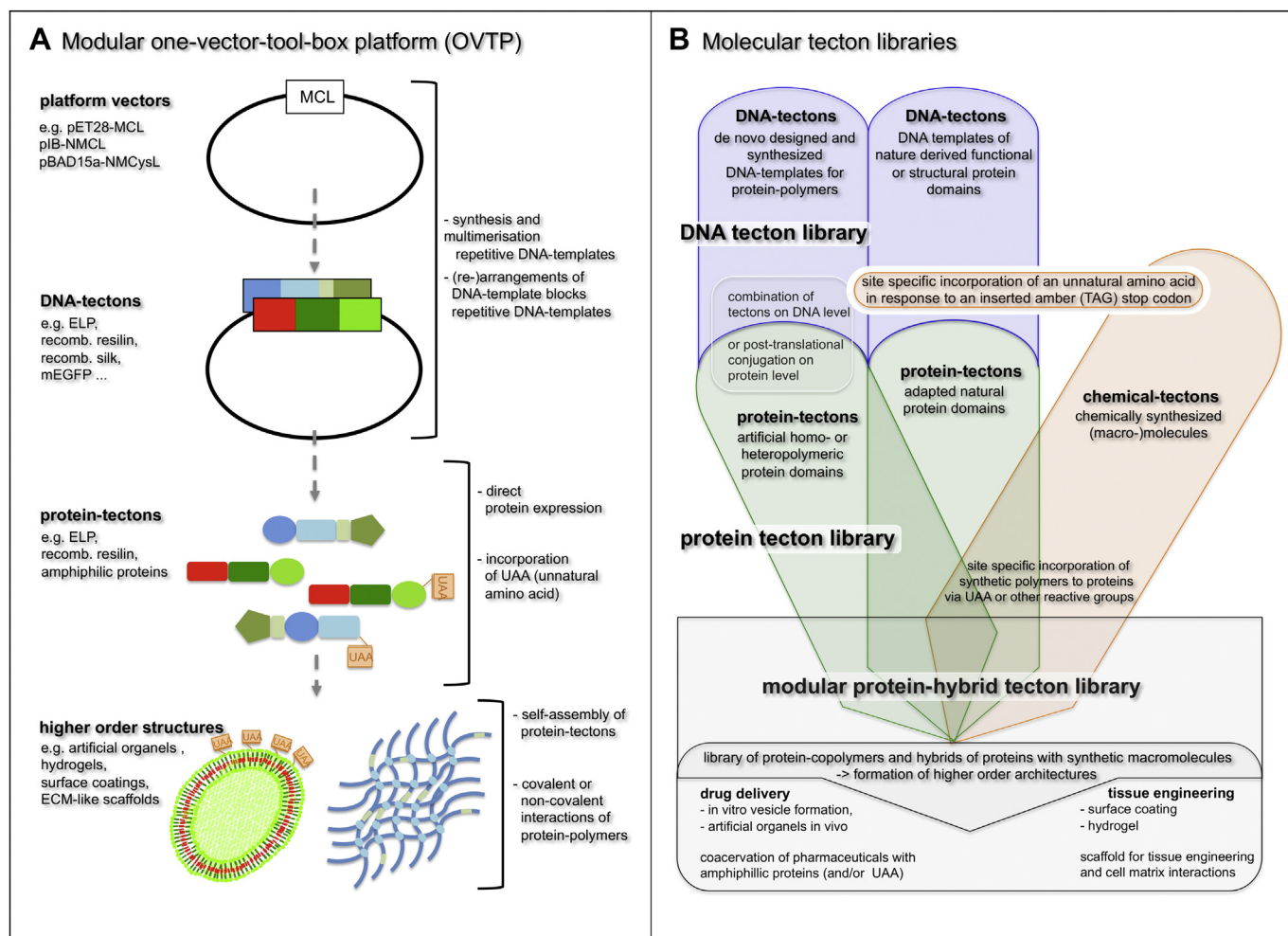


Fig. 1. Modular “one-vector-toolbox platform” (OVTP) for the generation of protein-based biohybrid-materials as molecular tecton libraries. A. Scheme of the modular one-vector-toolbox platform (OVTP) for the generation of molecular tecton libraries and succeeding steps from DNA to complex biomaterial architectures. The left column denominates different components of the OVTP along with the organizational level of this approach. The column in the middle displays illustrations of the components. The right column describes the executing processes along the consecutive organizational levels. B. Scheme of the different consecutive levels of molecular tectons (modular building block) visualizing the modular assembly and arrangement of biohybrid-materials. The blue region depicts the tecton library generated, adapted or assembled on the DNA level. The green area depicts the library of protein-tectons derived from the DNA-tectons which can be further arranged post-translationally with other protein-tectons or chemically synthesized molecules. The orange area displays the field of chemical-tectons which can be conjugated with the protein-tectons e.g. via genetically encoded unnatural amino acids (UAA). The overlapping arrows indicate the connectability of the different modular protein-tectons on the post-translational level with each other and with chemically synthesized molecules. Self-assembly, covalent and non-covalent interactions of tectons allow to constitute higher order structures and architectures to generate composite protein-biohybrid materials applicable in material science, pharmacy, nanotechnology and personalized medicine.

comprised of 40 eq CuSO₄, 100 eq Tris-[1-(3-hydroxypropyl)-1H-[1,2,3]-(triazol-4-yl)methyl]amine (THPTA) ligand and 200 eq Na-ascorbat (Fig. 4C.I). To remove excess reactants and the click solution the reaction mixture was purified by PD Spin Trap G-25 from GE Healthcare.

2.11. Fluorescence microscopy

In vitro imaging and documentation of fluorescent proteins pAzF-mEGFP-E20F20-His and mEGFP-E20F20-His was done by epifluorescence microscopy. After expression and purification of the desired constructs 5 µl protein solution was transferred onto a cover slip. Proteins were imaged using a NIKON eclipse TS100-F fluorescence microscope with DS-Qi1 camera and oil immersion objective CFI achromat 1.25 × 100. Images were processed using Image J 1.45 s.

