Enzyme-Responsive Hydrogels

10

Shah M. Reduwan Billah, Md. Ibrahim H. Mondal, Sazzad H. Somoal, M. Nahid Pervez, and Md. Obaidul Haque

Contents

S. M. Reduwan Billah (✉)
CCIRA UK Limited, Galashiels, UK

Department of Chemistry, Durham University, Durham, UK

School of Textiles and Design, Heriot-Watt University, Scottish Borders Campus, Galashiels, UK
e-mail: reduwan.shah@gmail.com

Md. I. H. Mondal
Department of Applied Chemistry and Chemical Engineering, University of Rajshahi, Rajshahi, Bangladesh
e-mail: mihmondal@gmail.com

S. H. Somoal
Institute for Environmental Sciences, University of Koblenz-Landau, Landau, Germany

M. Nahid Pervez
School of Textiles and Design, Heriot-Watt University, Scottish Borders Campus, Galashiels, UK

Md. O. Haque
Polymer and Textile Research Laboratory, Department of Applied Chemistry and Chemical Engineering, University of Rajshahi, Rajshahi, Bangladesh

Md. I. H. Mondal (ed.), *Cellulose-Based Superabsorbent Hydrogels*,
Polymers and Polymeric Composites: A Reference Series,
https://doi.org/10.1007/978-3-319-77830-3_62

Abstract

In an enzymatically responsive system, a suitable enzyme is used as a stimulus for a control release or delivery at a specifically targeted site where that enzyme is designed in such a way that can work at certain controlled conditions (such as temperature, pH). Enzyme-responsive hydrogels prepared from cellulose along with other materials have suitable macromolecular networks and can work in controlled environment. Specifically designed enzymatic stimuli-responsive system, one of the highly explored techniques, popularly explored to add a triggerable agent (such as a polymer or a lipid) that can encapsulate the active component in a protective manner. Usually, this active agent is responsive to degradation or swelling when it reaches at the target site. An enzymatic stimulus-responsive system is highly attractive field of research due to its many potential applications (e.g., in controlled release, drug delivery, and other areas of life and material sciences). This chapter gives a brief overview on the design and uses of enzyme-responsive hydrogels based on cellulose and other polymers for their various applications in different fields including in controlled drug delivery and other areas of biomedical and material sciences.

Keywords

Cellulose · Hydrogels · Enzyme · Enzyme-responsive hydrogels · Stimuli-responsive hydrogels · Drug delivery · Biomedical

1 Introduction

Hydrogels are substances that absorb significant quantities of water, and usually they are prepared from natural materials. They are widely studied all around the world for their enormous biomedical applications [1–4]. The attractive characters of hydrogels stimulate scientists for continuous investigation on novel biomaterials for their potential industrial applications. Hydrogels have the capability to absorb large quantities of water due to their three-dimensional polymer networks, and this morphological structure also modifies their characters to allow them to be soft and pliable as well as to retain structural water which makes them suitable for many conventional and potential biomedical uses. Recently, hydrogels are popular for a variety of biomedical

applications [5–12]. Because of the high water content of hydrogels, they are compatible to most of the living tissues, and their viscoelastic nature also contributes to reduce the damage to the surrounding tissue when implanted in the host system [13–15]. Besides this, the mechanical characters of hydrogels are parallel to those of soft tissues which are particularly attractive for tissue engineering applications. Bioactive materials are frequently used for hydrogel synthesis, and these materials can interact with the host tissues to assist and improve the healing process as well as to mimic functional and morphological properties of organ tissues [13–18]. Hydrogels can also be made by using biomaterials along with smart materials in order to offer natural adaptations (e.g., sensing devices, controlled actuations, regulation of target functions, control in feedback systems). This type of stimuli-responsive hydrogels is capable to adapt with the required changes in their surrounding environmental stimuli (for instance, surrounding composition, presence of enzyme, temperature, pH, light) [19–28]. Smart hydrogels are attractive for their practical and potential uses as biomimetic materials and for their applications in biosensors, processors, and also in activators for electrical responses [29–33]. As for example, the frequent uses of electroconductive hydrogels (such as in bio-recognition membranes for implantation of biosensors, fabrication of drug-eluting devices based on electrically stimulated hydrogels, and also in low interfacial impedance layers of neuronal prostheses based on hydrogels) have been successful to open new horizons in the fabrication of devices for biomedical detection purposes. One of the main reasons for this success is based on the fact that both biomolecular recognitions and responsive functions based on biomolecular targets and induced structural changes can be incorporated into the structural networks of hydrogels using meticulous preparation techniques and skillful design strategies [34–36]. In order for an effective therapeutic delivery, monitoring, and molecular imaging (using noninvasive techniques) to detect and treat different diseases, hydrogel-based drug delivery systems are popularly used since hydrogels provide the required feasibilities for the integration of smart systems and biomolecular imaging. As a result, this type of smart hydrogels can be used for self-regulation and controlling hydrogel-based devices for maintaining physiological variables (suitable for drug delivery and cell culture applications) [37, 38]. For drug delivery, hydrogel implantation can be carried out by preforming or injecting; however, the preformed hydrogels are usually processed by using active reagent in vitro before in vivo implantation. In biological sciences hydrogels are widely used in different areas, because both the intracellular cytoskeleton and extracellular matrix (ECM) are compatible with gel-phase materials. For example, biological gels can be designed to show responsive characters when they are exposed to suitable changes in their environment using different methods including specific enzymatic processes that involve adaption and reorganization of gel structures [39–47]. Both adaption and reorganization are very important to different processes within cell cultures (e.g., differentiation and cell division). Thus, the production of synthetic mimics of hydrogels is critically important as they can be used to measure and direct different biological processes. Enzyme-responsive hydrogels can be applied to mimic biological matrices, and different enzymatic processes have the feasibility to fabricate enzyme-assisted assembly of gels with precisely controlled characters [48–50].

