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Supramolecular Biomaterials. A Modular Approach towards Tissue Engineering

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Supramolecular chemistry is an exciting area of science that plays a central role in bringing different disciplines together, ranging from molecular medicine to nanotechnology. Materials science based on supramolecular interactions is an emerging field, which has made important steps forward in the past ten years. The self-assembly of small synthetic molecules into long-chain architectures gives rise to the careful design of supramolecular polymers or fibers based on highly directional, reversible, non-covalent interactions. Much afford is put into the development of supramolecular (polymeric) materials with true materials properties, both in solution and in the solid state. These supramolecular materials are beginning to reach the market in all kind of applications. The field of regenerative medicine in general and that of tissue engineering in particular is one of the most challenging areas in which supramolecular materials might have a high potential. In tissue engineering, the biological environment and the interactions of cells with the artificial biomaterial is of utmost importance for the functioning of the implant, i.e. the engineered tissue. Ideal biomaterials do not only have to fulfil the biomaterials trinity of tuneable mechanical properties, regulation of the degradability and the ease for bioactivity incorporation, but also have to mimic the natural environment where the materials are brought into. Therefore, a modular, self-assembly approach using several supramolecular building blocks is an exquisite way to produce such "responsive" biomaterials. It is proposed that the artificial materials described in this account have the same type of dynamic ability to adapt its biofunctionality as is so well known for the living cells in the host tissue. This account will highlight two systems, i.e. self-assembling oligopeptide fibers as pioneered by Stupp et al. and Zhang et al., and our hydrogen-bonded supramolecular polymers, to show the potential of a modular approach to dynamic biomaterials for tissue engineering.

1. Introduction

Starting with the pioneering work of Pederson, Cram, and Lehn, the field of supramolecular chemistry, which is defined as the "chemistry beyond the covalent bond," has emerged as a leading discipline.[1,2] Complex structures have been designed and studied, varying from protein and peptide assemblies, supramolecular catalysts and molecular sensors, to responsive supramolecular polymers. Novel supramolecular systems are the core for the further development of molecular medicine and the bottom-up approach in molecular nanotechnology. Characteristic for a supramolecular design is the specific use of non-covalent secondary interactions, ranging from hydrogen bonding, hydrophobic forces, and metal–ligand interactions. Especially, those interactions that provide a high degree of directionality to the assembly process of the different component are favourite elements in the design. Many excellent reviews and books are dedicated to the field of supramolecular chemistry and the reader is referred to those.[1,2]

Another emerging and very intriguing area of research is that of the regenerative medicine and especially the part which is called tissue engineering (TE).[3,4] Langer and Vacanti defined tissue engineering as "an interdisciplinary field that applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function or a whole organ."[3] One important component in TE is the design and development of scaffolds, i.e. biomaterials that have to support, guide and stimulate the developing tissue. The research on biomaterial implants started with the development of inert prostheses, via so-called second to third generation materials that were designed to be both resorbable and bioactive.[5] Degradation of the biomaterial, formation of new extracellular matrix (ECM) components and remodelling of the developing tissue by cells are key processes in the TE concept. However, the dynamics and adaptable nature of the biomaterial itself are not taken into

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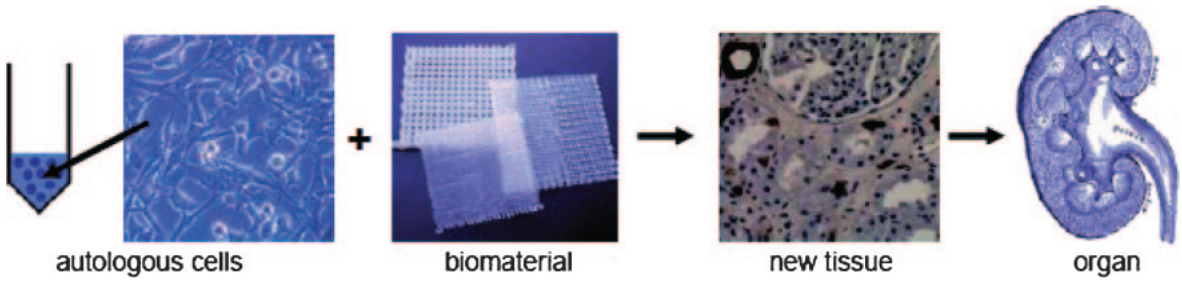

Fig. 1. The tissue-engineering concept. A biopsy is taken from a patient from which cells are isolated and expanded in vitro. Then, the cells are cultured on the ideal polymer scaffold with the right geometry. Ultimately, a new tissue, a part of an organ or a whole organ, is formed which can be transplanted into the patient.

account yet. This might be a disadvantage and shortcoming because a closer look at nature itself reveals that the interaction of cells with the ECM and vice versa is far from a static picture. Whereas the third generation materials are designed to be bioactive and able to elicit cellular responses, they cannot adapt their biofunctionality to the required properties of the living cells. Here, we propose that adaptable bioactive biomaterials based on supramolecular chemistry, therefore defined as the fourth generation of implants, might fulfil this dynamics requirement. This brings these two already interdisciplinary fields of supramolecular chemistry and tissue engineering together with a major challenge for the chemical biologists.

This account focuses on the recent disclosures on the interplay between supramolecular chemistry and tissue engineering by first describing shortly the state of the art in biomaterials for tissue engineering, with the conclusion that supramolecular materials have great promise in the field. Then, two subdivisions of supramolecular materials are presented: our supramolecular hydrogen-bonded polymers and the self-assembling peptide architectures as pioneered by Stupp et al. and Zhang et al. First, the general aspects of the systems will be presented with the focus on our own work, followed by the real applications of these supramolecular systems in tissue engineering, while we finally speculate on the impact of this combination.

2. Tissue Engineering and the Biomaterials Trinity

2.1 Tissue Engineering. The increasing incidence of organ failure in the world, mainly caused by ageing of the population, is responsible for rising healthcare expenses. The number of patients requiring organ replacement therapy is expected to steadily increase in the future. At present, patients with organ failure rely on organ transplantation or costly treatments that help correct the systemic effects of organ malfunction. However, the poor availability of donor organs, and the apparent side effects of conventional organ replacement therapies, has driven the quest for alternative and durable organ replacement solutions. For various tissues the development of bioartificial organs, i.e. hybrid constructs containing cells and biomaterials, is currently explored.[6] It is expected that these bioartificial organs have far-reaching implications in improving life expectancy and quality of life. Initially, biomaterials were used as inert prostheses to restore malfunctioning of organs in the human body, of which hip implants and spinal cord intervertebral disks are clear examples.[5] However, difficulties arise when soft tissues and organs, as heart valves and kidney have

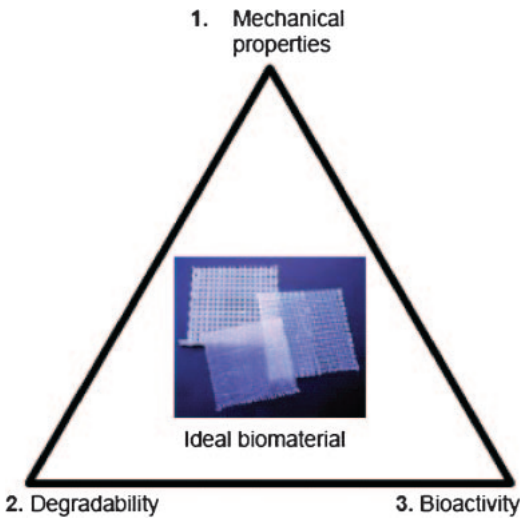

Fig. 2. The biomaterials trinity. The ideal scaffold has to meet three properties. It has to have the right mechanical properties, it has to be degradable and incorporation of bioactive molecules has to be possible.

to be transplanted. In these cases inert implants cannot be used. Therefore, tissue engineering,[3] in which devices specifically interact with the human body to achieve local regeneration, is a promising strategy to tackle this problem.

The tissue-engineering concept implies the formation of a new tissue by means of culturing of the patient's own cells on a polymer scaffold (Fig. 1). For correct tissue formation, the cells have to receive the right signals to form the tissue, while the polymer scaffold has to be degraded. These so-called signals can be present as soluble growth factors in the culture medium and as immobilized bioactive molecules in the scaffold. Furthermore, the newly formed tissue has to have ample mechanical strength to replace the scaffold in time. Besides that, the scaffold has to have the right geometry, because culturing cells on a simple piece of plastic will not result in for example a TE heart valve. To this end, it is important that the scaffolds, which have to guide and regulate the formation of the novel tissue, match with the tissue required. Therefore, we propose that ideal scaffolds have to consist of biomaterials, which are, next to being biocompatible and processable, able to fulfil the biomaterials trinity of tuneable mechanical properties, biodegradability and bioactivity (Fig. 2). Ultimately, the engineered tissue has to be transplanted into the patient. In this way, custom-made implants are produced.

2.2 The Biomaterials Trinity. Difficulties arise in the precise design of these so-called third generation materials[5] caused by synthetic reasons and lack of knowledge about what the exact properties have to be. Many examples have been disclosed in which at least two of the three standards are met. Here, a number of examples are shown in which mechanical properties, biodegradability and the incorporation of bioactivity are investigated (Fig. 2).

The mechanical properties of the scaffold are important because every tissue and organ in the human body shows different mechanical behaviour.[7] Mechanical properties of biomaterials can, for instance, be regulated by varying molecular weight or using co-polymers. As an example, the material properties of poly(trimethylene carbonate) (PTMC) are strongly dependent on the molecular weight of the polymers.[8] It has been shown that very high molecular weight PTMC with M_n above 200 kg mol^{-1} has excellent mechanical properties ($E = 6$ MPa, $\sigma_{break} = 12$ MPa; $\varepsilon_{break} = 830\%$). This PTMC displayed strain-induced crystallization in contrast to low molecular weight counterparts. In another example it has been shown that photopolymerization of hyaluronic acid modified with methacrylic anhydride into networks resulted in materials with different compressive moduli, ranging from 2 to over 100 kPa depending on the molecular weight (from 50 to 1100 kDa).[9] Furthermore, co-polymers of TMC and D,L-lactide (DLLA) display different mechanical properties than the homopolymers.[10]

Often, the mechanical properties and rate of degradation are connected. Control over the rate of degradation is of major importance, because the formation of new tissue has to occur simultaneously (Fig. 3). If the polymer matrix is already degraded before the tissue is formed, the construct will fall apart. It has been shown that different polymers have different degradation rates:[7] whereas degradation of polycaprolactone takes more than two years,[11] poly(glycolic acid) is degraded within weeks. To get control over degradation rates, many co-polymers have been investigated.[7] Also, the molecular weight of the polymers and morphology of the scaffolds[12] play an important role in the rate of degradation. Besides that, degradation rates can differ between in vitro and in vivo degradation studies. As an example, PTMC hardly degrades in vitro, but shows complete resorption after one year of subcutaneous implantation.[13] In addition, it is important to regulate the mechanism of degradation. Whereas surface erosion is accompanied with gradual mass loss, bulk erosion shows the accumulation of acid in the interior of the material which ultimately leads to disintegration accompanied by a burst release of acid.

It is beyond the scope of this account to give an overview of all polymer systems that have been investigated concerning their mechanical properties and degradation behaviour. However, it is important to keep in mind that both properties can be regulated by varying molecular weight, by applying different scaffold morphologies or by using several co-polymers.

Besides control over mechanical properties and degradability, the incorporation of certain bioactive factors is important, because in that way the cells can be guided to form the right tissue. Examples of bioactive molecules are peptide sequences and proteins.[14–16] In general, two methods are used to modify materials with bioactives. This can be done by simply mixing, which leads to dynamic systems which are rather unstable (Figs. 4A and 4C). However, this approach might be very useful in drug delivery systems, provided the rate of release can be controlled. In addition, covalent modification of polymers shows great promise (Figs. 4B and 4D). The latter results in stable materials of which the dynamics are limited. This lack of dynamics can be beneficial, because in this way the bio-

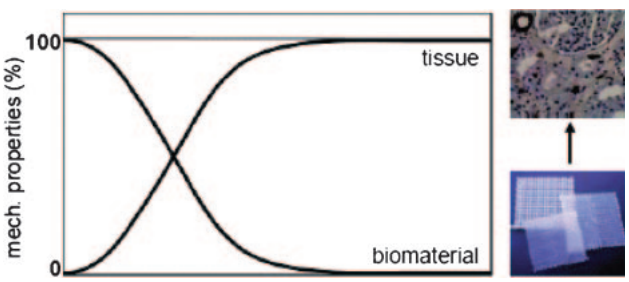

Fig. 3. Degradation of scaffolds has to be accompanied by simultaneous formation of new tissue.

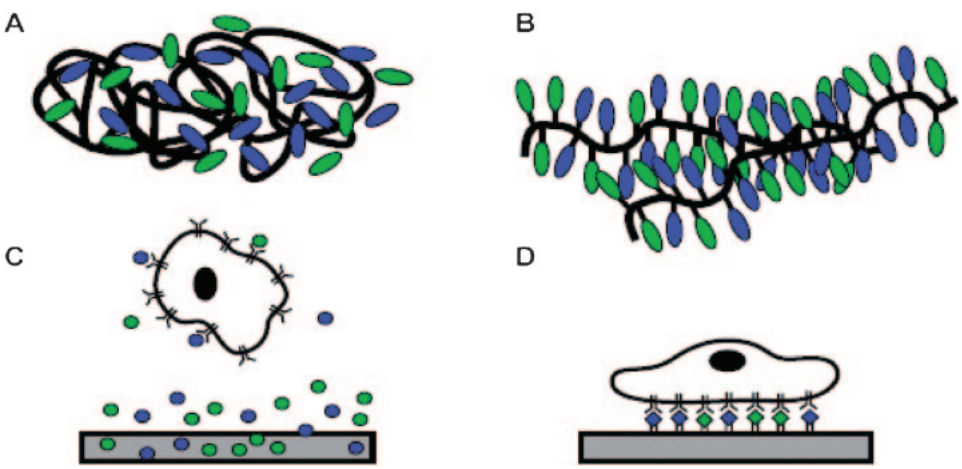

Fig. 4. Bioactive materials can be produced by two methods: **A.** non-covalent modification or, **B.** covalent coupling of bioactive molecules. **C.** Non-covalent modified materials are dynamic but rather unstable, which leads to delivery of the bioactives that can exert their function in the environment. **D.** Covalent functionalized materials are stable but not dynamic, which results in local functioning of the activity at the surface of the material.

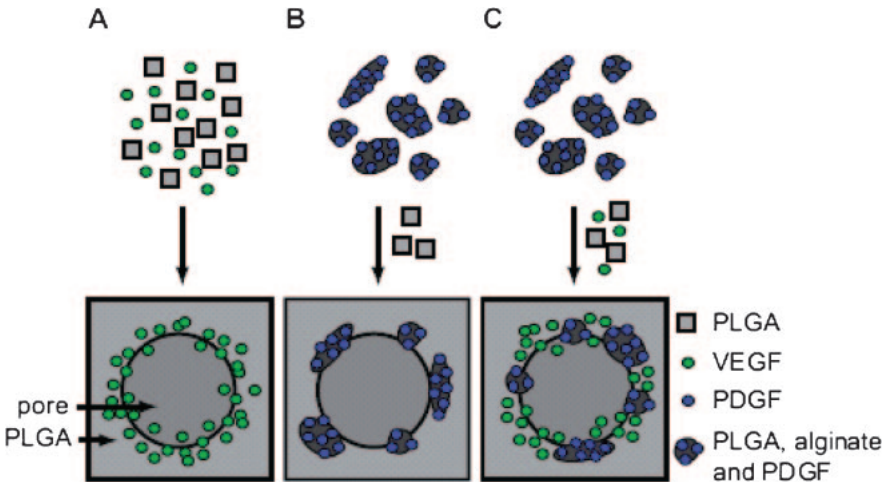

Fig. 5. Schematic representation of bioactive porous polymer scaffolds for growth factor release. The different scaffolds show distinct release kinetics.[17] **A.** VEGF was mixed with PLGA. **B.** PDGF was incorporated in PLGA–alginate microspheres before processing with PLGA into scaffolds. **C.** PDGF containing PLGA–alginate microspheres were processed with PLGA and VEGF.

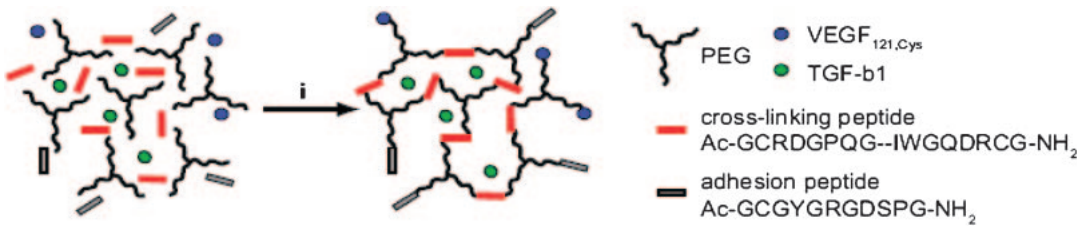

Fig. 6. Vinylsulfone–poly(ethylene glycol) (PEG) macromers were incubated with thiol-containing RGD peptides, TGF-β1, $VEGF_{121}$ with a C-terminal cysteine and dithiol–peptides containing a cleavage site for MMPs as cross-linking peptides. This resulted in the formation of a hydrogel network at (**i**=) physiological temperature and pH.[18]

active molecule has to exert its function localized at the surface of the material. However, it can also be a major drawback because the system cannot adapt to the environment. Additionally, the synthetic versatility remains limited and the polymers require rather high processing temperatures, which mostly results in deactivation of the bioactive species.

Many examples have been shown in which materials were made bioactive performed by one of both methods. The first example shows non-covalent modification of poly(lactic-co-glycolic) (PLGA) matrices with vascular endothelial growth vector (VEGF) and platelet derived growth factor (PDGF) (Fig. 5).[17] VEGF was mixed with the PLGA polymer and PDGF was incorporated into microspheres of PLGA and alginate before processing. A porous scaffold was formed which showed dual growth factor release, in which each growth factor showed distinct kinetics. Rapid formation of a mature vascular network was observed after subcutaneous implantation.

Secondly, a smart material was designed, that could spontaneously assemble into a growth factor bearing hydrogel network at physiological pH (Fig. 6). Vinylsulfone–poly(ethylene glycol) (PEG) macromers were incubated with thiol-containing RGD (Arg–Gly–Asp) peptides, transforming growth factor β1 (TGF-β1) and $VEGF_{121}$ with a C-terminal cysteine. After that, dithiol–peptides containing a cleavage site for matrix metalloproteinases (MMPs) were used as cross-linking moieties, resulting in the formation of the hydrogel network.[18] $VEGF_{121}$ was covalently cross-linked whereas TGF-β1 was non-covalently incorporated. This network could be proteolytically degraded in vitro by cell-derived MMPs. Endothelial cells could adhere and spread out after 3 days on these PEG–peptide hydrogels containing VEGF, whereas on the reference gels without VEGF hardly any adhesion and no spreading could be observed.