2.12. Cell adhesion and cell growth assays of hMSC

hMSC (human mesenchymal stroma cell) cultures of four different donors were investigated: SC02_11, SC003, SC099, SC109. The cultivated cells (passage 3 or 4) were trypsinized and counted with CASY technology. 10.000 cells were seeded per 100 µl for each well and incubated at 37 °C with 5% CO₂. The wells were previously coated with seven different ELP polymers: R20F20, mEGFP-E20F20, F20C1, (V2Y) F20, V20, E40-mEGFP, (V2Y)15. Therefore, the wells of 96-well plates were incubated with 40 µl of the respective peptide solutions with a concentration of 50 µg/ml over night at 37 °C. The supernatant was removed and single wells were washed 3 times with PBS. The primary adhesion was assessed by light microscopy and determined after 4 h by the quantification of the DNA amount per well with PicoGreen. The cell growth was determined by cultivating cells for another 72 h and by the quantification of the DNA amount per well. This was used as indirect measurement for the cell number and subsequently for cell growth. The data of the cell adhesion experiments represent the mean value of 2–3 different measurements (wells) with SEM (error bars), normalized against the uncoated well (polystyrene) control. The cell growth data are determined after 72 h of incubation by calculating the relative DNA amount of the adhered cells compared to the DNA amount after primary adhesion measurement. The data represent the mean value of 3–4 different measurements (wells) with SEM (error bars) normalized against the uncoated polystyrene plastic control.

3. Results and discussion

3.1. A DNA based toolbox platform as fundament for the modular synthesis of protein polymers

The informational origin of protein sequences can be engineered at the DNA level. In order to manipulate the protein level we implement an operating platform to generate and handle DNA-tectons via the development of specialized plasmid vectors. Classical expression vectors (e.g. pET28b) were adapted to our system or completely rebuilt by the arrangement of their essential constituents (e.g. pBAD15a) and complemented with defined linker-regions (Table 1A.I, Fig. 2A, Supplementary Table 1A). These linker-regions are the key element of the OVTP consisting of defined arrangements of unique restriction enzyme recognition sequences. They allow for the multimerization, directional ligation, the defined arrangement of DNA-tectons, the generation of standardized overhangs between DNA-tectons and the direct expression of the tectons in different host organisms (Fig. 2).

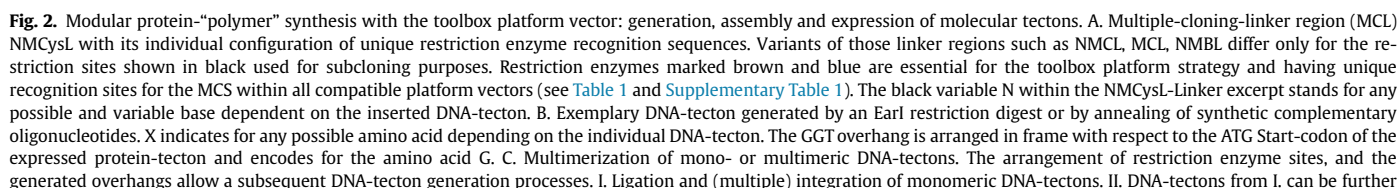
Therefore, all redundant enzyme recognition sequences had to be removed from the vector backbone. The integration of compatible and directional overhangs generated by type IIS restriction enzymes Earl and BspQI and also type II restriction enzyme SacI (Fig. 2A) allows for the defined multimerization of inserts (Fig. 2C). The standardized overhang generated by the restriction enzymes Earl or BspQI consists of 3 base pairs (GGT) coding for the amino acid glycine (G) (Fig. 2A&B). The overhang is set in frame with respect to the translational start codon in the linker regions to minimize error-proneness in project design and execution (Fig. 2A, Supplementary Table 3). The respective vectors were used as acceptor and donor vectors for any rearrangements of DNA-tectons (Fig. 2C). They are suitable for the direct expression of DNA-tectons with different levels of complexity (Fig. 2D). Based on the OVTP we generated multitudes of *de novo*

DNA-tectons or adapted native DNA-domains (Table 1B and Supplementary Table 1B). We also developed a set of basic vectors compatible with this platform, e.g. to allow for the implementation of an amber (TAG) Stop-codon for the site-specific integration of an UAA (Table 1A.III,B.IV and Supplementary Table 1A.III, 1B.IV). These UAAs allow for subsequent bio-orthogonal conjunction with chemically synthesized (macro)molecules and for the implementation of non-biological functions [28]. To expand the capabilities of our system we also engineered OVTP vectors that permit differential co-expression of protein-tectons in response to IPTG or arabinose induction (e.g. pET28 and pBAD15a vectors). Furthermore, we generated “helper” vectors to enable the expression of gene products in other organisms than *E. coli* (e.g. insect cells: pIB-NMCysL; *physcomitrella p.* or *arabidopsis t.*: pMAV4rc-Linker). Other OVTP vectors allow for the removal of all dispensable and objectionable amino acids N- or C-terminal to the desired protein (pIB-ELPL, pET28-NMBXL) (Table 1A.II, and Supplementary Fig. 4).

