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Perspective

Introduction of Enzyme-Responsivity in Biomaterials to Achieve Dynamic Reciprocity in Cell–Material Interactions

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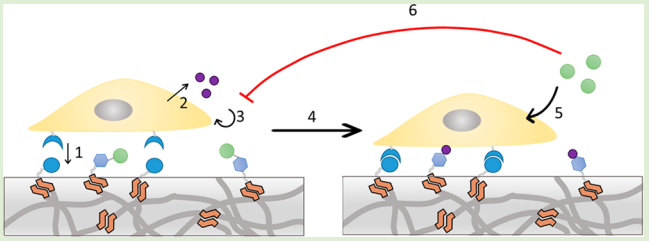
ABSTRACT: Much effort has been made in the development of biomaterials that synthetically mimic the dynamics of the natural extracellular matrix in tissues. Most of these biomaterials specifically interact with cells, but lack the ability to adapt and truly communicate with the cellular environment. Communication between biomaterials and cells is achieved by the development of various materials with enzyme-responsive moieties in order to respond to cellular cues. In this perspective, we discuss different enzyme-responsive systems, from surfaces to supramolecular assemblies. Additionally, we highlight their further prospects in order to create, inspired by nature, fully autonomous adaptive biomaterials that display dynamic reciprocal behavior. This Perspective shows new strategies for the development of biomaterials that may find broad utility in regenerative medicine applications, from scaffolds for tissue engineering to systems for controlled drug delivery.



1. INTRODUCTION

In the regenerative medicine field, synthetic biomaterials are being developed that interface with biology, that is, with cells and tissue. These biomaterials are mostly static, providing support to the cellular environment but not exposing dynamic interactions with the cells.[1−4] In order to regenerate the body, research has shifted to biomaterials that provide more than only architectural structure by incorporating functional groups, such as bioactive molecules that can be delivered to the cells to provoke cellular reactions.[5−11] A source of inspiration is the extracellular matrix (ECM), which is the material that surrounds the cells in our tissues.[12−14] This specialized biological material is composed of thousands of different molecules held together via noncovalent, supramolecular interactions and displays a life-like behavior by using dynamic reciprocity (DR).[15−17] DR refers to the bidirectional interaction between cells and their ECM.[18]

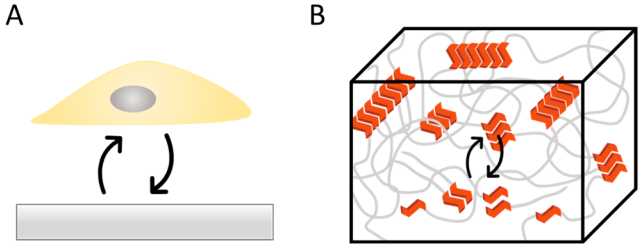


Figure 1. Schematic representations of (A) dynamic reciprocity between a cell and a biomaterial and (B) dynamic reciprocity in a dynamic, supramolecular material.

1.1. Dynamic Reciprocity in the Natural ECM. DR between the ECM and the cell is very important in many processes, such as tissue morphogenesis, angiogenesis, cancer, and wound healing.[19,20] During tissue development, the tissue is continuously being remodeled by changes in the degradation and structural organization of the ECM, regulating the mechanical properties of the tissue.[21] These changes in the ECM in its turn controls the survival, migration, proliferation, and differentiation of cells.[22] During these tissue remodeling processes, ECM-degrading enzymes such as matrix metalloproteinases (MMPs) are very important and their activity is highly regulated both temporally and spatially.[23] Normally, the activity of MMPs is low. However, when the tissue is diseased or inflamed, they become highly active.[24] During homeostasis, the activity between MMPs and their inhibitors, tissue inhibitor matrix metalloproteinases (TIMPs), is regulated.[25] However, under pathophysiological conditions, the balance between MMP activity and TIMP activity can be shifted, and excessive MMP activity can cause maladaptive changes to the tissue architecture.[26] This can eventually lead to uncontrolled

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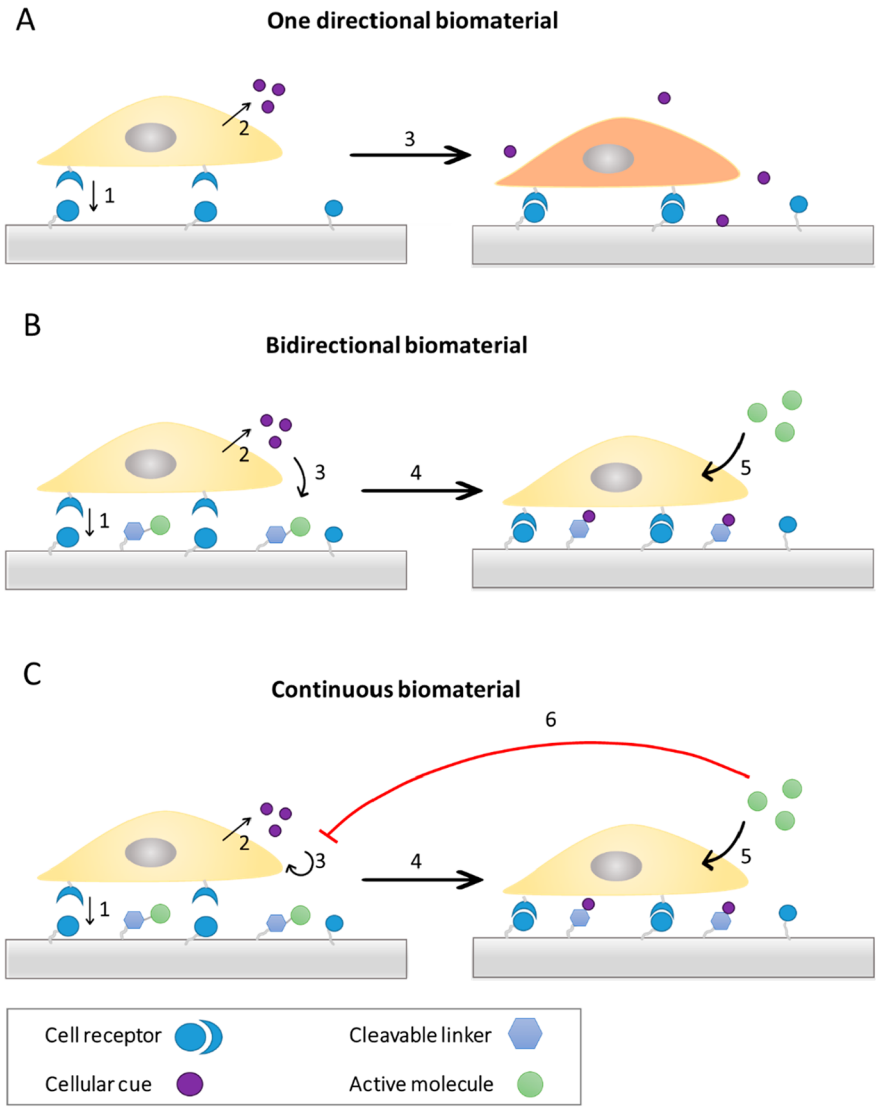

Figure 2. (A) One-way biomaterial response. The cell binds to the material (1) whereby cellular molecules are released (2). Released molecules have no effect on the biomaterial; however, the cell can, for example, differentiate upon binding to the material (3). (B) Bidirectional biomaterial response. The cell binds to the material (1) and the cell releases molecules (2) that will create a response to the biomaterial (3); it will release all its bioactive molecules (4). These molecules can be internalized in the cell or released into the environment (5). (C) Continuous biomaterial response. The cell binds to the material (1) and the cell releases molecules (2) that will create a response to the biomaterial (3); it will release some of its bioactive molecules (4). These molecules can be internalized in the cell (5). The bioactive molecules will in their turn inhibit the released molecules from the cell (6). This process can be repeated multiple times.

cell proliferation and growth of the tissue, and hence, the development of a cancerous microenvironment and tumor growth. Another process in which DR is very important is in the wound-healing process. Normally, the wound-healing process is coordinated with a series of molecular, cellular, and biomechanical events in order to restore the function of the damaged tissue.[27] During wound healing, DR is very important because it allows for the intense communication between cells and their microenvironment, the ECM. Through the interactions with the ECM, the cells differentiate, proliferate, migrate, or survive. The interaction between the ECM and the cells is equally important for the regulation of the mechanical properties of the tissue, such as changes in stiffness (e.g., stress-stiffening) and regulation of the (visco)-elasticity of tissues. Importantly, mechanical signals from the ECM have shown to regulate the (stem) cell characteristics and the commitment of cells to a certain lineage.[28] Besides that, DR allows for the cells to reorientate in the desired architecture needed for proper tissue repair. In some cases, for example, diabetes mellitus and immunosuppression, wounds can fail to recover and chronic wounds can appear.[18] During these wound repairing processes, DR ensures the active synthesis and deposition of ECM molecules, leading to new, possibly nonfunctional tissue. However, its inability to synthesize functional tissue can be caused by the disruption of normal interactions between the cells and their ECM, which can delay the healing of the tissue and can lead to the formation of chronic wounds. Research has shown that the mechanical environment at the wound site is important for the quality of wound healing, and chronic wounds often occur when there is mechanical stress in the tissue.[29] In order to create biomaterials that control, for example, wound-healing

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processes and tissue development, a biomaterial should be created that exploit DR like the natural ECM, allowing the application of these biomaterials for in situ tissue engineering.[30−32]

1.2. Dynamic Reciprocity in Model Systems. Although many researchers agree that it is important to mimic the DR of the natural system, little research has been performed on the exploration of DR in synthetic systems. There are research groups that focus on the understanding of DR of natural systems, in order to understand to process of disease development. For example, Bissell and co-workers describe the importance to engineer 3D models in order to understand cancer development and to predict the outcome of some treatments.[33] Other groups focus on the development of stimuli-responsive biomaterials, in order to mimic the DR between cells and the ECM. Stimuli-responsive materials are smart materials that can change their properties in response to changes in biological, physical, and chemical conditions such as temperature, pH, ionic strength, light, redox potential, and small (bio)molecules.[34−37] Nowadays, it is still challenging to control the cellular behavior on biomaterial scaffolds.[38−42] Cells cultured on a biomaterial can sense the mechanical and chemical properties of the scaffold and respond to these properties, by adapting for example their shape or morphology.[43] However, in many biomaterials currently being developed, this is a one-way response in which the cells adapt to the biomaterial, but the biomaterial does not adapt to the cellular response. A dynamic smart biomaterial should be able to adapt to cellular cues; for example the secretion of growth factors in response to enzyme activity in order to mimic the DR in the natural tissue (Figure 1A).[44−48] Smart biomaterials are therefore good candidates to modulate the complex and dynamic cell-biomaterial interactions. This is proposed to be done by incorporating functional groups that modulate the surface and/or bulk properties, thereby controlling the cellular behavior such as adhesion, growth, and migration and responding to cellular cues such as secretion of small (bio)molecules, enzymes, and proteins.[46] Besides that, the dynamic interactions within the biomaterial are also important, as the natural ECM continuously assembles and disassembles upon environmental cues (Figure 1B).

1.3. Cell−Biomaterial Interactions: One-Way, Bidirectional, and Continuous Responses. In general, different kinds of cell−biomaterial interactions can be distinguished. When the cell excretes bioactive molecules that will not provoke any changes to the biomaterial architecture (Figure 2A), then the biomaterial exhibits an one-way biomaterial response. These biomaterials are often used as a scaffold material to provide solely mechanical support. Nowadays, more advanced biomaterials are being developed that can provoke cellular reactions and stimulate a bidirectional biomaterial response (Figure 2B). Those biomaterials consist of bioactive molecules that are released upon a specific cue, which can be derived from the cell itself. The released cue can either be up taken by the cell or be released in the environment. Although these biomaterials are more dynamic, they often consists of one cycle in which all the bioactivity is consumed. An ideal biomaterial controls multiple cycles of bioactivity, in which the biomaterial releases active molecules when a cellular cue is applied. This active molecule in its turn will provoke a cellular reaction, which can for example inhibit the release of the cellular cue (Figure 2C). In this way, the DR of the natural systems is mimicked.

This Perspective aims to highlight recent studies on biomaterial development, from one-way biomaterial responses to continuous biomaterials. Although mechanics play an important role in dynamic reciprocity, this perspective will focus on biochemical processes rather than biomechanical processes. We focus on biomaterials that are regulated by enzymatic processes, as enzymes are very important in the DR of healthy tissue development, and also play a role in impaired tissue homeostasis. As mentioned above, ECM-degrading enzymes are highly active during tissue remodeling and the activity of the enzymes is highly regulated. This is important not only to remain tissue homeostasis, but also in processes such as angiogenesis, embryogenesis, and morphogenesis.