Hydrogels have been widely used for various applications for a long time. For example, since the 1960s, synthetic hydrogels have been applied in biomedical uses,

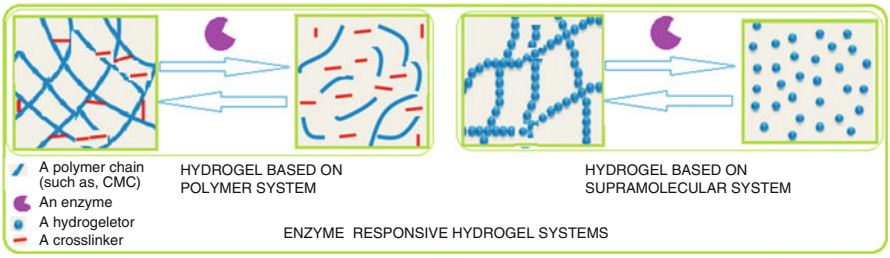

Fig. 1 A schematic representation of an enzymatic stimulus-responsive system

which at that time Wichterle and Lim illustrated the use of biocompatible hydrogels prepared by cross-linking poly(hydroxyethylmethacrylic)acid [poly(HEMA)] in contact lenses [49]. In 1980 naturally derived hydrogels (such as based on alginates) were reported and used to encapsulate pancreatic cells [50], which was then followed by the application of shark collagen in the dressings of burn wounds [51]. Both synthetic and natural materials are frequently used in hydrogel formations; however, currently hybrid systems are being rigorously investigated for their vivid benefits [52]. In previous studies on hydrogels, mostly structural properties of hydrogels that can effectively match natural tissues were used to be studied most rigorously; however, this focus has been shifted to the synthesis of materials that can match structurally and chemically as well as have the capability to mimic aspects of biological gels. As for instance, hydrogels are being designed in such a way which can retain short peptides, sugars, or other biomolecules in order to facilitate suitable interactions with biological systems. Recent progress in this field is mostly focused on the control degradation using enzymatic action to design biomaterials [48]. The application of enzymes in the fabrication of materials is significantly important because of their selectivity and synthetic capability under mild conditions [48]. Synthetics gels can be divided into two groups considering the chemical nature of their networks; they are (a) polymeric hydrogels and (b) supramolecular hydrogels (Fig. 1). Typically, polymeric hydrogels are covalently cross-linked networks that illustrate swelling characters because of the absorption and ability to trap water in their structures [49]. However, supramolecular hydrogels generally show reversible, non-covalent molecular interactions (such as hydrogen bonding, pi stacking, electrostatic and hydrophobic interactions, van der Waals forces) between self-assembling molecules (also termed as hydrogelators) to form nanofibers. Depending on their surface chemistry, these nanofibers are entangled into 3D networks that provide the capability to trap water and form hydrogels [52, 53]. This chapter briefly presents selective aspects of enzyme-responsive hydrogels and their applications in specific areas.

2 Enzymatic Stimuli-Responsive Hydrogels

Environmental stimuli-responsive materials have the capability to change form and/or function when exposed to environmental stimuli (or cues); some of the widely used environmental stimuli include pH, temperature, light, and electric fields

[49–51]. Suitable portable electronic devices based on stimuli-responsive materials have variety of applications in different branches of science and technology as well as in every-day life. These materials are highly sought for many advanced applications in a variety of areas (e.g., in electronics [52], healthcare [53, 54], and energy creation and storage) [55]. The stimulus or trigger used for induction on the stimuli-responsive materials causes a change or a combination of different changes on the characters of the exposed material which is a very important part of the responsive material technology suitable to design many controlled features of certain material properties. Enzymes have some sort of decisive control on different processes of living organisms, and thus enzymatic stimuli-responsive materials can be used to design a wide range of materials including artificial biological materials to perform desired activities [56, 57]. Enzyme-responsive materials (ERMs) change their functionality when they are exposed to the action of a particular enzyme or a combination of enzymes. Enzyme-responsive hydrogels are usually designed and synthesized for enzyme-responsive materials [4, 58–62]. The practical use of enzyme-responsive materials is a gradually increasing phenomenon in light of the inspiration for biologically compatible synthetic technologies to mimic natural environments. For example, in biological systems enzymes take part in a plethora of biochemical reactions which have effective influences on different nanoscale processes which include (a) protein expression, (b) formation of cellular adhesions, (c) signal transduction, and (d) macroscale processes such as (a) cell movement and (b) muscle contraction [57, 63]. In enzyme-responsive materials, enzymes control the specificity, selectivity, and the catalytic efficiency of the host materials [63–65]. Figure 1 shows a schematic presentation of enzyme-responsive materials based on polymeric (in the left side) and supramolecular hydrogels (in the right side). Enzymatic processes may be exploited in both the degradation and the controlled assembly of hydrogel materials. Recent investigations illustrated that the stem cell growth and differentiation (in addition to biochemical signals) are strongly influenced and show sensitivity toward suitable physical stimuli that exist within the range of their surrounding environment [56].

Enzyme as a stimulus is very often used to perform a certain piece of work in order to control material properties. A wide range of enzyme-responsive materials are used to carry out different ranges of selective activities. For example, different enzymes are used in enzyme-responsive materials where some of the most frequently used enzyme classes include (a) proteases, (b) kinases, (c) phosphatases, and (d) endonucleases. Proteases and endonucleases are capable to cleave peptides and oligonucleotides, respectively; they have their usual applications in the degradation or disassembly of enzyme-responsive materials. In addition, they are also capable to cleave functional groups from enzyme-responsive materials which make the materials suitable for a variety of sensing applications. On the contrary, enzymes with covalent bond formation capability (e.g., transglutaminases) can be applied for enhancing the structural strength of enzyme-responsive materials by allowing the formation of cross-links within the material [48, 56–59]. Generally, sophisticated methods are used to apply the hydrolytic properties of proteases and endonucleases as enzymatic triggers for initiating overall changes on the properties of the enzyme-

