The quest for function in systems with two dynamic covalent bonds

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2.

Synthetic Cell-Compatible Materials for Biomedical Applications

2.1 Introduction

Biomaterials have been defined as chemical composites other than food or drugs that come into contact with tissues or biological fluids. Their development encompasses efforts in the fields of medicine, chemistry, cell biology, and material engineering. The use of biomaterials dates back thousands of years to the employment of gold in dentistry, while today they find widespread use in everyday life, as contact lenses or dialysis tubing, and as essential parts of medical devices, for example, vascular grafts.[1] At the same time, new generations of (synthetic) biomaterials are extensively applied in modern approaches to drug delivery,[2] immuno-engineering,[3] regenerative medicine and tissue engineering.[4]

In the last 20 years, strategies for the design of modern synthetic biomaterials relied on breakthroughs in, and symbiosis of, synthetic supramolecular polymer chemistry[5], 3D molecular patterning techniques,[6] and biomimetic rational design, which integrates principles from cell and molecular biology.[4] These advances, in rather separate scientific areas, gave rise to materials in which the reversible nature of non-covalent interactions allows for sensing and responsiveness towards biological cues, or mimicking structural and functional aspects of biological signaling.

In this chapter, we aim to shortly summarize recent advances in the field of biomaterials, with emphasis on design principles, illustrative examples, and the overall progress of synthesis. We also highlight the role of the extracellular matrix (ECM), as its complexity has inspired the development of many biomimetic materials.

2.2 Extracellular Matrix (ECM) as an Inspiration for Biomimetic Materials

The extracellular matrix (ECM) is the non-cellular constituent present in all tissues and organs, that provides not only physical support for the cells, in the form of an interwoven fibrous scaffold, but also coordinates cell fate in a spatio-temporal manner employing many biochemical, and biomechanical signals. The ECM is composed of: (i) insoluble hydrated macromolecules (proteoglycans with covalently linked glycosaminoglycan side chains, and fibrous proteins, collagen, fibronectin, laminin, elastin), (ii) soluble macromolecules (growth factors, cytokines, and chemokines) and (iii) proteins on the surfaces of neighbouring cells (**Figure 2.1**).[4a]

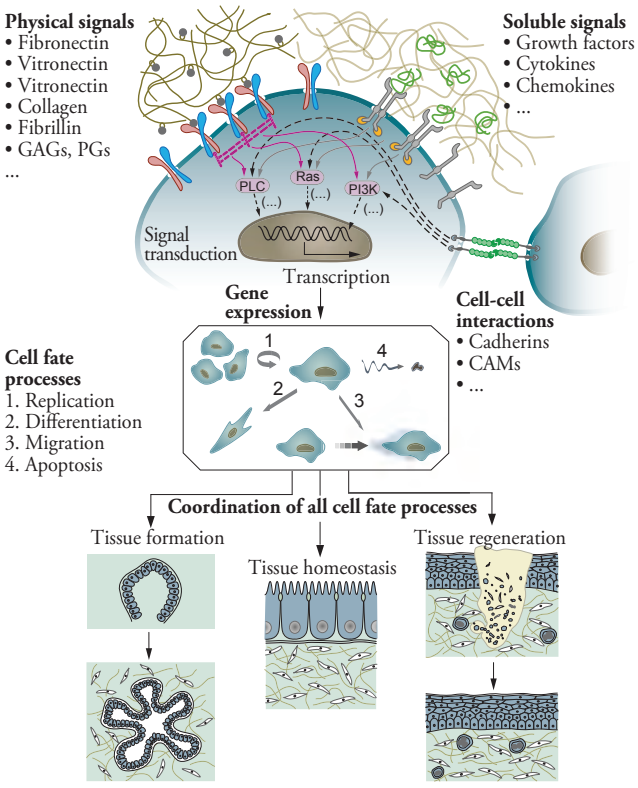

Figure 2.1 A cartoon representation of the ECM. Protein- and proteoglycan-based (physical signals) gel network containing soluble signals which interact with cell-surface receptors, which in turn induce processes such as replication, differentiation, migration, and apoptosis of the cells. Precise coordination of these processes directs tissue formation, homeostasis, and regeneration. GAGs, glycosaminoglycan; PGs, proteoglycans; PLC, phospholipase C; CAMs, cell adhesion molecules. Reprinted with permission from ref. 4a. Copyright©, 2005, Nature Publishing Group.

Collagen is the most abundant fibrous protein within ECM, found in the form of a triple-stranded helix. These helices, depending on the type of collagen molecules, further self-assemble into fibrils or networks, characterized by enormous tensile strength, to limit the distensibility of tissues. Fibrous, non-collagenous glycoproteins, such as laminin, fibronectin and elastin, can be found in many isoforms, due to a myriad of post-translational modifications, therefore having slightly different functions in a developmental stage- and tissue-specific processes. Nevertheless, general traits define each of the proteins mentioned above. For example, elastin is found associated with collagen, providing tissues the ability to resume their shape after repeated stretching. Fibronectin exists as a protein dimer, composed of two monomeric units, connected by two disulfide

bonds. It can bind to other ECM proteins and cell-surface receptors, i.e., integrins. At the same time, it plays an essential role in cell attachment as it contains an adhesive Arg-Gly-Asp (RGD) sequence that resides in a hydrophilic loop of the protein. Proteoglycans (PGs) are proteins containing carbohydrate-based polymer side chains, due to which these molecules possess a hydrophilic character, and can retain a high-water content. As PGs are present in the form of hydrated gel within the extracellular microenvironment, they provide resistance to compressive forces, facilitate diffusion of biological compounds, buffer and supply growth factors through facile binding.[7,8]

In addition to providing structural support, the extracellular microenvironment acts as a repository of soluble and secreted signals, which through communication with integrins, modulate signalling pathways and cellular responses. The transmission of information between cells and their surroundings is bidirectional, that is, intracellular changes are induced by extracellular stimuli and *vice versa*.[9] Effects of intracellular signalling come into play in activation of integrins themselves and processes of ECM remodelling and degradation.[4a]

Although all ECMs are mainly composed of water, proteins, and polysaccharides, their exact composition is highly tissue-specific and determined through a reciprocal dialogue between the cell constructs and the microenvironment. The large number of components, their interconnectivity, and the overall complexity of the system make designing materials that can orchestrate cell behaviour in a controlled manner highly challenging. In comparison with the ECM, present synthetic matrices are rather simple but represent a good starting point towards the development of comprehensive materials for clinical applications.