3.2. Generation and arrangement of DNA-tectons

DNA-tectons of natural structural or functional protein domains (Supplementary Table 1B.IV) were adapted and integrated into the OVTP by PCR based amplification and supplementation with compatible 3 bp overhangs to fit the respective sites of the platform vectors. These OVTP-compatible DNA-tectons complement artificial protein-material libraries. *De novo* generation of highly repetitive structural DNA-tectons and their spatial arrangement was achieved by using recombinant DNA-techniques and OVTP vectors. Multimerization starts with short annealed synthetic oligonucleotides of sense and antisense DNA strands encoding for the target monomer of repetitive protein or peptide motives (Fig. 2B), e.g. elastin-like-protein (ELP)-monomer, recombinant resilin- or spider silk DNA-template monomers (Table 1B.I). Possessed overhangs are compatible to the OVTP and monomers could be integrated into the linker-region in a first ligation step with 1–15 copies (Fig. 2B and C.I). As basic units for continuative multimerization and (re-) arrangement we generated a sequence verified library of DNA-tectons with typically 5, 10 or 20 multimers of the monomeric motive (Fig. 2C.II). For subsequent multimerization reactions the platform vectors can serve as donor or acceptor (Fig. 3A and C). Restriction digest with the type IIS enzyme Earl excises the DNA-tecton for subsequent ligation steps (Fig. 3A). Restriction digest with the type IIS enzyme BspQI opens the vector upstream to a DNA-tecton allowing for the seamless insertion of one or more excised DNA-tectons. Cyclic alteration of reaction temperature and the supplementation of reaction agents allows for a multi-cycle one-tube reaction generating multiple DNA-tectons. We denominate this process “cyclic-ligation” (CL) (Fig. 3A). The 3 bp overhangs at each end of the excised DNA-tecton are complementary to each other enabling directed integration of multiple DNA-tectons in one reaction. Depending on the individual sequence and length, these DNA-inserts tend to self-ligate and circularize reducing the cloning efficiency of long inserts significantly or completely [23]. In order to avoid this drawback especially for long DNA-tectons we complemented the BspQI restriction site with an overlapping SacI restriction site (Fig. 2A).

This arrangement enables an alternative processing route for long inserts starting from the same construct. The consecutive restriction digest with SacI and Earl generates DNA-tectons with terminal overhangs incompatible to each other but compatible to an appropriately prepared vector preventing the insert from circularisation [23,17] (Fig. 3C.III). The accepting platform vector has to be prepared by consecutive restriction digest with BspQI and SacI. This results in an accepting vector with compatible overhangs



upstream to a potentially present DNA-tecton within the OVTP vector (Fig. 3C.I). BspQI and Earl generate identical compatible overhangs outside their recognition sequences allowing for seamless ligation of DNA-tecton B upstream to the DNA-tecton A constituting the 5'-linker region for subsequent processing steps. We denominate this tecton duplication “sequential-directional ligation” (SDL) (Fig. 3C).

All OVTP vectors presented in Table 1A (and Supplementary Table 1A) allow for the alternative use as donor or acceptor vectors in the CL or the SDL process. Combinations of these procedures offer nearly unlimited extension of homo- or heterologous DNA-tectons and their unrestricted arrangement to complex DNA-tectons (Fig. 3C).

In Fig. 3B.I we present a series of ELP (VPGVG)₂₀ multimers (abbr. V20) as DNA-tectons. Therefore, ELP-V20 DNA-tectons were multimerized via CL yielding a pET28-MCL-V20 vector. Depending on the DNA-sequence of the inserted tectons typically 1 to 5 incorporations of the tectons were achieved in 3–5 cycles (Fig. 3A.I). The number of incorporated tectons does not correlate strictly with the number of temperature cycles. The products were verified by analytical restriction enzyme digest and DNA sequencing. To generate heterogeneous ELP multimers with a unique functional cysteine (C) near to the C-terminus as functional group for dimerization, surface immobilization or bioconjugation, we ligate Earl excised DNA-tectons of ELP multimers F/V/R10 ($n = 10$ and F, V, R = X in (VPGXG)_n) from an OVTP donor vector into a BspQI digested (VPGCG)₁ (abbr. C1) containing acceptor vector (Fig. 3B.II).

DNA-tectons for recombinant resilin or spider silk-“polymers” were prepared using a combination of CL to achieve suitable DNA-tectons followed by SDL to produce DNA-templates of the desired length. Fig. 3D presents the analytical restriction digests of DNA-tectons for recombinant resilin multimers ranging from 12mers to 30mers (Fig. 3D.I) as well as recombinant spider-silk motive multimers ranging from 10 to 80 mers (with up to 8.5 kb) (Fig. 3D.II and Table 1B.I).

The efficient combination of CL and SDL enables the generation of large numbers of DNA-tectons serving as templates for homo- or heterogeneous structural and functional protein block-copolymers (Table 1B and Supplementary Table 1B). Whereas Table 1 specifies the vectors and DNA-tectons used in this study, Supplementary Table 1 displays an extended selection of tectons generated so far for the use as basic entities for a large variety of applications.

3.3. Expression of proteins from OVTP DNA-tectons

DNA-tectons generated via the OVTP were expressed directly in *E. coli* or other organisms (Supplementary Table 1A.I&II). Fig. 4A.I presents a set of homopolymeric ELPs V20_n with $n = 1, 2, 3, 4, 5$ generated by CL. Proteins were expressed and purified via transition-temperature purification [29] and Ni-NTA His-tag purification [30] using the pET28 vector constructs (Table 1B.I). In order to demonstrate the expression of large *de novo* generated functional protein polymers we transform the vectors pET28-NMCL-(RGDser-NG)₈₀, pET28-NMCysL-(RGD-IEGR)₄₀ and pET28-NMCL-(fibroCS5-NG) coding for artificial multiple cellular adhesions peptide sequences (Table 1B.V) directly into BLR cells and demonstrate the IPTG induced high-level expression of these proteins via analytical SDS-PAGE (Fig. 4A.II). The multimeric proteins of these functional peptide sequences were chemically or enzymatically cleaved into monomers due to inserted proteolytic recognition

sequences. This allows to produce large amounts of defined short epitope peptides with a unique, reactive N-terminal cysteine (manuscript in preparation).

The design and generation of adapted OVTP vectors and the described arrangement of restriction sites within the linker-region enabling the combination of three previously separated methodical approaches for the *de novo* synthesis of artificial, repetitive proteins. Merging CL, SDL and the direct expression of the constructs into one unified platform, raises the overall system to a new quality by abrogating the limitations of the separate approaches. Thus, constituting the basis for a growing modular, molecular tecton library with expressed tectons ranges from elastine-, resilin, silk- to epitope sequence elements for bionanomaterials with inherent biological and nanotechnological functions.