The modification of materials with peptides is synthetically more accessible. The most extensively studied peptide is probably the cell adhesion RGD[19,20] sequence. Many polymers, materials, and surfaces have been modified with this sequence to study cell adhesion or as tissue-engineering application.[21] An elegant example is shown in which peptide graft co-polymers were synthesized using metathesis ring opening polymerization (ROMP) of peptide norbornene monomers.[22,23] The peptide norbornene monomers were synthesized on the solid support and consisted of the cell adhesion GRGDS (Gly–Arg–Gly–Asp–Ser) peptide sequence and its synergistic PHSRN (Pro–His–Ser–Arg–Asn) sequence (Fig. 7).[24,25] It has been shown that cell adhesion of fibroblasts to fibronectin was inhibited.

Another example in which surfaces have been modified with RGD peptides is shown in Fig. 8. Star-shaped PEG polymers containing isocyanate end-groups were reacted with amine-containing glass surfaces after which GRGDSC peptides were reacted with the remaining isocyanate groups.[26] Gradients of RGD, varying from 1 RGD to less than 0.1 RGD per star, were produced. The amount of human osteosarcoma cells adhered and spread on the film increased with increasing concentration of RGD. In vivo evaluation of poly(methyl methacrylate)

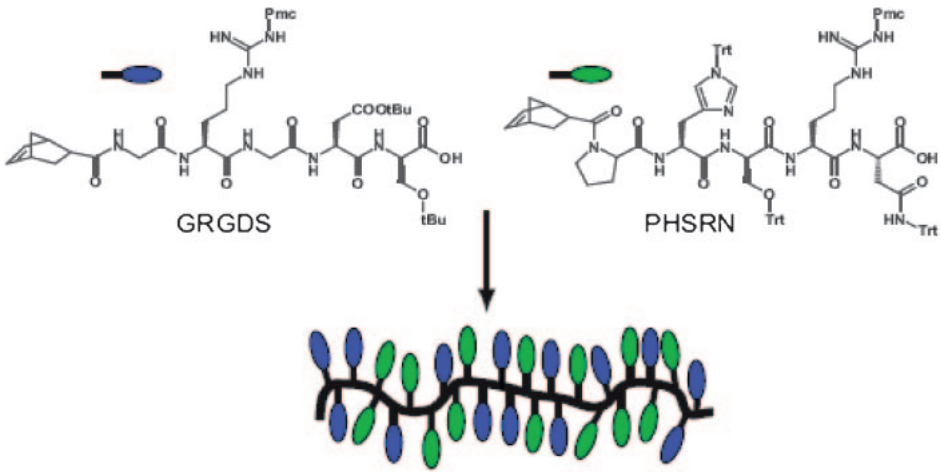

Fig. 7. Norbornene peptide monomers used for ring-opening metathesis polymerization (ROMP) consisting of the GRGDS peptide sequence or of the PHSRN peptide sequence. Co-polymerization results in the formation of a graft co-polymer containing both peptides.[22,23] The used protection groups are 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl (Pmc), *t*-butyl (*t*Bu), *t*-butoxy (O*t*Bu), and trityl (Trt).

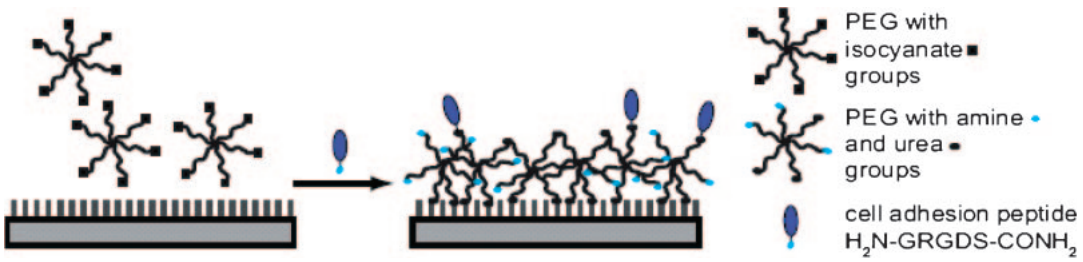

Fig. 8. Star-shaped poly(ethylene glycol) polymers on glass were modified with RGD peptides.[27]

(PMMA) beads covalently functionalized with acrylamide containing cyclic-RGD peptides after implantation in bones of rabbits showed enhanced bone ingrowth in the presence of the peptides.[27] This indicates that the RGD-peptides are also active in vivo.

Many research groups all over the world perform studies on non-covalent and covalent modification of biomaterials. Here, we would not like to give a whole overview on this area, but we only would like to indicate a few examples as illustration of the field. In conclusion, bioactive proteins can be incorporated in hydrogel systems or polymer scaffolds. However, the chemistry for this modification is limited, because of synthetic restrictions due to the size of the proteins, their high level of functional groups, their incompatibility with organic solvents and their folded tertiary structure, which have to be maintained. In addition, proteins can hardly be coupled during polymerization of the polymer chains. More convenient is the incorporation of bioactive oligopeptides that can also be coupled to solid polymers such as thermoplastic elastomers. These oligopeptides can be used in their protected form and can even be introduced during polymerization reactions. However, problems arise when the concentration and nature of the peptide has to be changed; all reactions and polymerizations have to be repeated. Furthermore, covalent biofunctionalization results in control over the stability of the biomaterial, however, this system is not dynamic. For non-covalent modification the opposite applies, the bioactive material is dynamic but not stable. Many of these difficulties can be overcome by the use of supramolecular chemistry, which enables to assemble the biomaterial after building all the separate blocks. Then, via supramolecular synthetic methods several biomaterials can be produced containing different compositions and concentrations of peptides. Supramolecular hydrogen-bonded polymers and self-assembling peptide architectures are interesting supramolecular systems, which have been extensively studied with respect to their materials properties both in solution and in the solid state. These two systems are eminently suitable for tissue-engineering purposes as stated in our introduction.[28,29] This is not only the result of convenient synthesis methods via assembly procedures and their ability to fulfil the biomaterials trinity, but also and evenly important, their dynamic behaviour might be beneficial when brought into contact with the living cells in the host tissue. In order to discuss their applicability as biomaterials for TE applications, we now first would like to briefly describe the relevant aspects of these supramolecular systems in general. Then, we propose a biomaterials trinity for ideal scaffolds.

3. Supramolecular Hydrogen-Bonded Polymers

3.1 Supramolecular Polymers—An Introduction. It was only after the pioneering work of Staudinger that it became evident that polymeric properties in both solution and solid state are the result of the macromolecular nature of the molecules.[30] A large number of repeating units are covalently linked into a long chain and the entanglements of the macromolecular chains are responsible for many of the typical poly-

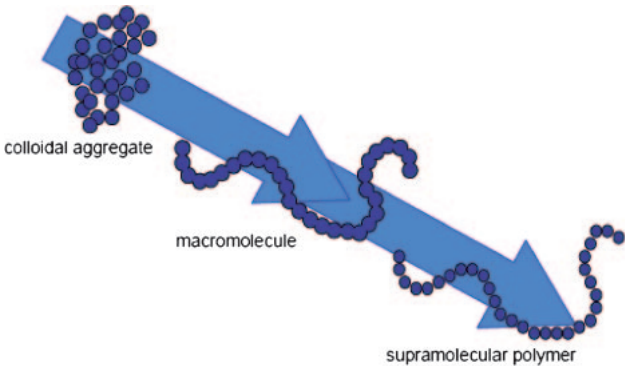

Fig. 9. Schematic representation of the evolution in time of macromolecular science from colloids, via the original work of Staudinger (who showed, for the first time, that high molecular weight polymers do exist), to supramolecular polymers that can be seen as a combination of both earlier concepts: a high molecular weight polymer held together by secondary interactions.

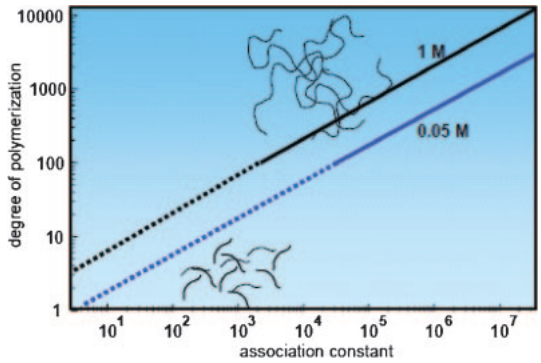

Fig. 10. A theoretical plot of the relation between association constant K_a and degree of polymerization DP as a function of concentration, using a simple isodesmic-association function.

mer properties. Before macromolecules were generally accepted, the majority of scientists was convinced that polymer properties were the result of the colloidal aggregation of small molecules or particles (Fig. 9).

The impressive recent progress in supramolecular chemistry, paved the way to design polymers and polymeric materials that lack the macromolecular structure. Instead, when the covalent bonds that hold together the monomeric units in a macromolecule are replaced by highly directional non-covalent interactions, supramolecular polymers are obtained (Fig. 9).[31] In recent years, a large number of concepts have been disclosed that make use of these non-covalent interactions. The term "supramolecular polymer" is rather popular and used for a variety of different structures, utilizing secondary (or supramolecular) interactions between chains or for the construction of polymer chains. In the following definition, the polymers that have secondary interactions between macromolecular chains only are ignored, since all polymers possess either hydrogen bonding (nylons), dipole–dipole (polyesters), or London-dispersion interactions (polyethylene), that determine their materials properties. Hence, supramolecular polymers are defined as polymeric arrays of monomeric units that are brought together by reversible and highly directional non-covalent interactions, resulting in polymeric properties in dilute and concentrated solution as well as in the bulk. The directionality and strength of the supramolecular interaction are important features of systems that can be regarded as polymers and that behave according to well-established theories of polymer physics.[32,33]

It is useful to review some of the general aspects of the supramolecular approach, taking into account the limitations of our definition of supramolecular polymers. Using a directional complementary couple (A–B) or a self-complementary unit (A–A), it is possible to form all known structures of polymers, including linear homo- and copolymers, cross-linked networks and branched structures.[31] Generally, the assembly of bi- or multifunctional monomers can be considered as a step-growth process, with the number average degree of polymerization (DP) of bifunctional monomers defined by the Carothers equation.[34] If the stepwise polymerization of bifunctional monomers is considered non-cooperative and the association constant for the non-covalent interaction is known, the DP can be calculated. The degree of polymerization is obviously dependent on the concentration of the solution and the association constant as shown in a theoretical relationship (Fig. 10). To obtain polymers with a high molecular weight, a high association constant between the repeating units is a prerequisite. In analogy with covalent condensation polymers, the chain length of supramolecular polymers can be tuned by the addition of monofunctional "chain stoppers."[34] This also implies that a high purity of bifunctional monomers is essential, since a small fraction of monofunctional impurity will strongly decrease the DP. Hence, as in traditional polymer synthesis, monomer purification is extremely important to obtain high molecular weight polymers.

There are three main categories of supramolecular polymers based on metal–ligand coordination, π–π stacking or hydrogen bonding (Fig. 11). Although most of the structures keep their polymeric properties in solution, it was only after the careful design of multiple-hydrogen-bonded supramolecular polymers that systems were obtained that show true polymer materials properties, both in solution and in the solid state.[32,35] Polymers based on this concept hold promise as a unique class of novel materials because they combine many of the attractive features of conventional polymers with properties that result from the reversibility of the bonds between the monomeric units.[35] Architectural and dynamic parameters that determine polymer properties, such as degree of polymerization, lifetime of the chain and its conformation, are a function of the strength of the non-covalent interaction, which can reversibly be adjusted. This results in materials that are able to respond to external stimuli in a way that is not possible for traditional macromolecules. Therefore, we will only describe multiple-hydrogen-bonded supramolecular polymers in this account.

3.2 Hydrogen-Bonded Structures. Hydrogen bonds are formed between atoms such as oxygen or nitrogen that have an electronegativity larger than hydrogen. The atom to which the hydrogen is connected, is referred to as the hydrogen-bond donor (D) and the other atom is called the hydrogen-bond acceptor (A). The typical bonding energy of a hydrogen bond is 10–80 kJ mol^{-1} in the gas phase. Combining several hydrogen

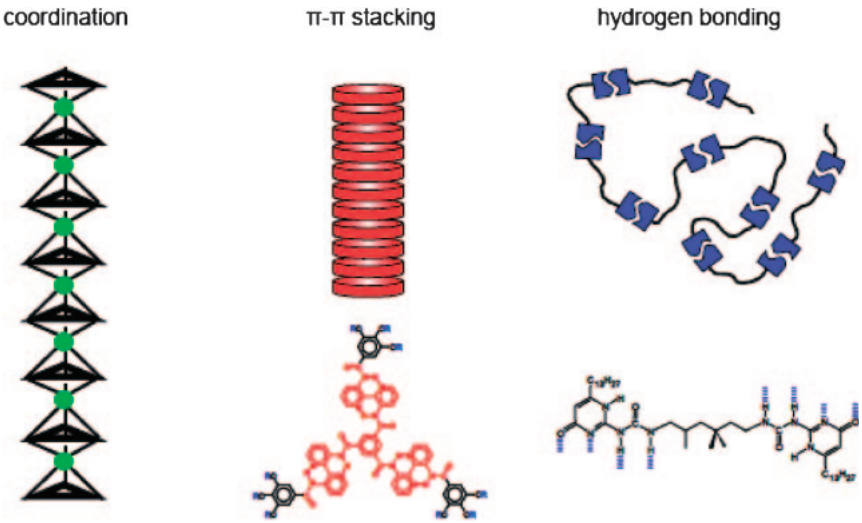

Fig. 11. The three main categories of supramolecular polymers, divided by the type of non-covalent interaction.

bonds in a functional unit is a valuable tool to increase the strength of this interaction and by employing a particular arrangement of the hydrogen-bonding sites to enhance its specificity. These arrays can be complementary or self-complementary. The strength of single hydrogen bonds basically depends on the nature of donor and acceptor, although it is influenced to a large extent by the solvent. Association strength between multiple-hydrogen-bonding units obviously depends on the same factors, as well as on the number of hydrogen bonds. It has also been shown that the particular arrangement of neighbouring donor and acceptor sites is an additional factor, which significantly affects the strength of the complexation.[36,37] This phenomenon was first recognized for the association of linear arrays of 3 hydrogen-bonding sites. Whereas complexes between the common ADA–DAD motif exhibits an association constant of around $10^2\,M^{-1}$ in chloroform, this value is around $10^4\,M^{-1}$ in complexes with a DAA–DDA motif, while AAA and DDD arrays exhibit association constants exceeding $10^5\,M^{-1}$. Detailed calculations by Jorgenson et al. showed that this effect is due to differences in secondary interactions between these motifs.[36,37] In the complexes, diagonally opposed sites repel each other electrostatically when they are of the same kind (both donor or both acceptor), while disparate sites attract each other. In the DDD–AAA motif, the number of attractive secondary interactions is maximized, while in the ADA–DAD motif the number of repulsive interactions is at its largest. Very stable complexes can be obtained when quadruple hydrogen-bonding units are employed.

We have reported on self-complementary quadruple H-bonding units based on mono-ureido derivatives of diaminotriazines[38] (DADA-array) with a dimerization constant of $K_{dim} = 2 \cdot 10^4\,M^{-1}$. Even more stable structures are based on 2-ureido-4[1*H*]-pyrimidinones (DDAA-array) with an even higher dimerization constant of $6 \cdot 10^7\,M^{-1}$ in chloroform, $1 \cdot 10^7\,M^{-1}$ in chloroform saturated with water and, $6 \cdot 10^8\,M^{-1}$ in toluene.[39–41] The UPy-moiety exists as a mixture of three tautomers of which two can dimerize, the keto and enol tautomer (Fig. 12A). Both dimerizing tautomers have different dimerization constants as a result of diagonal secondary electrostatic interactions.[36,37] The keto tautomer displays an AADD array, whereas the enol form consists of an DADA array. The electrostatic effects have been quantified and it has been shown that each primary hydrogen-bonding interaction showed a contribution of $\approx 8\,kJ\,mol^{-1}$ to the free energy of complexation.[42] Each attractive or repulsive secondary interaction increases or decreases the free energy with 2.9 $kJ\,mol^{-1}$, respectively. This implies that the keto UPy-tautomer has less repulsive secondary interactions and therefore a higher dimerization constant than the enol UPy-tautomer.

3.3 Supramolecular Hydrogen-Bonded Polymers. Supramolecular polymers are formed when hydrogen-bonding units are applied as associating end-groups of bifunctional molecules. The association constants must be sufficiently high to get a high degree of polymerization, which results in real polymer properties. The first supramolecular polymeric systems based on hydrogen bonding were published by the groups of Lehn[43,44] and Kato/Fréchet;[45,46] their liquid-crystalline examples together with those of the group of Griffin,[47,48] led to exploration of the many potential materials properties of supramolecular polymers (Fig. 13). The cooperativity between the association of the molecules and the anisotropy of the mesophase is used: directional head-to-tail association tends to align the molecules, whereas anisotropy increases the degree of association of the functional groups. Using this kind of cooperativity, it was possible to obtain materials that are connected by a single hydrogen bond between carboxylic acid and pyridyl functional groups. In all of these liquid-crystalline supramolecular polymers, the individual units are not liquid-crystalline, but the mesogens arise as a result of the formation of the hydrogen bond. In diluted solution, however, these structures dissociate into their individual building blocks and lack any property that is so typical for synthetic polymers.