2.3 Naturally Derived Models of ECM

Understanding of cell behaviour within complex multicellular tissues is vital for gaining insight into physiological (i.e., organogenesis) and pathological (i.e., tumour growth) cellular processes. The use of traditional two-dimensional (2D) cell culture models has provided a plethora of exciting findings; however, they are limited in replicating *in vivo* conditions such as the cellular microenvironment. Therefore, there is an increasing need for the development of *in vitro* systems that accurately reproduce such conditions, hence allowing the organization of cells in a three-dimensional (3D) manner.[10] Experimental models, currently used for this purpose, are biologically derived matrices such as Matrigel, collagen, and other ECM derived fibrous proteins, and more defined, synthetically derived materials where biologically relevant factors can be integrated with a modular fashion.

Matrigel is a commercialized gel-like protein mixture (contains fibrous proteins, but also growth factors and cytokines) obtained from Engelbreth-Holm-Swarm (EHS) mouse sarcoma cells, which resembles the extracellular microenvironment present in tissues. By mimicking cell-ECM interactions, Matrigel showed to be a potent scaffold for promoting cell differentiation due to its ability to maintain self-renewal and pluripotency of cells.[11] However, this naturally derived multicomponent matrix suffers from some drawbacks: batch-to-batch variability of the extract is often a cause of irreproducibility of experimental results, physical properties are hard to tune, and because of its ill-definition and tumour origin, constructs grown in this matrix are not suitable for clinical application.

Collagen, fibrin, and other ECM insoluble macromolecules also find application individually, as cell-culture microenvironments, and they can be obtained by decellularization of tissues or extraction and purification of the desired protein to yield functional scaffolds.[12] Collagen owes its widespread use as a naturally derived biomaterial to its biocompatibility and ability to undergo degradation by the enzymes secreted from the encapsulated cells. Additional chemical covalent cross-linking of hierarchically self-assembled fibers of collagen molecules allows for the control over physical properties to a certain extent. Hyaluronic acid (HA) is one of the glycosaminoglycan components of the extracellular matrix of human connective tissues, and plays a significant role in wound healing. HA is used in several biomedical applications thanks to its biocompatibility, ability to be formed into hydrogels by covalent cross-linking with a variety of hydrazide derivatives and degradability towards hyaluronidases, which are present in cells and serum. Aside from poor mechanical characteristics of HA-based hydrogels, another disadvantage is the necessity for thorough purification of raw HA material, before it can be safely used on patients.[13]

In general, the use of naturally vs. synthetically derived biomaterials for cell-culture often represents the trade-off between biocompatibility and consistency. Therefore, elaborate efforts are made towards mimicking biological biomaterials, in terms of their richness in biological signals, bidirectionality in information flow and mechano-responsiveness, while keeping control over chemical uniformity. Synthetic organic chemistry, and especially supramolecular chemistry, present us with an extensive toolbox to design new materials based on nature's principles, and these alternatives are discussed below.

2.4 Synthetic Approaches to Fabrication of Materials That Can Mimic the ECM

Taking the ECM as an inspiration, a few evident elements stand out, that are incorporated readily into the design of synthetic biomaterials.

The fibrous interwoven architecture of the ECM has prompted several research groups to develop materials with similar structures. These hydrogels are formed through the supramolecular self-assembly of building blocks that is followed by entanglement, bundling, association and cross-linking of individual fibers or fibrils (**Figure 2.2**).[14] Peptides,[15] block copolymers,[16] filamentous nanoparticles (NPs), e.g., cellulose[17] and chitin nanocrystals[18] have been used as components to build man-made networks that mimic fibrillar morphology and mechanical properties of the ECM.