3.4. Post-translational conjugation of protein-tectons

In order to further expand the functional and structural complexity of our library approach, we conceptionally implemented the conjugation of protein-tectons with synthetic molecules via natural or unnatural amino acids which are site-selectively introduced into the protein-tectons. For example, RGD or fibroCS5 cellular adhesion sequence motives supplemented site-selectively with cysteine can be conjugated to structural biopolymers that have the capacity to form hydrogels by oxidation reactions, for further use as surface coatings or scaffolds in tissue engineering approaches.

In classical protein conjugation the reactivities of the side chains of natural amino acids are often selectively introduced and used because of their rare presence in native proteins (e.g. cysteine) or the variety of possible modification reactions for primary amines (e.g. lysine) [28,31]. Here we demonstrate the use of a unique sulfhydryl-group for the post-translational conjugation of protein-tectons. The previously described X20C1 DNA-tectons ($X = F, R, V$) (Table 1B.II) were transferred into a OVTP-compatible pET28 vector constituting a His-tag N-terminal to the DNA-tecton. The expressed proteins were equipped with a unique sulfhydryl-group close to the C-terminal end of the ELP. Depending on the accessibility of the sulfhydryl-group two cysteine containing proteins can efficiently form disulfide-bonds under oxidative conditions constituting homodimers of ELP protein-tectons. Under reductive conditions the cysteine containing ELPs exhibit their reduced monomeric appearance (Fig. 4B). Conjugation of two protein-tectons with cysteine as C-terminal amino acid generates branched proteins with contrarily orientated branches. Furthermore, mixing different cysteine containing ELPs (e.g. V20C1, R20C1, F20C1 and R20C1F20) under oxidative conditions constitutes heterodimeric ELPs (Supplementary Fig. 2A). The resulting branched proteins were separated and purified by subsequent steps of differential precipitation and His-tag purification as demonstrated for the heterodimers of expressed proteins from pET28-His-V20C1 and pET28-MCL-F20C1 plasmids (Supplementary Fig. 2B).

3.5. Implementation of bioorthogonal chemical-tectons into the OVTP: functionalizing self-assembled higher order architectures - nanocompartments

Expanding the natural molecular toolbox, bioorthogonal chemical reactions offer a great potential to introduce new functionalities into natural and biosynthetic products [32]. This

multimerized by the CL process (see Fig. 3A). III. To prevent circularisation of DNA-tectons SDL can be performed. The DNA-tectons can be prepared alternatively using the enzymes SacI and Earl or BspQI to generate incompatible overhangs. Those tectons can be cloned seamlessly into an appropriate vector (see Fig. 3B). D. All vectors can be directly transformed into expression cells of appropriate organisms without any subcloning step.