The group of Rebek, Jr., has developed an ingenious way to form supramolecular polymers by utilizing the hydrogen bonding between urea functionalized calixarenes (Fig. 14).[49–52] Association of bifunctional molecules consisting of two covalently connected calixarene moieties, results in the formation of "polycaps." The association between the monomers is based upon hydrogen bonding in cooperation with complexation of a small guest; the polymerization of the assembly is driven

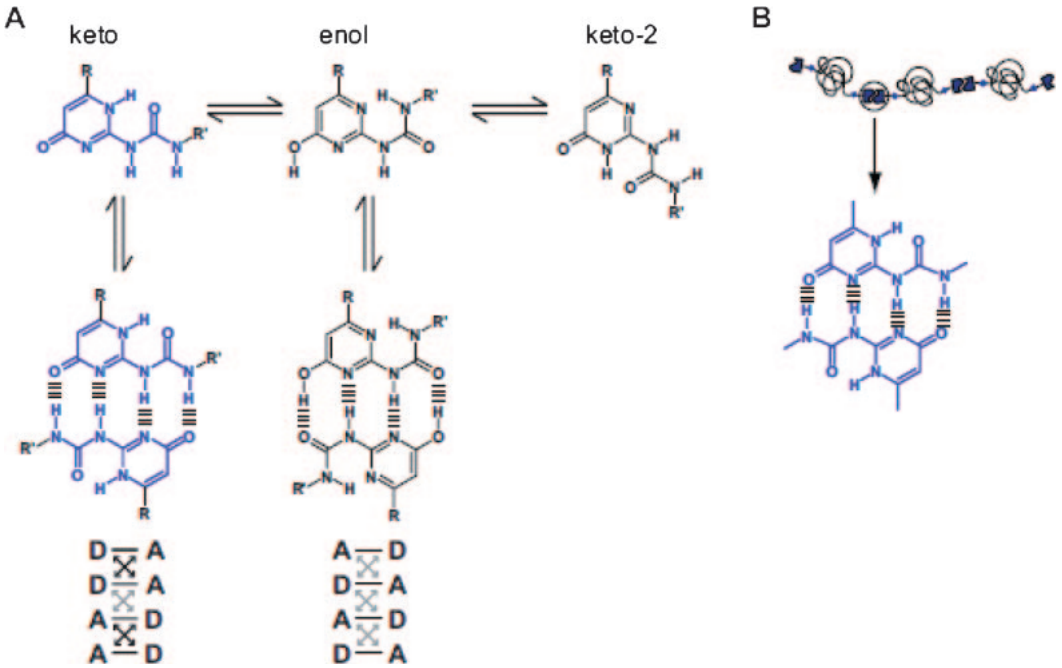

Fig. 12. The ureido–pyrimidinone (UPy) unit. **A.** The different tautomers in which the UPy can exist; the self-complementary forms that are able to dimerize via quadruple hydrogen bonding (keto and enol), and the tautomer that cannot dimerize (keto-2). The hydrogen-bonding arrays are indicated showing next to the primary hydrogen bonds (solid line), also the attractive (black arrow) and repulsive (grey arrow) secondary electrostatic interactions. **B.** Schematic representation of UPy-functionalized prepolymers. End-modification of telechelic prepolymers results in chain extension via UPy–UPy dimerization.

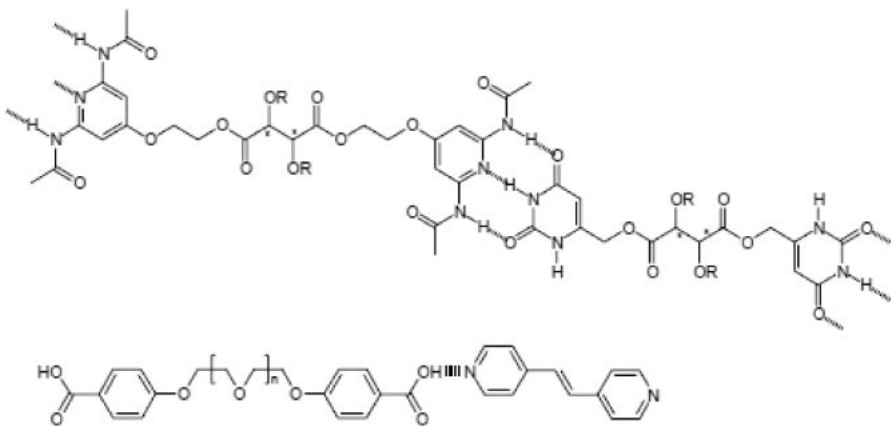

Fig. 13. Two examples of supramolecular polymeric systems, using liquid-crystalline phases to stabilize the hydrogen-bonding interactions.

by encapsulation of small guests such as benzene. The physical integrity of the supramolecular polymers under shear was demonstrated by the strong normal forces in rheometry experiments. Additionally, the "polycaps" can be drawn into fibers with a tensile strength in the order of 10^8 Pa.

Another nice example of supramolecular polymer aggregates based on complementary units is shown by Craig et al., in which several oligonucleotides are used to produce self-assembled A–B-type polymers via base pairing (Fig. 15).[53,54] Remarkably, incorporation of short spacers for X within the monomer results in an increased probability for cyclization and increased time scales of equilibration. Furthermore, for other interesting examples and their physical properties we refer to the many nice reviews that have been written.[32,33,55–58]

Although these supramolecular polymers possess intriguing new properties, synthetic barriers hamper extensive study of the mechanical properties of these materials. The supramolecular polymers discussed above are the products of multistep synthesis, and it is a daunting task to prepare sufficient amounts of material for e.g. melt-rheological experiments and tensile testing.

The development of the UPy-functionality, a synthetically very accessible quadruple hydrogen-bonding unit with a very high association constant, has helped enormously to open the way to complete exploration all aspects of supramolecular polymers. The UPy-unit can be made in a one-step procedure from commercially available compounds.[38,40] Difunctional compounds, possessing two of these UPy-units form very

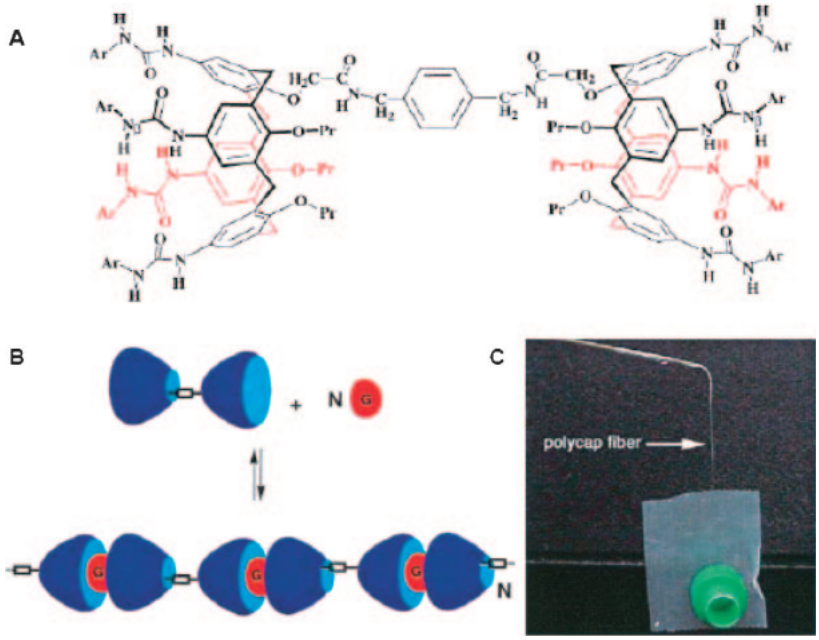

Fig. 14. **A.** Ditopic calixarene which can form **B.** "polycaps" by complexation with quest molecules. **C.** These "polycaps" can be drawn into fibers.[49,52] Reprinted with permission from *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 7132, and **2000**, *97*, 12418. Copyright 1997, 2000 National Academy of Sciences, USA.

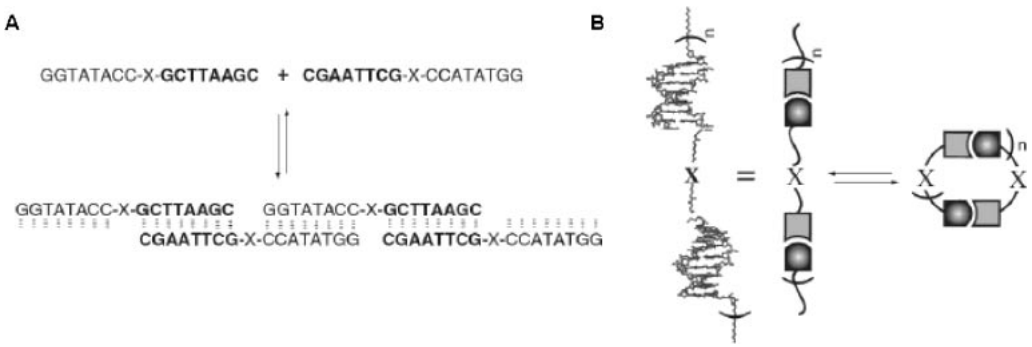

Fig. 15. Supramolecular polymerization of complementary oligonucleotides. **A.** Self-assembly of an oligonucleotide without spacer. **B.** Schematic representation of the dynamic equilibrium in nucleotide-based polymers.[53,54] Reprinted with permission from *Macromolecules* **2004**, *37*, 1863. Copyright 2004 American Chemical Society.

stable and long polymer chains in solution as well as in the bulk. Dissolving a small amount of this low molecular weight compound in chloroform, results in solutions with a high viscosity. It can be calculated that polymers with chain lengths of the order of 10^6 Dalton can be formed when highly purified monomers are used. The presence of monofunctional impurities is expected to lead to a dramatic reduction in DP, because they will act as "chain stoppers." In fact, deliberate addition of small amounts of monofunctional compounds results in a sharp drop in viscosity, proving the reversibility and uni-directionality of association. The reversibility of the linkages between the building blocks is instrumental in the development of materials that change their properties in response to environmental changes, so-called "smart materials." Application of a light sensitive monofunctional compound yielded a material from which the degree of polymerization in solution could be tuned by UV-irradiation.[59] Although the supramolecular polymers based on bifunctional UPy-derivatives in many ways behave like conventional polymers, the strong temperature dependence of their mechanical properties really sets them apart from macromolecular polymers. At room temperature, the supramolecular polymers show polymer-like viscoelastic behavior in bulk and solution, whereas at elevated temperatures liquid-like properties are observed. These changes are due to a threefold effect of temperature on the reversible polymer chain. Due to the temperature dependence of the K_a value of UPy association, the average DP of the chains is drastically reduced at elevated temperatures. Simultaneously, faster dynamics of the scission–recombination process leads to faster stress relaxation in an entangled system. These two effects occur in addition to the temperature-dependent stress relaxation processes that are also operative in melts or solutions of conventional polymers. Similar to the behaviour in the melt, solution viscosities of UPy-based supramolecular polymers are also strongly temperature dependent.

Although the supramolecular hydrogen-bonding polymers

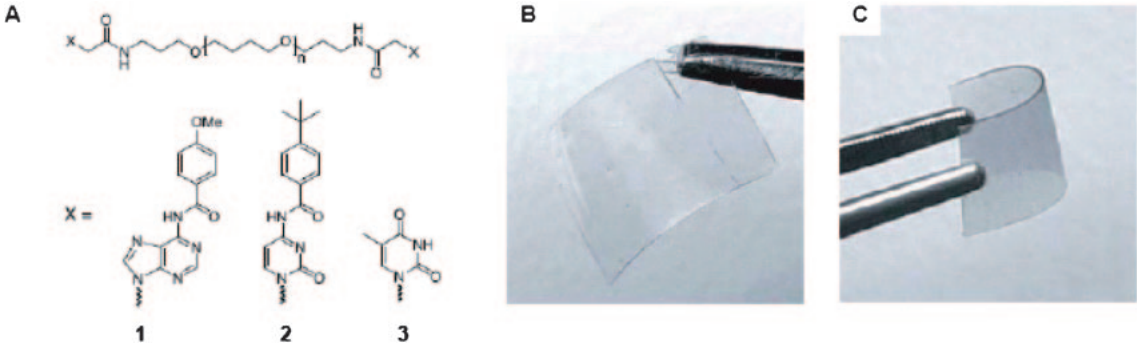

Fig. 16. **A.** Bifunctional prepolymers modified with nucleobase derivatives and pictures of films formed from **B. 1** and **C. 2**.[64,65] Reprinted with permission from *J. Am. Chem. Soc.* **2005**, *127*, 18202. Copyright 2005 American Chemical Society.

based on small building blocks possess intriguing new properties, they have some disadvantages; small building blocks have an increased tendency to form small discrete assemblies through cyclization.[60] Besides that, in some cases the recognition unit itself is over several kDa in size and contributes significantly to the materials properties observed. Therefore, in order to obtain supramolecular polymers with tuneable and macroscopic polymer properties, the supramolecular functionalities need to be separated by polymeric spacers (Fig. 12B). In this way, chain extension or functionalization of macromonomers or prepolymers can be accomplished. This results in supramolecular materials with real mechanical properties, which are prerequisites for many real polymer applications, and especially for tissue engineering purposes.

Lenz and co-workers were the first to acknowledge that the physical properties of linear polymers are dramatically changed when modified with associating end-groups. They proposed that the liquid-crystalline behaviour and the ability to form elastomeric films of polyglycols terminated with diacids could be explained by dimerization of the carboxylic end-groups.[61] Lillya et al. have also shown that dimerization of the carboxylic acid units in benzoic acid-modified poly(tetrahydrofuran) (PTHF) results in significant improvement of the material properties owing to the formation of large crystalline domains of the hydrogen-bonding units.[62] Only a slight change in material properties was observed when poly(dimethylsiloxanes) (PDMS) were functionalised with benzoic acid groups.[63]

Nucleobases were used as recognition motifs to functionalize low molecular weight prepolymers by the groups of Rowan[64,65] and Long.[66–68] Upon modification of the bisamino end-groups of telechelic PTHF with a molecular weight around $2\,\mathrm{kg\,mol^{-1}}$ with adenine- or cytosine-derivatives, respectively polymers **1** and **2**, the material properties changed dramatically from a soft waxy solid to flexible materials with enough mechanical stability to be processed into fibers and films (Fig. 16).[64,65] Both materials show extreme temperature sensitivity which results in the formation of very low viscosity in the melt. Interestingly, the thymine-modified PTHF **3** did not exhibit such properties which was attributed to its high crystallization temperature. Long and co-workers have functionalized poly(styrenes) (PS) with adenine, thymine, or uracil.[66,67] They found assemblies of adenine–PS and thymine–PS in solution. Besides that, they also showed the synthesis of uracil-modified poly(*n*-butyl)acrylate. Interestingly, they found strong associations between four-arm star-shaped adenine and thymine-containing poly(D,L-lactides).[68] Furthermore, the group of van Hest showed the synthesis of nucleobase-functionalized block copolymers via atom-transfer radical polymerization of thymine, adenine, cytosine, and guanine nucleobase-functional methacrylates.[69,70]

Table 1. UPy-Modified Polymers as Published in Literature

Compound	Polymer[a)]	Coupling	# UPy	M_n/kg mol^{-1}	References
4a/**4b**	PDMS	direct	di	0.18/6.0	41, 71
6a	PEB	HDI	di	3.5	72–74
6b	PEO–PPO	HDI	di	2.0	72
6c	PTHF	HDI	di	1.0	76
6d	PHMC	HDI	di	2.2	72
6e/**6f**	PTMC	HDI	di	2.0/4.0	77
6g	PBE	HDI	di	2.3	72
6h	PBT	HDI	di	5.3	81
6i	PBI	HDI	di	5.0	81
6j	PCL	HDI	di	2.1	28, 80
6k/**6l**	PE-*co*-PP	HDI	di	12/16	79
7a/**7b**/**7c**	PS	IPDI	mono	3.3/5.7/31	78
7d/**7e**/**7f**	PI	IPDI	mono	3.8/6.9/23	78
7g/**7h**	PS-*b*-PI	IPDI	mono	8.4/53	78
7i	PE-*co*-PP	IPDI	mono	19	79
8a	PEO–PPO	IMCI	tri	6.0	75
8b/**8c**/**8d**	PTMC	HDI	tri	1.9/3.7/13	77
8e	PE-*co*-PP	IPDI	star	88	79

a) The compound numbers correspond to the numbers in Fig. 18. The prepolymer, the coupling method of the UPy to the polymer, the amount of UPy-moieties and the M_n of the polymers is given. Abbreviations: PDMS = poly(dimethylsiloxane), PEB = poly(ethylene butylene), PEO–PPO = poly(ethylene)–poly(propylene), PTHF = poly(tetrahydrofuran), PHMC = poly(hexamethylene carbonate), PTMC = poly(trimethylene carbonate), PBE = poly(butylester), PBT = poly(butylene terephthalate), PBI = poly(butylenes isophthalate), PCL = poly(caprolactone), PE-*co*-PP = poly(ethylene-*co*-propylene), PS = poly(styrene), PI = poly(isoprene), PS-*b*-PI = poly(styrene)-*block*-poly(isoprene), IMCI = 3(4)-isocyanatomethyl-1-methylcyclohexyl isocyanate, IPDI = isophorone diisocyanate, HDI = hexamethylene diisocyanate.

The quadruple hydrogen-bonding UPy-unit, developed in our group, has been further employed in the functionalization of several low molecular weight polymers, such as poly(dimethylsiloxanes) (PDMS),[41,71] poly(ethylene butylenes) (PEB),[72–74] poly(ethers),[72,75,76] poly(carbonates),[72,77] poly(styrenes) (PS),[78] poly(isoprenes) (PI),[78] poly(ethylene-*co*-propylenes) (PE-*co*-PP),[79] and poly(esters)[28,72,80,81] (Table 1).

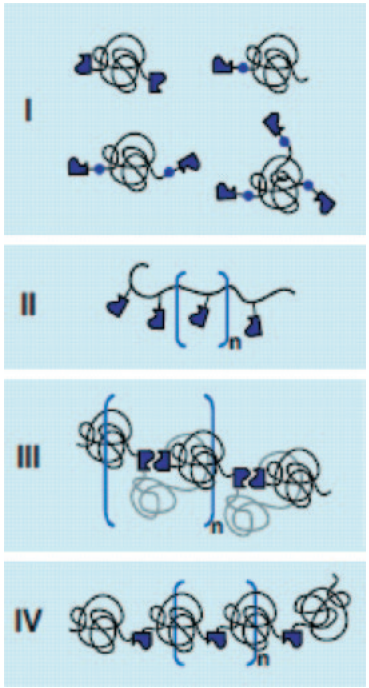

Fig. 17. Four classes of UPy-modified prepolymers. **I.** end-functionalized UPy–polymers, **II.** grafted UPy–polymers, **III.** so-called "modular-domain" UPy–polymers and **IV.** chain-extended UPy–polymers.

The UPy-moiety can be coupled to hydroxy- or amine-terminated oligomers and prepolymers in several manners. Therefore, we have divided the UPy-modified polymers in four classes: **I.** end-functionalized UPy-polymers, **II.** grafted UPy-polymers, **III.** so-called "modular-domain" UPy-polymers and **IV.** chain-extended UPy-polymers (Fig. 17).

The first class of UPy-polymers comprises of prepolymers that have been end-functionalized, directly at the 6-position of the pyrimidinone ring, or via the reaction of a diisocyanate with the amine of methyl-isocytosine. This resulted in several UPy-architectures; mono-, bi-, trifunctional and star-like UPy-polymers. Solution viscosity studies and bulk rheological measurements on UPy-modified oligo-dimethylsiloxane **4a** indicated the formation of high molecular weight PDMS.[71] UPy-PDMS **4b** exhibited viscoelastic bulk properties that differed from the non-modified PDMS which behaves as a Newtonian fluid (Fig. 18A).[41,71] As demonstrated before, the purity of the supramolecular materials is of great importance. Therefore, UPy-synthon **5c** containing a highly reactive isocyanate functionality was designed which is synthetically accessible on large scale by reaction of commercially available isocytosines and hexamethyl diisocyanate (HDI) (Fig. 18B). Convenient reaction of this synthon with amino- or hydroxy-terminated polymers allows for an easy work-up procedure to obtain several UPy-functionalized polymers (Figs. 18C–18E).[72] Furthermore, other commercially available isocyanates, such as IMCI (3(4)-isocyanatomethyl-1-methylcyclohexyl isocyanate) **5a** and IPDI (isophorone diisocyanate) **5b** have been used via first reaction with the hydroxy end-group of the polymer and subsequent reaction with the methyl-isocytosine (Fig. 18B).