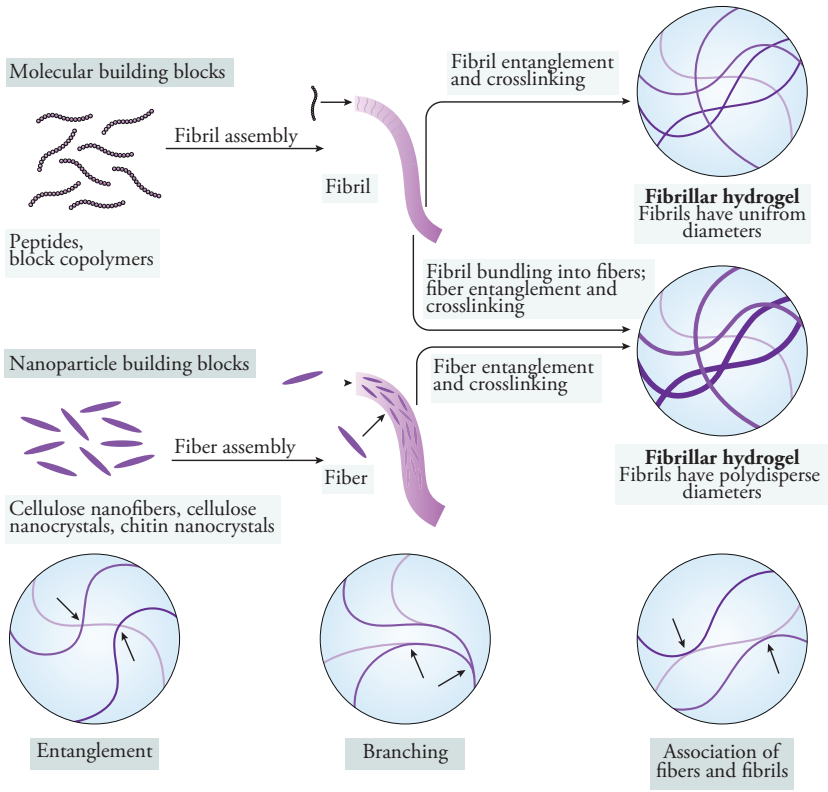

Figure 2.2 Formation of fibrous synthetic hydrogels. Molecular and nanoparticle building blocks self-assemble into supramolecular polymers (fibrils and fibers), which can further associate, branch, form entanglements, and bundles to yield hydrogels. Adapted and reprinted with permission from ref. 14. Copyright©, 2019, Nature Publishing Group.

However, cell-compatible hydrogels are not only characterized by the presence of a fibrous scaffold; the ability to mediate cell adhesion and movement via receptor-ligand interactions, to stabilize and present growth factors, and the capability of various bioactive molecules to diffuse also affect the applicability of the material. For example, the porosity of the material should be such to allow cell infiltration and interconnectivity, while densely packed architectures with pores <10 μm hamper interactions at the cell-material interface, hence, cell motility, and transport of soluble signals (**Figure 2.3**).[19]

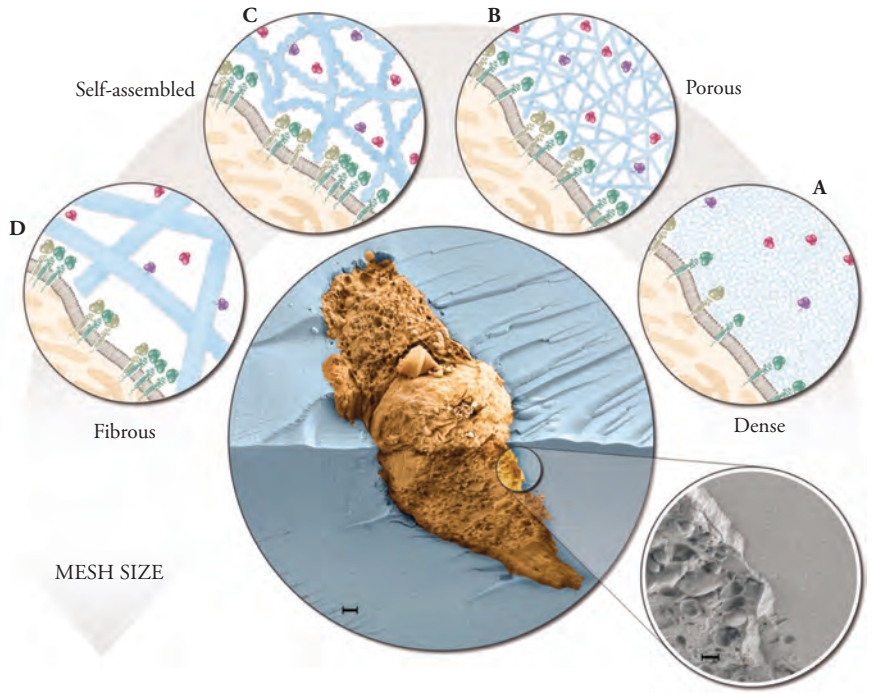

Figure 2.3 A false-coloured high-resolution cryogenic scanning electron micrograph displaying the interface between hydrogel (blue) and an embedded cell (brown) (scale bar 1 μm). The bottom right insert shows an enlarged view of the cell membrane in contact with the hydrated gel (scale bar 300 nm). Inserts A-D schematically illustrate gel phase (blue, fibrous network, and white, void space) with different mesh sizes, increasing from right to left. The extracellular network presents the cell (brown) with soluble bioactive compounds (red) and growth factors (purple). The communication between cells and their surroundings occurs through cell receptors, i.e., integrins (green) which lie on the cell membrane (grey). The porosity of the network can control movement and transport of soluble ligands. The structure depicted in D (micrometer-sized pores) promotes cell migration in comparison to the densely packed scaffold with nanometer-sized pores showed in A. Clustering of integrins on the cell surface is readily occurring in structures with larger void spaces between the fibers, similar to C and D, which enables focal adhesion contact between cells and the network. Reprinted with permission from ref. 19. Copyright©, 2012, AAAS.

Identification of short peptide sequence in fibronectin[20] responsible for the cell-adhesion properties of the protein opened up many research pathways towards the creation of ligand-functionalized materials.[21] RGD, and many other specific biomolecular domains that control not only cell adhesion, but also cell differentiation[22], or are susceptible to degradation by proteases secreted by cells,[23] have been incorporated into synthetic biomaterials, using top-down rational design. **Figure 2.4** summarizes design strategies for the fabrication of biomaterials that mimic the complexity of naturally-derived extracellular matrix.