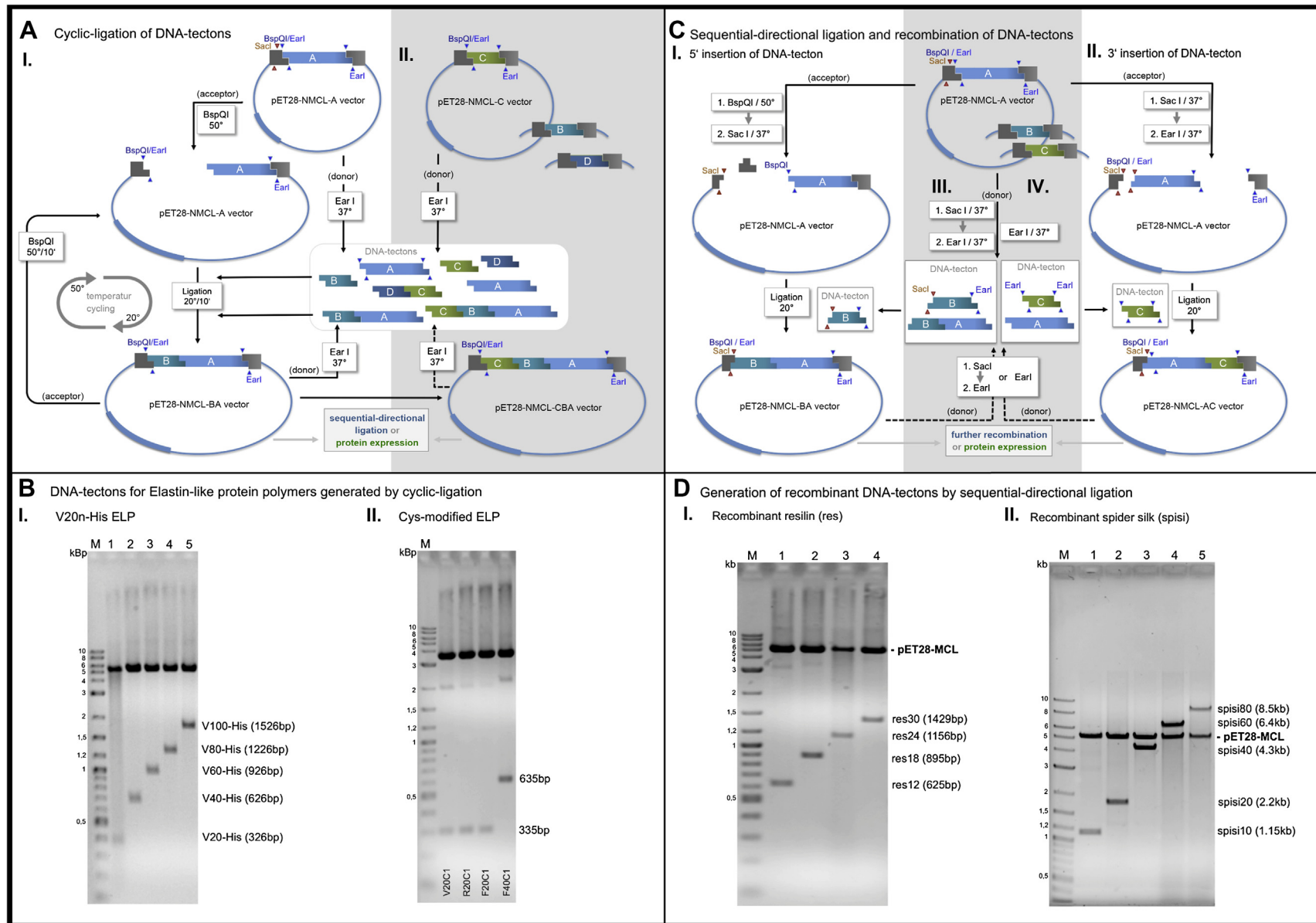


Fig. 3. A. Cyclic-Ligation (CL) of DNA-tectons. In order to generate repetitive protein multimers a CL reaction was performed by alternating reaction temperatures with alternating enzymatic activity by an “one-tube reaction”. I. The CL process of DNA-tectons is illustrated schematically. Different DNA-tectons are indicated with different colours and the letters A to D. II. OVTP vectors with different DNA-tectons were used as donors and acceptors for the preparation and insertion of other DNA-tectons. Tectons were added successively/serial to each cycle as a uniform fraction or parallel as a mixed fraction at each cycle to generate homogeneous or randomly configured complex DNA-tectons. B. DNA-tectons assembled *de novo* and multimerized via cyclic-ligation and sequential-directional ligation (SDL). I. Multimerization of elastin-like-protein (ELP) (VPGVG)₂₀ (= V20) to homopolymeric multimers. Here we show an 1.5% agarose gel with an analytical restriction digest with SacI and HindIII that excise the resulting multiples of a V20-DNA-tecton generated within one CL process as shown above. M stands for a DNA-ladder with the respective numbers of base pairs (bp) as indicated on the left side. The lanes 1 to 4 mark for different clones resulting from the transformation of the CL reaction with the V20-DNA-tectons in a prepared pET28-MCL vector. II. Arrangement and functionalization of different DNA-tectons (ELP-V20, -R20, -F20, -F40) with a C-terminal DNA-template encoding for a Cys-modified ELP-monomer (VPGCG)₁. Analysed constructs were cloned into a platform compatible pIB-ELPL vector.

DNA-tectons were analysed on a 1.5% agarose-gel by a restriction digest with NcoI and EcoRI and were sequenced and transferred to the desired compatible pET28 vector. C. Sequential-directional ligation and recombination of DNA-tectons to generate defined block-copolymers and DNA-tecton arrangements. I. Schematic illustration of the SDL process. The DNA-tectons prepared from the identical OVTP vectors presented in part A., (e.g. NMCL-A) by defined sequential action of the restriction enzymes SacI and EarI (see III. - donor). By the sequential action of the restriction enzymes BspQI and SacI the same plasmid were used as an acceptor for compatible DNA-tectons. II. Combinations of defined preparations of OVTP vectors in combination with fitting DNA-tectons as illustrated allows for the precise composition of DNA-tectons within the OVTP vector. Exemplarily an EarI prepared DNA-tecton (IV: tecton C) could be integrated 3' to an internal primary DNA-tecton (A). Resulting vectors (e.g. AC) now served as acceptor or donor vector for further combinations on DNA level or for protein-tecton expression. D. Assembly and multimerization of DNA-tectons encoding for different homopolymeric protein polymers via SDL. I. Multimerization of the DNA-template for recombinant resilin-like protein (res12 - res30) to homopolymeric multimers. Analytical restriction digest of the resulting multiples of a res6 DNA-tecton generated via SDL reactions. M (DNA-ladder). Lanes 1 to 4 mark different clones resulting from the SDL of res6 DNA-tectons after transformation into XL1-blue cells. Products visualized via analytical restriction digestion with XbaI and XhoI. II. Multimerization of the DNA-template for a recombinant spider silk-like protein (spisi10 - spisi80) to large homopolymeric multimers. Lanes 1 to 5 the different clones resulting from SDL of spisi10 and spisi20-DNA-tectons into OVTP vectors. The plasmid DNA was digested with the enzymes XbaI and XhoI and visualized on a 1.5% agarose-gel.

facilitates the generation of individual composite protein-materials with specialized features [32,33]. A subset of OVTP vectors with implemented amber-stop codons (TAG) allow for a simple modular incorporation of UAA presented in Table 1A.III and Supplementary Table 1A.III and 1B.VII. In the context of bioorthogonal and site-specific reactions the copper catalysed [3 + 2] cycloaddition (CuAAC) between azides and alkynes represents one of the most versatile bioconjugation reactions to date. Genetically encoding azidophenylalanine as UAA into a protein-tecton of interest allows to introduce the non-natural azide-group efficiently at defined positions within the protein of interest.

As an expansion to native reactive groups unnatural reactivities can be used for individual and defined functionalization of biomaterials. To investigate the potential of directing the formation of programmable higher order structures by engineering self-assembling amphiphilic protein block-copolymers we designed the composite ELP mEGFP-(VPGE)20(VPGFG)20-His (abbrev. mEGFP-E20F20). This amphiphilic protein reveals the capability to form vesicle-like structures *in vitro* as demonstrated in Supplementary Fig. 3 (compare existing supramolecular structure forming systems [34–36]) and *in vivo* (manuscript submitted). This offers a path towards generating artificial organelles as an important goal in chemical synthetic biology [37]. In order to demonstrate the programmability we show the ability to functionalize the vesicle-constituting protein-tectons and subsequently the higher order vesicular structures *in vitro* via site-specific chemical modification. Therefore, we transferred the mEGFP-E20F20 construct into the pET28-(TAG)NMBL OVTP vector (Table 1B.IV) to insert an UAA into the protein-tecton.

In order to ensure an accessible modification site of the vesicle, the UAA pAzF is introduced at a hydrophilic N-terminal position of the protein (pAzF-mEGFP-E20F20) [38]. Consequently the green fluorescent pAzF-mEGFP-E20F20 is successfully modified with red chrome alkyne dye 642 (Fig. 4C). The reaction was carried out via copper catalysed 1,3 dipolar azide-alkyne [3 + 2] cycloaddition (CuAAC) with Tris-[1-(3-hydroxypropyl)-1H-[1,2,3]triazol-4-yl)methyl]amine (THPTA) as water soluble ligand (Fig. 4C.I, Supplementary Scheme 1) [39]. Thus, we could site-specifically introduce a new chemical function into the desired protein-tecton *in vitro* to generate a functionalized bioconjugate.