The upscaling of the reaction of hydroxy-terminated PEB with synthon **5c** resulted in supramolecular polymer **6a** with less than 0.2% residual hydroxy end-groups.[73] The mechanical properties of the UPy-polymer changed dramatically; whereas the starting material was a viscous liquid, **6a** turned out to be a rubber-like material with a Young's modulus of 5 MPa (Fig. 19). Similarly, functionalization of more polar prepolymers with synthon **5c** also resulted in improved materials properties. Modified polyether **6b** displays a rubber plateau in dynamic mechanical thermal analysis and a storage modulus of 10 MPa.[72] UPy-polyether **6c** and UPy-polycarbonate **6d** were used in "supramolecular" PIPS (polymerization-induced phase separation), as described below (Section **3.4**).[76] Upon UPy-functionalization of PTMC prepolymers, the viscous liquids become strong and flexible materials, **6e** and **6f**. Tunability of the mechanical and thermal properties was achieved by mixing these bifunctional UPy–PTMCs with different trifunctional UPy–PTMCs, **8b–8d**.[77] The UPy-modified polyesters **6g**, **6h**, **6i**, and **6j** are semi-crystalline polymers, whereas the starting materials are brittle solids (Fig. 19).[28,72,80,81] The UPy-functionalized poly(caprolactone) PCL **6j**, was shown to be eminently suitable for tissue-engineering applications, as described below (Section **5.2**).[28,80,82]

Furthermore, monofunctional UPy-modified PS **7a–7c**, PI **7d–7f**, and PS-*b*-PI **7g** and **7h**, synthesized via living anionic polymerization and end-group modification, showed interesting melt viscosities at constant shear rates. These viscosities were more than 100 times higher than the unfunctionalized polymers.[78] In addition, differential scanning calorimetry and rheological characterization suggested the formation of aggregates, and not simple dimers, in the melt state. Also, poly(ethylene-*co*-propylene) prepolymers were coupled to UPy-units.[79] Next to monofunctional PE-*co*-PP **7i**, bifunctional polymers **6k** and **6l** and even a star-shaped structure **8e** were synthesized. Trifunctional UPy–PEO–PPO copolymer **8a** has been shown to assemble into supramolecular networks. Solution viscometry measurements, including chain stopper studies, indicated formation of a reversible network.[75] In addition, due to the formation of reversible cross-links, a higher plateau modulus was observed in dynamic mechanical analysis.

Class II UPy-modified polymers consist of polymers with pendant UPy-groups (Fig. 20). They were prepared via free radical polymerization using UPy-modified methacrylate **9a** and several acrylates or methacrylates resulting in UPy-grafted polymers **9b**.[83,84] Solution and bulk experiments showed great influence of the UPy-units. In addition, strong hydrogen bonding between the UPy-groups in relatively nonpolar solvents increased the apparent molecular weight which resulted in significant larger electrospun fibers than of the unfunctionalized counterparts.[84] Furthermore, branched structures **9c** could be made by the addition of ethylene glycol dimethacrylate to the radical polymerization reaction.[85]

The third class of UPy-group containing supramolecular polymers, the "modular-domain" UPy-polymers, were proposed by Guan et al. (Fig. 21).[86,87] Inspired by titin, a giant protein of the muscle sarcomere that has more than 100 repeating modules and displays high strength, toughness, and elasticity, they synthesized a PTHF containing UPy-functionalities in the main chain. They used a UPy with hydroxy-functionalities at the 6-position and at the urea, compound **10**, which was

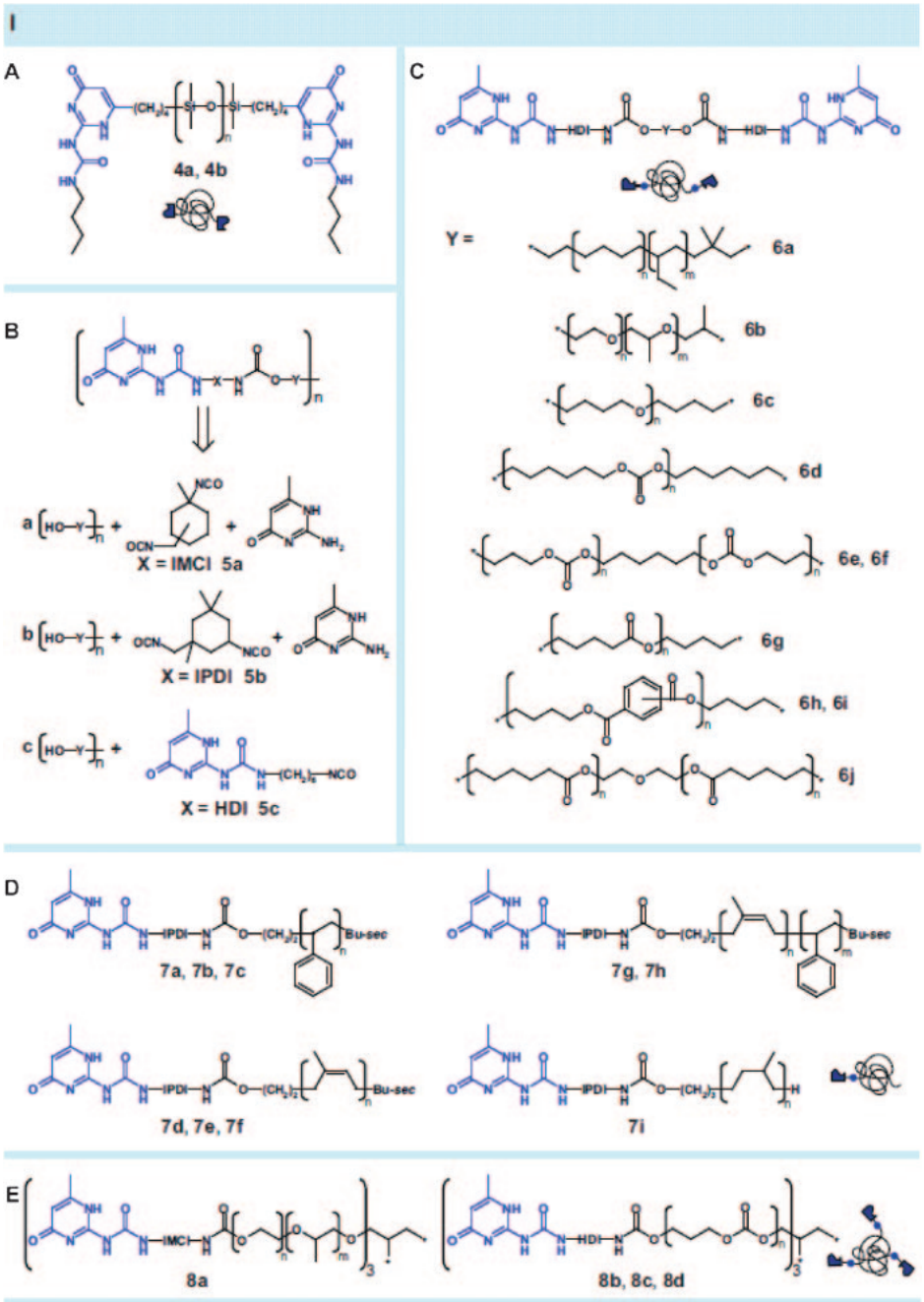

Fig. 18. Class I UPy-modified polymers as published in literature (see also Table 1). **A.** Directly coupled PDMS, **B.** Hydroxy-terminated polymers can be functionalized with UPy-units using three different isocyanates, IMCI, IPDI, or HDI. For the latter a convenient synthesis method is developed using a UPy–isocyanate synthon. **C.** Bifunctional UPy–polymers. **D.** Monofunctional UPy–polymers. **E.** Trifunctional UPy–polymers.

reacted with a HDI end-capped PTHF. Analogous to biopolymers, single-molecular nanomechanical properties were studied with atomic force microscopy (AFM) and demonstrated the sequential breaking of UPy dimers. Stress–strain profiles of solution-cast films of the UPy-based polymer revealed that the polymer was very elastic as evidenced by the high strain up to 900% and complete recovery to its original length. However, the proposed structure was not proven and they already mentioned in their following paper that the UPy-units can randomly bind inter- and intramolecularly because they are self-complementary.[88] Therefore, they have redesigned the system without UPy-moieties, but with a complementary double closed loop formed by a peptidomimetic β-sheet motif.[88]

Meanwhile, the company SupraPolix has designed a UPy-synthon with two isocyanate functionalities **11**, which can be used in easy synthesis of chain-extended UPy-polymers with UPy-moieties in the main chain (Fig. 22).[80] This is proposed to be the IVth class of UPy-modified polymers. However, in our opinion the "modular-domain" UPy-polymer reported by Guan et al., i.e. the class III UPy-polymers, has a similar struc-

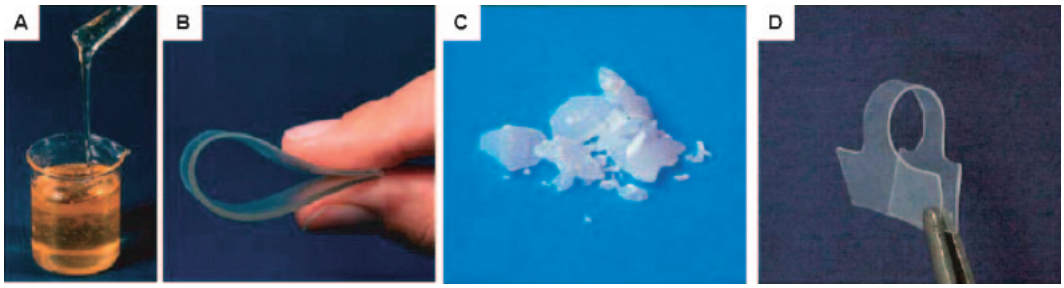

Fig. 19. The material properties of low molecular weight prepolymers change dramatically upon functionalization with UPy-groups. **A.** Hydroxy-terminated PEB, **B.** UPy-modified PEB **6a**, **C.** hydroxy-terminated PCL and **D.** UPy-functionalized PCL **6j**.[28,72]

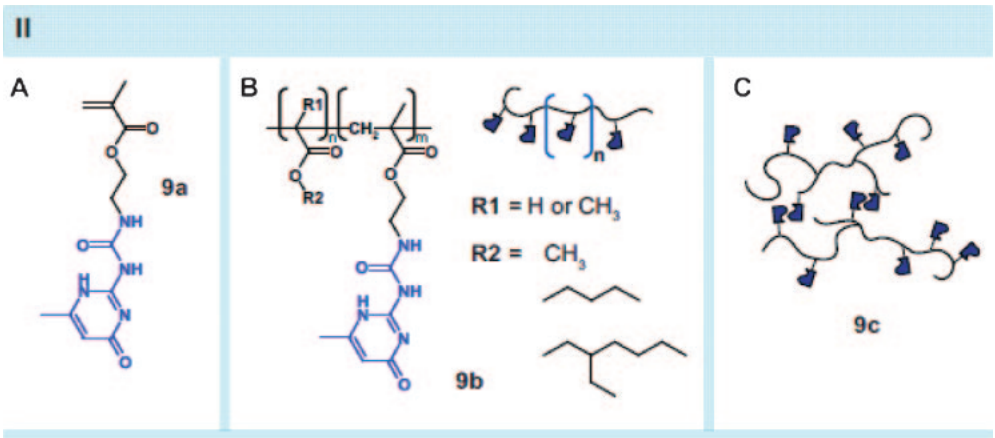

Fig. 20. Class II UPy–polymers. **A.** UPy-modified methacrylate. **B.** Grafted UPy–polymer prepared via radical polymerization. **C.** UPy-network as a result of the addition of EGDMA (ethylene glycol dimethacrylate) to the radical polymerization reaction.[83–85]

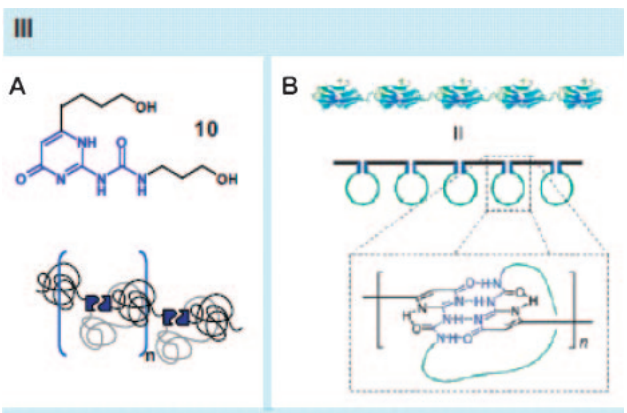

Fig. 21. Class III "modular-domain" UPy–polymers. **A.** UPy-moiety with two hydroxy-functionalities which can be used in the chain-extension of a telechelic PTHF with isocyanate end-groups. **B.** The titin protein and the proposed UPy-dimerization in the main-chain of the UPy-modified PTHF.[86,87] Reprinted with permission from *J. Am. Chem. Soc.* **2004**, *126*, 2058. Copyright 2004 American Chemical Society.

ture as our chain-extended UPy-polymers. Application of our convenient route of modification of low molecular weight PCL led to UPy-materials with improved properties. The chain-extended UPy–PCL was used for tissue-engineering applications, as described below in more detail (Section **5.2**).

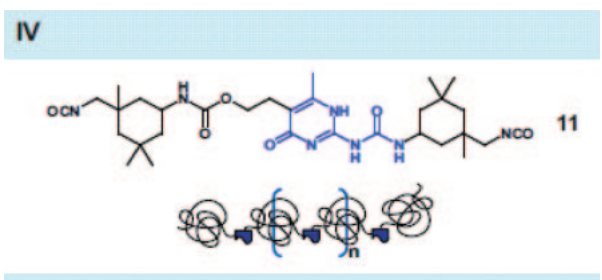

Fig. 22. Class IV, the chain-extended UPy–polymers consisting of UPy-moieties in the main chain.[80]

Besides the four UPy-polymer classes proposed, our group has also investigated the effect of additional non-covalent interactions next to the UPy–UPy dimerization. We have shown that additional hydrogen bonding in the lateral direction dramatically influences the morphology of UPy–PEBs (Fig. 23).[74] UPy–PEBs **12b** and **12c**, coupled via a urethane or urea functionality, respectively, show a rod-like morphology in atomic force microscopy (AFM) caused by lateral association of the additional hydrogen bonds and UPy-dimer stacking. The directly coupled UPy–PEB **12a** is clearly free of structure, indicating the importance of additional hydrogen bonding between polymer chains.

In conclusion, we have shown that the functionalization of telechelic polymers with UPy-moieties really improves the

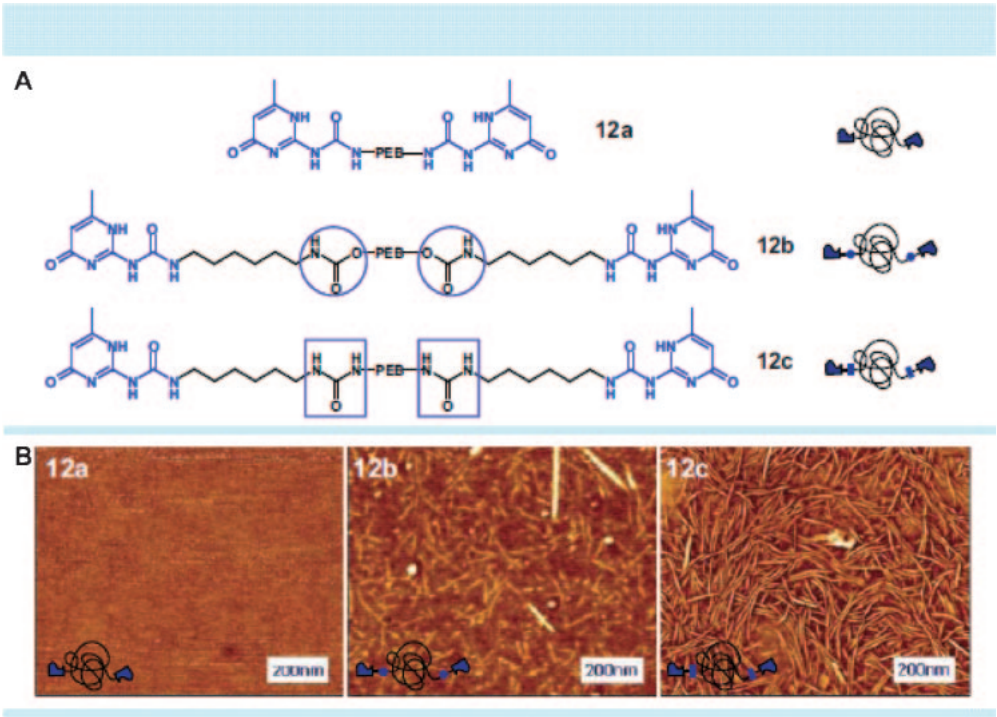

Fig. 23. **A.** Three methods of UPy-modification of PEB resulted in **B.** different morphologies as shown by AFM.[74]

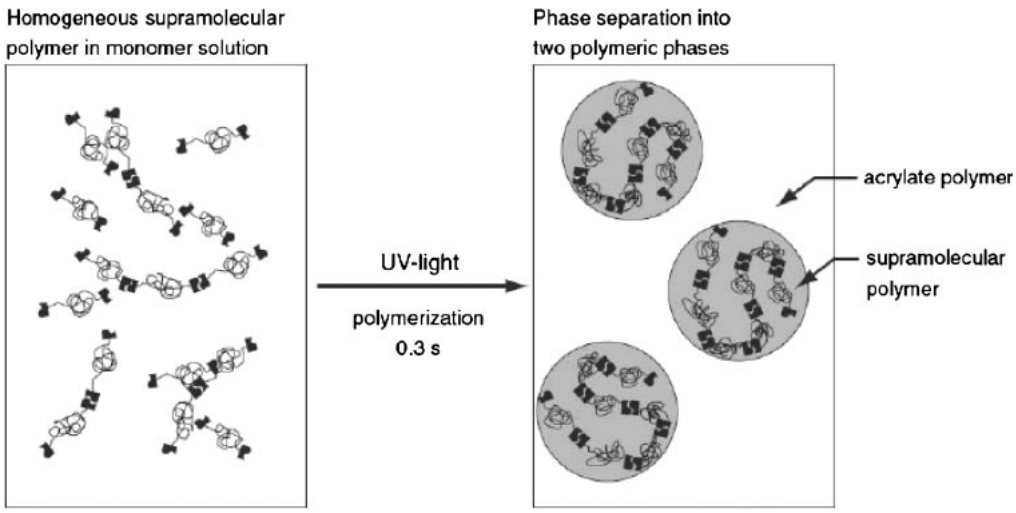

Fig. 24. Schematic representation of PIPS using UPy-modified supramolecular polymers.[76]

material properties. This has also many benefits in processablity and usability as will be described below.