responsive materials. Additionally, phosphatases and kinases are also frequently used because of their complementary catalytic actions. For example, kinases are popular enzymes for phosphorylation of molecules during the presence of adenosine-5′-triphosphate and phosphatases are used for dephosphorylation reaction. Antagonistic interplay of this type of enzymes provides the scope of designing reversible enzyme-responsive materials with dynamic properties. The level of enzymatic interaction between enzymes and different enzyme-responsive materials is sometimes distinctly different from enzymatic reactions which involve solubilized substrates. As for instance, the usual use of kinetic models for stating the enzymatic conversion of solubilized substrates (such as Michaelis-Menten kinetics) cannot be employed for enzyme-responsive materials when the materials are significantly large in size (and show relatively lower mobility compared to that of the enzyme). Most enzyme-responsive materials (such as hydrogels, surfaces, particles) where the enzymes are expected to move toward the substrates show similar behavior. Moreover, it is also possible that aspects relating to diffusion kinetics where substrates bound to other materials may also exhibit a difference in characters than from the properties of solubilized substrates with respect to enzyme specificity and steric effects. As a result, for designing efficient enzyme-responsive materials, different important factors are usually considered, some of which are (a) the chemical and physical characters of the material, (b) the concentration of the enzyme present in substrate/material, and (c) the method in which the substrate is anchored to the material [64–66]. In addition, a longtime goal of the research activities on hydrogel is focused on the way to develop stimuli-responsive hydrogels with potential controls on external features relating to cell encapsulation or release of actives. Stimuli-responsive smart or intelligent hydrogels are those gels which may display property or functionality changes in response to variations in the external environment. Typically, these changes in their surroundings involve solvent polarity, temperature, pH, supply of electric field, light, etc. [4, 59]. More generally, materials based on stimuli-responsive technologies are increasingly attracting attention due to their potential applications in everyday life, offering improvements in many technologies [48, 60, 61]. There are excellent reviews on this topic that discusses the design, advantages, and challenges of stimuli-responsive hydrogels [56, 62]. In cellular environments, most stimuli-responsive mechanisms take place under the control of enzymes [63]. Compared with physical or conventional chemical stimuli (e.g., pH, temperature, ionic strength, ligand-receptor interactions), enzymatic regulation of material properties shows much promise because it enables responsiveness to biological signals, which are highly selective and involve catalytic amplification to enable fast response times [64]. Both polymeric and supramolecular hydrogels are useful [65] which can be used to degrade matrices in a controlled fashion using suitable methods, such as by incorporating enzyme-responsive materials into the structural compositions of the hydrogels. So, by doing this it is possible to design hydrogels with the capability of controlling degradation by using different ways including the breaking of chemical cross-links (polymeric hydrogels) or controlled disassembly (supramolecular hydrogels) (Fig. 1). As it is possible to carry out enzymatic degradation selectively and precisely using suitable techniques and

materials, enzyme-responsive hydrogels are highly attractive field of current active research for their huge application potentials in different areas of science and technology [66, 67].

2.1 Biocatalytic Assembly of Supramolecular Polymer-Based Enzyme-Responsive Hydrogels

In general, dynamic processes in biological systems can be controlled by two spatially confined molecular mechanisms; they are (a) catalysis and (b) molecular recognition. The working principles are a bit different for the biological system compared to that of other traditional methods usually used to control supramolecular synthesis that typically uses the change in one or more of the environmental conditions (e.g., pH [68], temperature [69], solvent polarity [66], and/or ionic strength [67]). For over the last two decades or so, enzymes have been popularly used for direct supramolecular assembly [48, 70, 71] due to a number of reasons including the relative ease in biocatalysis. Some advantages of biocatalysis performed in this way are (a) easy responsive assembly under constant, physiological conditions [72], (b) exploitation of biocatalytic reactions particularly relating to certain cell types or diseased states [64, 73], (c) easy inherent catalysis involving molecular amplification (turnover numbers approx. 10^3–10^7) that facilitates fast response time, (d) new tools for bottom-up nanofabrication by taking advantage of the ability to spatially and kinetically control the self-assembly process [72–74], and (e) thermodynamical control systems with routes toward the discovery of peptide-based nanostructures by using reversible exchange of amino acid sequences in dynamic peptide libraries. Additionally, biocatalytic self-assembly forms gelators by using enzymatic reactions (either through hydrolysis or condensation of precursors) followed by the self-assembly of these molecules to form supramolecular structures. These assemblies, in turn, entangle to form different nanostructures (such as 1D, 2D, or 3D nanostructures) (Fig. 2) through different non-covalent interactions that include (a) π-π interactions, (b) hydrogen bonding, and (c) electrostatic interactions [75–79].

2.2 Designing Enzyme-Responsive Materials

Generally, a wide range of issues are properly considered for the design of high-quality enzyme-responsive materials very selectively, and two of them are briefly included here.

2.2.1 Operational Capability

Enzyme-responsive materials are expected to maintain enzymatic activity under specific operational conditions or given environmental conditions (such as aqueous environments at neutral, basic, acidic pH, other selected ionic conditions). Particular types of synthetic materials (e.g., polymers or inorganic particles) sometimes can

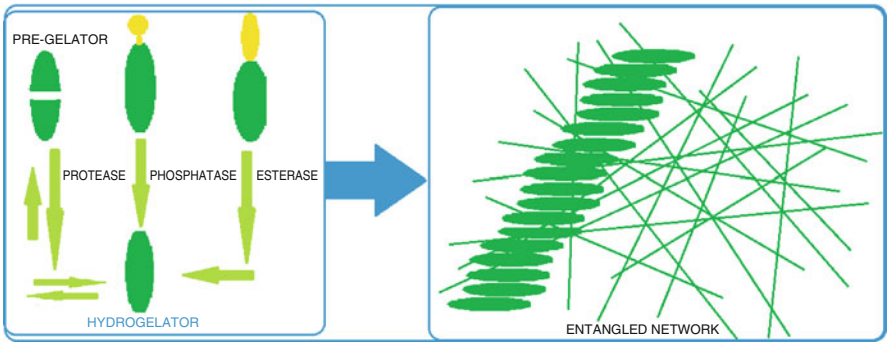

Fig. 2 Schematic illustration of enzyme-assisted self-assembly. The enzyme action results in the formation of hydrogelators which are able to self-assemble to form supramolecular structures and then entangle to form a network

work under this type of environmental condition. As for instance, poly(ethylene glycol) [66], dextran [67, 70], amylose [80], gold [81, 82], and silica [71, 72] nanoparticles and selected peptide-based materials [73, 74] are successfully used to design environmental stimuli-responsive materials.

2.2.2 Meeting Specific Design Requirements

Environmental stimuli-responsive materials are expected to carry out enzymatic activity and other stipulated functions by fulfilling certain design requirements. Some of these requirements include (a) the presence of an active enzyme-sensitive part, (b) translation of enzymatic action of the enzyme-sensitive part to the rest of the material, and (c) sufficient contribution for an effective change in the overall character of the material.