2. SMART BIOMATERIALS BASED ON ENZYME-RESPONSIVE SYSTEMS

Enzyme-responsive biomaterials to regulate enzyme activity in tissue development are useful for the regenerative medicine and tissue engineering field. Enzymes are highly selective macromolecular globular proteins that can catalyze a rate of biochemical reactions.[49,50,35] Enzymes can work under mild conditions, that is, low temperatures, pH 5−8, and an aqueous environment.[51−53] Due to their high specificity and activity, these are extensively studied in responsive hydrogel systems.[54−59] Enzyme-responsive biomaterials are developed based on three different mechanisms: (1) Enzyme-responsive surfaces,[60] in which a small molecule or polymer is cleaved from the surface upon cellular cues; (2) Hydrogel systems with enzyme-cleavable bonds or cross-links;[62−64] (3) Enzyme-responsive supramolecular assemblies,[61] in which a non-self-assembling precursor self-assembles after enzymatic cues.

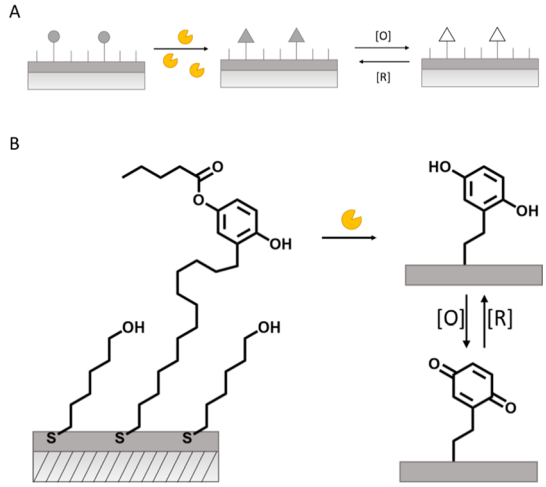

Figure 3. (A) An enzyme-responsive molecule is immobilized to a self-assembled monolayer. An enzyme (cutinase) converts the substrate into a redox-active molecule. The molecule can be oxidized ([O]), and this is reversible by reduction ([R]). (B) Self-assembled monolayer with 4-hydroxphenyl valerate as a substrate. When the enzyme cutinase is applied, the 4-hydroxphenyl valerate is hydrolyzed to a hydroquinone. The hydroquinone can be oxidized ([O]) to yield a benzoquinone and this process can be reversed by reduction ([R]). Reprinted with permission from ref 67. Copyright 2003 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

2.1. Enzyme Responsive Surfaces. *2.1.1. One-Way Surfaces Responses.* Enzyme-responsive surfaces have been of great interest because of the ability to locally release a

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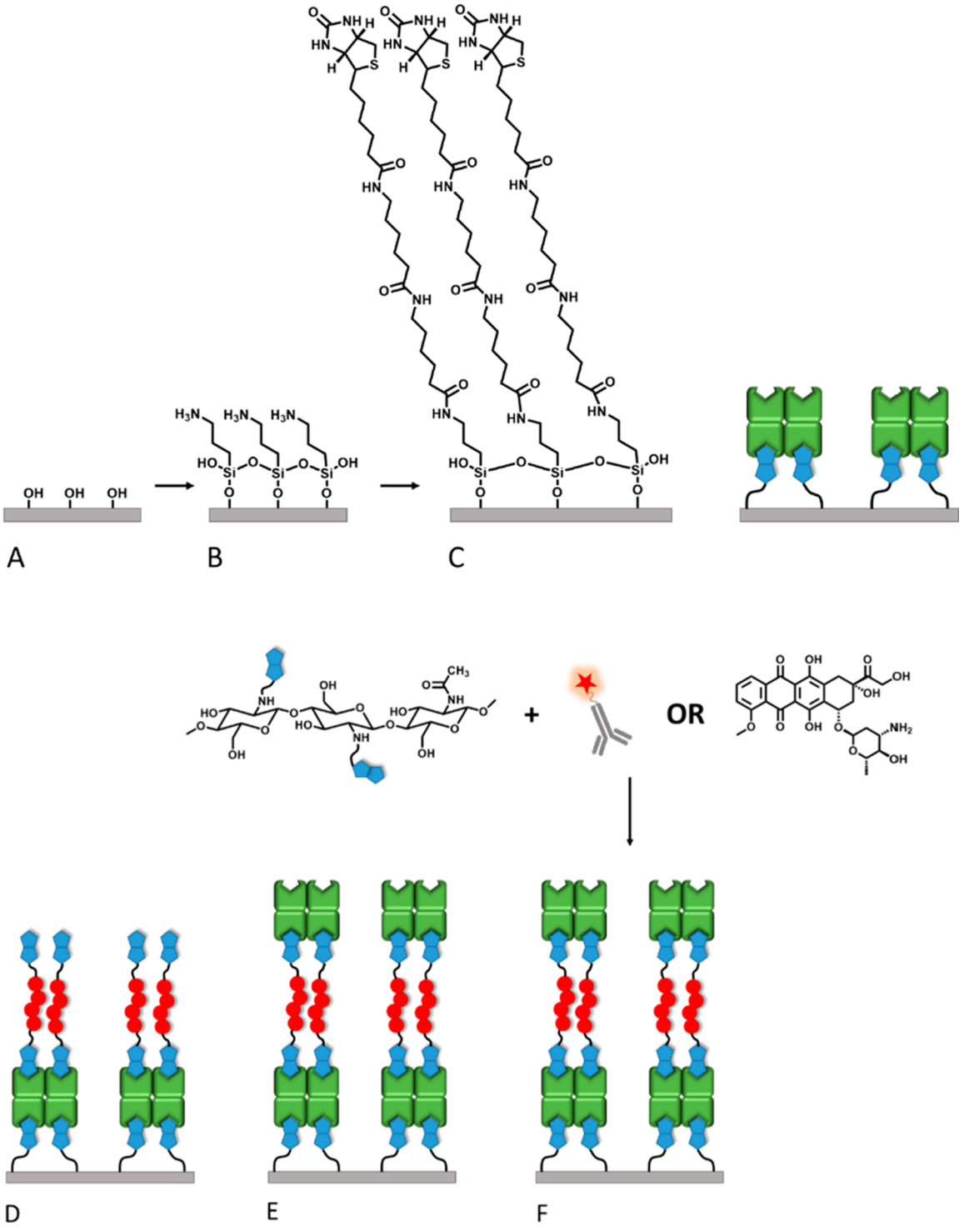

Figure 4. An enzyme-responsive multifunctional surface for the release of biomolecules was created as follows: (A) A glass substrate (B) was silanized with 3-aminopropyltriethoxysilane (APTES); (C) the biotinylated substrate is then modified with sulfo-NHS-LC-LC-biotin and further functionalized with streptavidin; (D) Streptavidin was modified with enzyme-cleavable biotinylated peptides (H-Lys(biotinyl)-Pro-Ile-Ser-Phe-Phe-Arg-Leu-Gly-Lys(byotinil)-OH); (E) and a layer of streptavidin was deposited. (F) The layers were modified with either biotinylated depolymerized chitosan and FITC-labeled antihuman CD4MAbs or biotinylated depolymerized chitosan and doxorubicin (DOX). When the enzyme Cathepsin D was applied, the enzyme-cleavable peptide was cleaved and either the FITC-labeled antihuman CD4MAbs or the doxorubicin are released. Reprinted with permission from Mortato, M.; Argentiere, S.; De Gregorio, G.L.; Gigli, G.; Blasi, L. Enzyme-Responsive Multifunctional Surfaces for Controlled Uptake/Release of (Bio)Molecules. *Colloid Surf. B* **2014**, *123*, pp 89−95. Copyright 2014 Elsevier.

biological molecule or diagnostic agent to a target tissue or to measure and control the activity of cells on a biomedical surface.[26,65,66] In addition, the surface can change its chemical or mechanical properties upon enzymatic cues, for example, changing from adhesive to nonadhesive surfaces or changing its redox properties.[35] Of the latter, Mrksich and co-workers designed a strategy in which self-assembled monolayers could convert enzymatic activities to electrical signals (Figure 3A).[67]

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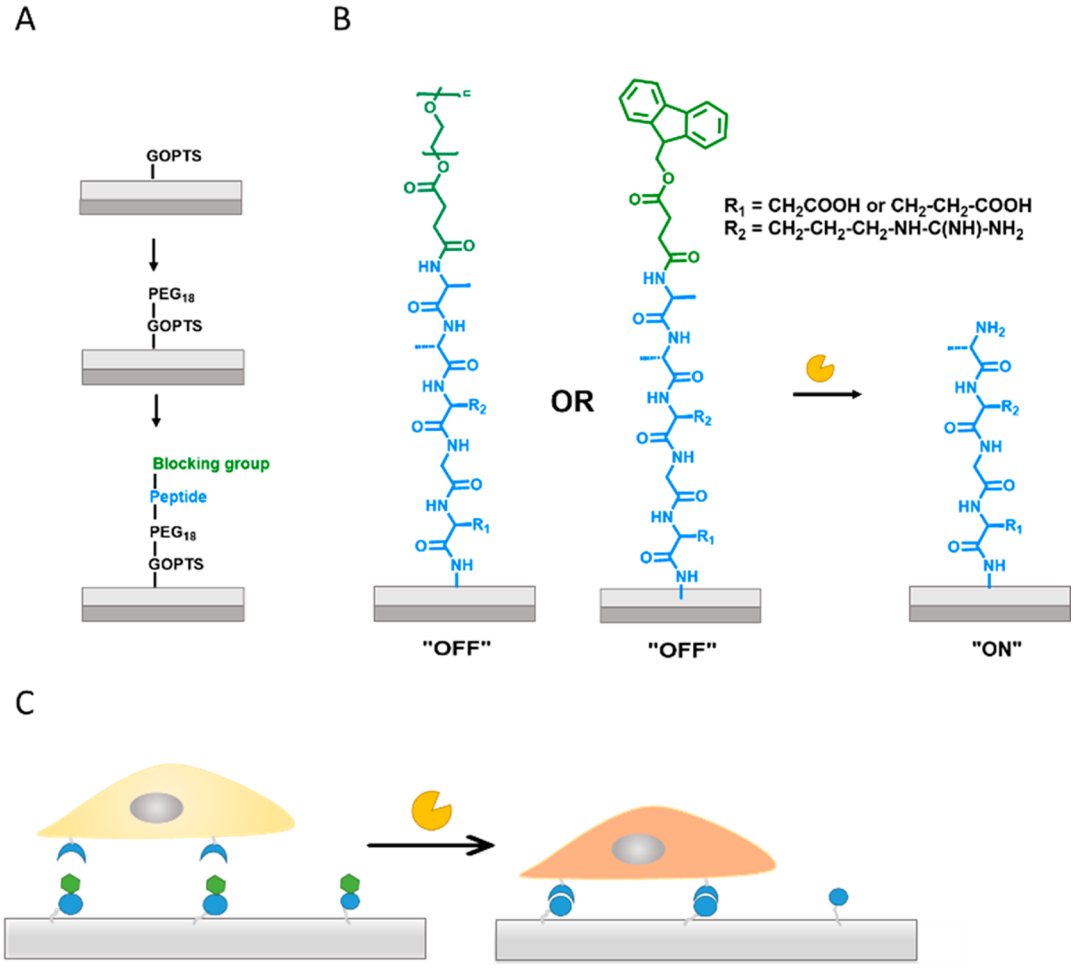

Figure 5. (A) An enzyme-cleavable surface is developed by salinization of glass substrates with (3-glycidyloxypropyl)trimethoxysilane and reacted with a diamine-functionalized polyethylene glycol (PEG). Then, SPPS was used to functionalize the surface with a peptide chain (D- or E-GRAA). One was modified with a PEG blocking group (PEG-AARGD-) and the other one with a FMOC-blocking group (FMOC-AARGD-). (B) The surface was "OFF", meaning the blocking group was present and the RGD sequence shielded for the cells. When the enzyme elastase was added, the peptide sequence was cleaved between the alanine−alanine, thereby removing the blocking group. (C) Schematic representation of the concept. When the blocking group (green hexagonal) was present, the cells could not adhere that well. When the enzyme elastase was added, the blocking group was removed and the cells adhered and differentiated. Reprinted with permission from ref 79. Copyright 2016 American Chemical Society.