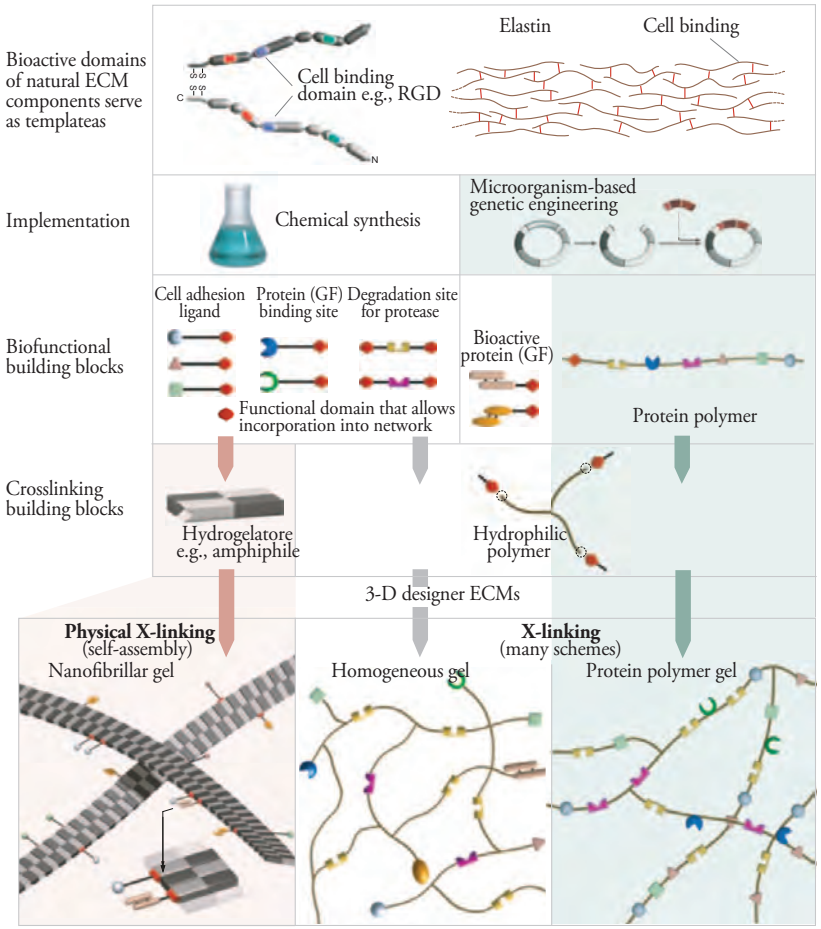

Figure 2.4 Graphical summary of workflow in fabrication of biomimetic materials. Firstly, domains that control cell response are identified (many are already known, like RGD and VPGVG sequences) in the cell's *in vivo* microenvironment, followed by their incorporation into building blocks. Those building blocks can subsequently self-assemble into fibers or undergo chemical cross-linking, to obtain synthetic networks. Reprinted with permission from ref. 4a. Copyright©, 2005, Nature Publishing Group.

Alternatively, a bottom-up approach provides the opportunity to build synthetic hydrogels block by block and implement crucial features for biomedical applications. Desired features that are most commonly engineered into a hydrogel are: (i) biodegradability – the ability to undergo proteolysis, which is often required for cell migration; (ii) bioactivity – the ability to mediate morphogenesis and differentiation due to external delivery of growth factors; (iii) bioadhesion – the ability of cells to adhere to gel; (iv) transport of hydrophobic/hydrophilic molecules – determined by the porosity of the material and (v) mechanical properties – relevant for control over mechanotransduction pathways that further regulate cell fate processes. Identical to the top-down approach, the building of hydrogels through a bottom-up approach relies on comprehension of the operating principles of biofunctional ligands present in naturally derived ECMs.[19]

2.4.1 Hydrogels Based on Synthetic Polymers

The central idea behind synthetic hydrogels as matrices for cell growth is a structure-property relationship, which means that molecular characteristics such as chemical composition, molecular weight, and organization are in direct connection with the chemical and physical properties of the macroscopic material. Synthetic materials include poly(ethylene glycol) (PEG), poly(vinyl alcohol) (PVA), poly(acrylic acid) (PAA), poly(propylene fumarate-co-ethylene glycol) (P(PF-co-EG), polypeptides and others.[24]

Poly(ethylene glycol) (PEG) is often used in synthetic hydrogels because the bioinertness of these building blocks prevents non-specific protein adhesion. At the same time the outstanding versatility of PEG macromers permits the incorporation of chemical and physical signals for cell adhesion and controlled differentiation.[25] The seminal work of Hubbell and coworkers demonstrated that PEG macromers, specifically functionalized for the events of cell adhesion and proteolytic degradation, can promote cell migration, thus conduct tissue regeneration.[23a]

Recently, methacrylated dextran (DexMA) modified with cell-adhesive moieties was used for the fabrication of a fibrous matrix, where fiber diameter, density, anisotropy, and interconnectivity can be tuned to investigate how cells sense stiffness (**Figure 2.5a-b**). The authors reported a previously unknown mechanism of nearby fiber sequestration (**Figure 2.5c**), as a cellular response to the architecture and mechanics of the fibrillar network. Recruitment of the fibers led to an increase in local adhesive ligand density at the cell surface (**Figure 2.5d**), which, in return, enhanced adhesion signaling, cell spreading and proliferative signaling.[26]