3.4 Non-Biomedical Applications of UPy-Based Materials. The strong secondary interactions of the UPy-unit combined with the ease of their synthesis is probably the main reason that numerous patent applications have been filed following the first publication in 1997 by Sijbesma et al. Applications, making use of the dynamics of these supramolecular architectures[35,89] are found in fields ranging from coatings,[90] adhesives,[91] printing,[91–96] electronic devices,[97] and personal care[98] to cosmetics.[99,100] The added value from these materials to these everyday applications is based on their improved processability in the melt or solution while maintaining excellent material properties in the solid state, the ease of synthesis, the compatibility with existing polymeric systems, and the dynamic nature which makes the materials responsive and adaptable to external stimuli. In this section, several examples of these applications will be discussed that take advantage of the possibilities originating from the unlocking of the processing properties of the material properties by using supramolecular interactions. Moreover, this unambiguously shows that supramolecular polymers are clearly not restricted to the laboratory.

The dynamic nature in supramolecular polymers was explored by Keizer et al. in polymerization-induced phase separation (PIPS) with hydrogen-bonded supramolecular polymers (Fig. 24).[76] In PIPS, a polymer is dissolved in a reactive monomer which is subsequently polymerized, e.g. by UV-irradiation, to cause phase separation resulting in two polymeric

phases with certain morphology. This process is currently used to produce high-impact composite materials. It avoids the use of solvent and consequently results in fast and clean production of high-performance multiphase composite materials. The rate of phase separation in PIPS is generally limited by the mobility of the dissolved polymer. Supramolecular polymers, however, may dissociate when dissolved in a reactive monomer, resulting in strongly enhanced diffusion. Hence, macroscopic phase separation of supramolecular polymers can be reached within a very short reaction time. The mechanical behaviour of materials obtained by fast UV-curing (0.3 s) of solutions of UPy-modified polymers in acrylates was comparable to high molecular weight analogues.[76]

A different application in which the dramatic differences in phase behaviour of supramolecular polymers in a relatively narrow temperature range can be used is ink-jet printing. By the ejection of ink droplets through a small orifice, images can be created on a certain substrate, i.e. paper. Although the ink needs to display low viscosity before ejection, it needs to be highly viscous, almost solid, when it is printed to the substrate. Otherwise, the ink will smear out through capillary action of the paper, resulting in blurry images. To this end, the dynamic features of supramolecular polymers seem eminently suitable. Indeed, patents have been filed in which supramolecular polymers are used in ink compositions.[92] The thermosensitivity of supramolecular polymers can also be used to produce a coating for a printing plate.[96]

Another promising application area of supramolecular polymers are hot-melt adhesives.[91] Hot melt adhesives are solvent-free adhesives made of thermoplastics that are characteristically solid at temperatures below 90 °C, become fluid at higher temperatures, and rapidly set upon cooling. Just by cooling they quickly form a bond. They are compatible with most materials, and are clean and easy to handle. Because the UPy-chemistry is complementary to the chemistry used to make thermoplastic polyurethanes (TPUs), UPy-moieties can be easily added to TPU based hot-melt formulations that are synthesized from polyether diols, diisocyanates, and low molecular weight diol chain extenders. Partially replacing these chain extenders with methyl-isocytosine, results in TPUs with lower molecular weights that are end-functionalized with UPy-groups capable of forming the supramolecular interactions.

The use of supramolecular UPy-polymers in cosmetic applications is described in patents as well.[100] In general, cosmetic formulations are applied to keratinous materials such as hair, nail and lips in order to increase their aesthetic appearance or to apply specific care. Hence, good film forming, mechanical stability, adherence to the substrate, colour-stability and removability are important features for cosmetic formulations. An important issue in the design of UPy-materials for cosmetics and personal care products is their biocompatibility, which leads us to the last, most novel and innovating application of UPy-polymers; as supramolecular biomaterials in the biomedical field.[28,101] These UPy-biomaterials not only have to fulfil similar restrictions as the supramolecular materials described above, but even more.

4. Self-Assembling Peptide Systems

In recent years the importance of peptides as building blocks in supramolecular architectures has been demonstrated. Many examples of oligo-peptide based self-assembled aggregates have been disclosed in which hybrid conjugates are synthesized by the combination of peptide sequences with for example all kinds of polymers,[102–104] long alkyl-chains or phospholipids.[105] These conjugates have great potential in the biomedical field. However, none of these systems has been used for real tissue-engineering applications, except for two (as will discussed below). Nevertheless, these supramolecular architectures are discussed as examples of possible supramolecular biomaterials because they are able to operate in water or at the polymer–water interface. Therefore, they might find their application as TE or drug delivery scaffolds. Another advantage of the use of supramolecular building blocks is their controlled way of synthesis; therefore the synthesis method is also mentioned. Here, we only describe a few examples of peptide amphiphiles (PAs), for more detailed information on PAs we refer to the review of Löwik and van Hest.[105] The applications of biomimetic and bioactive PAs are nicely reviewed by Kokkoli and co-workers.[106]

The first example shows the self-assembly of various amphiphilic peptides by varying the length of the N-terminal alkyl tail to the GANPNAAG (Gly–Ala–Asn–Pro–Asn–Ala–Ala–Gly) sequence, known for its preferred β-hairpin conformation.[107] This leads to several types of aggregates with different peptide conformations varying from random-coil to β-sheet architectures. Also, these GANPNAAG peptides have been non-covalently incorporated in liposomes using peptide sequences that were functionalized with alkyl chains on both the N-terminus and C-terminus.[108] The folding of the peptide was stabilized into a β-hairpin conformation owing to the two alkyl-tails (Fig. 25A). In contrast, functionalization of the peptide with only one alkyl-tail resulted in the formation of a random-coil instead of a β-hairpin (Fig. 25B). These GANPNAAG-alkyl conjugates were entirely synthesized using solid-phase techniques. A polymer–peptide conjugate that forms spherical aggregates was also completely synthesized using solid-phase chemistry (Fig. 25C).[109] First an amine-functionalized polystyrene polymer was coupled to a resin, after which the GANPNAAG peptide was built up.[109]

Furthermore, micellar structures with antimicrobial activity have been created from block copolymers that were synthesized on solid supports loaded with peptides (Fig. 26A).[110] Living free radical polymerization (LFRP) initiators were coupled to the antimicrobial tritrypticin VRRFPWWWPFLRR (Val–Arg–Arg–Phe–Pro–Trp–Trp–Trp–Pro–Phe–Leu–Arg–Arg) peptide on the resin. Subsequently, nitroxide-mediated or atom-transfer radical polymerizations were performed to form the tritrypticin–poly(acrylic acid-*block*-PS) conjugates.

Polymer–peptide conjugates of poly(ethylene glycol)[111] or poly(*n*-butyl acrylate)[112] with strong β-sheet forming peptides have been shown to assemble into tape structures (Fig. 26B). The peptide sequence, GWT$(VT)_4$VG (Gly–Trp–Thr–$(Val–Thr)_4$–Val–Gly) was built up on a PEO-conjugated solid support.[111] A similar peptide structure was directly built on the resin after which the poly(*n*-butyl acrylate) was attached.[112]

Klok and co-workers showed that the self-assembly of PEG-based peptide hybrids containing α-helical coiled-coil peptide sequences can be regulated by varying the amino acid resi-

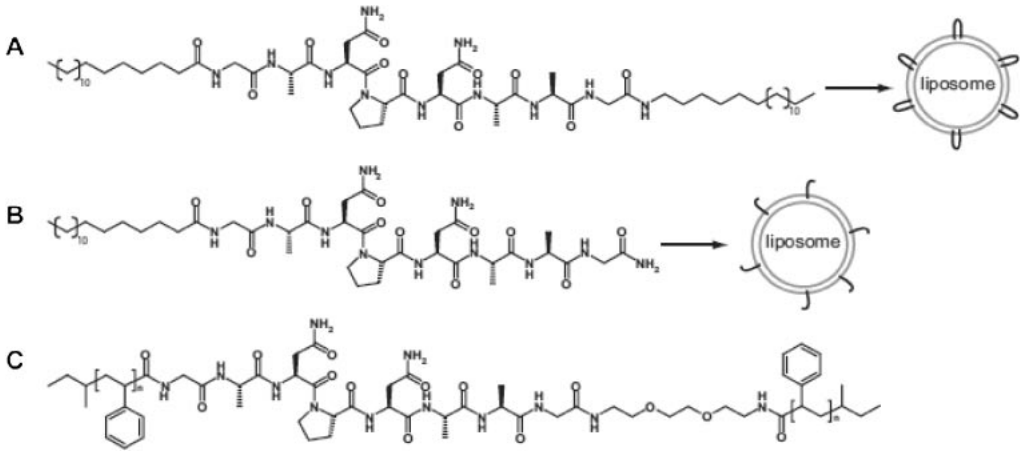

Fig. 25. GANPNAAG peptide conjugates. **A.** The GANPNAAG sequence modified with two alkyl chains assembles into a β-hairpin while **B.** the GANPNAAG peptide modified with one alkyl tail shows a random-coil conformation when incorporated in liposomes.[108] **C.** GANPNAAG-modified polystyrene was entirely synthesized on the solid support.[109]

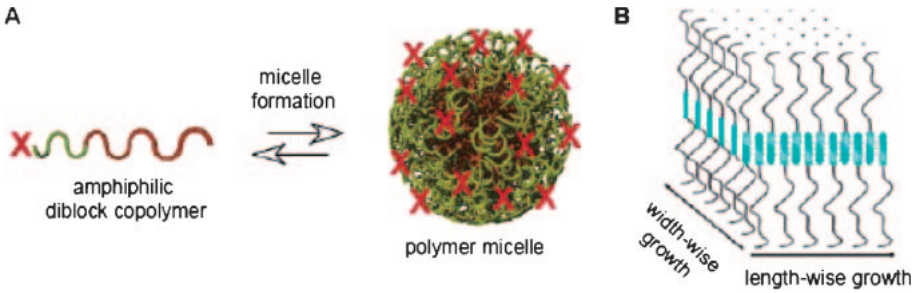

Fig. 26. **A.** Well-defined micelles were formed by the tritrypticin–poly(acrylic acid-*block*-PS) conjugate.[110] Reprinted with permission from *Biomacromolecules* **2005**, *6*, 220. Copyright 2005 American Chemical Society. **B.** Proposed structure of the aggregates of the GWT(VT)$_4$–VG–poly(ethylene oxide) conjugate.[111] Reprinted with permission from *J. Am. Chem. Soc.* **2006**, *128*, 7722. Copyright 2006 American Chemical Society.

dues.[113] Organized nanostructures based on these PEG–peptide hybrids have been found both in solution and in solid state.[114] Also, the biological activity can be correlated to this self-assembly behaviour.[113] Functionalization of the peptide sequence with PEG was performed on the resin.

Finally, peptide amphiphiles containing an alkyl tail and the integrin binding domains of the extracellular matrix protein fibronectin, i.e. the cell adhesion GRGDS (Gly–Arg–Gly–Asp–Ser) sequence and its synergistic PHSRN (Pro–His–Ser–Arg–Asn) peptide, were designed by Kokkoli et al.[115] The RGD and PHSRN sequence were separated by 4 to 5 Ser–Gly residues, which resulted in two different linker distances of 29.6 and 37 Å, respectively. These spacers approach the real distance from which the RGD and PHSRN are apart in the fibronectin protein. They found that human umbilical vein endothelial cells behave comparably on the PAs as on fibronectin with respect to their adhesion behaviour. Interestingly, the cells show even stronger cytoskeleton organization and focal adhesion formation on the PAs.

As shown in the last example, PAs are eminently suitable for biological experiments and applications. Therefore, we would like to discuss their properties with respect to tissue engineering in the next section.

5. Supramolecular Biomaterials

High control over both stability and dynamics of bioactive materials might be accomplished by using supramolecular chemistry. The adaptablitity of the biomaterial to the host tissue is of major importance for good interaction between cells (with their cell membrane receptors) and the bioactives on the biomaterial. Therefore, it is important to mimic the natural environment; the cell in its natural environment, its niche (Fig. 27). Tissues are not static; signals are being turned on and off, receptors are moving over the cell membrane, cells are moving on the ECM, cell membrane receptors adjust to the ECM and vice versa, pathways will be activated, and so on. These are very dynamic events, in which (almost) all interactions are based on recognition and on specific non-covalent, supramolecular interactions. Therefore, there is a need for a new materials design; supramolecular materials that can adapt to its environment.

The importance of synthetic biomaterials as instructive extracellular environments,[116] the engineering of the cell surface interface,[117] the importance of control over the nano-scale of biomaterials[118,119] and the design of novel biomimetic materials by self-assembly,[120] is nicely pointed out in these excellent reviews.

5.1 Self-Assembling Peptide Biomaterials. A beautiful supramolecular system which is applied for tissue engineering has been developed by Stupp et al. This system consists of peptide-based amphiphilic molecules that form three-dimensional nanofibers and are versatile hydrogel scaffolds for the

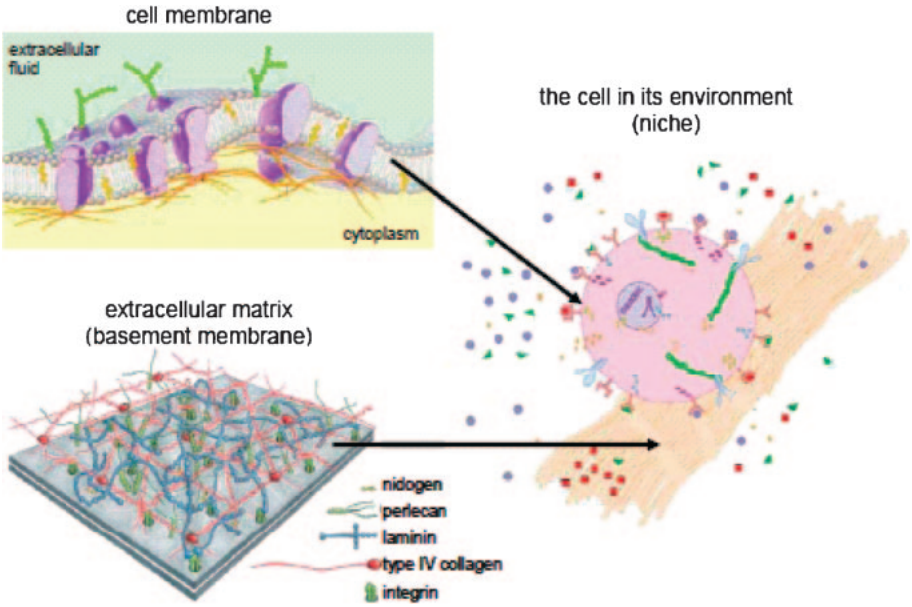

Fig. 27. The cell in its natural environment, its niche. All bioactives and factors involved interact via specific non-covalent, supramolecular interactions. Modified pictures taken from references.[121–123] Reprinted with permission from *Mater. Today* **2006**, *9*, 26. Copyright 2006 Elsevier. And reprinted with permission from Molecular Biology of the Cell 2002. Copyright 2002 Garland Publishing Group.

preparation of self-assembling nanomaterials with varying morphology, surface chemistry and bioactivity.[124] In addition, at least three different modes of self-assembly can be used: pH control, divalent ion induction and concentration. These peptide amphiphiles (PA) were entirely synthesized on the resin which indicates their ease to be synthesized. In general, these peptide amphiphiles are constructed of at least three important regions; a long alkyl tail, a (flexible) linker region consisting of amino acids and the bioactive part.[29,125] Additionally, the PAs that were applied for the mineralization of hydroxyapatite were defined in more detail and consist of five key structural features (Fig. 28).[125] Cysteine residues were built in between the alkyl tail and bioactive part, which could be oxidized to form disulfide bonds resulting in polymerization of the supramolecular structure. Furthermore, the bioactive region was divided into two parts: a single serine residue for strong interaction with calcium ions and the RGD sequence for induction of cell adhesion. The self-assembly was induced by lowering the pH below 4. The fibers were cross-linked by formation of intermolecular disulfide bonds via oxidation, which resulted in a chemically robust fiber. The cross-links could be reversed by reduction of the disulfides to thiols. Furthermore, it was shown that hydroxyapatite crystals grew with their *c* axes oriented along the long axes of the nanofibers, which resembles the hydroxyapatite crystallization in bone in which the crystals also grow in a similar way along the collagen fibrils.[125] This system is very promising for the engineering of bone.

Besides the pH inducible self-assembly, it has been shown that two bioactive PA molecules were able to co-assemble into nanofibers by electrostatic interactions at a certain pH (Fig. 29). While the negatively charged PA **13** and **15** self-assemble in acidic pH, PA **14** and **16** with a positive charge self-assemble at a basic pH. The molecule pairs **13**/**14** and **15**/**16** co-assemble at neutral pH.[126] Furthermore, PAs with a free N-terminal amine and the alkyl tail at the C-terminus are synthesized, which makes it possible to incorporate peptides that need a free N-terminus because of activity reasons.[127] This new design made it possible to study modulation of the fluorescence by coupling the fatty acid tail to the C-terminus and a chromophore to the N-terminus.