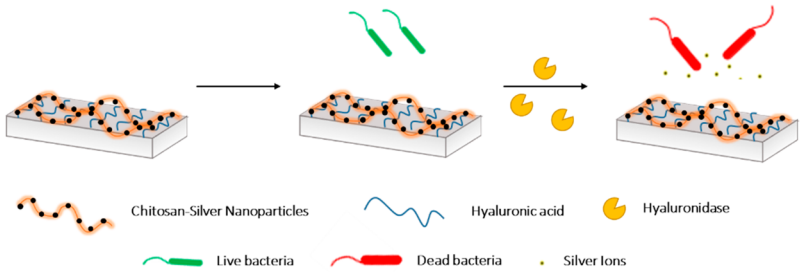

Figure 6. Schematic representation of an enzyme-responsive substrate. Via a layer-by-layer assembly method, a substrate was modified with chitosan-silver nanocomposites (orange) and hyaluronic acid (blue). When bacteria approach the surface (green), the enzyme hyaluronidase (HAase) (yellow) will be released from the bacteria, which degrades the hyaluronic acid and releases the silver ions from the surface (black). Those silver ions will kill the approaching bacteria (red). Reprinted with permission from Liu, P.; Hao, Y.; Ding, Y.; Yuan, Z.; Liu, Y.; Cai, K. Fabrication of Enzyme-Responsive Composite Coating for the Design of Antibacterial Surface. *J. Mater. Sci. Mater. Med.* **2018**, *29*, 160. Copyright 2018 Springer Nature.

Self-assembling monolayers (SAM) that consist out of an alkanethiolate terminated by either tri(ethylene glycol) or 4-hydroxyphenyl valerate moieties were incubated with the enzyme cutinase. The cutinase hydrolyzed the redox-inactive 4-hydroxyphenyl to the redox active hydroquinone (Figure 3B). When exposed to iron(III) chloride, the hydroquinone was efficiently converted to benzoquinone groups, which could produce an electrical signal. This work is a great example of both a one-way biomaterial response, namely, the hydrolyzation of the 4-hydroxyphenyl valerate to a hydroquinone

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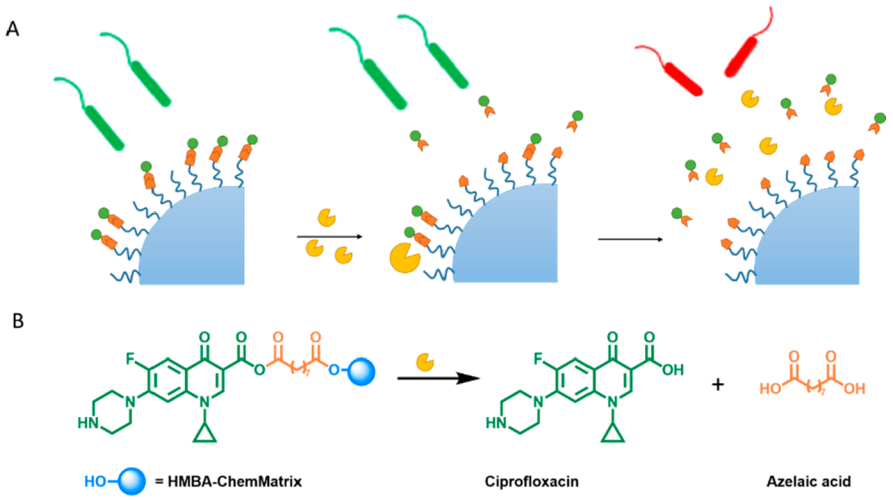

Figure 7. (A) Schematic representation of a bacteria-triggered enzyme-responsive release of antibiotics. PEG polymers are modified with an antibiotic attached to an enzyme-sensitive linker. When bacteria approach the surface, the bacteria secrete Lipase A (LipA) and Lipase C (LipC), which will cleave the enzyme-sensitive linker and release the antibiotic. (B) Chemical structure of the enzymatic cleavage of azelaic acid by LipA and LipC, resulting in the release of the antibiotic (ciprofloxacin). Reprinted with permission from ref 83. Copyright 2014 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

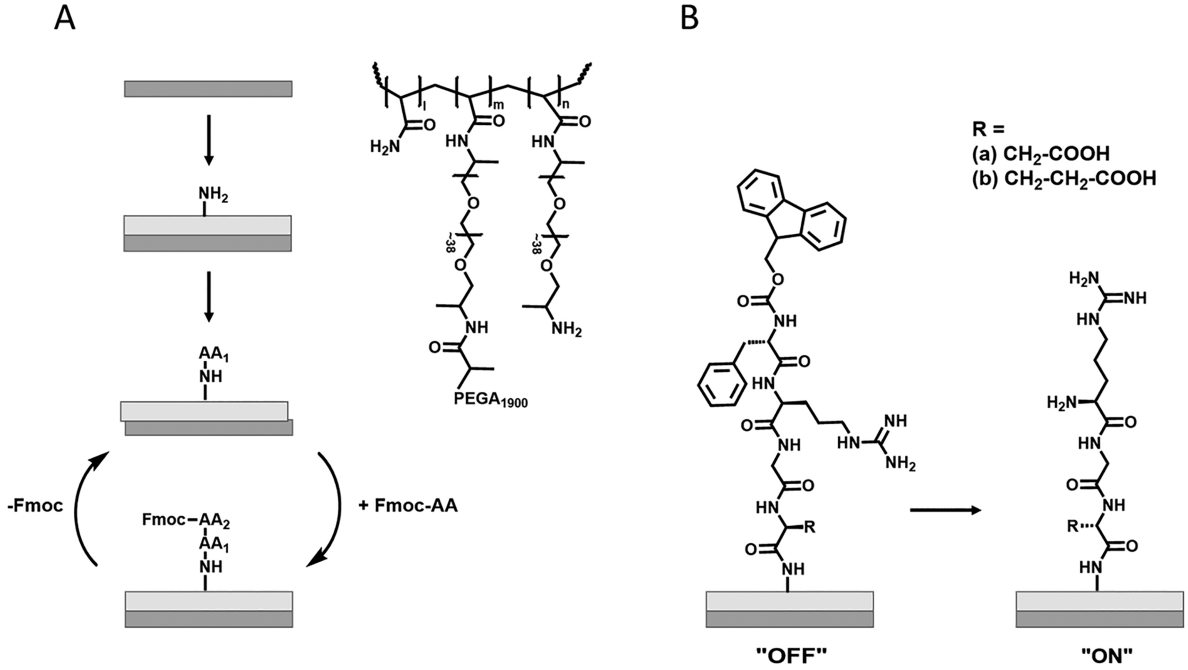

Figure 8. (A) A surface out of PEGA monomers with a photoinitiator was modified with RGD peptides. First, the surface was functionalized with PEGA monomers. Then, the RGD peptide was synthesized by standard Fmoc-based peptide synthesis. (B) Chemical structure of the "OFF" (non-cell-adhesive) and "ON" (cell-adhesive) state of the peptide-modified PEGA surface. Republished with permission from Todd, S. J.; Farrar, D.; Gough, J. E.; Ulijn, R. V. Enzyme-triggered cell attachment to hydrogel surfaces. *Soft Matter* **2007**, *3* (5), 547−550; permission conveyed through Copyright Clearance Center, Inc. Copyright 2007 Royal Society of Chemistry.

and a bidirectional response, namely, the oxidation and reduction of the hydroquinone to a benzoquinone and vice versa, and can be used as a system to study enzymatic activity or develop cell-based sensors. Cell-based sensing is an alternative to biosensing techniques, in which biomolecular markers are measured in bodily fluids and tissues.[68] Cell-based sensing has an advantage over biosensing as it has high sensitivity to a broad range of chemically active substances and it can identify very low concentrations of environmental cues.[69]

2.1.2. Bidirectional Surface Responses. Besides cell-based sensing, enzyme-responsive surfaces are also used as local drug delivery systems. These systems are advantageous over conventional drug administration methods, such as intravenous or oral administration, because they have less side effects, require little dosages and less administration of the drug to stimulate a therapeutic effect.[70] The delivery of drugs in a local and specific way has been explored by a great amount of research and has made a seminal impact to the drug delivery field.[71,72] Drug delivery from a scaffold has been used for the regeneration of cartilage tissue,[73,74] heart tissue,[75,76] and

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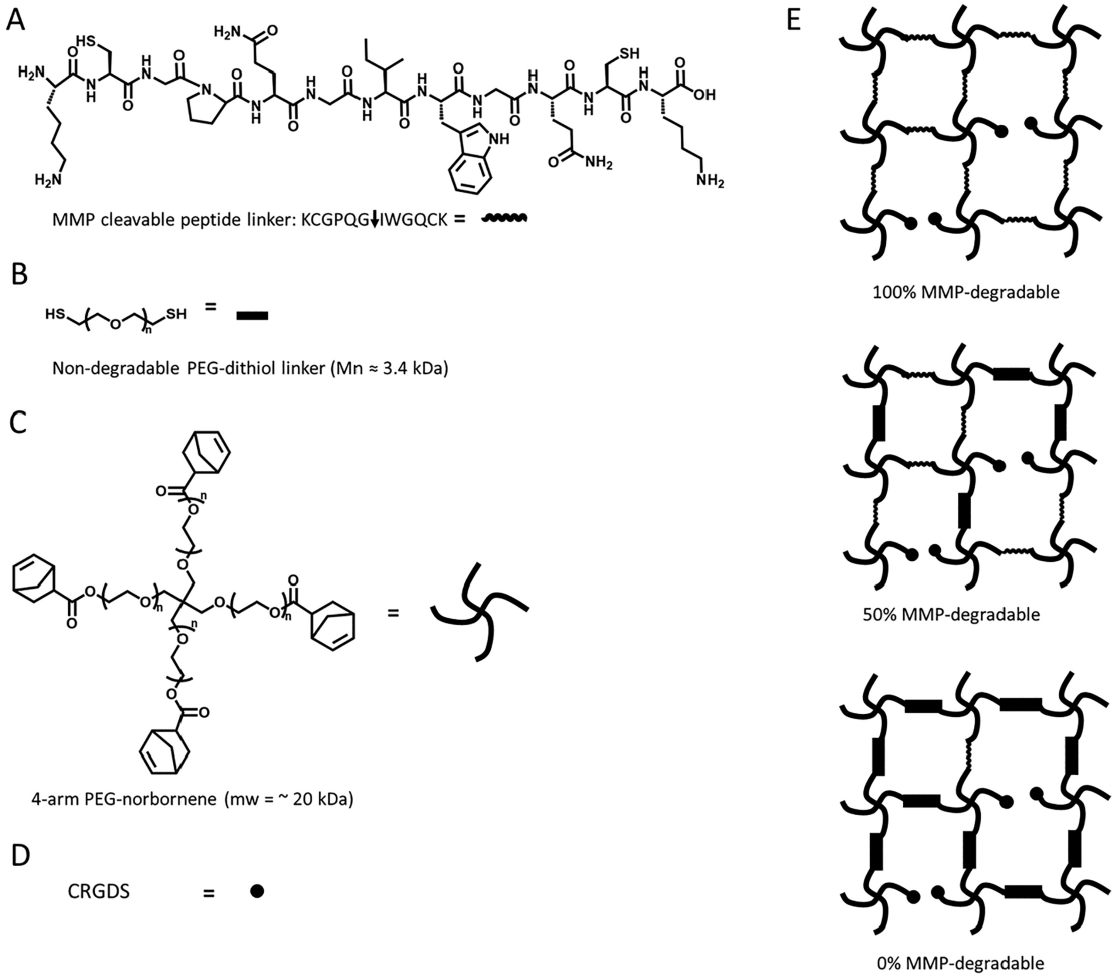

Figure 9. (A) Chemical structure of MMP-cleavable peptide linker. (B) Chemical structure of nondegradable PEG-dithiol linker. (C) Chemical structure of 4-armed PEG-norbornene cross-linker. (D) CRGDS were incorporated to enhance cell attachment. (E) Schematic presentation of hydrogels with different composition. Reprinted with permission from Anderson S.; Lin C.; Kuntzler D.; Anseth K. The Performance of Human Mesenchymal Stem Cells Encapsulated in Cell-Degradable Polymer−Peptide Hydrogels. *Biomaterials* **2011**, *32* (14), 3564−3574. Copyright 2011 Elsevier.

abdominal tissue.[77,78] In the work of Blasi et al., an enzyme-responsive surface was developed for the use of biotin−streptavidin binding for the local delivery of chemotherapeutic drugs (Figure 4).[34] An enzyme-cleavable biotinylated peptide was synthesized and reacted on a biotinylated glass substrate that was covered with streptavidin. This biotinylated enzyme-cleavable peptide was then reacted with streptavidin. At the end, the functionalized glass substrates were incubated with functional molecules such as FITC-labeled antihuman CD4MAbs and doxorubicin, a chemotherapeutic drug, to create a reactive surface. When exposed to Cathepsin D, the enzyme-cleavable peptide is cleaved and the attached biomolecules are released. In this way, a reactive surface is created which could release multiple biological cues, which could be applied as a local drug delivery system. However, this is a clear example of a bidirectional biomaterial response, in which a cellular cue (enzyme) causes a reaction to the biomaterial (release of biomolecules), which in its turn has a biologic effect. However, all the bioactivity is consumed once the biomaterial is exposed to the enzyme. In the ideal case, the biomolecules are released in a sustained fashion, so that the biomaterial stays bioactive for a longer period of time and has a longer therapeutic effect.