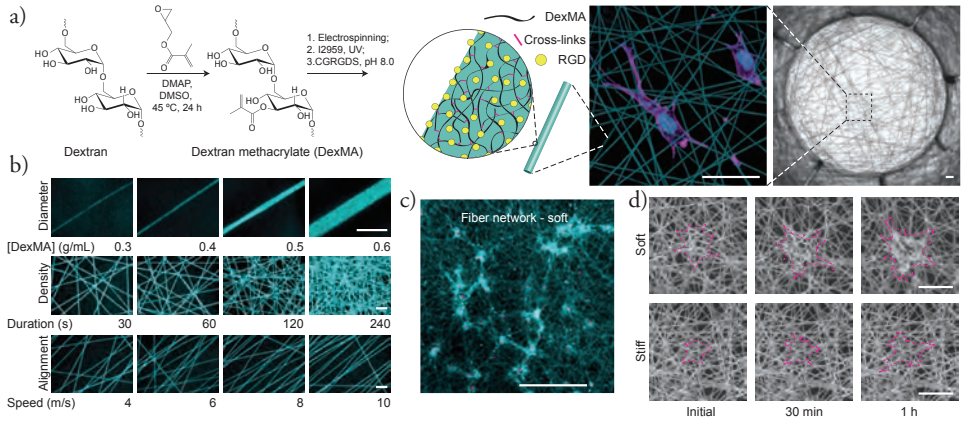

Figure 2.5 a) Stepwise fabrication of RGD-functionalized DexMA fibrous networks. Dextran reacts with glycidyl methacrylate to yield dextran methacrylate, followed by the addition of photoinitiator and electrospinning onto substrates. Into the networks of suspended fibers, RGD is incorporated to promote cell adhesion. Scale bar 100 μm; **b)** Through the modulation of different properties (diameter, density and alignment of fibers) a control over physical properties is achieved. Scale bar 10 μm; **c)** Formation of densely packed clusters of fibers due to the recruitment of fibers by cells. Fibers are imaged by coupling with rhodamine methacrylate (cyan), and cell nuclei are labeled with Hoechst 3342 (magenta). Scale bar 500 μm; **d)** Time-lapse confocal imaging of fiber networks functionalized with fluorescently tagged RGD shows an increase of local RGD density. Cell outlines are shown in magenta. Scale bars 50 μm. Reprinted and adapted with permission from ref. 26. Copyright©, 2015, Nature Publishing Group.

2.4.2 At the Interface Between Covalent and Supramolecular Hydrogels - Materials That Make Use of Adaptable Linkages

As already discussed, many synthetic systems that mimic (to a certain extent) *in vivo* conditions, have been developed. Employed design strategies often rely on the integration of moieties susceptible to protease-mediated dissolution[23,27] or hydrolytic degradation of the material,[28] to facilitate cell migration, spreading, and signalling. Since the aforementioned types of degradation are irreversible, several limitations arise with the use of strictly covalent-based hydrogels that incorporate these bioactive domains. For example, a permanent breakdown of gel leads to the material disappearance over a prolonged period of time, which is of particular importance in experiments related to tissue engineering.[29] Also, a confined dissolution of material generates local alteration of physical properties; therefore, cellular processes at the local level cannot be regarded as a consequence of materials bulk characteristics, which are the readily determined ones.[30] At the same time, traditional synthetic soft materials are static, and in that sense, inefficient in recapitulating the dynamicity of natural extracellular matrices.

As a solution to these limitations, hydrogels that make use of dynamic covalent bonds or non-covalent associations (calcium coordination,[31] hydrogen-bonding,[32] and host-guest interactions[33]) have been developed, since these interactions provide local lability and adaptability, while long-term bulk stability of the system is maintained. A common denominator of the two types of adaptable linkages is reversibility; however, it is preferred that they show their reversible nature at physiological conditions and without external stimuli (or with stimuli of minimal invasiveness to the cells), to be suitable for use in adaptable materials.[34] The processes of formation and dissociation (breakage) of both non-covalent interactions and dynamic covalent bonds are determined by the thermodynamic equilibrium constant (K_{eq}) as well as kinetic rate constants (**Figure 2.6**). For example, if the K_{eq} is high, the material might exhibit mechanics analogous to non-degradable gels, thus preventing cell spreading and migration. In contrast, a low K_{eq} translates into a material susceptible to fast erosion leading to cell sedimentation.[35] The rates at which bonds are formed (k_{on} and k_1) and broken (k_{off} and k_{-1}) play an important role as they directly govern processes of gelation and gel relaxation, respectively.[36] A further detailed, excellent discussion on thermodynamic and kinetic design criteria for adaptable hydrogels is given in ref. 35.

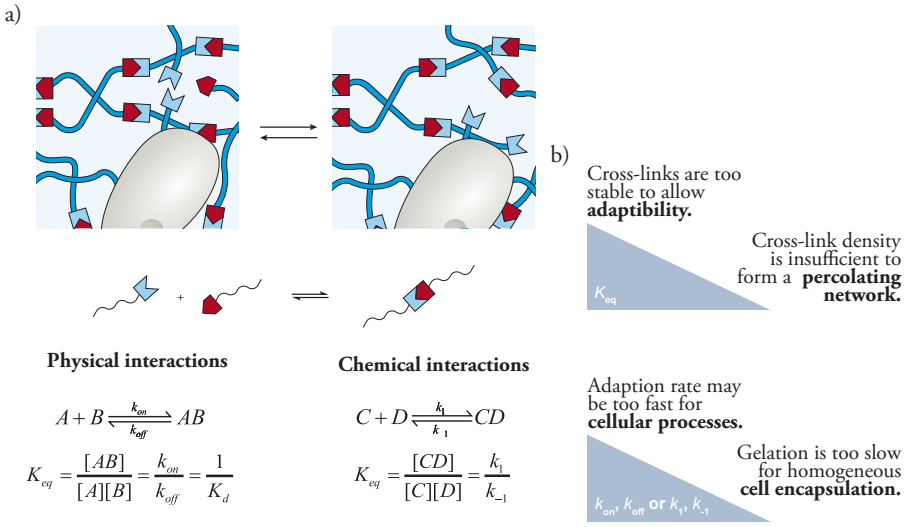

Figure 2.6 a) Cartoon representation of formation and breakage of adaptable bonds. Their reversibility facilitates material adaptation at the local level while maintaining the bulk stability. Kinetic constants of both non-covalent and dynamic covalent interactions determine the material's properties. Reprinted and adapted with permission from ref. 34. Copyright©, 2016, Nature Publishing Group. **b)** Graphic summary of the relationship between thermodynamic and kinetic parameters and characteristics of materials that make use of adaptable linkages. Reprinted and adapted with permission from ref. 35. Copyright©, 2015, Wiley-VCH.