The modularity of the PAs is shown in the many applications of PA nanofibers that have been investigated, varying from mineralization of hydroxyapatite crystals on cross-linked PA nanofibers,[125] oligonucleotide binding via introduction of oligonucleotide moieties,[128] magnetic resonance imaging using attached contrast agent molecules (like DOTA derivatives),[129,130] templated assembly of lipophilic inorganic nanoparticles on the PA nanofibers via base-pairing,[131] to avidin binding to biotin presenting PA fibers.[132] Also the presentation of integrin binding epitopes on PAs have been studied.[133] Branched PAs were designed using orthogonal protection group chemistry on additional lysine residues. Several PAs with the cell adhesion RGD sequence were synthesized, as well as PAs containing both RGD and its synergistic PHSRN peptide. Furthermore, also the laminin-derived IKVAV (Ile–Lys–Val–Ala–Val) and YIGSR (Tyr–Ile–Gly–Ser–Arg) sequences were incorporated.[133]

PA nanofibers containing the laminin IKVAV sequence were also studied for the possibility to be used for tissue engineering of nerves. Selective differentiation of neural progenitor cells was accomplished via incorporation of this IKVAV peptide[29] (Fig. 30). The PA nanofiber gels with this bioactive sequence induced very rapid differentiation of encapsulated neural progenitor cells into neurons while discouraging the development of astrocytes. Besides that, heparin binding PA nanostructures were built consisting of a peptide sequence with strong binding affinity to heparin, i.e. the LRKKLGKA (Leu–

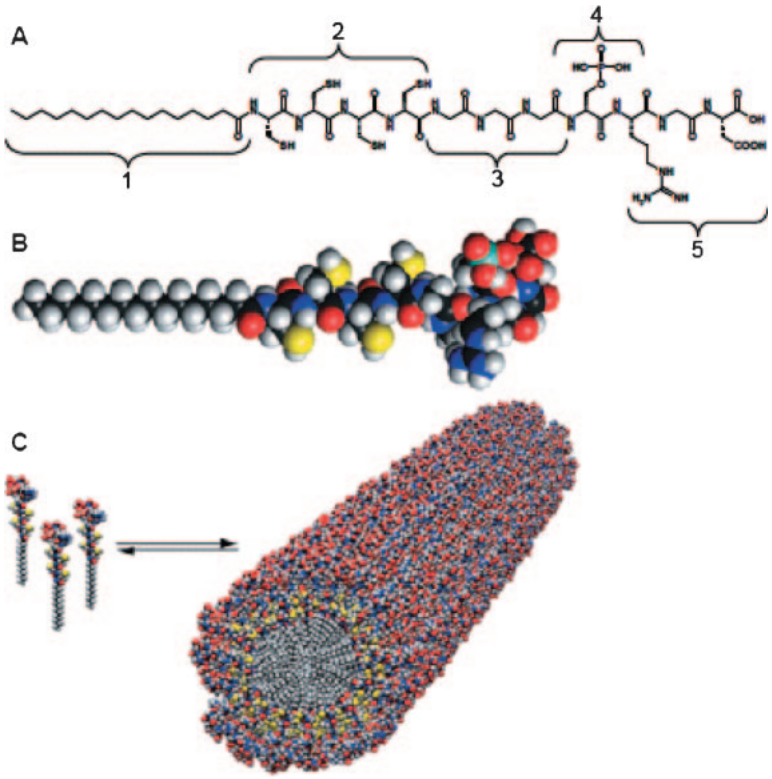

Fig. 28. **A, B.** The "Stupp" PA used for hydroxyapatite mineralization can be divided into five regions: an alkyl tail (1), four cysteines for cross-linking (2), a flexible linker region (3), a phosphorylated serine for calcium ion binding (4) and the RGD sequence for cell adhesion (5). **C.** The self-assembly of the PAs into a cylindrical micelle.[125] Reprinted with permission from *Science* **2001**, *294*, 1684. Copyright 2001 AAAS.

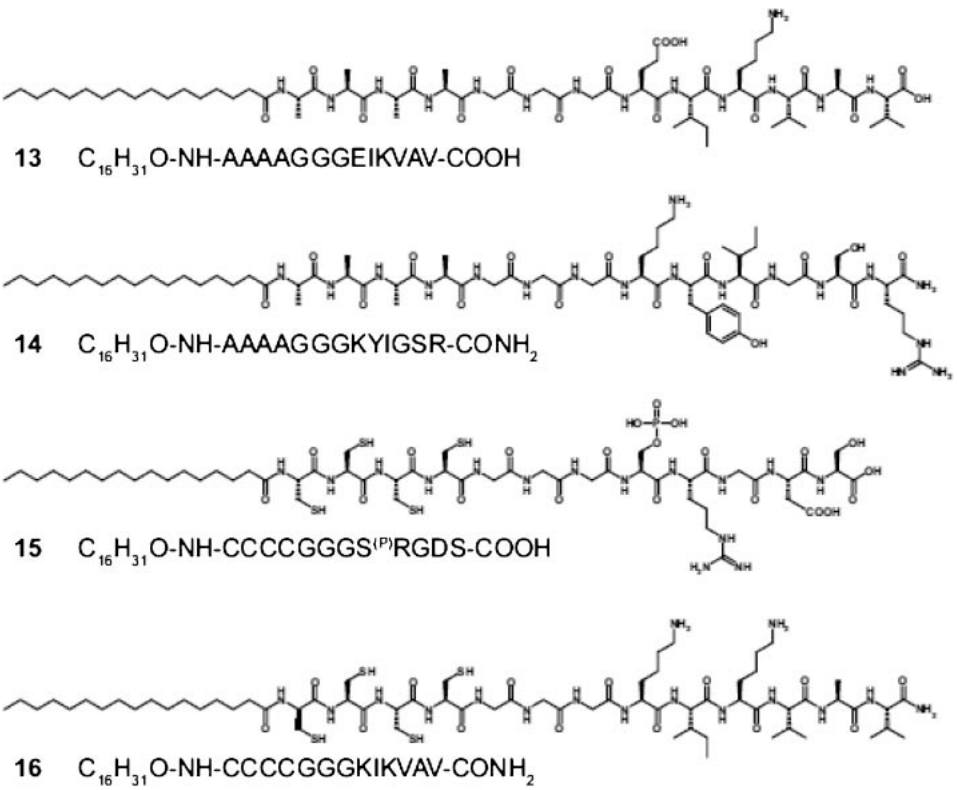

Fig. 29. Chemical structures of four PAs. PA **13** and **15** self-assemble at acidic pH and PA **14** and **16** at basic pH. PA-couples of **13**/**14** or **15**/**16** co-assemble at neutral pH.[126]

Arg–Lys–Lys–Leu–Gly–Lys–Ala) peptide (Fig. 31).[134] Heparin was used to nucleate the self-assembly of the PAs, yielding rigid nanofibers that display heparin chains to orient proteins for cell signalling. Extensive new blood vessel formation was stimulated by these heparin decorated PAs in vivo in the rat cornea.[134]

Another interesting design based on self-assembling peptide sequences is shown by Zhang and co-workers.[135,136] They introduced β-sheet forming peptides inspired by nature to produce nanofiber scaffolds. Importantly, these peptides were synthesized using standard Fmoc solid-phase chemistry. The first member of this family, the EAK16 (Ala–Glu–Ala–Glu–Ala–Lys–Ala–Lys)$_2$ peptide, was found in a part of alternating hydrophobic and hydrophilic amino acid residues in the yeast protein zuotin.[137] Through ionic interactions between Glu and Lys residues, the peptides-formed networks of interwoven filaments of approximately 10 nm in diameter which resulted in stable macroscopic membranes.

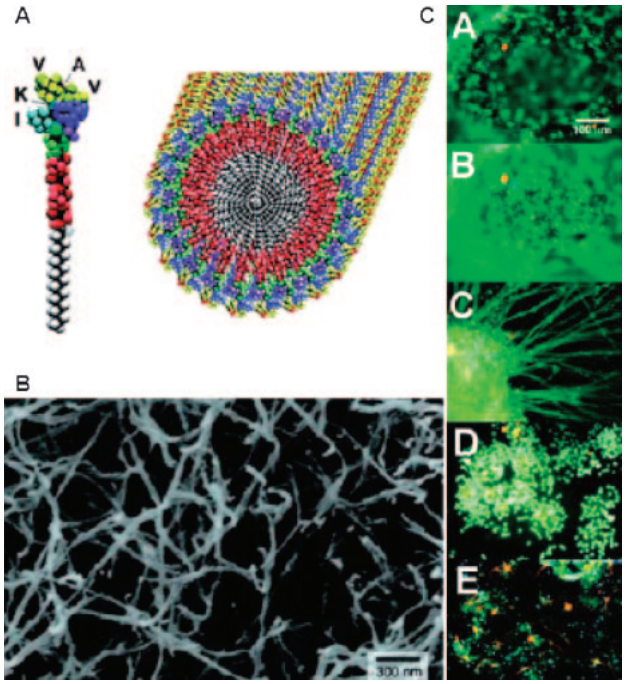

Fig. 30. Self-assembly of peptide amphiphiles (PA) into nanofibers. **A.** Representation of an IKVAV-containing PA. **B.** Scanning electron micrograph of a PA nanofiber network formed after addition of cell culture medium. **C.** Neural progenitor cells (A–C) encapsulated in IKVAV–PA gels at day 1 (A and B) and at day 7 (C). Neural progenitor cells (D and E) cultured on laminin-coated cover slips at day 1 (D) and at day 7 (E). All cells were Hoechst stained (blue), differentiated neurons were labeled for β-tubulin (green) and differentiated glial cells were labeled for GFAP (orange).[29] Reprinted with permission from *Science* **2004**, *303*, 1352. Copyright 2004 AAAS.

The physical properties as well as the biological behaviour of RADA16 (Arg–Ala–Asp–Ala)$_4$ nanofiber scaffolds were studied in great detail (Fig. 32A). The reassembly mechanism after mechanical breakage through sonication of the RADA16 nanofiber gels, containing more than 99.5% water, was investigated.[138] Using atomic force microscopy, rheology measurements and circular dichroism, it was shown that they could quickly reassemble indistinguishable from the original one. Hippocampal neural cells have been entrapped in these 3-dimensional hydrogel scaffolds.[139] Furthermore, it has also been shown that these scaffolds support neural cell adhesion and differentiation, as well as extensive neurite outgrowth.[140] Rat neurons form active synapses on the surface of these scaffolds (Fig. 32B). Also, putative liver progenitor cells seeded in these 3D-scaffolds differentiated into hepatocyte-like spheroid structures.[141] Besides that, mouse embryonic stem cells and mouse embryonic fibroblasts have shown to differentiate into osteoblast-like cells possibly caused by the unique 3D-microenvironment originated from these nanofiber hydrogels.[142]

Furthermore, the RADA16 hydrogels have been injected in the myocardium which led to the recruitment of progenitor cells that expressed endothelial markers.[143] Also vascular smooth muscle cells were recruited to the injected microenvironment, showing the formation of functional vascular structures. When these hydrogel carriers were loaded with exogenous neonatal cardiomyocytes and were injected into the myocardium, it was seen that the transplanted cells survived and even recruited more endogenous cells. In addition, this RADA16 scaffold has been functionalized with several bioactive peptide sequences to enhance endothelial cell function.[144] YIGSR and RYVVLPR (Arg–Tyr–Val–Val–Leu–Pro–Arg) which are derived from laminin 1, and TAGSCLRKFSTM (Thr–Ala–Gly–Ser–Cys–Leu–Arg–Lys–Phe–Ser–Thr–Met) which is a mimic of one of the collagen IV strands were attached to the RADA16 peptide (Fig. 33).

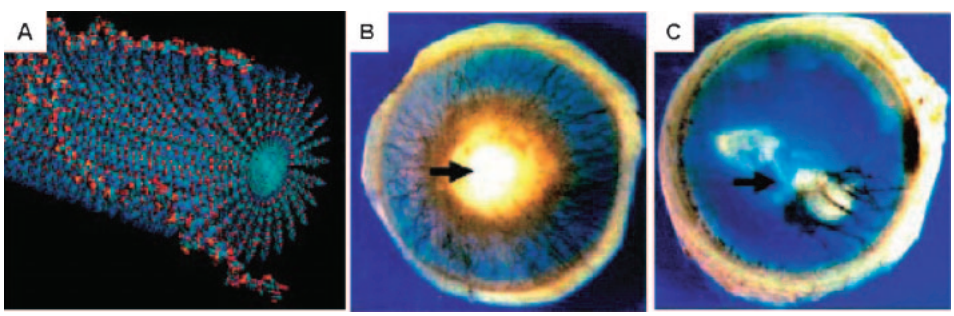

Fig. 31. **A.** Schematic representation of heparin-nucleated PA nanofiber. Rat cornea 10 days after implantation at the site indicated with the black arrow, of the **B.** heparin-nucleated PA nanofiber networks with growth factors or **C.** as control, a collagen gel with heparin and growth factors.[134] Reprinted with permission from *Nano Lett.* **2006**, *6*, 2086. Copyright 2006 American Chemical Society.

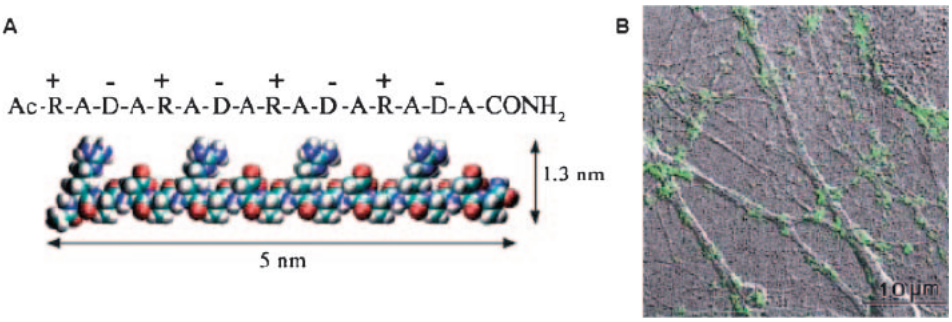

Fig. 32. **A.** Schematic representation of the RADA16 nanofiber.[138] **B.** Primary rat hippocampal neurons form active synapses on the peptide nanofiber scaffolds, indicated by the bright discrete green labelling.[140] Reprinted with permission from *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 6728 and **2005**, *102*, 8414. Copyright 2000, 2005 National Academy of Sciences, USA.

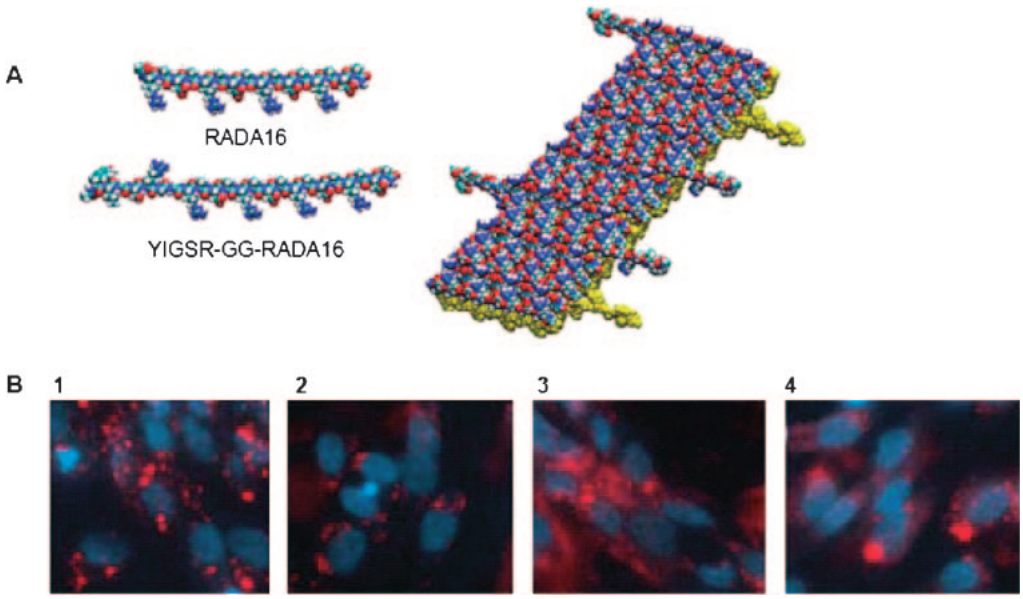

Fig. 33. **A.** Schematic representation of the RADA16 and the YIGSR-functionalized RADA16 peptides, which assemble into β-sheet tapes when mixed in a ratio of 9:1. The YIGSR sticks out of the tape. **B.** Human aortic endothelial cells cultured on peptide scaffolds of RADA16 (**1**) and of 9:1 mixtures of RADA16:functionalized RADA16; YIGSR (**2**), RYVVLPR (**3**), and TAGSCLRKFSTM (**4**). Fluorescent staining with di-ac-LDL (red) and DAPI (blue).[144] Reprinted with permission from *Biomaterials* **2005**, *26*, 3341. Copyright 2005 Elsevier.

It was shown that human aortic endothelial cells (HAEC) behave like endothelial cells when seeded on a 9:1 mixture of the RADA16 peptide and the functionalized RADA16 sequence, respectively. They showed LDL uptake activity, enhanced nitric oxide release and an elevated deposition of laminin I and collagen IV. The functionalized peptides even seem to behave better.[144]

Another peptide sequence, the KLD12 $(Lys–Leu–Asp)_4$ peptide, has shown to foster chondrocyte ECM production and cell division. Therefore, this hydrogel has potential in cartilage TE.[145] For more information on these self-assembling β-sheet forming scaffolds with respect to their application in regenerative medicine we refer to the interesting recent review of Zhang and co-workers.[135]

These well-designed hydrogel systems have high potential as application for tissue engineering. In addition, it would be nice to have a system which is also based on supramolecular interactions, but displays strong, elastomeric material properties. Therefore, we developed, as inspired by the work on self-assembling peptide systems, supramolecular biomaterials based on ureido–pyrimidinone moieties.

5.2 Ureido-Pyrimidinone-Functionalized Supramolecular Biomaterials. Our supramolecular biomaterials are based on the quadruple hydrogen-bonding ureido–pyrimidinone (UPy) moieties (Fig. 34). This UPy-unit strongly dimerizes in organic solvents and takes care of chain extension of relatively short end-functionalized UPy-prepolymers in the bulk ($K_a = 10^6–10^7\ M^{-1}$) as discussed above. The reversible nature of these hydrogen-bonding interactions (with lifetimes between 0.1–1 s) creates responsive materials and allows for a modular approach. These new materials show mechanical properties similar to conventional polymers, without losing their reversible nature. We showed that we could produce both passive and active scaffolds by introducing a supramolecular and modular approach using these UPy–UPy interactions (Fig. 34). The creation of a toolbox with different UPy-modi-

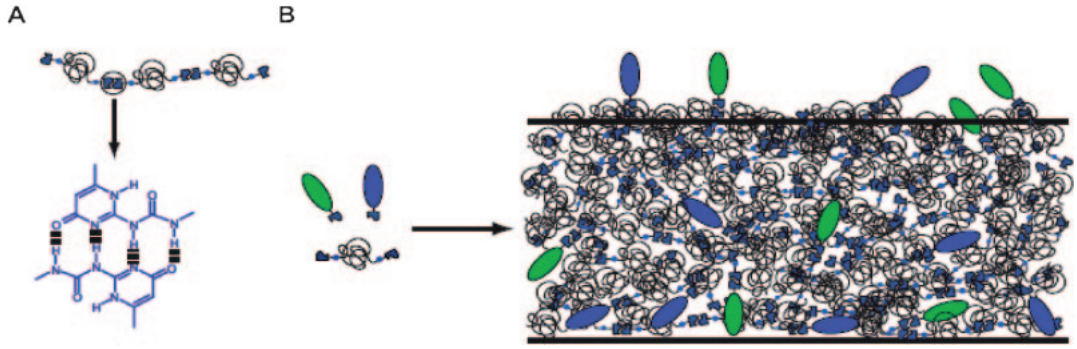

Fig. 34. The modular approach to bioactive supramolecular biomaterials. **A.** The UPy-moiety in a supramolecular polymer. **B.** The modular approach to constructing bioactive materials with various properties via simply mixing different UPy-functionalized biomolecules (green and blue moieties) with UPy–polymers.[28]

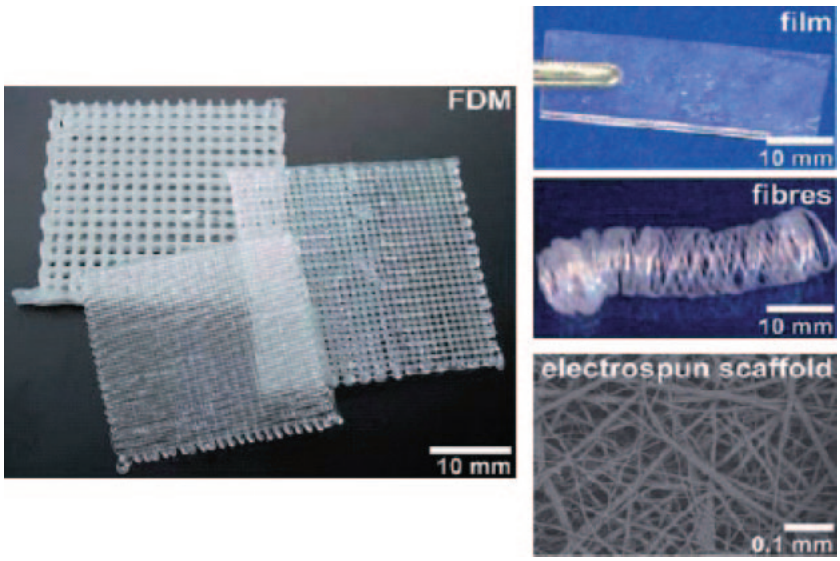

Fig. 35. Processability of UPy–oligocaprolactone into passive scaffolds with several morphologies.[28] Reprinted with permission from *Nat. Mater.* **2005**, *4*, 568. Copyright 2005 Nature Publishing Group.

fied polymers, bioactive molecules and imaging probes allows for the off-the-shelf assembly of biomaterials by easy mixing without the need for additional elaborated synthesis steps. Furthermore, this supramolecular concept bridges the gap between simply (non-covalent) mixing and covalent modification of polymers with bioactive molecules and is therefore very promising. A modular approach to passive scaffolds was used by intimate mixing of UPy-modified prepolymers to tune the mechanical properties and biodegradability of the biomaterials. Active scaffolds were made by incorporation of UPy-modified bioactive compounds such as bioactive peptides and proteins using the same mix-and-match principle.