Incubation of these bioconjugates at conditions allowing mEGFP-E20F20-His to form vesicle-like structures lead to the appearance of higher order structures visualized by epifluorescence microscopy (Fig. 4C.II). Although the fine structure is under the resolution limit the red fluorescent chrome alkyne dye and the green fluorescent pAzF-mEGFP-E20F20 is clearly co-localized indicating for the formation of a functionalized higher order structure *in vitro*. This scheme is also applicable to introduce libraries of clickable small molecules expanding this approach towards chemically functionalized protein-hybrid libraries.

3.6. Protein-modules creating individualized environments for differentiating cells

We demonstrated the efficiency of the modular OVTP to generate multitudes of structural modules and to arrange them on the DNA and protein level. For tissue engineering applications several extraordinary properties highlight composite “ELP based materials” (EBM) as very attractive building blocks. Their tunable sequence dependent viscoelastic and physico-chemical properties are key parameters for materials in tissue engineering [40,41]. They can easily be associated to non-covalent or crosslinked macroscopic scaffold materials. Moreover, homo- or heterogeneous ELP-polymers are known to be biodegradable, non-cytotoxic and non-immunogenic and can easily be modified via genetic

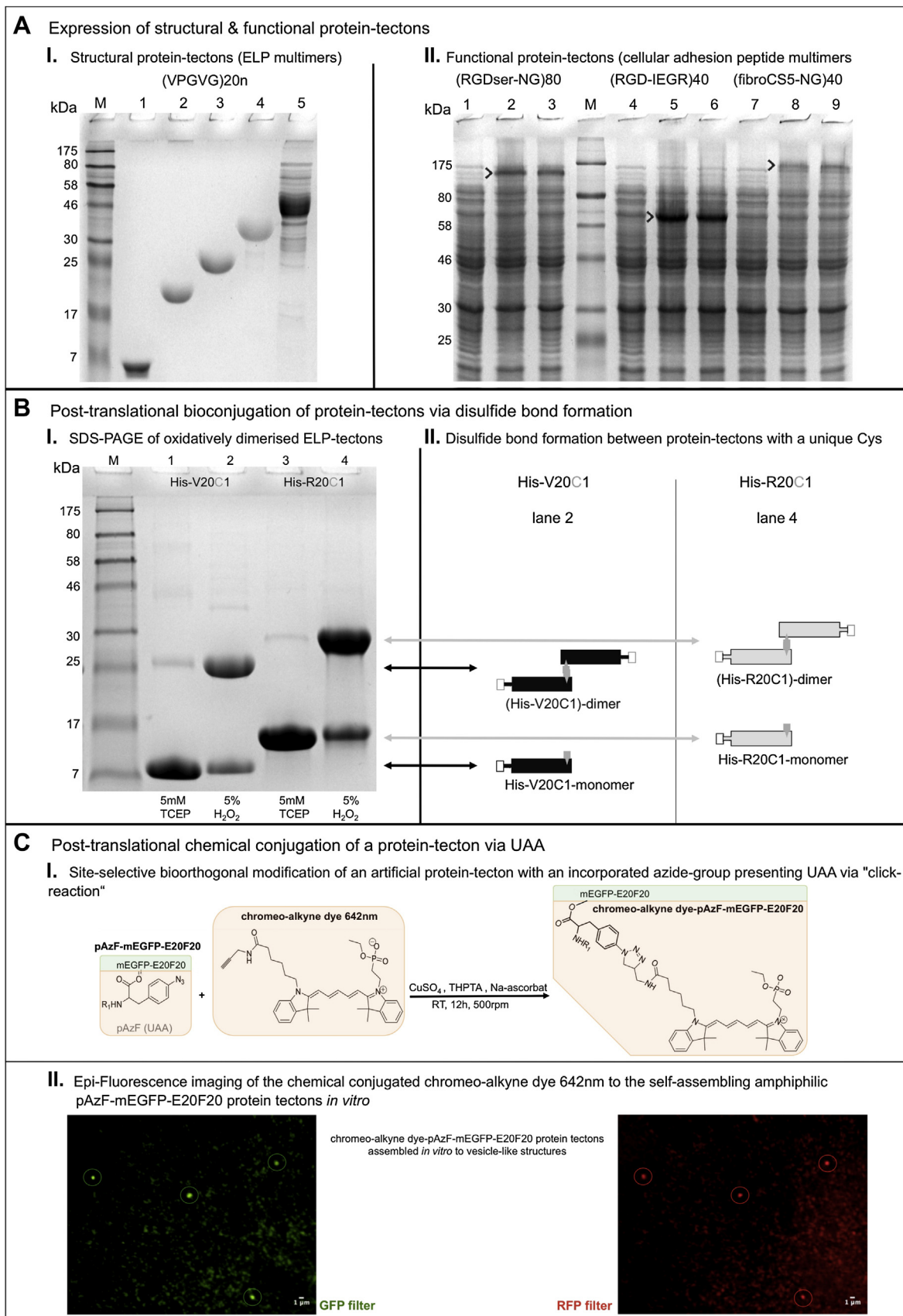


Fig. 4. Expression of protein-tectons and post-translational conjugation. A. Expression of structural & functional protein-tectons derived from cyclic-ligation (CL) and sequential-directional ligation (SDL). I. CL generated structural protein-tectons. *De novo* generated ELP (VPGVG)20 protein-tectons (= V20) integrated in pET28-NMCL expression vectors and expressed in BLR *E. coli* cells. Recombinant protein are analysed with PAGE on a 15% PAG (M marks for a 7–175 kDa protein-ladder (NEB)). Lanes 1 to 5 indicate the recombinant

incorporation of cellular recognition sequences [42,43]. Thus, EbM were investigated intensively during the last decade for various tissue engineering applications on the basis of individual sequences [44,45]. The modular organization of the OVTP facilitates the fast, efficient and reliable generation of a large number of defined composite EbMs. To demonstrate the potential of composite EbM as building blocks mimicking extracellular matrix (ECM) proteins towards cost-efficient individual environments for different cell types [46], we investigate the compatibility and interaction of various EbMs with different cell types or clonal progenitor cell populations.