Aside from being used for the fabrication of adaptable materials (**Figure 2.7b**),[35,36] reversible interactions also find application in a dynamic presentation of biologically relevant ligands (**Figure 2.7a**)[37,38] and alteration of mechanical properties as a consequence of a change in cross-linking density or conformation (**Figure 2.7c**).[39,40]

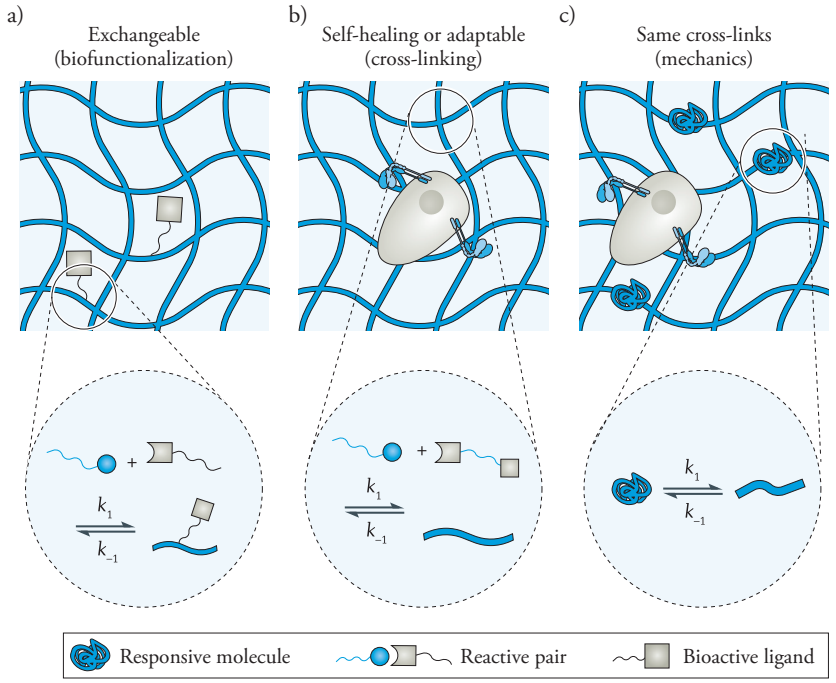

Figure 2.7 Reversible interactions can be used for **a)** biofunctionalization of materials; **b)** cross-linking that can be locally modified while preserving materials integrity; and **c)** modulation of mechanical properties *in situ*. Reprinted and adapted with permission from ref. 34. Copyright©, 2016, Nature Publishing Group.

The combination of bioorthogonal, light-based chemistries has been exploited to decorate synthetic hydrogels with vitronectin, to regulate the osteogenic differentiation of human mesenchymal stem cells.[38] A strain-promoted azide-alkyne cycloaddition was used for hydrogel formation and uniform incorporation of photocaged alkoxyamines, that can be irreversibly deprotected, upon spatially defined irradiation. Subsequently, liberated alkoxyamines undergo oxime ligation with aldehyde-functionalized proteins, resulting in their immobilization within the hydrogel network. Aside from a carbonyl functionality, the proteins also carry a photolabile linker that enables their irreversible release upon subsequent irradiation. In both the photodegradation-oxime ligation sequence and the photoscission reaction for protein removal, the exposure of the gel to patterned UV light allows spatial and temporal control over the presentation and removal of biomolecular signals in biomimetic materials, similarly to the ECM.

Hydrogels synthesized through hydrazone bond formation showed adaptable behaviour, i.e., the rates of hydrogelation and cell encapsulation, and gel relaxation were found to be intrinsically connected with the rate of bond formation and hydrolysis, respectively.[36] By careful choice of the type of macromers (aliphatic or aromatic, number of PEG arms) and stoichiometry of hydrazine and aldehyde reactive groups, the physical characteristics of the hydrogels were tuned to fabricate an adaptable gel on a timescale compatible with cytoskeletal outgrowth.

The cell morphology is coupled to substrate stiffness; cells spread to a greater extent on surfaces with higher moduli.[41] In a recent study, a hydrogel that incorporates photoswitchable azobenzene cross-linkers, which can reversibly modulate its stiffness upon irradiation with light at cytocompatible wavelengths, has been developed.[40] Mesenchymal stem cells (MSC) were cultured on a previously UV-irradiated hydrogel. As *trans-cis* azobenzene isomerisation induces gel softening, cells remained small, compared to a non-irradiated, stiff gel control. Upon irradiation with cytocompatible blue light, azobenzene cross-linkers isomerized back to the *trans* form, which resulted in hydrogel stiffness being regained. The change in hydrogel moduli promoted cell spreading, leading to cell morphology identical to one of the cells cultured in a non-irradiated control. These modular (in terms of stiffness) hydrogels pave the way towards a better understanding of cellular mechanotransduction, i.e., the conversion of physical inputs into biochemical responses.

Hydrogels made by cross-linking of polymeric precursors via hydrogen bonding, electrostatic or hydrophobic interactions, or through host-guest complexation are considered as supramolecular biomaterials,[5,42] since they make use of supramolecular recognition motifs.