The feasibility of UPy-units in passive biomaterials was investigated in first instance studying class I UPy-polymers. It was shown that supramolecular polymers consisting of FDA approved oligocaprolactones, end-functionalized with UPy-moieties, are eminently suitable as biomaterials. They can be easily processed into several scaffold morphologies varying from meshes, to films and grids, on which fibroblast cells were able to proliferate (Fig. 35).[28] Next to that, this UPy-polymer and other specially designed water-soluble UPy-moieties were shown to be biocompatible using several direct and indirect in vitro toxicity studies. The oligocaprolactone UPy-polymer did not degrade in vitro during a period of more than 100 days, however, the degradation was accelerated when lipase enzymes were used.[28]

Comparable results were found for UPy-oligo(trimethylene carbonates) which changed from amorphous materials to semi-crystalline polymers owing to UPy-modification.[77] This UPy-modification allowed for easy processing at slightly elevated temperatures into stable 3D-scaffolds that did not flow at temperatures below 50 °C because of the formation of supramolecular UPy-crosslinking, in contrast to HMW–PTMC scaffolds (Fig. 36). Furthermore, the materials properties could be tuned by mixing bifunctional and trifunctional UPy–PTMCs.

Then, a modular approach was introduced to produce co-polymeric UPy-systems of class I and class IV UPy-polymers (Fig. 37). Co-polymeric systems of bifunctional (class I) and chain-extended oligocaprolactones (class IV) were made to tune the mechanical properties and tissue response in vivo.[80] Surprisingly, a 20:80 mixture of both polymers with the chain-extended UPy-polymer in excess shows flexible proper-

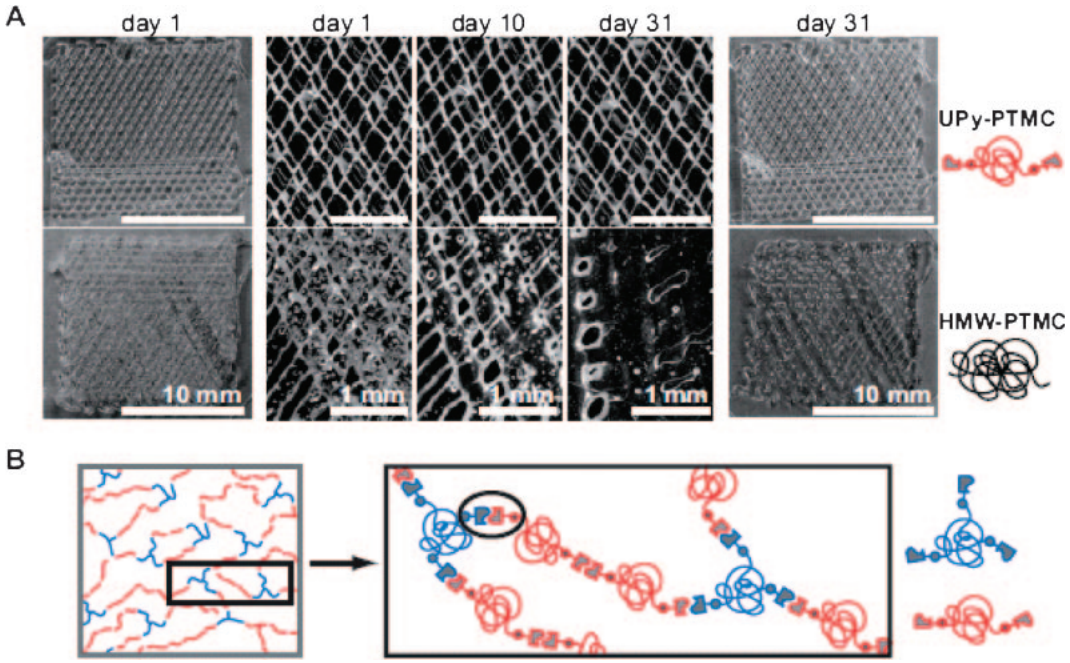

Fig. 36. **A.** Fused deposition modelling (FDM) scaffolds of bifunctional UPy–PTMC and HMW–PTMC followed in time. **B.** Bifunctional and trifunctional UPy–PTMC polymers were mixed to obtain materials with varying materials properties.[77]

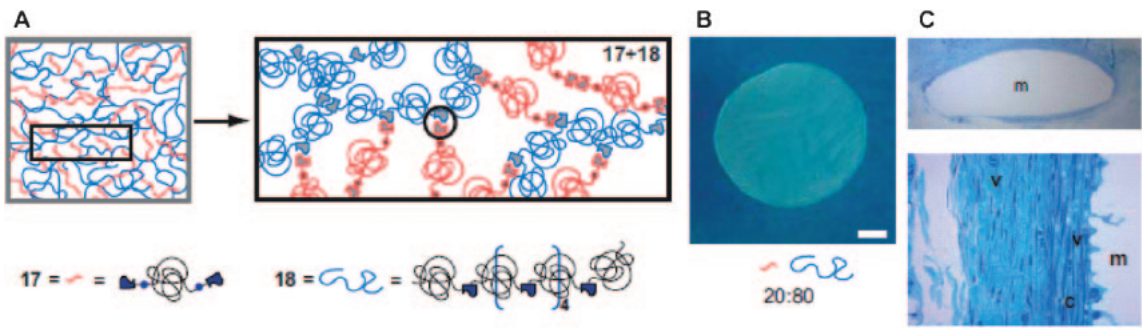

Fig. 37. **A.** Intimate mixing of bifunctional (**17**) and chain-extended (**18**) UPy–oligocaprolactones results in co-polymeric materials. **B.** The explanted disk of the 20:80 mixture of **17**:**18** and **C.** its histology after subcutaneous implantation with **m** = biomaterial, **v** = blood vessel, **c** = capsule.[80]

ties without visible deformation upon implantation for 42 days. This mixture, a blend formed by intimate mixing through UPy–UPy interaction, shows a mild tissue response accompanied with the formation of a thin capsule. The material does not become more crystalline upon implantation. Hence, this mixture might be an ideal scaffold material for soft tissue engineering due to its flexibility and diminished fibrous tissue formation, and illustrates the strength of the modular approach.

A toolbox was designed containing different building blocks, varying from several UPy-polymers, to UPy-modified dyes, biotins, bioactive peptides and proteins. Several UPy-modified peptide sequences and two model proteins that can be used for the introduction of bioactivity into the UPy-modified polymeric materials were synthesized. A convenient solid-phase synthesis method was developed to functionalize peptide sequences with UPy-moieties on the solid support.[146] Two different methods were used to couple the UPy-unit to the peptide sequence: via the free N-terminal amine, or via an additionally incorporated lysine, with an orthogonal protection group, at the C-terminus resulting in a UPy-peptide with a free N-terminal amine. UPy-peptides with an N-terminal cysteine were used to successfully functionalize green fluorescent protein mutants with UPy-moieties using native chemical ligation (unpublished results).

In order to prove the modular concept to bioactive biomaterials the UPy-functionalized oligocaprolactones were simply mixed with UPy-modified cell adhesion promoting GRGDS (UPy–Gly–Arg–Gly–Asp–Ser) and synergistic PHSRN (UPy–Pro–His–Ser–Arg–Asn) peptide sequences (Fig. 38). The in vitro results indicated strong and specific cell binding of fibroblasts to the UPy-functionalized bioactive materials containing both UPy-peptides. An even more striking effect was seen in vivo where the formation of single giant cells at the interface between bioactive material and tissue was triggered.[28]

An important property of bioactive polymer films is their stability in an aqueous environment. We investigated what happens at the water–supramolecular polymer interface. It

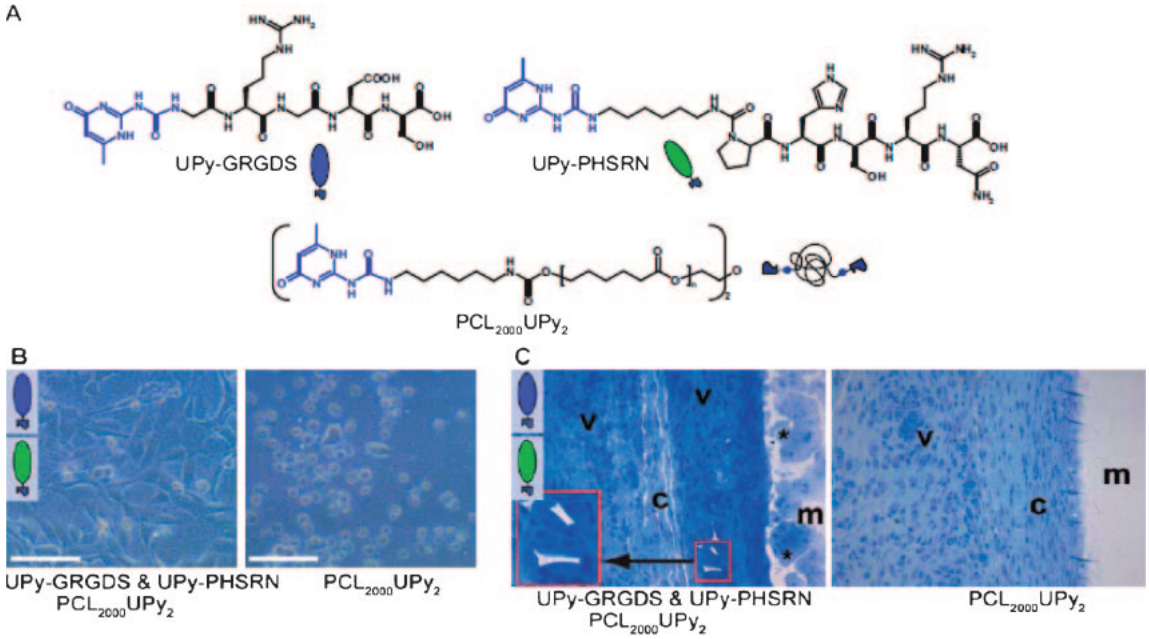

Fig. 38. Bioactive supramolecular biomaterials have been produced consisting of **A.** the UPy–GRGDS and UPy–PHSRN peptides, and the UPy-modified oligocaprolactone ($PCL_{2000}UPy_2$). **B.** The behaviour of the active scaffolds is shown in vitro and **C.** in vivo, with **m** = biomaterial, **v** = blood vessel, **c** = capsule, ∗ = giant cell.[28]

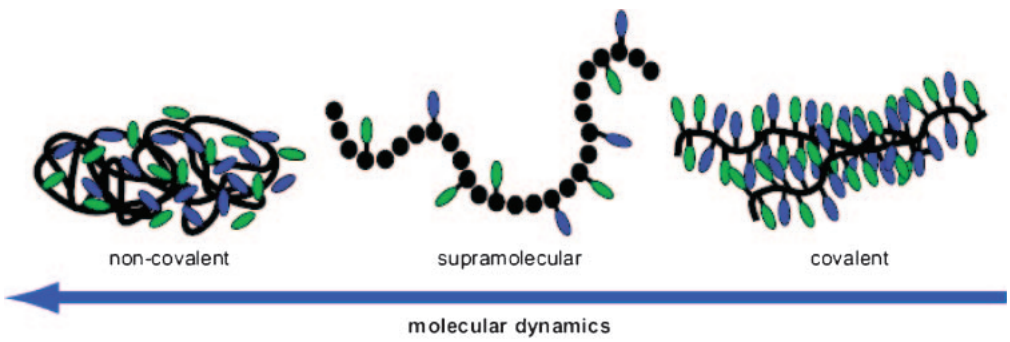

Fig. 39. Control over structure and dynamics in a supramolecular way.

has been shown that cells can adhere to cell adhesion UPy–peptides incorporated in the polymer films, indicating that the peptide–polymer interaction is strong enough to make this happen. Besides that, also in vivo experiments showed the presence of UPy-modified cell adhesion peptides at the surface. It is assumed that the water-soluble UPy–peptides are partially incorporated in UPy–UPy dimer stacks and that the polymers provide a hydrophobic shield around the UPy-bound peptide. These phenomena prevent total dissolution of the peptides in water. Similar results have been found when UPy-modified biotin was incorporated in UPy–polymer films, showing that avidin could bind to the biotin (unpublished results). In conclusion, all experiments prove the modular concept of supramolecular (bioactive) biomaterials.

6. Conclusion and Discussion

In this account, we have described a new area of biomaterials for tissue engineering (TE) based on supramolecular chemistry. We have discussed two families of supramolecular biomaterials, i.e. our own hydrogen-bonded supramolecular polymers and the self-assembling peptide nanofibers of Stupp and Zhang. These systems have in common that their properties can be tuned by changing the nature of the building blocks. We proposed that biomaterials for TE purposes have to fulfil the biomaterials trinity of regulation of the mechanical properties, the degradability and the amount and nature of the bioactivity. Besides that, these biomaterials have to be able to behave dynamically. They should have the possibility to adapt their biofunctionality in a temporal and spatial way to the tissue the material is brought into. We propose that these supramolecular systems are eminently suitable for this purpose. Furthermore, the binding strength, i.e. the association constants of the bioactives to the material can be regulated by using supramolecular interactions. This allows for the design of materials that can be tuned on the axis between non-covalent and covalent modification (Fig. 39). Besides the dynamics of the biomaterial, also the three-dimensional geometry is important. It has been shown that a nano-fiber topology is important in mimicking the basement membrane, because the ECM is composed of many nano-fibers formed by colla-

gens, laminins, elastins, and other ECM molecules.[119] The peptide amphiphiles already form nanofibers. In our materials this is also seen when additional hydrogen-bonding interactions are introduced in the lateral direction of the UPy–UPy dimers. In this way the UPy–UPy dimers can stack upon each other which has been shown for UPy-modified poly(ethylene butylenes)[74] and for poly(trimethylene carbonates).[77]

Furthermore, where the PA hydrogels are eminently suitable for applications where mechanical strength is not the main issue in the biomaterials trinity,[118] our UPy-materials do effectively sustain and transfer mechanical loading to cells, since they are mechanically very strong, despite their supramolecular nature. However, an ideal combination would possibly be a composite of the two systems described. An ideal scaffold might be produced in which different bioactive molecules are immobilized on different places in a composite 3D-material consisting of a combination of these PA hydrogels and hydrogen-bonded polymeric matrices, which show different mechanical properties and degradation behaviour. In this way the scaffold might be able to regulate several cellular processes at the same time but also successively depending on the localization of the bioactive molecules.