2.3 Specific Characteristics of Enzyme-Responsive Materials for Market Applications

2.3.1 Typical Natures of Enzymatically Responsive Systems

Enzymes have one of the unique control mechanisms usually observed within natural substances in order to regulate the complex biological processes which are by far not matched by the artificially developed systems. As a result one of the main objectives to design enzyme-responsive materials is to synthesize suitable materials with capabilities to replace or at least a comprehensive interaction with natural biological systems. The strength of enzymatically controlled material characteristic depends on the feasibility to design the required material response which is totally influenced and controlled by the biological systems present in the surrounding environment for an ultimate goal to assimilate the environmentally responsive materials into a biological process. However, due to biological complexity, there are tough challenges to develop enzyme-responsive materials with tremendous

application potential to carry out particular jobs on cue when prompted by an enzyme. Enzyme-responsive systems have been widely used in different areas of science and technology including (a) enzyme diagnostic systems [56, 86], (b) drug delivery systems [9, 74, 83–88], and (c) different areas of regenerative medicine [74]. For example, in order to detect different enzymes and their enzymatic activities, different types of enzyme-responsive particles have been developed. Additionally, because of aggregation or dispersion of particles and also due to a particular type of control, the quenching activity of quantum dots, usually the detection of enzyme, depends on the change in magnetic or spectroscopic characters [56, 89] where some of these systems are sensitive even in low level of enzyme concentration [9]. Enzymatic stimuli-responsive hydrogels are frequently used for targeted drug delivery, while the strength of enzymatically controlled drug delivery depends on the ability to allow drug release when it is required in the presence of specific enzymes. Higher levels of specific enzymes are related to different diseases; hence suitable markers and stimuli for the spatially and temporally targeted delivery of therapeutics are important. Thus, for a simultaneous treatment of the diseased tissue and the measurement of the effect of the treatment, the impact of environmentally responsive hydrogels, especially enzymatic stimuli-responsive hydrogels, has many special significance. As for instance, regenerative medicine constitutes a wide range of biomedical research where enzymatic stimuli-responsive materials are frequently used along with the association of many functional polymers or nanomaterials or biomaterials. Some specific applications out of many other important uses of enzymatic stimuli-responsive materials and enzymatic stimuli-responsive hydrogels include (a) applications of enzymatic stimuli-responsive materials or enzymatic stimuli-responsive hydrogels in the formation and degradation of hydrogels as artificial cell supports [74], (b) the enzymatic control of surface properties to control cell response [9, 85], and (c) the enzyme-triggered release of bioactive molecules (such as growth factors) [48, 53–55, 58, 59].

2.3.2 Matching Enzymes and Materials to Specific Applications

Selection of right enzymes or ESM exerts challenges during the design of environmentally responsive materials; however, different model enzymes are sometimes conveniently used to illustrate the mechanism of an ERM. This type of system is useful when the material used can show good performance under the specific conditions present in its surrounding environment. The target enzymes should have the ability to affect only the ESF in the material in order to leave the rest of the material unchanged for minimizing the chances of non-specific responses. Additionally, different strategies which have been developed for realizing the same material response should be reflective of the need to have a versatile repertoire of ERMs that can be adapted for a particular application. Undesirable effects of the biological environment on the material should be avoided, apart from some responsiveness characters, such as response to the marker enzyme and inertness of the environmentally responsive materials to other components present in the system (such as other enzymes, pH, and temperature). In this context, many ERMs have been synthesized and developed that have the capability to respond to an enzyme

with a high specificity to a particular substrate [26, 90]. On the contrary, when enzyme-responsive materials are unable to display the required specificity to a particular enzyme, they have limited application potential. For enzyme-responsive materials where longer peptide or DNA sequences are used that may be recognized by other enzymes and to ensure high impact applications of ERMs, these types of issues need to be resolved. For example, several ERMs with simple ESF (di- or tripeptides that are only sensitive to very specific enzymes) or intentionally broad enzyme specificity have been reported to perform well in in vitro studies that include the formation of supramolecular hydrogels inside cells and polymer hydrogel degradation [26–28, 66, 87, 91].

2.3.3 Choosing the Enzyme Response Mechanism

In case when a particular type of mechanism employed to translate enzymatic action into a material, response has to be tailored to the enzyme catalytic action if a target enzyme has been selected for a specific application. Since both bond formation and cleavage have been employed to date, the bond cleavage has been the more popular choice for existing ERMs. Besides this, some new methods have been applied in order to have opposite material responses with the similar type of enzymatic reaction, for example, enzymatic cleavage of peptide sequences on metal nanoparticles has been used to cause both aggregation and dispersion of the particles [28, 30]. In addition, current trends to enhance different targeted properties of enzyme-responsive materials are more focused on relatively higher dynamic or reversible systems that illustrate a shift from a single-time use of enzyme-responsive materials. Moreover, the introduction of functional characters with a capability to be controlled reversibly and/or on an on-demand basis is another level of progress for a relatively more precise type of incorporation of ERMs into a biological system. Different targeted applications where phosphatases and kinases play significantly important roles are useful for this due to their natural design to catalyze opposite reactions. Reversible enzyme-responsive materials are usually based on a phosphatase/kinase system, although nucleases and ligases perform similar functions (cleavage and bond formation between nucleotides); one example of an enzyme-responsive material is well known where the DNA strands are chemically reconnected after enzymatic cleavage [26–28, 40].

2.3.4 A Brief Discussion on the Enzyme Kinetics of Enzyme-Responsive Hydrogels

A full understanding on enzyme-substrate interactions and the manipulations of these interactions relating to enzymatic control on the suitable drug delivery systems based on enzyme-responsive hydrogels requires a basic understanding on certain aspects of enzyme kinetics, which is briefly outlined here in Fig. 3. The enzyme and substrate must interact to bind together for a conversion of a substrate (S) into a desired product (P), and in that stage the substrate gradually starts to convert into to the product by the enzymatic reaction depending on a range of influencing factors (such as temperature and pH of the surrounding environment, nature of the substrates and enzymes used, amount of both substrates and enzymes used, time of enzymatic

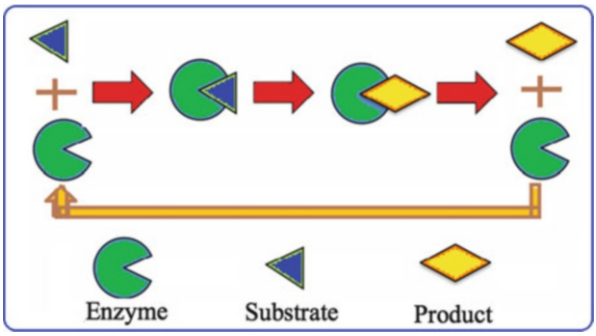

Fig. 3 A schematic representation of enzyme-substrate interactions and the manipulations of these interactions relating to enzymatic control on the suitable drug delivery systems based on enzyme-responsive hydrogels

reaction, etc.). At the final stage, the product is released by the action of enzyme (as demonstrated in Fig. 3). Figure 3 also demonstrates the mechanism by which enzyme specificity occurs using the "lock and key" mechanism. In this case, each enzyme has an active site (termed as lock) to which substrates of proper conformation (termed as key) must bind together and interact to cause a change in chemical structure. However, this change in chemical structure contributes to regulate the specificity of the enzymes in such a way to ensure that only specific substrates with the proper conformations have the capability to bind to the lock and undergo enzymatic conversion into product [27, 28, 92]. For instance, Fig. 3 exhibits a circular substrate would not be converted to product as it would not properly fit in the triangular lock.