2.4.3 Hydrogels Formed Through Molecular Self-Assembly

Biomimetic materials formed through molecular self-assembly show to have a high potential in recapitulating both structural and functional features of natural ECMs. These specific advantages arise from the characteristics of non-covalent interactions that hold together molecular constituents; in return, materials are often described as dynamic, modular and with tunable physical properties.[43]

The ability of small molecules to undergo self-assembly is determined by the presence of stacking motifs that drive their association through reversible non-covalent interactions. Therefore, the design of building blocks is crucial, as it determines emergent properties, e.g., mechanical characteristics and dynamic behavior of filamentous nanostructures.[5]

One-dimensional assemblies can further entangle, associate, bundle or cross-link chemically, to result in a three-dimensional scaffold (**Section 2.4**, **Figure 2.2**).[14] The fibrous morphology of these networks is an advantage as they can embed and support cells, similar to natural matrices. As discussed earlier, mechanical properties are governed by thermodynamics and kinetics of binding interactions,[35] while responsiveness to external physical, chemical, or biological stimuli[44] is engineered in supramolecular biomaterials to allow the remodeling of the environment in real-time. The modularity of supramolecular assemblies, and therefore, their ability to incorporate biologically relevant ligands, can be understood through the simple analogy with Lego™ interlocking plastic bricks. Stacking of the yellow bricks on top of each other results in a structure; utilizing the same complementary interactions –pegs and holes – a differently colored brick can be incorporated. Thus, through the design of self-assembling molecule and its biofunctionalized variation, and alteration of component formulation ratios, cell-adhesion regions and growth factors can be tuned into materials. Although supramolecular biomaterials have found application *in vivo* and *in vitro*, a challenge to design a generalizable molecular platform, yet with optimized parameters for a unique cell type and cellular response for regenerative medicine, remains.[45]

Driven by the serendipitous discovery of a self-assembling peptide by Zhang and collaborators, a library of self-complementary ionic peptide sequences that can stack to form β-sheet structures in aqueous media, has been designed (**Figure 2.8a-b**).[46] The fiber-like assembly formation is driven by electrostatic interactions between positively and negatively charged amino acid residues, alongside hydrophobic interactions between neutral amino acid residues.[47] In a recent study, one of the ionic peptides, RADA16-I was functionalized with the integrin-binding sequence derived from angiopoietin-1, QHREDGS.[48] The mesenchymal stem cells (MSCs) were cultured in the functionalized hydrogel and transplanted together onto the border of the affected area to evaluate the potential towards stem cell therapy for myocardial infarction. It was found that these gels promote MSCs proliferation, and facilitate the formation of new blood vessels and cell-to-cell communication through secretion of IGF-1 and HGF in a rat model. Overall, this led to a reduction of scar size and improvement of cardiac function.

Peptide-amphiphiles (PA) designed by Stupp and coworkers, comprise of an amino acid segment and alkyl tail and can self-assemble into nanofibers (**Figure 2.8c-d**). Structurally versatile constituents determine the fiber morphology, which demonstrates the robustness of the system towards self-assembly despite the chemical modification.[49] One of the PA molecules, containing IKVAV sequence, which is known to direct neurite

growth and promote neurite sprouting, was used as a component of the fibrous scaffold to induce differentiation of neural progenitor cells (NPCs).[22] The addition of cell suspension triggers the process of fiber self-assembly, and at the same time, it is gentle enough to not affect cell survival.

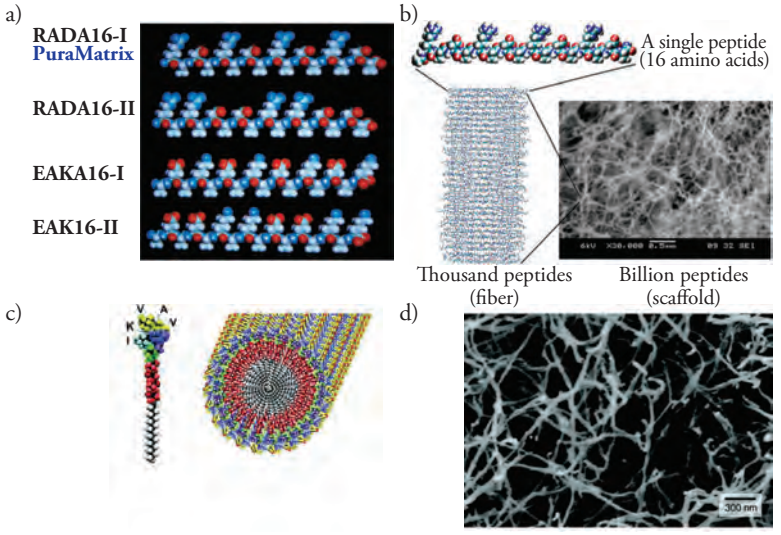

Figure 2.8 a) Molecular models of ionic self-assembling peptides that form nanofibers. These designer peptides have two distinctive sides: one hydrophobic and one hydrophilic surface, where the latter one is made of alternating negatively and positively charged amino acid residues; **b)** The single peptide chain (RADA 16-I) is approximately 6 nm long. Thousands of individual peptides assemble into a fiber, to ultimately organize into a fiber scaffold. Reprinted and adapted with permission from ref. 46. Copyright©, 2010, The Royal Society of Chemistry. **c)** Molecular model of a peptide-amphiphile functionalized with the IKVAV sequence that self-assembles into nanofibers; **d)** SEM micrograph of the fibrous hydrogel. The process of hydrogelation is triggered by adding cell media. Reprinted and adapted with permission from ref. 22. Copyright©, 2004, AAAS.