In a study of Roberts et al., a surface was developed that could change the adhesion of cells and initiate differentiation, by the immobilization of peptides on the surface (Figure 5).[79] These peptides are susceptible to the enzyme elastase, which cleaves the blocking group of the RGD-peptide, allowing cells to adhere to the surface. In this way, a switch is created. Moreover, this study showed mesenchymal stem cells (MSCs) can differentiate into osteoblast after adherence on the surface, depending on the adhesion points.

Other groups focus on the control of bioinert versus bioactive surfaces using enzyme-responsiveness.[80] For example, enzymatic cues can release functional molecules from the surface that control cell or bacterial growth. Bacterial colonization on surfaces can cause severe infections and illnesses, and hence, it is very important to minimize the bacterial growth on a surface.[81−83] Liu and co-workers created an enzyme-responsive substrate that releases silver (Ag) ions from the surface upon enzyme cleavage for an antibacterial effect (Figure 6).[84] First, they synthesized chitosan-silver nanocomposites and created an antibacterial coating using a

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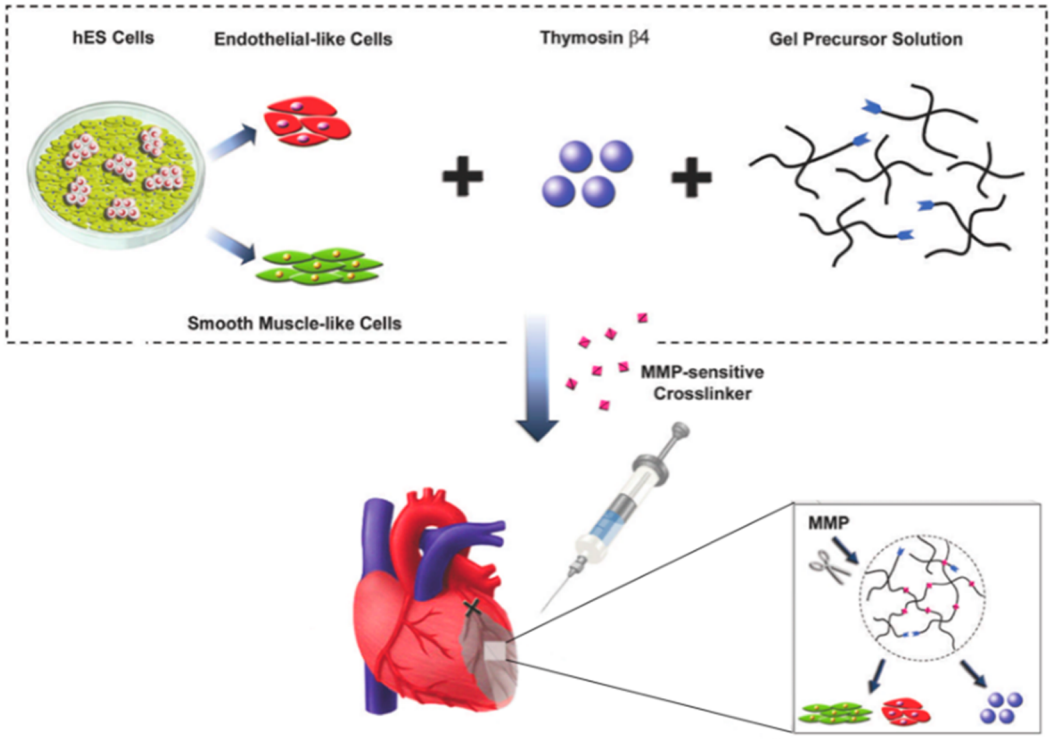

Figure 10. A gel precursor solution, consisting of vinyl sulfone-functionalized branched poly(ethylene glycol) matrix was cross-linked with a MMP-sensitive cross-linker, while thymosin $\beta 4$ and hES cells were encapsulated. As the gel gelates in a few minutes, the gel could be injected into infarcted tissue in liquid form, followed by in situ gel formation. Reprinted with permission from Kraehenbuehl, T. P.; Ferreira, L. S.; Hayward, A. M.; Nahrendorf, M.; van der Vlies, A. J.; Vasile, E.; Weissleder, R.; Langer, R.; Hubbell, J. A. Human Embryonic Stem Cell-Derived Microvascular Grafts for Cardiac Tissue Preservation after Myocardial Infarction. *Biomaterials* **2011**, *32* (4), 1102−1109. Copyright 2011 Elsevier.

layer-by-layer self-assembling method with hyaluronic acid (HA). Then, the Ag-ions were rapidly released from the substrate when exposed to the enzyme hyaluronidase (HAase), which showed an inhibitory effect on the growth *S. aureus* and *E. coli*. This study shows the antibacterial potency of enzyme-cleavable substrates, which, as stated before, is of great clinical importance.

Another example of an enzyme-mediated release of antimicrobial compounds for the control of bacterial populations is shown in the work of Komnatnyy et al. In this study, poly(ethylene glycol) (PEG) materials were modified with lipase-sensitive linkages.[85] They coupled ciprofloxacin, which is an antimicrobial agent, to the lipase-labile bonds and mixed this in the system. When exposed to the bacteria *P. aeruginosa*, which secretes the two lipases, Lipase A and Lipase C, the lipase-labile linker is cleaved and the ciprofloxacin is released. In this way, the bacteria triggers the release of antimicrobial agents, which kills the bacteria that approaches the surface (Figure 7). Those examples, that control bacterial growth on a biomaterial surface, are both bidirectional biomaterial responses. This means that, on the first contact with the bacteria, the bacteria will be killed. However, as all the antimicrobial activity is consumed there is no second line of defense, meaning over time the implant can be infected by bacteria.

The examples discussed so far exploit the use of enzymes to control surface properties and show that enzyme-responsive surfaces can be used to deliver functional molecules to the local environment. In this way, drugs can be delivered locally or bacterial growth can be controlled. However, most of these systems are bidirectional biomaterial responses, which means that, although the cellular cue gives a biomaterial response, all the bioactivity is consumed upon first contact with the enzyme. Therefore, research has shifted to biomaterials that show continuous responses. In these biomaterials, bioactivity is remained over multiple cycles of enzyme cleavage.

2.2. Enzyme-Responsive Hydrogels. The systems described above are 2D biomaterial networks; however, to make the biomaterials more physiologically relevant and predictive to the natural systems, 3D biomaterial networks are preferred. Hydrogels are water-swollen 3D networks of cross-linked polymer chains and are used in a variety of biomedical applications due to their similarity to the natural tissue.[86] In order to mimic the dynamic properties of the natural system, it is important for a hydrogel to respond to biochemical cues.[87] The physical and mechanical properties of a hydrogel system can be modified by the incorporation of specific groups within the hydrogel.[88−90] By the incorporation of reversible cross-link methods, the hydrogel can change its properties when an external trigger is applied, such as temperature, electric field, or small biomolecules.[41] This concept is often used to create injectable hydrogels that can be used to deliver drugs.[91] One example is the work of Lutolf et al., who developed MMP-sensitive hydrogels that form degradable networks for cell invasion. Changing the physical and mechanical properties of a hydrogel system using enzymatic cues could also be used to create injectable hydrogels that gelate on demand.[92] The dynamic character of the hydrogel and the possibility of modifying the physical, chemical, and biological properties of the hydrogel makes them great candidates for stimuli-responsive, continuous biomaterials. In order to show the variety of systems and the versatility

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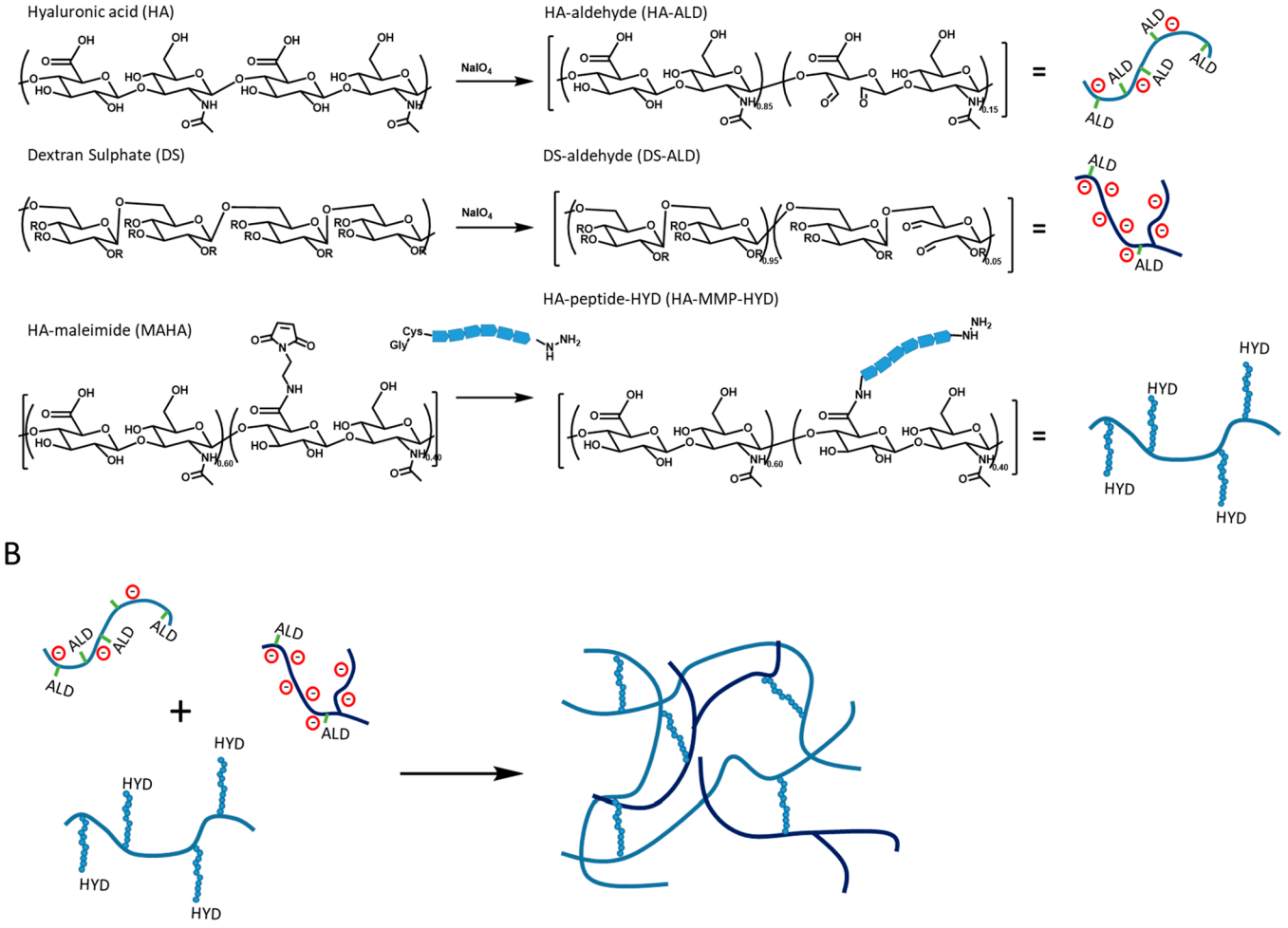

Figure 11. (A) Hyaluronic acid (*HA*) was modified with aldehyde groups (*ALD*) or hydrazide groups (*HYD*) with a MMP-cleavable sequence, and dextran sulfate (*DS*) polymers were modified with aldehyde groups (*ALD*). (B) A hydrogel was developed by the cross-linking of the *ALD* and *HYD* groups. Reprinted with permission from Purcell, B. P.; Lobb, D.; Charati, M. B.; Dorsey, S. M.; Wade, R. J.; Zellars, K. N.; Doviak, H.; Pettaway, S.; Logdon, C. B.; Shuman, J. A.; Freels, P. D.; Gorman, J. H.; Gorman, R. C.; Spinale, F. G.; Burdick, J. A. Injectable and Bioresponsive Hydrogels for On-Demand Matrix Metalloproteinase Inhibition. *Nature Materials* **2014**, *13*, 653−661. Copyright 2014 Springer Nature.

of enzyme-responsive hydrogels, the following section will describe a few examples of enzyme-responsive hydrogels, starting from simplistic bidirectional hydrogel systems, either used as surface coating or as a 3D hydrogel network, followed by more complicated injectable enzyme-responsive hydrogel systems that evoke a continuous response to the tissue.