2.4 Selective Examples of the Synthesis of Enzyme-Degradable Self-Assembled Hydrogels

Nowadays hydrogels are rigorously studied due to their different applications in various areas of science and technology; some applications include well-defined controlled release of bioactive molecules (such as proteins) and encapsulation of living cells. Biodegradability of hydrogels is one of the very interesting features of controlled drug delivery systems where the original three-dimensional structure has the capability to be disintegrated into nontoxic substances to ensure biocompatibility of the gel. Chemical cross-linking is a frequently used popular method to synthesize mechanically robust hydrogels, but the cross-linkers used in hydrogel preparation require to be extracted from the hydrogels before any suitable application because of toxic nature of the used cross-linkers. In this context, physically cross-linking techniques are preferred alternatives for preparation of hydrogels. Additionally, enzymes are also used for cross-linking to perform specific operations where most of the enzymes are usually used for biodegradation. The generation of a range of star-shaped block copolymers composed of a biocompatible poly(ethylene glycol) (PEG) core tethered to a polyalanine (PAla) shell that possesses the capability to (reversibly) self-assemble in water is a very interesting and important way of producing biodegradable hydrogels. The hydrogels formed in this way offer a

hydrophilic environment that is compatible to different biological processes involving proteins and are able to withhold albumin for prolonged periods before its triggered release following the targeted material degradation by a proteolytic enzyme (such as elastase). Thus, the hydrogels prepared in this way provide a promising opportunity to deliver proteins (and inhibitors, to some extent) in response to a proteolytic enzyme overexpressed in chronic wounds [27, 28, 46–48, 88, 92].

3 Applications of Enzyme-Responsive Systems

3.1 Biomedical Applications of Enzyme-Responsive Hydrogels

Enzyme-responsive hydrogels especially based on peptide self-assembly are attractive biomaterials because of their huge application potentials where some of the principal uses include (a) cell culture, (b) drug delivery, (c) biosensing, and (d) proving the scope for a dynamic control and regulation of cell fate by using systems with intracellular operation capability. So, in brief, the potential biomedical applications of enzyme-responsive hydrogels include (a) control and direction of cell fate, (b) imaging and biosensing, (c) controlled drug release, and (d) applications in cell scaffolds and tissue engineering [93–107]. Regenerative medicine, is a fast-growing area of science and technology. Different researcher groups are constantly working in different issues where devising and upgrading useful techniques and methods for repairing diseased or injured tissues are continuously investigated. Currently, stem cells are being rigorously studied because of their different aspects relating to embryogenesis, homeostatic turnover, and normal tissue repair. In order to realize the potential, stem cell-based therapies are still required to meet criteria in a clinical setting because of different issues including (a) number limitation, (b) immunogenicity, (c) tumor formation, and (d) ethical considerations surrounding their usages. Additionally, the stem cell differentiation mechanisms are complicated and very difficult to understand; hence expanding stem cell numbers and predictably directing their commitment to a desired lineage is very challenging (particularly, to devise tissue regeneration strategies). Different research groups are engaged with continuous research activities to realize the full potentials of enzyme-responsive hydrogels for tissue engineering applications particularly in the area of regenerative medicines [93–107].

3.2 Application of Enzyme-Responsive Hydrogels in Drug Delivery and Bioelectronics

Usually, enzyme-responsive materials have an enzyme-sensitive part along with another part that can direct or control the level of interactions in order to lead macroscopic transitions. Enzyme-responsive materials have many advantages over pH- and temperature-responsive materials, and all these types of materials can be used for stimuli-responsive hydrogels using suitable chemistry and methods. When

an enzyme-responsive hydrogel is applied on a target substrate, the catalytic action of the enzyme on the substrate can lead to different changes that include (a) changes in supramolecular architectures and (b) swelling/collapse behavior or the surface transformation of the substrate. In addition, the sensitivity of the enzyme as a stimulus is unique as enzymes are highly selective in their reactivity and they have the capability to operate under mild conditions present in vivo (a vital case for many biological pathways). The stimulus does not need to be added externally but can be supplied by the biological environment itself as many enzymes are already present in the body. When the naturally present enzyme matches with the triggering enzyme of the responsive material-based hydrogel system, it is more effective for particular type of uses such as controlled drug delivery in a particular location. Both enzyme-responsive materials and hydrogels produced by using them are important for drug development. For example, peptide-based nanocapsules and nanoparticles and hydrogel based on these products are useful for the delivery of bioactive molecules due to its cleavability by protease [107, 108]. Besides this, enzyme-responsive materials and hydrogels produced by using these materials are also suitable to apply in the fabrication of smart antibacterial devices [109], where the principal idea of smart antibacterial devices is based on the release of an antibacterial agent by the presence of the bacteria themselves [5–9].

3.3 Enzyme-Responsive Hydrogels for Biocomputing

Enzyme-responsive hydrogels are useful tools for their potential applications in bio-based computing systems that have wide scale uses in biomedical applications (such as monitoring wound healing and other physiological monitoring). For example, quick and accurate detection of injury (more specifically in accidents or battle fields) is challenging which is further complicated by time and logistics required to deliver emergency medicines. In addition, injuries that cause internal bleeding (particularly, when the effected individual fails to provide any information about his own conditions due to the severity of the accident) expose terrible challenge to diagnose the exact problems, and this type of situations requires advanced diagnostic measures in order to determine the injuries to the soft tissues with a sufficient reliability. In typical cases, pieces of sophisticated diagnostic equipment are usually used to study these conditions; some examples include (a) magnetic resonance imaging and (b) electromyography, which are costly and time-consuming. Besides this, there are many issues to operate these pieces of sophisticated equipment in the actual situation when the delivery of an immediate therapeutic intervention is vitally important. Moreover, in situations where such imaging equipment or laboratory tests are unavailable or impossible to timely deliver the results, the diagnosis is generally carried out by a medical professional using different physical tests. But this technique sometimes can lead to misdiagnosis leading to inaccurate treatments that may encumber the healthcare provider with an extra burden on the patient. In such situations, effective diagnostic tools which can be reliably used to rapidly detect to devise a plausible targeted treatment are most likely to offer great promise that can

contribute to enhance the prognosis of injury (such as in accidental or battlefield conditions). Most recently, biochemical computing technology based on sophisticated enzyme-based cascades has been drawing active research interest to develop systems that can rapidly and reliably work in the diagnosis of injuries and provide useful information to prescribe appropriate emergency medicine to save lives. In this type of systems, leverage from Boolean principles is used to emulate electronic logic gates in the biochemical domain. Enzyme-based logic gates have promising capability to integrate complex patterns of biological and chemical inputs in order to provide required information for relatively rapid diagnosis on a real-time scenario. This type of advanced diagnostic systems can be engineered and leveraged to realize desired diagnosis objectives and to provide required treatment for accidental injuries in an autonomous fashion which have potentials to develop an integrated "Sense-Act-Treat" field [74, 87, 88, 106].