Nature's biological scaffolds are constituted of blocks with different sterical and physico-chemical characteristics such as collagen, fibronectin or (tropo)elastin in combination with functional sequence elements (e.g. cellular adhesion sequences) [15,47]. The interplay between structural and functional elements of the ECM determines its individual microenvironmental functions [48,49]. Surface-interaction, cellular adhesion or signalling epitopes and mechanical or physical properties of the surrounding materials define and guide cellular behaviour of many primary cells and cell lines [50,48]. To mimic the environmental requirements and compositions *in vivo* and *in vitro* the effects of signal and cellular recognition sequences together with different structural backbone and scaffold materials have to be investigated in detail [40]. At the same time multipotent primary cell lines, as promising cellular components for tissue engineering approaches, reveal strong heterogeneity with respect to proliferation and differentiation [51,52].

Due to their regenerative properties and multi-potentiality hMSC (human mesenchymal stroma or stem cells) are considered to be an auspicious candidate for cell therapies and tissue engineering [53]. Furthermore, they are capable to differentiate into multiple cell types such as adipocytes, osteoblasts, and chondrocytes both *in vitro* and *in vivo*. MSC have been reported to be used to reconstruct damaged tissues upon scaffold supported transplantation [54].

Depending on the origin of the tissues and different cell donors, single-cell-derived clonal MSC populations are known to be highly heterogeneous in their proliferative and differentiation potential [53]. With respect to their adhesive, proliferative and differentiation potential, cell culture conditions (e.g. culture media, culture dish surfaces) are very important parameters [55,56]. Moreover, the influence of cell–matrix interactions integrating biophysical properties of 2D surfaces and 3D environments are known to regulate stem cell fates [57,50]. Thus, optimization with regard to cell adhesion, proliferation and differentiation is essential for creating individualized cellular environments of diverging cell lines or primary cell cultures [48].

To monitor the suitability and influence as constituents of cellular environments we investigated the potential for cellular adhesion and proliferation of *de novo* generated protein-tecton EbMs. Fig. 5 displays the results of cell adhesion assays (Fig. 5A) and cell growth (Fig. 5B) of hMSC cultures on seven different EbM surface coatings. Cell cultures of different human donors were

investigated and shown to adhere in a comparable manner to uncoated surfaces. The adhesion of the different cell populations to distinct EbM-coatings was varying with maximally 20% compared to the uncoated wells and with up to 40% to each other. This demonstrates that all investigated EbM principally mediate efficient surface adhesion of the respective cell population independently of their individual origin (Fig. 5A). In contrast to this finding the cell growth of these cell populations was varying remarkably up to 50% in comparison to the uncoated plastic surface. We can show that each individual cell population exhibit significant preferences to different EbM coatings (Fig. 5B). Hence, each individual cell population has its own optimal surface-structure requirement with respect to cellular growth. In addition preliminary cell growth experiments with human and mouse fibroblast cell lines affirm these results (Supplementary Table 2). These results emphasizing EbMs as promising candidates for ECM mimicking materials. OVTP offers the efficient generation of combined structural and functionalized surface niches for clonally derived cells with proliferative and individual differentiation potential. These cells requiring distinct stimuli for the processes of cellular proliferation and differentiation which could be presented by EbMs. Our results underlines the potential of OVTP in generating precisely engineered individual EbMs enabling specific cell interactions with an interesting potential for personalized regenerative medical approaches.

4. Conclusion

The future demand for adjustable and programmable composite biomaterials is an indisputable challenge. Here we address the creation of defined, rationally designed building blocks for combinatorial biomaterial libraries. We introduce the concept of a modular one-vector-toolbox platform (OVTP), illustrating the principles, workflow and abilities. Merging previously separated techniques into one integrative system offers new possibilities and manageability of the overall system. The feasibility and efficiency of this approach is demonstrated by the generation of multitudes of homo- or heteropolymeric modular DNA-tectons. Synthesized repetitive DNA-tectons span the range from short repetitive DNA sequences up to large artificial and complex tectons. The OVTP allows for the unlimited recombination of modular tectons and their direct expression as protein-tectons. Combining designed or nature-derived modules of structural or functional tectons and the implementation of site-selective integration sites for UAA and their subsequent modification with synthetic molecules extends the combinatorial protein-tecton library towards a protein-hybrid library. Conjugation and self-assembly of modifiable protein-tectons demonstrates the potential to constitute functionalized higher order architectures such as nanocompartments/nanoreactors. To date the growing modular-tecton library presents a toolbox of hundreds of building blocks for the variable and reliable rational design of adjustable ECM mimicking biomaterials and for composite protein-hybrid materials.

proteins V20-His (10.7 kDa), V40-His (18.9 kDa), V60-His (27 kDa), V80-His (35.2 kDa) and V100-His (43.4 kDa). II. Functional protein-tectons generated via SDL. *De novo* generated, multimerized RGD sequence derivatives (as depicted in SI Table 1). Recombinant proteins were expressed in *E. coli* BLR cells and analysed with PAGE on a 10% PAG. Lanes 1 to 3 show proteins from pET28-NMCysL-(RGDser-NG)80 (–, +, + IPTG induction: 146.2 kDa), lanes 4 to 6 show proteins for pET28-NMCysL-(RGD-IEGR)40 (–, +, + IPTG induction: 84.4 kDa), lanes 7 to 9 show proteins from pET28-NMCysL-(fibroCS5-NG)40 (–, +, + IPTG induction: 112.3 kDa). Black arrows indicate for the expressed recombinant protein in the IPTG induced fractions. B. Post-translational bioconjugation of protein-tectons via disulfide-bond formation. I. SDS-PAGE of oxidatively dimerized ELP DNA-tectons. Homodimerization of His-(VPGVG)20(VPGCG)1 (=His-V20C1) protein-tecton and His-(VPGRG)20(VPGCG)1. (=His-R20C1) protein-tecton via oxidative disulfide-bond formation. II. C-terminal disulfide-bond formation between native sulfhydryl-groups of cysteine containing protein-tectons schematically depicting the resulting branched homodimeric proteins. C. Post-translational chemical conjugation of a protein-tecton via unnatural amino acids (UAAs). I. Site-selective bioorthogonal modification of an artificial protein-tecton with an azide-group presenting UAA via “click-chemistry”. The reaction scheme of amphiphilic pAzF-mEGFP-E20F20 protein containing the unnatural amino acid (UAA) pAzF provides the bioorthogonally reactive azide-group to allow for site-specific copper catalysed 3 + 2 cycloaddition of the depicted alkyne dye. Green background box represents the amphiphilic block-copolymeric protein-tecton. The orange background box represents the unnatural amino acid and the chemical alkyne dye as an example for a chemical functionalization. II. Epi-Fluorescence imaging of the chemically conjugated chromeo-alkyne dye 642 nm to the self-assembling amphiphilic pAzF-mEGFP-E20F20 protein-tectons *in vitro*.