2.2.1. Bidirectional Hydrogel Systems. In the study of Todd et al., a hydrogel system was developed that could control cell attachment on demand.[93] They tethered a well-known cell-adhesive bioactive peptide, arginine-glycine-aspartic acid (RGD) to a polyethylene glycol acrylamide (PEGA) surface to control cell attachment (Figure 8). The RGD peptide is capped by a Fmoc-F blocking group, whereby the F amino acid serves as a recognition motif for different enzymes, namely, chymotrypsin, thermolysin, and proteinase K. Although proteinase K showed the highest cleavage of the three enzymes, it was not selective, whereas the chymotrypsin was highly selective and used for further studies. Before cleavage of the Fmoc-F by the chymotrypsin, the surface was non-cell-adhesive for osteoblasts and in the "OFF" state. When chymotrypsin was added for a couple of hours, the Fmoc-F blocking group was cleaved off and the surface was in the "ON" state, allowing cells to attach to the surface. In this study it was shown that enzymes can be used to direct cell behavior on a hydrogel system. The downside of this study is that, once the hydrogel is in the "ON" state, it cannot go back into its "OFF" state. Therefore, the biomaterial shows one cycle of bidirectional response and not a continuous response, as desired in this system.

A MMP-degradable PEG-based hydrogel system was developed by Anderson and co-workers.[94] In their work, a thiol−ene photopolymerized PEG-peptide hydrogel was developed. The PEG was cross-linked with different combinations of MMP-degradable or nondegradable monomers and modified with cell-adhesive peptide ligands in order to create degradable and nondegradable cell-adhesive networks (Figure 9). Human mesenchymal stem cells (hMSCs) were encapsulated in these MMP-degradable hydrogels, and their survival, proliferation, and cell spreading were monitored. It was shown that the hydrogel was degradable by MMPs (MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, and MMP-9) and that the hMSCs survived in all hydrogels, despite the presence or absence of the MMP-degradable linkers. However, when there were fewer MMP-degradable linkers, the cell spreading was less compared to the completely degradable systems. In conclusion, the study showed that a hydrogel system could be formed that can be

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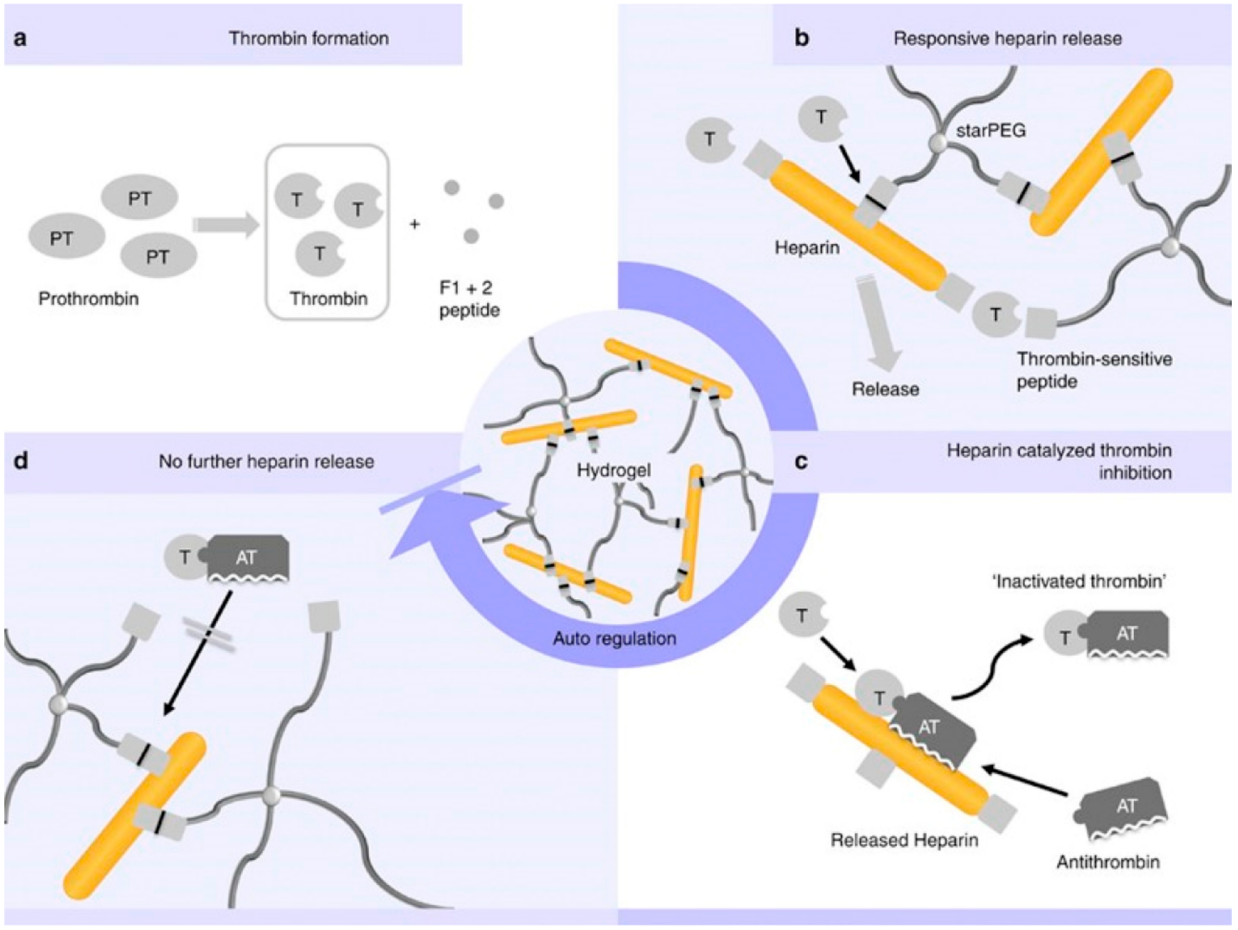

Figure 12. (A) Prothrombin is cleaved, which will generate thrombin. (B) Thrombin cleaves the peptide (NH_2-Gly-Gly-(D)Phe-Pip-Arg-Ser-Trp-Gly-Cys-Gly-$CONH_2$) of the linker unit between the arginine and the serine. Thereby, heparin is released. (C) The released heparin serves as a scaffold for the complexation of thrombin with its plasma-based inhibitor, resulting in the inactivation of thrombin. (D) The inactivation of thrombin results in the inhibition of heparin release. Reprinted with permission from Maitz, M. F.; Freudenberg, U.; Tsurkan, M. V.; Fischer, M.; Beyrich, T.; Werner, C. Bio-Responsive Polymer Hydrogels Homeostatically Regulate Blood Coagulation. *Nature Communications* **2013**, *4*, 2168. Copyright 2013 Springer Nature.

used for the expansion of hMSCs. The degradable linkers could be used as a drug-delivery system, providing cues to the hMSCs in situ. A disadvantage of the study is that the cleavable linkers are not selective, as they are cleaved by multiple MMPs of the body. Besides that, the current system is not a continuous biomaterial, where a bioactive cue is released in multiple cycles of activity, as the hydrogel is only tested for cell attachment and not for drug delivery purposes.

2.2.2. Continuous Hydrogel Systems. A more continuous system was designed in a study of Kraehenbuehl et al.[95] They used synthetic injectable hydrogels to deliver thymosin β4 to an infarcted heart region (Figure 10). The synthetic hydrogel consists of a vinyl sulfone-functionalized branched PEG with a matrix metalloproteinase (MMP)-cleavable peptide sequence. They showed that the hydrogels were formed within a few minutes in situ due to cross-linking of the vinyl sulfone of the PEG hydrogel. Moreover, the thymosin β4 was released over time by the degradation of the gel by MMP-2 and MMP-9, showing a decreased end systolic volume compared to the control group, which indicates better cardiac infarction healing. This was emphasized by the improved preservation of the cardiomyocytes and the collagen deposition in the heart when treated with the thymosin β4. In addition, the cardiomyocytes were better aligned and there were more microvessels present for the treated rats compared to the control. Overall, this hydrogel shows great potential for use as a stem-cell therapy to improve tissue healing in situ. Due to the sustained release of the thymosin β4, multiple cycles of bioactivity can be achieved.

The group of Burdick et al. developed an injectable hydrogel system that could regulate the MMP-activity at a MI site by adding a recombinant TIMP-3 in a hydrogel with MMP-cleavable cross-links.[26] The hydrogel was based on a polysaccharide backbone (hyaluronic acid (*HA*)) modified with either an aldehyde (*ALD*) or hydrazide (*HYD*) functional group. The MMP-degradable peptide (GGRMSMPV) was functionalized with a *HYD* group at the N-terminus to react to the *ALD* and modified with a thiol group at the C-terminus to react to a maleimide containing *HYD* group (Figure 11). The negatively charged polymer backbone was used to tether the recombinant TIMP-3 to the hydrogel. When the hydrogel was injected in the myocardium, the TIMP-3 was released from the hydrogel due to the elevated MMP activity in the myocardium. As a consequence, the MMP activity was inhibited by the TIMP-3. This research shows the regulation of enzyme activity by incorporating inhibiting molecules in the hydrogel and has great advantages in the regenerative medicine field and demonstrates a way to a continuous biomaterial response development.

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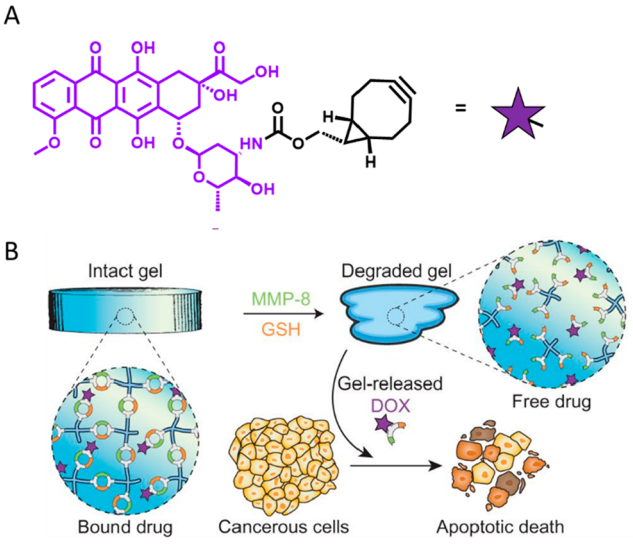

Figure 13. (A) Chemical structure of a doxorubicin functionalized with BCN for drug delivery purposes. (B) Schematic overview of a hydrogel with cross-linkers (redox- and enzyme-responsive). When both the enzyme is present and redox conditions apply (AND-gate), the gel is degraded, thereby releasing the doxorubicin. The doxorubicin will induce apoptosis in cervical cancer-derived HeLa cells. Reprinted with permission from Badeau, B. A.; Comerford, M. P.; Arakawa, C. K.; Shadish, J. A.; DeForest, C. A. Engineered Modular Biomaterial Logic Gates for Environmentally Triggered Therapeutic Delivery. *Nature Chemistry* **2018**, *10* (3), 251−258. Copyright 2018 Springer Nature.