3.4 Smart Bandage and Wound Healing

Enzyme switchable systems have the potential for biomaterial-based display applications with potential uses in a wide range of applications including in smart bandage systems for monitoring the state of injuries and wounds. For example, switchable biomaterials such as enzyme-responsive hydrogels have the potential for producing biomonitoring devices that benefit from lightweight form factors and have the feasibility to make conformal contact with the body. Additionally, a smart bandage can be designed to detect and monitor tissue wounds in vivo, and a flexible electronic device can be used to develop noninvasive maps of pressure-induced tissue damage or even provide some information when a noticeable damage is difficult to observe visually. For example, it has been reported that by using impedance spectroscopy across flexible electrode arrays in vivo on a rat model, the frequency spectra of impedance measurements showed a correlation in a robust way with the state of the underlying tissue across multiple animals and wound types. In addition, tissue damage has also been detected by using the impedance sensor and represented visually as a wound map that helped to identify regions at risk of developing a pressure ulcer which contributed to allow intervention. So, these results illustrate the feasibility of an automated, noninvasive "smart bandage" for early monitoring and diagnosis of pressure ulcers and for improving patient care and outcomes where enzyme-responsive hydrogels can be used [87–90, 108].

3.5 Enzyme (Such as Elastase)-Responsive Hydrogel Dressing for Chronic Wounds

Chronic wounds exert an expensive economical and clinical problem by causing the deaths of millions every year where the overexpression of enzyme (such as elastase) is a main factor that prolongs the normal wound repair process within chronic wounds. Many active research groups are engaged to overcome this situation, as

for instance, specific research activities are employed for designing hydrogel-based responsive chronic wound dressing systems. In this case, polymers such as PEGA (polyethylene glycol acrylamide) in the form of particles are used to prepare hydrogels for mopping up excess elastase by exploiting polymer collapse in response to elastase hydrolytic activity within sample fluids for mimicking the environment of chronic wounds. Besides this, many other investigations have focused on the functionalization of PEGA particles by enzyme-cleavable peptides (ECPs) with charged residues in order to control polymer swelling with a consequent elastase entrapment to cause a cleavage of the charge balance changes [74, 87, 88, 110].

3.6 Complex Bioactive Fiber Systems Incorporating Enzyme-Responsive Systems by Means of Electrospinning

Enzyme-responsive hydrogels have the potentials for producing complex bioactive fibers using different techniques including electrospinning. Generally, enzyme-responsive systems have a wide range of biomedical applications including in the production of complex bioactive fibers for a variety of applications in different areas of life sciences. As for instance, the prolonged life expectancy is a direct contribution of welfare and medical developments, and this prolongation often leads to an increased physical stress on the human body that sometimes requires to replace nonfunctional and damaged tissues or organs. Artificial complex electrospun bioactive fibers have many applications in tissue engineering. During tissue engineering, in vitro cell culture is usually carried out in an artificial three-dimensional scaffold, and there are different ways of doing this. For example, modern approaches toward three-dimensional scaffolds using bioactive interfaces are frequently used in tissue engineering that also provides the opportunity to use bio-integrated and biomimetic systems. Advanced understanding on how materials passively interact or actively communicate with biological systems via designed material-biology interfaces requires precise methods for fabricating macroscopic and nanostructured materials. Recently, modern materials and technology platforms have been developed for producing bioactive scaffolds for serving a number of purposes including for providing spatial control of mechanical, chemical, and biochemical signals at the bio-interface together with the tailored pore architecture and surface topology. Enzyme-responsive hydrogels or enzyme-responsive systems can be used to establish straightforward approaches to fabricate complex structures using electrospinning in order to produce structurally and chemically bioactive fiber suitable for relatively easier cell infiltration due to the scope for a control of the mesh porosity of the electrospun scaffolds. These electrospun scaffolds also provide the scope for direct crystallization of fibers suitable to be used for the investigation of the cell ingrowth for biomedical applications. Additionally, these types of electrospun bioactive fibers can further be functionalized for the decoration of the fiber surface using biomolecules (such as peptides and sugars) or other functional materials to serve specific purposes suitable for control delivery or other related biomedical applications. Additionally, electrospun bioactive fibers have promising biomedical

applications to replicate features of the natural extracellular matrix (ECM). However, most of the electrospun scaffolds are either nondegradable or degrade hydrolytically, whereas natural ECM degrades proteolytically, often through matrix metalloproteinases (MMPs). There are reports for the synthesis of reactive macromers that contain protease-cleavable and fluorescent peptides and are able to form both isotropic hydrogels and electrospun fibrous hydrogels through a photoinitiated polymerization. In addition, biomimetic scaffolds are susceptible to protease-mediated cleavage in vitro in a protease dose-dependent manner and in vivo in a subcutaneous mouse model using transdermal fluorescent imaging to monitor degradation. This type of systems has many biomedical applications [74, 88, 95–99, 105–109].

4 Perspectives and Trends in Future Developments

One of the main strengths of enzyme-responsive materials compared to other stimuli-responsive materials clearly depends on their ability to interact with a biological environment with the same communication mechanism used by nature. In principle, enzyme-responsive materials have the ability to perform their functions with high specificity to their target enzymes. These functions are not largely undisturbed by the multitude of other processes in the biological environment. Until today, research activities on ERMs are principally aimed at developing of enzyme-responsive mechanisms and the translation into a material response. Over the last couple of decades, notable development has been realized in the form of new translational mechanisms and the incorporation of ESFs into artificial materials. Although the high attraction of enzyme-responsive systems to act dynamically and on more than one enzymatic stimulus has been recognized, this area of ERM development still holds great potential to develop new materials with unprecedented possibilities. Enzyme-responsive systems are widely studied for their potential uses in the fabrication of next-generation biological devices. For example, different areas of biomaterials widely use functionalized hydrogels for their huge potentials in different areas of biomedical applications including in cell culture [53, 54] and biosensing [55] platforms. Hydrogels are popularly used in instructive matrices for stem cell growth. In addition, the incorporation of biochemical signals that have matrix protein-specific peptidic motifs (such as fibronectin-derived arginine-glycine-aspartic acid or RGD) which can be used to facilitate cellular adhesion is particularly important. As for instance, the mechanical [57] (such as gel stiffness) and structural/topographical factors [111] of the cell-contacting matrix have special impact on biochemical signals produced from the hydrogels, and they can be regulated to control different features of hydrogels' structure in order to have control of cell fate when these hydrogels are used in cell culture or similar areas. Besides this, the preparation with particular definitions of physical characters and chemical composition may need new synthetic protocols in order to control different properties that include (a) stiffness, (b) gel network structure, and (c) chemical functionalization.