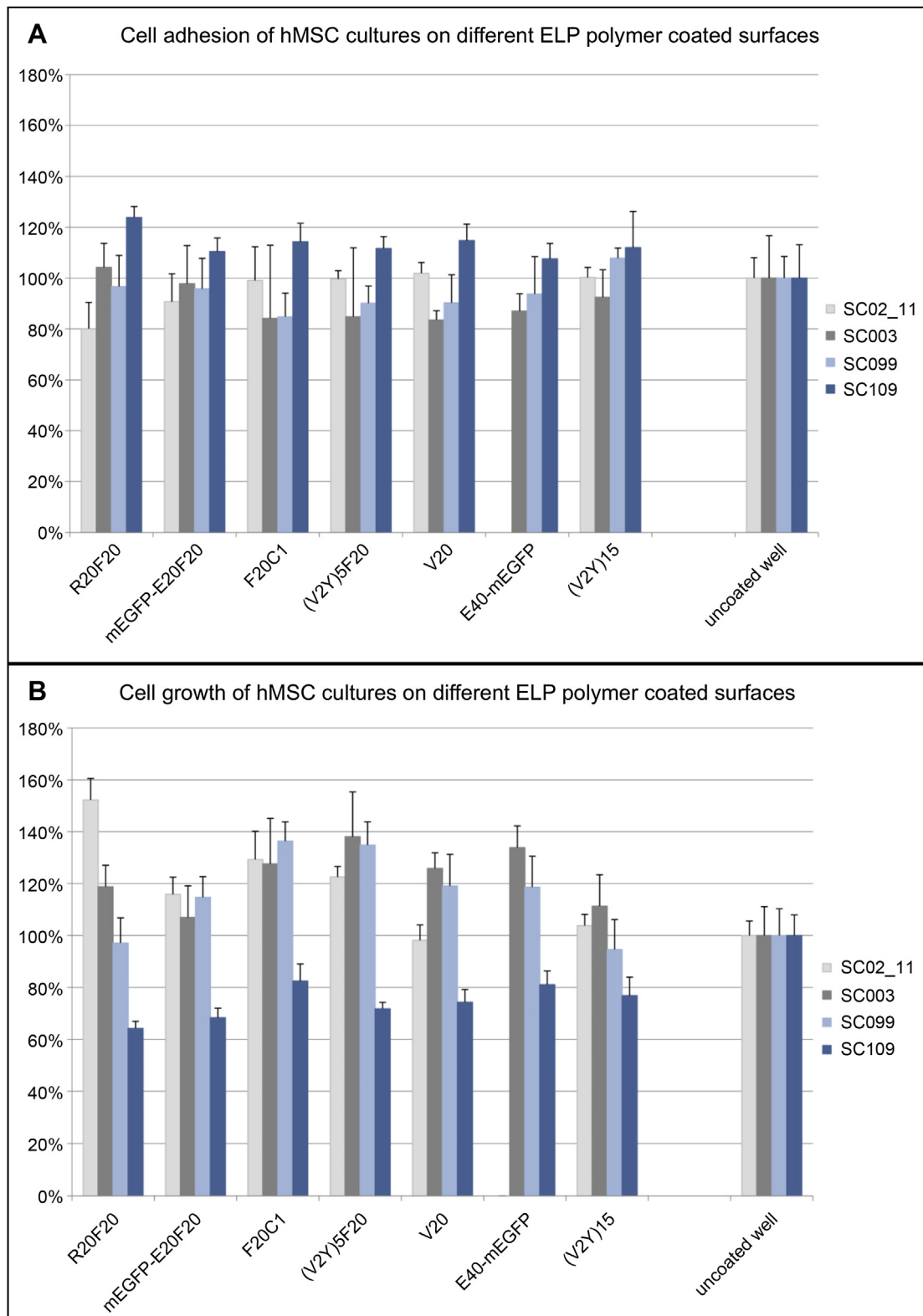


Fig. 5. Cell adhesion and cell growth of hMSC cultures on ELP coated surfaces. Comparison of cell adhesion and cell growth profiles of four different hMSC (human mesenchymal stroma cell) cultures on ELP coated surface. hMSC of four donors were investigated: SC02_11, SC003, SC099, SC109 and incubated on seven different ELP polymer coatings as indicated: R20F20, mEGFP-E20F20, F20C1, (V2Y)F20, V20, E40-mEGFP, (V2Y)15 and uncoated well. A. Cell adhesion of four hMSC cultures on seven different ELP polymer coated surfaces. Cell adhesion was monitored by microscopy and determined after 4 h by calculating the relative DNA amount of the adhered cells. B. Cell growth of four hMSC cultures on seven different ELP polymer coated surfaces. Cell growth was monitored by microscopy and determined after 72 h of incubation by calculating the relative DNA amount of the adhered cells compared to the DNA amount after primary adhesion was completed (4 h).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.biomaterials.2014.06.048>.

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