2.3. Smart Hydrogels (Based on Enzymes). Smart hydrogels function as many different functional systems, such as actuators,[96] sensors,[97] drug delivery systems,[98] smart surfaces,[52] self-healing hydrogels,[99] and controlled biodegradation in materials.[100] Although these systems are very dynamic, these lack a continuous interaction between the hydrogel system and the cell environment, and these systems are mostly bidirectional. In the field of tissue engineering, biomaterials are required that promote the novo formation of functional tissues and, thus, mimic the continuality of the natural system.[101] As discussed in the Introduction of this Perspective, cells explore DR to communicate with the ECM in order to keep tissue homeostasis.[102] In order to mimic the dynamic reciprocity of the natural system, new biomaterials are required that not only can respond to cell stimuli, but also adapt and communicate to the cells. One beautiful example of such a system is the work of Werner and co-workers who developed a blood coagulation-responsive hydrogel.[103,104] They cross-linked a starPEG-heparin material with a thrombin-cleavable peptide (NH_2-Gly-Gly-(D)Phe-Pip-Arg-Ser-Trp-Gly-Cys-Gly-$CONH_2$). When thrombin was presented to the hydrogel, the peptide was cleaved between the arginine and serine amino acid and releases heparin (Figure 12b). The released heparin inactivates the thrombin by complexation of thrombin with its inhibitor (antithrombin; Figure 12c). In this way, degradation of the system was inhibited and a negative feed-back loop is created (Figure 12d). As a proof of concept, they incubated the hydrogel with whole blood and compared the results with clinically applied polytetrafluoroethylene (PTFE) vascular grafts coated with and without heparin. When whole blood was incubated on the PTFE grafts, solid blood clots formed on the gels both without the heparin coating and with the heparin coated, whereas the blood stayed a liquid on the responsive hydrogels, indicating that blood-coagulation can be regulated with dynamic synthetic hydrogels. This work is a great example for a continuous biomaterial, in which bioactivity remains in multiple cycles of biomaterial response.

Responsive hydrogels have also been engineered by using logic gates.[59] A recent study showed that Boolean logic operations, such as YES, OR, and AND could be used to create a hydrogel that is responsive to multiple stimuli.[105] In this work, logic-based responsive cross-linkers were synthesized that were either enzymatically degradable, respond to reducing conditions, or that were responsive to light. To create the enzyme-degradable cross-linker, a small peptide was synthesized (GPQGIWGQ) that is cleaved between the glycine and isoleucine residue in the presence of MMPs. A disulfide bond was used to create a bond that is responsive to reducing conditions. Last, *ortho*-nitrobenzyl ester (oNB) was synthe-

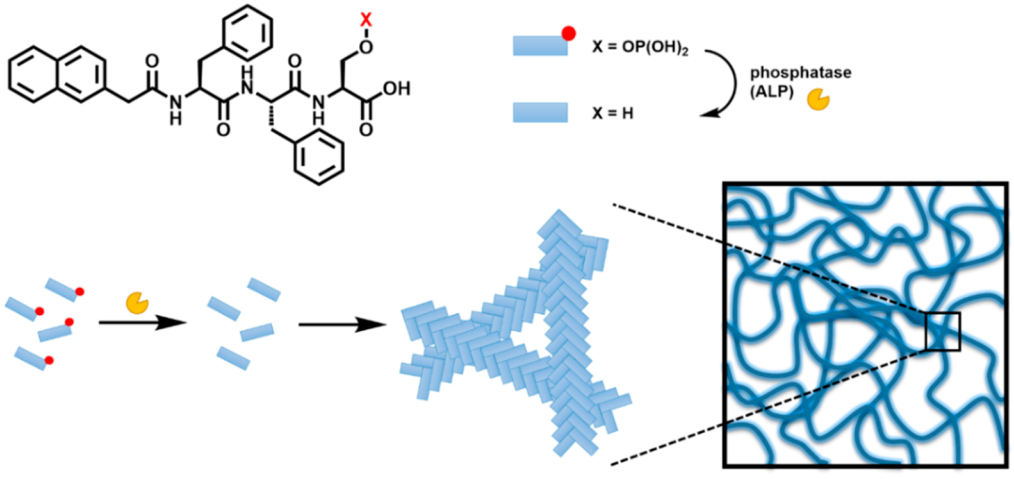

Figure 14. Schematic illustration of the enzyme-instructed self-assembly (EISA) using small molecules that are modified with a phosphate. When the phosphate is cleaved by phosphatase, the molecules can self-assemble into nanofibers that results in supramolecular hydrogels. Reprinted with permission from Zhou, J.; Du, X.; Wang, J.; Yamagata, N.; Xu, B. Enzyme-Instructed Self-Assembly of Peptides Containing Phosphoserine to Form Supramolecular Hydrogels as Potential Soft Biomaterials. *Front. Chem. Sci. Eng.* **2017**, *11*, 509−515. Copyright 2017 Springer Nature.

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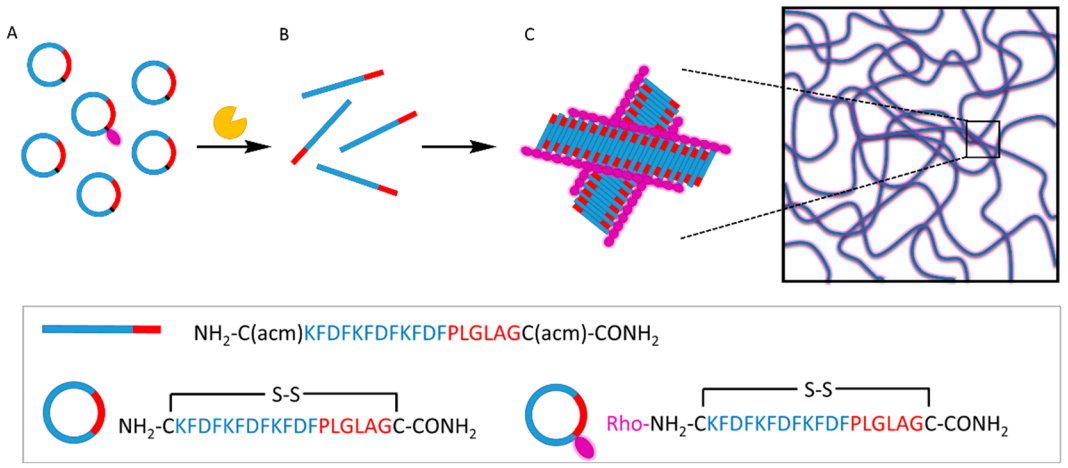

Figure 15. Schematic representation of enzyme-responsive self-assembling peptides. (A) A progelator peptide consisting of a MMP-recognition site (red) and a gelling sequence (blue). (B) The enzyme cleaves the progelator peptide, resulting in the linearization of the peptides into self-assembling peptides (SAPs). (C) At physiologic conditions, the SAPs hydrogelate into a hydrogel. Reprinted with permission from ref 116, licensed under the Creative Commons Attribution 4.0 International License. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

sized, which undergoes photoscission upon cytocompatible near-ultraviolet light exposure. With those three components, a library was created with 17 different cross-linkers, modified with reactive azide moieties to enable the cross-linkers to react with a four-arm poly(ethylene glycol) tetrabicyclononyne (PEG-tetraBCN) by means of a strain-promoted azide−alkyne cycloaddition (SPAAC) reaction. They used different combinations of enzyme, reducing components, and light to degrade the hydrogel with the different cross-linkers and characterized the reaction products with MALDI-TOF analysis. It was shown that indeed the YES gate was working, as it degraded when the programmed cue was presented to the hydrogel. The OR-gated hydrogels only degraded when one of the cues was present, where the AND gate only degraded when both of the cues were present, indicating that all the gates worked. In their work, multiple gates were coupled to each other to get more complicated (three-input) hydrogel systems, in which six out of eight of the systems behaved as expected. The versatility of the system was tested by adding a doxorubicin (DOX) to the AND gate system with a target application of the specific and local death of cancer cells (Figure 13). It was shown that when the cancer cells were incubated in the DOX hydrogel and the right cues were present, the DNA content decreased, indicating cell death. This work shows a great example of a next level hydrogel system, in which multiple cues are needed to provoke a reaction and can be used to mimic the dynamic reciprocity present in the natural system.

2.4. Enzyme-Responsive Supramolecular Assemblies. Introduction of DR inside a material (Figure 1B) is proposed to be achieved using supramolecular assemblies. A supramolecular aggregate is an assembly of molecules held together by directed, specific, noncovalent bonds, such as hydrogen bonding, van der Waals forces, hydrophobic, $\pi-\pi$, and electrostatic interactions.[106−108] The dimensions of assembly range from two molecules on the nanometer scale to large complexes of the micrometer scale.[109,110] Recent studies focus on exploration of enzymes in supramolecular assemblies, in which the enzyme can disrupt the supramolecular interactions or trigger the supramolecular assembly.[111−115] These methods are of interest because of the ability to make a hydrogel on demand, for example to trigger in situ gelation. One example in which a hydrogel can be formed in situ by supramolecular assembly is by Zhou et al. They developed an enzyme-instructed self-assembly (EISA) method using phosphoserine-containing small peptides to form hydrogels (Figure 14).[113] In their study a self-assembling unit, Nap-Phe-Phe sequence, was modified with different phosphorylated serine precursors. The Nap-Phe-Phe sequence self-assembles upon aromatic−aromatic interactions. The study showed that when alkaline phosphatase (ALP) is added, the phosphoserine is dephosphorylated, which resulted in the self-assembling of the small units into nanofibers, which formed a hydrogel. They also explored the effect of using D-amino acids instead of L-amino acids and showed that when D-amino acids are used, the hydrogels are stable against proteolysis. These hydrogels can be used in different applications, such as drug delivery systems and in situ hydrogel formation for cell therapy, showing the versatility of their work.

The group of Gianneschi et al. developed a different method in which a hydrogel can be formed in situ.[116] Sterically constrained pro-gelator peptides were developed that flow freely until cleaved by disease-associated enzymes. The cyclic peptides form self-assembling peptides which self-assemble into viscoelastic hydrogels (Figure 15). These hydrogels were than tested on their ability to hydrogelate when injected in the myocardium after myocardial infarction (MI). The SAPs were therefore modified with recognition sites for MI-associated proteases. It was shown that hydrogel formation occurred and that the viscoelastic hydrogels were stable against excess proteolysis. In this way, tissue can be repaired locally and also drugs can be loaded in the hydrogel to be released locally. When designed in such a way that the drugs are released in a sustained fashion, a continuous biomaterial can be designed.

One example of such a drug-release hydrogel is by Li et al., who focused on the development of drug-release hydrogels to prevent HIV transmission.[117] In this study hydrogelators were used, that self-assemble in water through supramolecular interactions (Figure 16). Three components were coupled to the hydrogelators; an anti-HIV and anti-inflammatory drug and a phosphate group, whereby the phosphate group allowed the