Enzymatic fabrication techniques are useful to achieve some of these goals [48, 58, 74, 87].

In addition, mimicking biological systems is a hot topic for current active research as an effort to have more decisive control over the bottom-up fabrication process. Very often different aspects of biological systems are effectively influenced and controlled using different spatially confined molecular mechanisms, some of which include (a) catalysis and (b) molecular recognition. Thus, enzymes have been used as important tools to regulate suitable nanofabrication methods with aims to develop relatively complex and highly selective next-generation biomaterials for a wide range of conventional and high-tech applications in various fields including in material and life sciences. Different important characters of enzyme-assisted formation and dismantling of supramolecular structures include (a) self-assembly under constant conditions, (b) spatiotemporal control of nucleation and structure growth, and (c) control of mechanical characters (such as stiffness). In addition, systems that assemble under thermodynamic control also show distinctive natures that include (a) defect correcting and (b) component-selecting abilities. Currently, more focus is concentrated on the effective design of suitable materials for specific applications that cover particular areas which include (a) cell culture, (b) drug delivery, (c) imaging, (d) biosensing, and (e) cell fate control. Dynamic processes in biological systems are usually controlled by enzymes. Ongoing extensive research activities aim to have better understandings of different processes at their molecular levels using enzymatic controlled self-assembly approaches. Some of the most challenging tasks in this context include (a) effectively controlling nucleation and structure growth, (b) regulating the access structures which represent non-equilibrium assemblies, and (c) producing asymmetric, dynamic, and multicomponent structures with expected functionalities and characters. An enzyme-responsive hydrogel has a wide range of continuous active research interests in order to form supramolecular structures and also to devise suitable ways to investigate the ability of these structures to perform specific activities, some of which include (a) recognition, (b) adaptation, (c) correction, and (d) interactions of the complex ways related to evolving behaviors [8, 9, 26–28, 38–41, 103–105].

5 Conclusion

Enzyme-responsive hydrogels prepared from cellulose along with other materials have suitable macromolecular networks and can work in controlled environment. An enzymatic stimulus-responsive system is a highly attractive field of research due to its many potential applications (e.g., in controlled release, drug delivery, and other areas of life and material sciences). This chapter has provided a brief overview on different selective features of enzyme-responsive hydrogels based on different polymers, and it has also stated various applications of enzyme-responsive hydrogels in specific fields including in controlled drug delivery and other areas of biomedical and material sciences. However, due to the limited scope available within the perimeter of this chapter, it is almost impossible to explain all features of different

enzymes usually used in the formulation of enzyme-responsive hydrogels. Efforts have been made to provide enough references which can be used to have more comprehensive information for advanced readers.

References

1. Hoffman AS (2004) Applications of "Smart Polymers" as biomaterials, 2nd edn. Elsevier Academic Press, London
2. Kopecek J (2003) Smart and genetically engineered biomaterials and drug delivery systems. Eur J Pharm Sci 20:1–16
3. Mano JF (2008) Stimuli-responsive polymeric systems for biomedical applications. Adv Eng Mater 10:515–527
4. Schmaljohann D (2006) Thermo- and pH-responsive polymers in drug delivery. Adv Drug Deliv Rev 58:1655–1670
5. Roy D, Cambre JN, Sumerlin BS (2010) Future perspectives and recent advances in stimuli-responsive materials. Prog Polym Sci 35:278–301
6. Ghadiali JE, Stevens MM (2008) Enzyme-responsive nanoparticle systems. Adv Mater 20:4359–4363
7. Williams RJ, Mart RJ, Ulijn RV (2010) Exploiting biocatalysis in peptide self-assembly. Biopolymers 94:107–117
8. Zelzer M, Ulijn RV (2010) Next-generation peptide nanomaterials: molecular networks, interfaces and supramolecular functionality. Chem Soc Rev 39:3351–3357
9. Ulijn RV (2006) Enzyme-responsive materials: a new class of smart biomaterials. J Mater Chem 16:2217–2225
10. Ghadiali JE, Cohen BE, Stevens MM (2010) Protein kinase-actuated resonance energy transfer in quantum dot−peptide conjugates. ACS Nano 4:4915–4919
11. Privman M, Tam TK, Pita M, Katz E (2008) Network analysis of biochemical logic for noise reduction and stability: a system of three coupled enzymatic and gates. J Am Chem Soc 131:1314–1321
12. Bonomi R, Cazzolaro A, Sansone A, Scrimin P, Prins LJ (2011) Detection of enzyme activity through catalytic signal amplification with functionalized gold nanoparticles. Angew Chem Int Ed 50:2307–2312
13. Zhao WR, Zhang HT, He QJ, Li YS, Gu JL, Li L, Li H, Shi JL (2011) A glucose-responsive controlled release of insulin system based on enzyme multilayers-coated meso porous silica particles. Chem Commun 47:9459–9461
14. Gordijo CR, Shuhendler AJ, Wu XY (2010) Glucose-responsive bioinorganic nanohybrid membrane for self-regulated insulin release. Adv Funct Mater 20:1404–1412
15. Hahn ME, Gianneschi NC (2011) Enzyme-directed assembly and manipulation of organic nanomaterials. Chem Commun 47:11814–11821
16. Welser K, Adsley R, Moore BM, Chan WC, Aylott JW (2011) Protease sensing with nanoparticle based platforms. Analyst 136(1):29–41
17. Mura S, Nicolas J, Couvreur P (2013) Stimuli-responsive nanocarriers for drug delivery. Nat Mater 12:991–1003
18. Cheng R, Meng F, Deng C, Klok HA, Zhong Z (2013) Dual and multi-stimuli responsive polymeric nanoparticles for programmed site-specific drug delivery. Biomaterials 34:3647–3657
19. Place ES, Evans ND, Stevens MM (2009) Complexity in biomaterials for tissue engineering. Nat Mater 8:457–470
20. Tibbitt MW, Rodell CB, Burdick JA, Anseth KS (2015) Progress in material design for biomedical applications. Proc Natl Acad Sci 112:14444–14451
21. Howes PD, Chandrawati R, Stevens MM (2014) Colloidal nanoparticles as advanced biological sensors. Science 346:1247390–1247390