Building blocks based on the self-complementary quadruple hydrogen-bonding motif, 2-ureido-4[1*H*]-pyrimidinone (UPy) unit (**Figure 2.9a**), were used to form linear supramolecular polymers, with tunable properties such as viscosity, chain length, and composition.[50] Simple mixing of UPy-functionalized polymers with UPy-modified biomolecules resulted in the formation of bioactive material (**Figure 2.9b**).[32] Presentation of GRGDS and PHSRN sequences, in *in vitro* and *in vivo* conditions, led to specific cell adhesion and spreading (**Figure 2.9c**), and formation of single giant cells at the interface between the material and tissue, respectively.

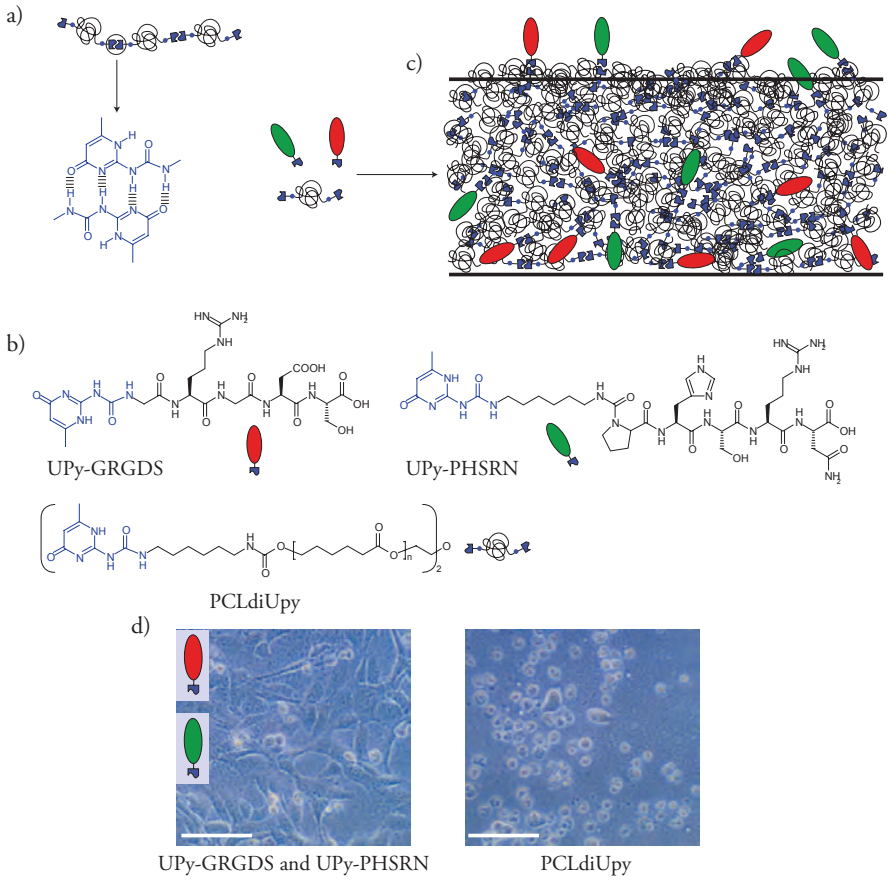

Figure 2.9 a) The self-complementary quadruple hydrogen-bonding motif, 2-ureido-4[1*H*]-pyrimidinone (UPy) unit was used to to yield supramolecular polymers; **b)** The chemical structures of UPy-functionalized peptides (UPy-GRGDS and UPy-PHSRN) and UPy-modified polycaprolactone; **c)** Formation of bioactive material through simple mixing of self-complementary building blocks, out of which, some carry biologically relevant functionalities; **d)** Optical microscopy visualization of fibroblast cells on drop-cast films of a mixture of PCLdiUPy, UPy-GRGDS and UPy-PHSRN (left panel) and PCLdiUPy alone (right panel). Cells cultured on the blend of 3 UPy-functionalized molecules exhibit adhesion and spreading. Reprinted and adapted with permission from ref. 32. Copyright©, 2005, Nature Publishing Group.

2.5 Conclusion

In conclusion, different types of synthetic soft materials for biomedical applications are being developed at a rapid pace. A collection of design strategies and illustrative models described here represents just a small selection of the remarkably diverse range of examples present in the literature. The diversity of the performed research is not surprising considering the structural and functional complexity and demands posed by the natural materials that are to be recapitulated.

Synthetic materials that encompass a fibrillar scaffold are considered eminently suitable as biomimetic materials, due to a resemblance with the natural ECM in terms of structural support, as well as allowing of cell migration by squeezing through pores or fiber recruitment and deformation. In order to promote cell processes such as replication, migration, differentiation, and, consequently, regulation of complex morphogenetic process in tissue formation and regeneration, synthetic materials must contain biological signals, derived from natural microenvironments. These components are readily built directly into the biomaterial by means of covalent linking, dynamic covalent bonds or specific functionalization of self-assembling building blocks. Even though considerable progress has been made, the remaining challenges lie in spatial-temporal control over the presentation and removal of biomolecular signals. Furthermore, the current state of the art still lacks robust design solutions for encompassing dynamic reciprocity. In other words, synthetic materials, although able to present cells with functional epitopes, which potentially direct their behaviour, often do fall behind the ECM in terms of ability to adapt upon information inflow originating from cells. Such adaptability is of importance as it would allow remodelling of the synthetic matrix to further support tissue morphogenesis and functional differentiation. Overall, the balance between mimicking the ECM's complex structure and the requirement for a simple system for clinical translation is an essential factor and continues to be challenging up to date. Advances in the understanding of cell biology and biochemistry, alongside collaborations at the interface of medicine and material science, could notably accelerate the progress towards synthetic candidates for versatile medical applications.

2.6 Acknowledgments

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