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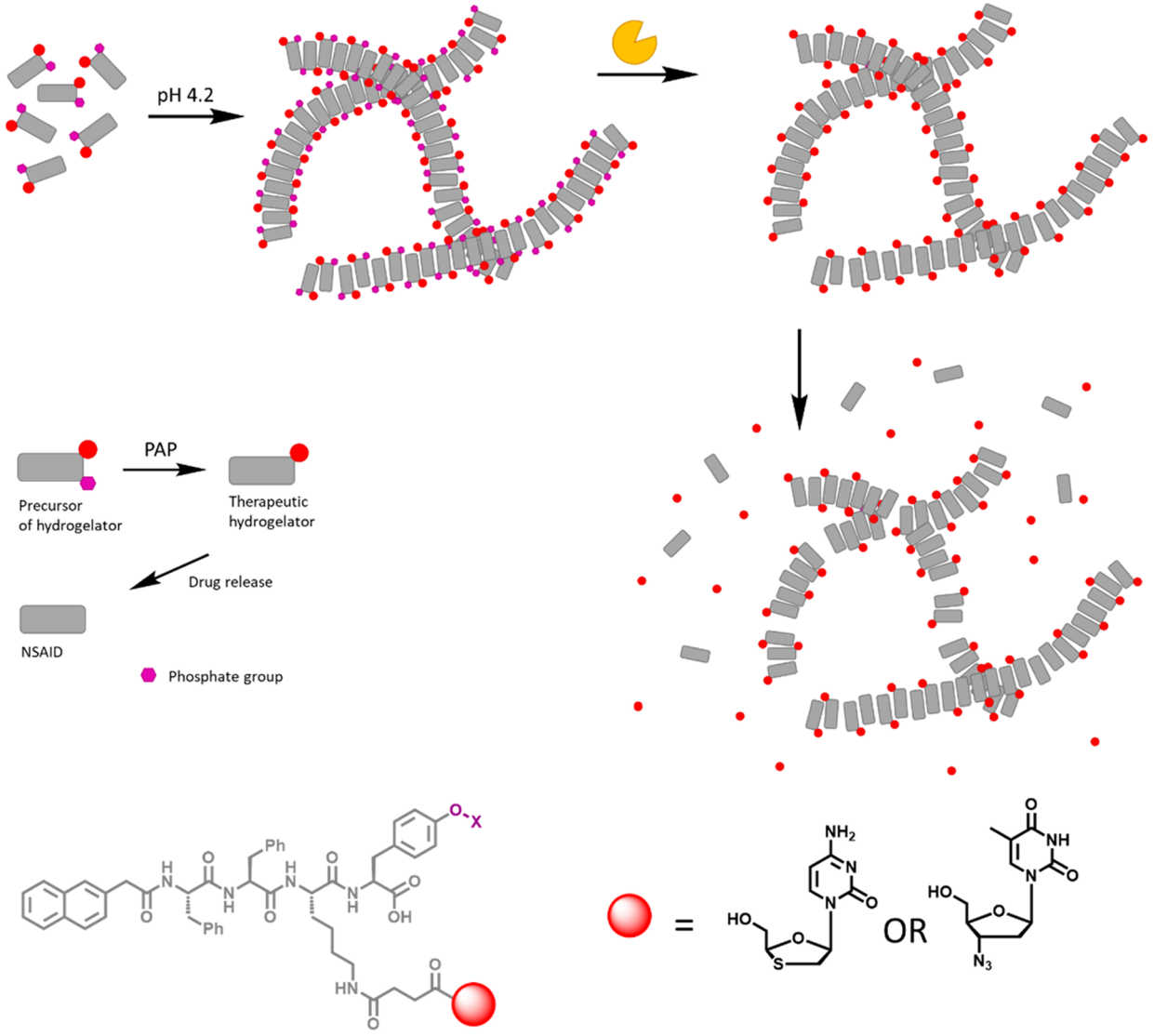

Figure 16. Schematic representation of the enzyme-responsive hydrogel system. Prostatic acid phosphatase (PAP) will cleave the phosphate group of a small precursor hydrogelator, which allows the precursor to self-assemble into a hydrogel. Therapeutic drugs can be attached to the hydrogelator to create a drug-releasing hydrogel. Reprinted with permission from ref 117. Copyright 2003 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

hydrogelation to occur at physiological pH. In acidic conditions, the hydrogelator self-assembles into supramolecular nanofibers. Upon the presence of the enzyme prostatic acid phosphatase (PAP), the cleavage of the phosphate group is catalyzed which resulted in a stronger network of nanofibers with enhanced elasticity. At physiological pH (pH 7.4) the ester bond in the hydrogelator is cleaved and the anti-inflammatory drug and anti-HIV drug are released from the hydrogel in a sustained matter. This study shows an approach for the development of enzyme-regulated drug release hydrogels preventing HIV transmission. As the drug is released in a sustained matter, the hydrogels come close to continuous biomaterials which mimic the DR in the natural ECM.

Another example of the delivery of drugs to a target site by the use of supramolecular assemblies is by Kalafatovic and co-workers.[118] In this research, peptide micelles were developed that formed fibrillar nanostructures upon hydrolysis by MMP-9 (Figure 17). The peptide micelles consisted of short β-sheet forming peptides that are susceptible to MMP-9 cleavage. They encapsulated a hydrophobic drug molecule (doxorubicin) in the hydrophobic core of the peptide micelles and showed that the doxorubicin could be slowly released from the fibrillar nanostructures both in vitro and in vivo upon cleavage by MMP-9. In this way, a micelle was formed that could be used as nanocarrier, which could locally release drugs at the target site for the inhibition of tumor growth, which is of great clinical importance.

In the work of Pieszka et al., control over supramolecular assembly and disassembly, rather than the formation of in situ hydrogels, was the focus of study.[119] A boronic acid-carbamate bond containing depsipeptide sequence was designed which could assemble into β-sheets upon hydrolysis and disassemble again by oxidation of the peptide (Figure 18). The peptide sequence consisted of the KIKISQINM amino acid sequence in which the serine allowed for the formation of an ester-bond oligopeptide, which could undergo O,N-acyl rearrangement (Figure 18A). This, in combination with the hydrolysis of the carbamate bond by enzymes, allowed for the self-assembly of

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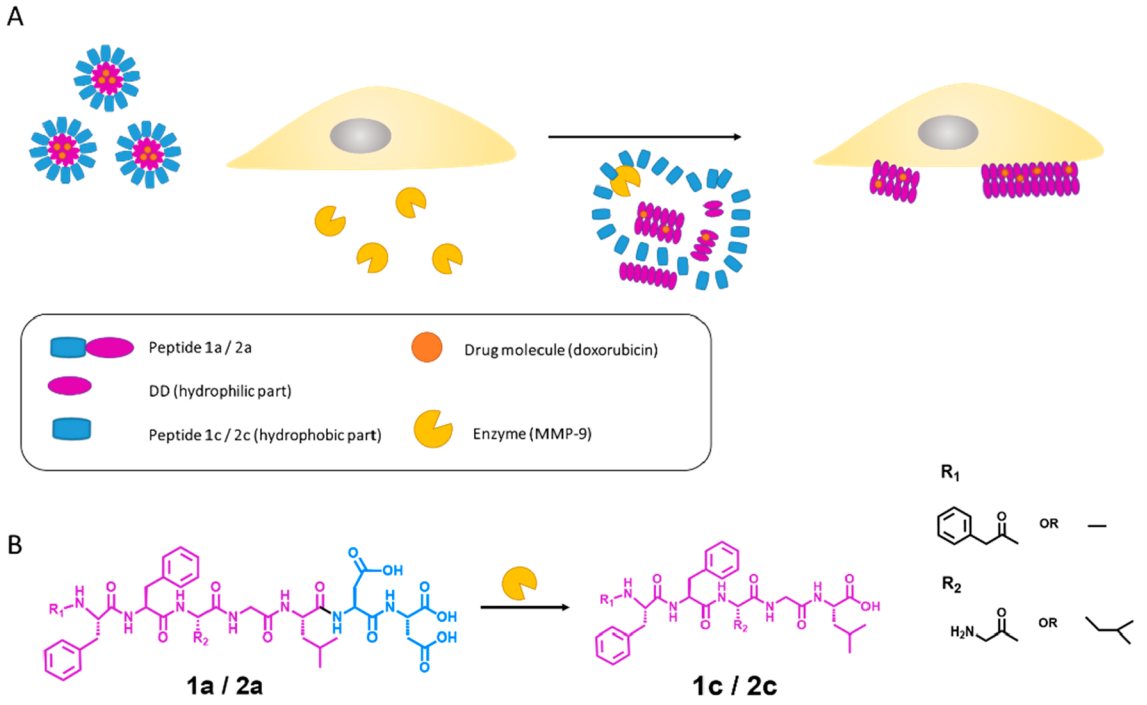

Figure 17. (A) Schematic representation of the transformation of micelles to supramolecular fibers in the presence of cancer cells. When the micelles approaches the cell, where there is an elevated level of MMP-9, the micelles are hydrolyzed and the doxorubicin will be trapped in the fibrillar structures. These fibrillar structures will release doxorubicin in a sustained matter to the cancer cell, which will cause the cell to die. (B) Chemical structures of the MMP-9 responsive peptide amphiphile. Reprinted with permission from Kalafatovic, D.; Nobis, M.; Son, J.; Anderson, K. I.; Ulijn, R. V. MMP-9 Triggered Self-assembly of Doxorubicin Nanofiber Depots Halts Tumor Growth. *Biomaterials* **2016**, *98*, 192−202,. Copyright 2016 Elsevier.

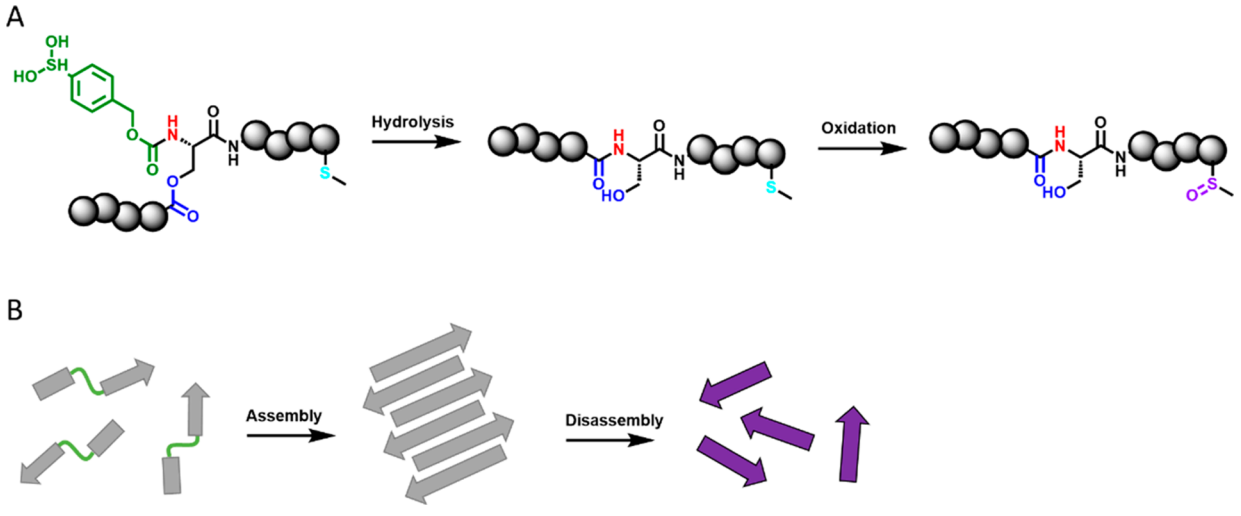

Figure 18. (A) Chemical structure of the depsi-oligopeptide (KIKISQINM), which was modified with a boronic acid carbamate bond. Hydrolysis of the carbamate bond and O,N-acyl rearrangement of the serine resulted in a linear peptide. The methionine of the linear peptide could be oxidized to form methionine sulfoxide. (B) Schematic representation of the assembly and disassembly of the depsi-oligopeptides. Sterically constrained depsi-oligopeptides could self-assemble into β-sheets and fibrils after hydrolysis. Then, the fibers could disassemble again after oxidation of the methionine. Reprinted with permission from ref 119. Copyright 2019 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

the peptide into β-sheets (Figure 18B). The self-assembly was directed by the hydrogen bonds between the amide groups in the backbone of the peptide and the hydrophobic interactions of the amino-acid side chains. The methionine in the amino-acid sequence could undergo oxidation by H_2O_2, which caused electrostatic repulsion between the residues and therefore resulted in the disassembly of the peptide into single strands. This concept of the external control on assembly and disassembly of oligopeptides could have a great impact in the biomedical field, for example, as hydrogel systems or drug delivery vehicles.