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References

1 J.-M. Lehn, *Supramolecular Chemistry*, Wiley-VCH, Weinheim, **1995**.
2 J.-M. Lehn, *Chem. Soc. Rev.* **2007**, *36*, 151.
3 R. Langer, J. P. Vacanti, *Science* **1993**, *260*, 920.
4 L. G. Griffith, G. Naughton, *Science* **2002**, *295*, 1009.
5 L. L. Hench, J. M. Polak, *Science* **2002**, *295*, 1014.
6 A. J. Strain, J. M. Neuberger, *Science* **2002**, *295*, 1005.
7 B. D. H. Ratner, A. S. Hoffmann, F. J. Schoen, J. E. Lemons, *Biomaterials Science*, Elsevier Academic Press, New York, **2004**.
8 A. P. Pego, D. W. Grijpma, J. Feijen, *Polymer* **2003**, *44*, 6495.
9 J. A. Burdick, C. Chung, X. Jia, M. A. Randolph, R. Langer, *Biomacromolecules* **2005**, *6*, 386.
10 A. P. Pego, A. A. Poot, D. W. Grijpma, J. Feijen, *J. Mater. Sci.: Mater. Med.* **2003**, *14*, 767.
11 G. G. Pitt, M. M. Gratzl, G. L. Kimmel, J. Surles, A. Schindler, *Biomaterials* **1981**, *2*, 215.
12 L. Lu, S. J. Peter, M. D. Lyman, H. L. Lai, S. M. Leite, J. A. Tamada, S. Uyama, J. P. Vacanti, R. Langer, A. G. Mikos, *Biomaterials* **2000**, *21*, 1837.
13 A. P. Pego, M. J. A. Van Luyn, L. A. Brouwer, P. B. van Wachem, A. A. Poot, D. W. Grijpma, J. Feijen, *J. Biomed. Mater. Res., Part A* **2003**, *67A*, 1044.
14 T. Boontheekul, D. J. Mooney, *Curr. Opin. Biotechnol.* **2003**, *14*, 559.
15 Y. Hirano, D. J. Mooney, *Adv. Mater.* **2004**, *16*, 17.
16 S. A. Maskarinec, D. A. Tirrell, *Curr. Opin. Biotechnol.* **2005**, *16*, 422.
17 T. P. Richardson, M. C. Peters, A. B. Ennett, D. J. Mooney, *Nat. Biotechnol.* **2001**, *19*, 1029.
18 D. Seliktar, A. H. Zisch, M. P. Lutolf, J. L. Wrana, J. A. Hubbell, *J. Biomed. Mater. Res., Part A* **2004**, *68A*, 704.
19 E. Ruoslahti, *Annu. Rev. Cell Dev. Biol.* **1996**, *12*, 697.
20 E. Ruoslahti, M. D. Pierschbacher, *Science* **1987**, *238*, 491.
21 U. Hersel, C. Dahmen, H. Kessler, *Biomaterials* **2003**, *24*, 4385.
22 H. D. Maynard, S. Y. Okada, R. H. Grubbs, *Macromolecules* **2000**, *33*, 6239.
23 H. D. Maynard, S. Y. Okada, R. H. Grubbs, *J. Am. Chem. Soc.* **2001**, *123*, 1275.
24 S. Aota, M. Nomizu, K. M. Yamada, *J. Biol. Chem.* **1994**, *269*, 24756.
25 D. J. Leahy, I. Aukhil, H. P. Erickson, *Cell* **1996**, *84*, 155.
26 J. Groll, J. Fiedler, E. Engelhard, T. Ameringer, S. Tugulu, H.-A. Klok, R. E. Brenner, M. Möller, *J. Biomed. Mater. Res., Part A* **2005**, *74A*, 607.
27 M. Kantlehner, P. Schaffner, D. Finsinger, J. Meyer, A. Jonczyk, B. Diefenbach, B. Nies, G. Holzemann, S. L. Goodman, H. Kessler, *ChemBioChem* **2000**, *1*, 107.
28 P. Y. W. Dankers, M. C. Harmsen, L. A. Brouwer, M. J. A. Van Luyn, E. W. Meijer, *Nat. Mater.* **2005**, *4*, 568.
29 G. A. Silva, C. Czeisler, K. L. Niece, E. Beniash, D. A. Harrington, J. A. Kessler, S. I. Stupp, *Science* **2004**, *303*, 1352.
30 H. Staudinger, *Die Hochmolekulare Organische Verbindungen*, Springer, Berlin, **1932**.
31 A. Ciferri, *Supramolecular Polymers*, CRC Press, Boca Raton, **2005**.
32 L. Brunsveld, B. J. B. Folmer, E. W. Meijer, R. P. Sijbesma, *Chem. Rev.* **2001**, *101*, 4071.
33 G. B. W. L. Ligthart, O. A. Scherman, R. P. Sijbesma, E. W. Meijer, in *Macromolecular Engineering. Precise Synthesis, Materials, Properties, Applications*, ed. by K. Matyjaszewski, Y. Gnanou, L. Leibler, Wiley-VCH Verlag, Weinheim, **2007**, pp. 351–399.
34 P. J. Flory, *Principles of Polymer Chemistry*, Cornell University Press, Ithaca, New York, **1953**.
35 A. W. Bosman, R. P. Sijbesma, E. W. Meijer, *Mater. Today* **2004**, *7*, 34.
36 W. L. Jorgensen, J. Pranata, *J. Am. Chem. Soc.* **1990**, *112*, 2008.
37 J. Pranata, S. G. Wierschke, W. L. Jorgensen, *J. Am. Chem. Soc.* **1991**, *113*, 2810.
38 F. H. Beijer, R. P. Sijbesma, H. Kooijman, A. L. Spek, E. W. Meijer, *J. Am. Chem. Soc.* **1998**, *120*, 6761.
39 F. H. Beijer, H. Kooijman, A. L. Spek, R. P. Sijbesma, E. W. Meijer, *Angew. Chem., Int. Ed.* **1998**, *37*, 75.
40 S. H. M. Söntjens, R. P. Sijbesma, M. H. P. van Genderen, E. W. Meijer, *J. Am. Chem. Soc.* **2000**, *122*, 7487.
41 R. P. Sijbesma, F. H. Beijer, L. Brunsveld, B. J. B. Folmer, J. H. K. K. Hirschberg, R. F. M. Lange, J. K. L. Lowe, E. W. Meijer, *Science* **1997**, *278*, 1601.
42 J. Sartorius, H.-J. Schhneider, *Chem. Eur. J.* **1996**, *2*, 1446.
43 C. Fouquey, J. M. Lehn, A. M. Levelut, *Adv. Mater.* **1990**, *2*, 254.
44 J. M. Lehn, *Macromol. Symp.* **1993**, *69*, 1.
45 T. Kato, J. M. J. Fréchet, *Macromolecules* **1989**, *22*, 3818.
46 T. Kato, P. G. Wilson, A. Fujishima, J. M. J. Fréchet, *Chem. Lett.* **1990**, 2003.
47 C. B. St. Pourcain, A. C. Griffin, *Macromolecules* **1995**, *28*, 4116.

48 C. M. Lee, A. C. Griffin, *Macromol. Symp.* **1997**, *117*, 281.
49 R. K. Castellano, R. Clark, S. L. Craig, C. Nuckolls, J. Rebek, Jr., *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 12418.
50 R. K. Castellano, C. Nuckolls, S. H. Eichhorn, M. R. Wood, A. J. Lovinger, J. Rebek, *Angew. Chem., Int. Ed.* **1999**, *38*, 2603.
51 R. K. Castellano, J. Rebek, Jr., *J. Am. Chem. Soc.* **1998**, *120*, 3657.
52 R. K. Castellano, D. M. Rudkevich, J. Rebek, Jr., *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 7132.
53 E. A. Fogleman, W. C. Yount, J. Xu, S. L. Craig, *Angew. Chem., Int. Ed.* **2002**, *41*, 4026.
54 J. Xu, E. A. Fogleman, S. L. Craig, *Macromolecules* **2004**, *37*, 1863.
55 M. J. Serpe, S. L. Craig, *Langmuir* **2007**, *23*, 1626.
56 A. J. Wilson, *Soft Matter* **2007**, *3*, 409.
57 L. Bouteiller, *Adv. Polym. Sci.* **2007**, *207*, 79.
58 W. H. Binder, R. Zirbs, *Adv. Polym. Sci.* **2007**, *207*, 1.
59 B. J. B. Folmer, E. Cavini, *Chem. Commun.* **1998**, 1847.
60 A. T. ten Cate, R. P. Sijbesma, *Macromol. Rapid Commun.* **2002**, *23*, 1094.
61 H. Hoshino, J. I. Jin, R. W. Lenz, *J. Appl. Polym. Sci.* **1984**, *29*, 547.
62 C. P. Lillya, R. J. Baker, S. Hutte, H. H. Winter, Y. G. Lin, J. Shi, L. C. Dickinson, J. C. W. Chien, *Macromolecules* **1992**, *25*, 2076.
63 S. Abed, S. Boileau, L. Bouteiller, N. Lacoudre, *Polym. Bull.* **1997**, *39*, 317.
64 S. J. Rowan, P. Suwanmala, S. Sivakova, *J. Polym. Sci., Part A: Polym. Chem.* **2003**, *41*, 3589.
65 S. Sivakova, D. A. Bohnsack, M. E. Mackay, P. Suwanmala, S. J. Rowan, *J. Am. Chem. Soc.* **2005**, *127*, 18202.
66 K. Yamauchi, J. R. Lizotte, T. E. Long, *Macromolecules* **2002**, *35*, 8745.
67 B. D. Mather, J. R. Lizotte, T. E. Long, *Macromolecules* **2004**, *37*, 9331.
68 A. S. Karikari, B. D. Mather, T. E. Long, *Biomacromolecules* **2007**, *8*, 302.
69 H. J. Spijker, A. J. Dirks, J. C. M. Van Hest, *J. Polym. Sci., Part A: Polym. Chem.* **2006**, *44*, 4242.
70 H. J. Spijker, F. L. van Delft, J. C. M. van Hest, *Macromolecules* **2007**, *40*, 12.
71 J. H. K. Hirschberg, F. H. Beijer, H. A. van Aert, P. C. M. M. Magusin, R. P. Sijbesma, E. W. Meijer, *Macromolecules* **1999**, *32*, 2696.
72 B. J. B. Folmer, R. P. Sijbesma, R. M. Versteegen, J. A. J. van der Rijt, E. W. Meijer, *Adv. Mater.* **2000**, *12*, 874.
73 H. M. Keizer, R. van Kessel, R. P. Sijbesma, E. W. Meijer, *Polymer* **2003**, *44*, 5505.
74 H. Kautz, D. J. M. van Beek, R. P. Sijbesma, E. W. Meijer, *Macromolecules* **2006**, *39*, 4265.
75 R. F. M. Lange, M. Van Gurp, E. W. Meijer, *J. Polym. Sci., Part A: Polym. Chem.* **1999**, *37*, 3657.
76 H. M. Keizer, R. P. Sijbesma, J. F. G. A. Jansen, G. Pasternack, E. W. Meijer, *Macromolecules* **2003**, *36*, 5602.
77 P. Y. W. Dankers, Z. Zhang, E. Wisse, D. W. Grijpma, R. P. Sijbesma, J. Feijen, E. W. Meijer, *Macromolecules* **2006**, *39*, 8763.
78 K. Yamauchi, J. R. Lizotte, D. M. Hercules, M. J. Vergne, T. E. Long, *J. Am. Chem. Soc.* **2002**, *124*, 8599.
79 C. L. Elkins, K. Viswanathan, T. E. Long, *Macromolecules* **2006**, *39*, 3132.
80 P. Y. W. Dankers, E. N. M. van Leeuwen, G. M. L. van Gemert, A. J. H. Spiering, M. C. Harmsen, L. A. Brouwer, H. M. Janssen, A. W. Bosman, M. J. A. van Luyn, E. W. Meijer, *Biomaterials* **2006**, *27*, 5490.
81 K. Yamauchi, A. Kanomata, T. Inoue, T. E. Long, *Macromolecules* **2004**, *37*, 3519.
82 P. Y. W. Dankers, D. J. M. van Beek, A. T. ten Cate, R. P. Sijbesma, E. W. Meijer, *Polym. Mater. Sci. Eng.* **2003**, *88*, 52.
83 K. Yamauchi, J. R. Lizotte, T. E. Long, *Macromolecules* **2003**, *36*, 1083.
84 M. G. McKee, C. L. Elkins, T. E. Long, *Polymer* **2004**, *45*, 8705.
85 M. G. McKee, C. L. Elkins, T. Park, T. E. Long, *Macromolecules* **2005**, *38*, 6015.
86 Z. B. Guan, J. T. Roland, J. Z. Bai, S. X. Ma, T. M. McIntire, M. Nguyen, *J. Am. Chem. Soc.* **2004**, *126*, 2058.
87 Z. B. Guan, *Polym. Int.* **2007**, *56*, 467.
88 J. T. Roland, Z. B. Guan, *J. Am. Chem. Soc.* **2004**, *126*, 14328.
89 R. P. Sijbesma, F. H. Beijer, L. Brunsveld, E. W. Meijer, U.S. 6320018, **2001**.
90 J. A. Loontjens, J. G. F. A. Jansen, B. J. M. Plum, EP 1031589, **2002**.
91 B. Eling, WO 02046260, **2002**.
92 H. B. S. Goodbrand, D. Popovic, D. A. Foucher, T. W. Smith, K. M. McGrane, U.S. 20030105185, **2003**.
93 Y. Ishizuka, E. Hawakawa, Y. Asawa, S. P. Pappas, WO 02053627, **2002**.
94 J. Locculier, L. L. Vanmaele, E. W. Meijer, P. Fransen, H. Janssen, EP 1310533, **2002**.
95 D. J. Luca, T. W. Smith, K. M. McGrane, U.S. 2003079644, **2003**.
96 A. Monk, S. Saraiya, J. Huang, S. P. Pappas, WO 02053626, **2002**.
97 J. W. Hofstraat, E. W. Meijer, A. P. H. J. Schenning, F. J. M. Hoeben, WO 111153, **2004**.
98 D. Kukulj, F. Goldoni, WO 02092744 A1, **2002**.
99 M. D. Eason, E. Khoshdel, J. H. Cooper, B. J. L. Royles, WO 03032929, **2003**.
100 A. Livoreil, J. Mondet, N. Mougin, WO 02098377, **2002**.
101 P. Y. W. Dankers, G. M. L. van Gemert, H. M. Janssen, E. W. Meijer, A. W. Bosman, WO 200611846, **2006**.
102 H.-A. Klok, *J. Polym. Sci., Part A: Polym. Chem.* **2005**, *43*, 1.
103 C. J. Hawker, K. L. Wooley, *Science* **2005**, *309*, 1200.
104 H. G. Borner, H. Schlaad, *Soft Matter* **2007**, *3*, 394.
105 D. W. P. M. Löwik, J. C. M. van Hest, *Chem. Soc. Rev.* **2004**, *33*, 234.
106 E. Kokkoli, A. Mardilovich, A. Wedekind, E. L. Rexeisen, A. Garg, J. A. Craig, *Soft Matter* **2006**, *2*, 1015.
107 D. Löwik, J. Garcia-Hartjes, J. T. Meijer, J. C. M. van Hest, *Langmuir* **2005**, *21*, 524.
108 D. W. P. M. Löwik, J. G. Linhardt, P. J. H. M. Adams, J. C. M. van Hest, *Org. Biol. Chem.* **2003**, *1*, 1827.
109 I. C. Reynhout, D. W. P. M. Löwik, J. C. M. van Hest, J. J. L. M. Cornelissen, R. J. M. Nolte, *Chem. Commun.* **2005**, 602.
110 M. L. Becker, J. Liu, K. L. Wooley, *Biomacromolecules* **2005**, *6*, 220.
111 J. Hentschel, E. Krause, H. G. Borner, *J. Am. Chem. Soc.* **2006**, *128*, 7722.
112 J. Hentschel, H. G. Borner, *J. Am. Chem. Soc.* **2006**, *128*, 14142.

113 G. W. M. Vandermeulen, D. Hinderberger, H. Xu, S. S. Sheiko, G. Jeschke, H.-A. Klok, *ChemPhysChem* **2004**, *5*, 488.
114 H.-A. Klok, W. M. Vandermeulen Guido, H. Nuhn, A. Rosler, W. Hamley Ian, V. Castelletto, H. Xu, S. Sheiko Sergei, *Faraday Discuss.* **2005**, *128*, 29.
115 A. Mardilovich, J. A. Craig, M. Q. McCammon, A. Garg, E. Kokkoli, *Langmuir* **2006**, *22*, 3259.
116 M. P. Lutolf, J. A. Hubbell, *Nat. Biotechnol.* **2005**, *23*, 47.
117 M. M. Stevens, J. H. George, *Science* **2005**, *310*, 1135.
118 M. Goldberg, R. Langer, X. Q. Jia, *J. Biomater. Sci., Polym. Ed.* **2007**, *18*, 241.
119 Y. C. Toh, S. Ng, Y. M. Khong, X. Zhang, Y. J. Zhu, P. C. Lin, C. M. Te, W. X. Sun, H. R. Yu, *Nano Today* **2006**, *1*, 34.
120 S. G. Zhang, *Nat. Biotechnol.* **2003**, *21*, 1171.
121 N. D. Evans, E. Gentleman, J. M. Polak, *Mater. Today* **2006**, *9*, 26.
122 G. Geibel, http://sun.menloschool.org/˜cweaver/cells/c/cell_membrane/cellmemb.gif.
123 B. Alberts, A. Johnson, J. Lewis, M. Raff, K. W. Roberts, P. Walter, *Molecular Biology of the Cell*, Garland Publishing Group, New York, **2002**.
124 J. D. Hartgerink, E. Beniash, S. I. Stupp, *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 5133.
125 J. D. Hartgerink, E. Beniash, S. I. Stupp, *Science* **2001**, *294*, 1684.
126 K. L. Niece, J. D. Hartgerink, J. J. J. M. Donners, S. I. Stupp, *J. Am. Chem. Soc.* **2003**, *125*, 7146.
127 H. A. Behanna, J. Donners, A. C. Gordon, S. I. Stupp, *J. Am. Chem. Soc.* **2005**, *127*, 1193.
128 M. O. Guler, J. K. Pokorski, D. H. Appella, S. I. Stupp, *Bioconjugate Chem.* **2005**, *16*, 501.
129 S. R. Bull, M. O. Guler, R. E. Bras, T. J. Meade, S. I. Stupp, *Nano Lett.* **2005**, *5*, 1.
130 S. R. Bull, M. O. Guler, R. E. Bras, P. N. Venkatasubramanian, S. I. Stupp, T. J. Meade, *Bioconjugate Chem.* **2005**, *16*, 1343.
131 L.-s. Li, S. I. Stupp, *Angew. Chem., Int. Ed.* **2005**, *44*, 1833.
132 M. O. Guler, S. Soukasene, J. F. Hulvat, S. I. Stupp, *Nano Lett.* **2005**, *5*, 249.
133 M. O. Guler, L. Hsu, S. Soukasene, D. A. Harrington, J. F. Hulvat, S. I. Stupp, *Biomacromolecules* **2006**, *7*, 1855.
134 K. Rajangam, H. A. Behanna, M. J. Hui, X. Q. Han, J. F. Hulvat, J. W. Lomasney, S. I. Stupp, *Nano Lett.* **2006**, *6*, 2086.
135 F. Gelain, A. Horii, S. G. Zhang, *Macromol. Biosci.* **2007**, *7*, 544.
136 S. G. Zhang, T. C. Holmes, C. M. Dipersio, R. O. Hynes, X. Su, A. Rich, *Biomaterials* **1995**, *16*, 1385.
137 S. G. Zhang, T. Holmes, C. Lockshin, A. Rich, *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 3334.
138 H. Yokoi, T. Kinoshita, S. G. Zhang, *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 8414.
139 C. E. Semino, J. Kasahara, Y. Hayashi, S. G. Zhang, *Tissue Eng.* **2004**, *10*, 643.
140 T. C. Holmes, S. de Lacalle, X. Su, G. S. Liu, A. Rich, S. G. Zhang, *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 6728.
141 C. E. Semino, J. R. Merok, G. G. Crane, G. Panagiotakos, S. G. Zhang, *Differentiation* **2003**, *71*, 262.
142 E. Garreta, E. Genove, S. Borros, C. E. Semino, *Tissue Eng.* **2006**, *12*, 2215.
143 M. E. Davis, J. P. M. Motion, D. A. Narmoneva, T. Takahashi, D. Hakuno, R. D. Kamm, S. G. Zhang, R. T. Lee, *Circulation* **2005**, *111*, 442.
144 E. Genove, C. Shen, S. G. Zhang, C. E. Semino, *Biomaterials* **2005**, *26*, 3341.
145 J. Kisiday, M. Jin, B. Kurz, H. Hung, C. Semino, S. Zhang, A. J. Grodzinsky, *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 9996.
146 P. Y. W. Dankers, P. J. H. M. Adams, D. W. P. M Löwik, J. C. M. van Hest, E. W. Meijer, *Eur. J. Org. Chem.* **2007**, *22*, 3622.

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