Another study in which control over assembly and disassembly was obtained was in a study of Webber et al.[120] Protein kinase A (PKA) was used as an enzymatic trigger to control the assembly of peptide amphiphile (PA) nanostructures (Figure 19). PKA is an important enzyme in the cell,

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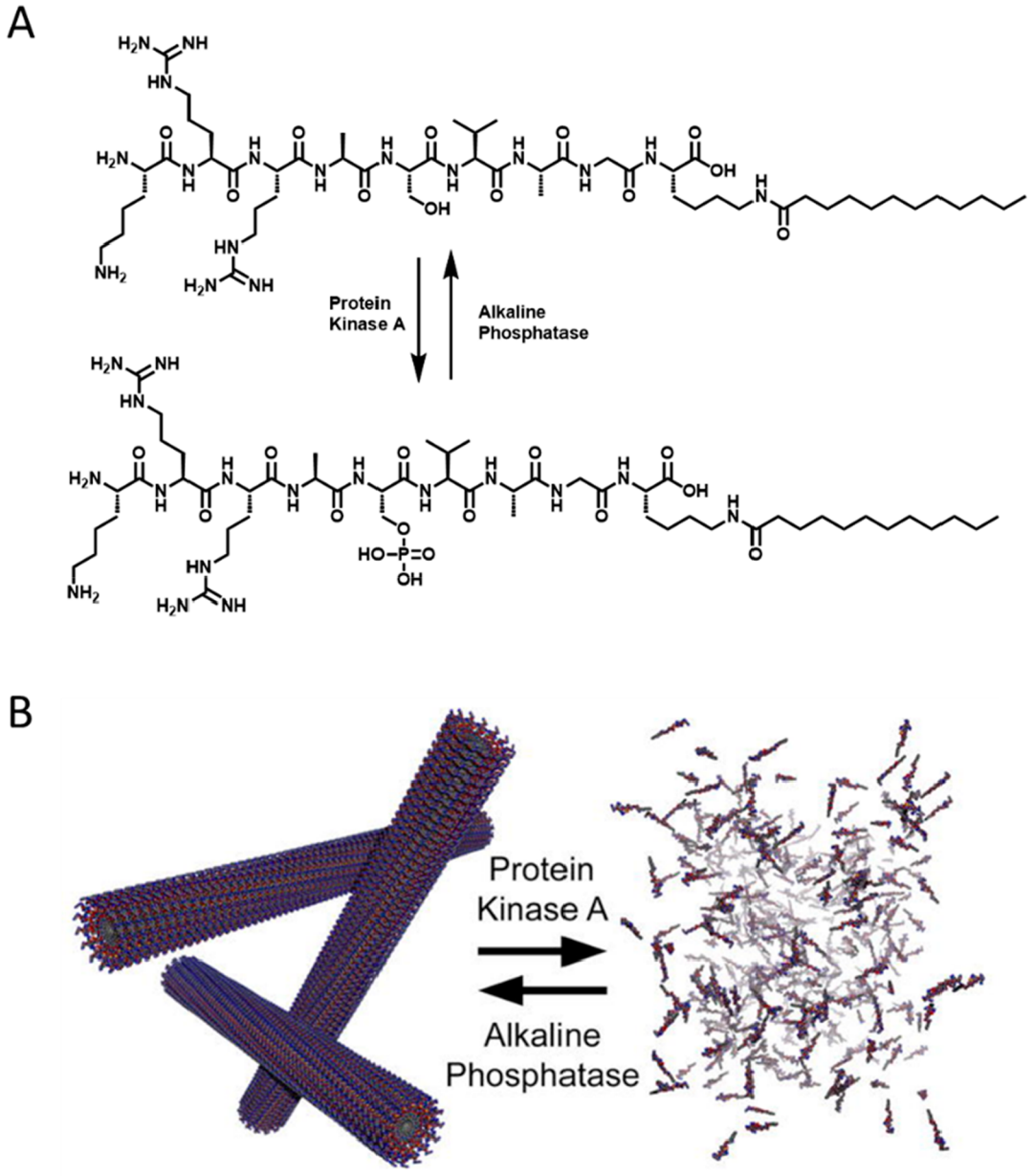

Figure 19. (A) Chemical structure of the peptide amphiphile (PA). The serine amino-acid of the PA could be phosphorylated by protein kinase A and this process could be reversed by the dephosphorylation by alkaline phosphatase. (B) Schematic representation of the assembly and disassembly of the PA upon treatment with protein kinase A or treatment with alkaline phosphatase. Republished with permission from Webber, M. J.; Newcomb, C. J.; Bitton, R.; Stupp, S. I. Switching of Self-Assembly in a Peptide Nanostructure with a Specific Enzyme. *Soft Matter* **2011** 7 (20), 9665−9672; permission conveyed through Copyright Clearance Center, Inc. Copyright 2011 Royal Society of Chemistry.

which has been involved in numerous signaling processes.[121] Besides that, it is also expressed by tumor cells and is, therefore, a good cancer biomarker.[122] PAs are peptide sequences that can self-assemble into bioactive nanostructures. The PA consists out of a hydrophobic tail, a β-sheet forming peptide sequence and a bioactive epitope (Figure 19A). The hydrophobic collapse of the hydrophobic tail and the hydrogen bond formation within the β-sheet forming peptide sequence causes the bioactive epitope to become present at the surface of the of the filamentous nanostructures. This allows for modification of the bioactive epitope with, for example, enzymes. Upon treatment of the PA with PKA, phosphorylation of the PA at the serine residue caused the peptide to disassemble (Figure 19B). This process could be reversed by adding phosphatase that cleaved the phosphate group, which resulted in the formation of filamentous nanostructures again (Figure 19B). Moreover, in this study a hydrophobic drug (DOX) was loaded into the core of the PA. It was shown that the drug, loaded in the PA, could be released upon phosphorylation by PKA, which was excreted by the cancer cells. The released DOX induced cytotoxicity to the cells. This study is a great example in which a drug could be specifically released at the site of interest. The diversity of the peptide sequences that can be used to design a PA allows for the development of PA-based enzyme-responsive networks for a wide range of biological applications.

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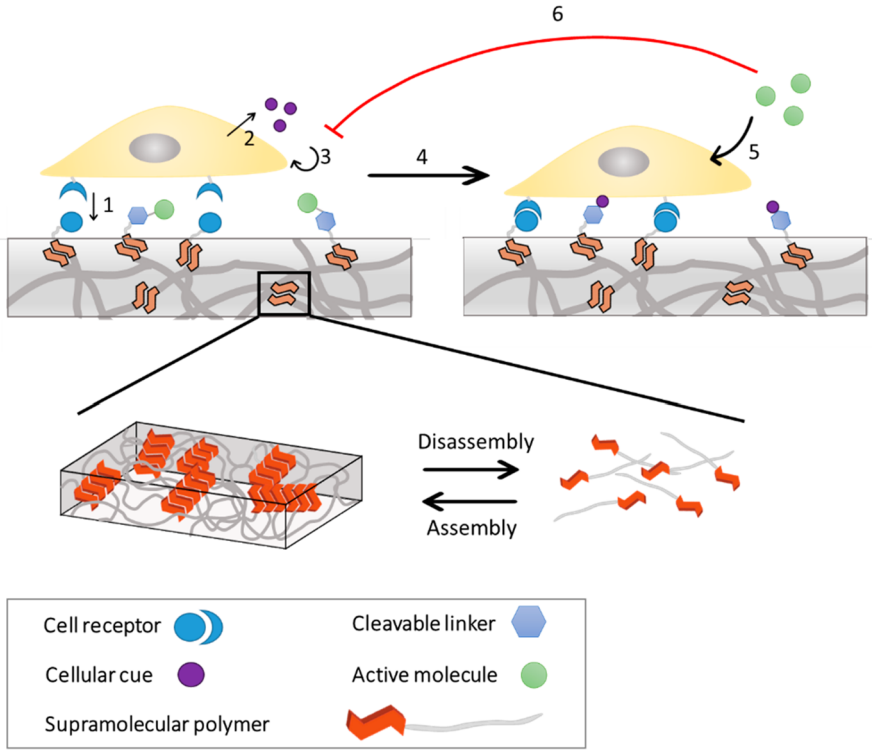

Figure 20. A continuous supramolecular biomaterial response with both surface reactivity and intrinsic dynamics. The cell binds to the material (1) and the cell releases molecules (2) that will create a response to the biomaterial (3); it will release some of its bioactive molecules (4). These molecules can be internalized in the cell (5). The bioactive molecules will in their turn inhibit the released molecules from the cell (6). This process can be repeated multiple times. At the same time, the supramolecular material is constant remodeling by assembly and disassembly of the supramolecular fibers.

3. EPILOGUE

In the past century, the biomaterials field has enormously evolved. In the beginning of the 20th century, biomaterials were developed that provide only static support to the extracellular environment. At the end of the 20th century, research moved from solely static materials to more dynamic materials by adding small peptide sequences to the materials, incorporating bioactivity.[123] These bioactivated biomaterials showed improved interaction with the tissue environment and could enhance biomaterial performance.[124] The last years, more complex biomaterials are being developed as highlighted in this perspective. Complex biomaterials have arisen that have control over cell adhesion, antimicrobial activity and biomaterial degradation. However, there are still some challenges that must be addressed. Despite the complexity of the enzyme-responsive systems, the systems are mostly bidirectional in their response.[2] In the ideal case, the biomaterial should be more complex and better mimic the ECM, by having a true interaction with the tissue environment. When we can develop biomaterials that act autonomous, we can mimic the biological system better. This could be accomplished by incorporating cues in the biomaterial that are responsive to multiple biological stimuli. The biomaterial can give a different response when each of the stimuli is present. In order to create the ideal autonomous biomaterial that mimic the DR of the natural ECM, supramolecular systems could be used (Figure 20). The advantage of supramolecular systems over covalent systems is that the molecular building blocks in supramolecular materials can be mixed-and-matched in order to combine the different properties of the building blocks in one material. Another advantage is that supramolecular materials mimic the natural system better due to their intrinsic dynamics. Supramolecular materials constantly assemble and disassemble, like the natural ECM (Figure 20). By the use of a modular approach, the intrinsic dynamics of the supramolecular system can be tuned in order to create responsive biomaterials.[125] Eventually, an unprecedented, learning response could be created by mixing in different biofunctional molecules, like enzyme-responsive cues, bioactive epitopes and drug molecules. For example, in the work of Putti et al. a multicomponent supramolecular fiber was developed, by coaxial electrospinning of a elastomeric ureido-pyrimidinone (UPy)−poly(hexa methylene carbonate) (UPy-PC) core with a hydrophilic shell of poly(ethylene-glycol) (UPy-PEG).[126] A hydrophilic and hydrophobic drug was encapsulated in the hydrogel and it was shown that, depending on the hydrophilicity of the drug, a burst release was observed (hydrophilic drugs) or a sustained release (hydrophobic drugs). When these drugs are modified that they are released upon certain cellular cues, an autonomous hydrogel could be created, in which a drug is released when a cellular cue is present. The drug in its turn, will be uptaken by the cell and provoke a cellular response. This response could be inhibition of the drug release, or has an effect on cell behavior, i.e. cell differentiation, cell migration, cell apoptosis (Figure 20). By the use of a modular approach, enzyme-responsive moieties could be mixed into a mechanically stable biomaterial in order to create a biomaterial that allows for both the degradation of the material by cells and the maintenance of the cell commitment by tuning the mechanical properties of

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the material.[127] In a study of Lueckgen et al. an enzyme-degradable peptide-cross-linked hydrogel was developed using norbornene and cysteine terminating peptides (RGD peptides and MMP-cleavable peptides). They were able to decouple the mechanical and rheological properties of the hydrogel from the degradation behavior, thereby allowing the cells to migrate through the material. This could be used for example cell-mediated drug delivery systems or tissue healing applications.

The challenge remains to overcome aspecific responses and crosstalk between different pathways. Another challenge is to design a biomaterial that has the right mechanical properties as well as the desired chemical and biological properties. Most continuous biomaterials being developed are hydrogel systems, which is not suitable for all tissue engineering applications. A challenge remains to develop a solid biomaterial that is able to show some continuous biomaterial response as described above (Figure 18). One way to overcome this problem is by mimic the design and complexity of natural systems by combining multiple natural systems in one, like done in supramolecular systems. Building blocks could be designed that are cleavable by enzymes from the body. On the cleavable linker a functional molecule is placed, that can, for example, induce apoptosis in tumor cells or inhibit the secretion of enzymes that cleave the linker. In this way, a multicomponent system is designed that has biological activity and can inhibit its own process.

In the end, biomaterials should be developed that show true interaction with the host tissue, being able to adapt and response to cellular cues, being fully autonomous in order to mimic the natural systems and thereby treating diseases that are challenging to address.

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Author Contributions

The manuscript was written through contributions of both authors. Both authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

ECM, extracellular matrix; DR, dynamic reciprocity; MMP, matrix metalloproteinase; TIMP, tissue inhibitor matrix metalloproteinase; SAM, self-assembling monolayers; APTES, 3-aminopropyltriethoxysilane; DOX, doxorubicin; HAase, hyaluronidase; HA, hyaluronic acid; PEG, poly(ethylene glycol); LipA, lipase A; LipC, lipase C; EISA, enzyme-instructed self-assembly; ALP, alkaline phosphatase; MI, myocardial infarction; SAP, self-assembling peptides; PAP, prostatic acid phosphatase; PEGA, polyethylene glycol acrylamide; RGD, arginine-glycine-aspartic acid; hMSC, human mesenchymal stem cell; ALD, aldehyde; HYD, hydrazide; PTFE, polytetrafluoroethylene; oNB, *ortho*-nitrobenzyl; BCN, bicyclononyne; SPAAC, strain-promoted azide−alkyne cycloaddition; UPy, ureido-pyrimidinone; PC, poly(hexamethylene carbonate); PKA, protein kinase A; PA, peptide amphiphiles;

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