22. Su J, Chen F, Cryns VL, Messersmith PB (2011) Catechol polymers for pH-responsive, targeted drug delivery to cancer cells. J Am Chem Soc 133:11850–11853
23. Park I-K, Singha K, Arote RB, Choi Y-J, Kim WJ, Cho C-S (2010) pH-responsive polymers as gene carriers. Macromol Rapid Commun 31:1122–1133
24. Jochum FD, Theato P (2013) Temperature- and light-responsive smart polymer materials. Chem Soc Rev 42:7468–7483
25. Ercole F, Davis TP, Evans RA (2010) Photo-responsive systems and biomaterials: photochromic polymers, light-triggered self-assembly, surface modification, fluorescence modulation and beyond. Polym Chem 1:37–54
26. Chandrawati R, Städler B, Postma A, Connal LA, Chong SF, Zelikin AN, Caruso F (2009) Cholesterol-mediated anchoring of enzyme-loaded liposomes within disulfide-stabilized polymer carrier capsules. Biomaterials 30:5988–5998
27. Phillips DJ, Gibson MI (2012) Degradable thermoresponsive polymers which display redox-responsive LCST behaviour. Chem Commun 48:1054–1056
28. Chen W, Du J (2013) Ultrasound and pH dually responsive polymer vesicles for anticancer drug delivery. Sci Rep 3:2162–2162
29. Roy R, Yang J, Moses MA (2009) Matrix metalloproteinases as novel biomarkers and potential therapeutic targets in human cancer. J Clin Oncol 27:5287–5297
30. Park J, Yun HS, Lee KH, Lee KT, Lee JK, Lee S-Y (2015) Discovery and validation of biomarkers that distinguish mucinous and nonmucinous pancreatic cysts. Cancer Res 75:3227–3235
31. Khademhosseini A, Langer R (2007) Microengineered hydrogels for tissue engineering. Biomaterials 28:5087–5092
32. Ulijn RV, Bibi N, Jayawarna V, Thornton PD, Todd SJ, Mart RJ, Smith AM, Gough JE (2007) Bioresponsive hydrogels. Mater Today 10:40–48
33. Tibbitt MW, Anseth KS (2009) Hydrogels as extracellular matrix mimics for 3D cell culture. Biotechnol Bioeng 103:655–663
34. Singh SP, Schwartz MP, Tokuda EY, Luo Y, Rogers RE, Fujita M, Ahn NG, Anseth KS (2015) A synthetic modular approach for modeling the role of the 3D microenvironment in tumor progression. Sci Rep 5:17814–17814
35. McCall JD, Anseth KS (2012) Thiol–ene photopolymerizations provide a facile method to encapsulate proteins and maintain their bioactivity. Biomacromolecules 13: 2410–2417. 45
36. Phelps EA, Enemchukwu NO, Fiore VF, Sy JC, Murthy N, Sulchek TA, Barker TH, García AJ (2012) Maleimide cross-linked bioactive PEG hydrogel exhibits improved reaction kinetics and cross-linking for cell encapsulation and in situ delivery. Adv Mater 24:64–70
37. Khetan S, Guvendiren M, Legant WR, Cohen DM, Chen CS, Burdick JA (2013) Degradation-mediated cellular traction directs stem cell fate in covalently crosslinked three-dimensional hydrogels. Nat Mater 12:458–465
38. Vandenbroucke RE, Libert C (2014) Is there new hope for therapeutic matrix metalloproteinase inhibition? Nat Rev Drug Discov 13:904–927
39. Chwalek K, Tsurkan MV, Freudenberg U, Werner C (2014) Glycosaminoglycan-based hydrogels to modulate heterocellular communication in in vitro angiogenesis models. Sci Rep 4:4414–4414
40. Turk BE, Huang LL, Piro ET, Cantley LC (2001) Determination of protease cleavage site motifs using mixture-based oriented peptide libraries. Nat Biotechnol 19:661–667
41. Hsu C-W, Olabisi RM, Olmsted-Davis EA, Davis AR, West JL (2011) Cathepsin K-sensitive poly(ethylene glycol) hydrogels for degradation in response to bone resorption. J Biomed Mater Res A 98:53–62
42. Brubaker CE, Messersmith PB (2011) Enzymatically degradable mussel-inspired adhesive hydrogel. Biomacromolecules 12:4326–4334
43. Vandamme TF, Lenourry A, Charrueau C, Chaumeil JC (2002) The use of polysaccharides to target drugs to the colon. Carbohydr Polym 48:219–231
44. Chourasia MK, Jain SK (2004) Polysaccharides for colon targeted drug delivery. Drug 11:129–148

45. Yao X, Liu Y, Gao J, Yang L, Mao D, Stefanitsch C, Li Y, Zhang J, Ou L, Kong D, Zhao Q, Li Z (2015) Nitric oxide releasing hydrogel enhances the therapeutic efficacy of mesenchymal stem cells for myocardial infarction. Biomaterials 60:130–140
46. Martino MM, Briquez PS, Ranga A, Lutolf MP, Hubbell JA (2013) Heparin-binding domain of fibrin(ogen) binds growth factors and promotes tissue repair when incorporated within a synthetic matrix. Proc Natl Acad Sci 110:4563–4568
47. Thornton PD, Billah SMR, Cameron NR (2013) Enzyme-degradable self-assembled hydrogels from polyalanine-modified poly(ethylene glycol) star polymers. Macromol Rapid Commun 34:257–262
48. Zelzer M, Todd SJ, Hirst AR, McDonald TO, Ulijn RV (2013) Enzyme responsive materials: design strategies and future developments. Biomater Sci 1:11–39
49. Wichterle O, Lim D (1960) Hydrophilic gels for biological use. Nature 185(4706):117–118
50. Lim F, Sun AM (1980) Microencapsulated islets as bioartificial endocrine pancreas. Science 210:908–910
51. Yannas IV, Lee E, Orgill DP, Skrabut EM, Murphy GF (1989) Synthesis and characterization of a model extracellular matrix that induces partial regeneration of adult mammalian skin. Proc Natl Aca Sci USA 86:933–937
52. Ratner B, Hoffman AS, Schoen F, Lemons JE (2004) Biomaterials science: introduction to materials in medicine, vol 2004, 2nd edn. Elsevier Academic Press, San Diego, pp 162–164
53. Silva GA, Czeisler C, Niece KL, Beniash E, Harrington DA, Kessler JA, Stupp SI (2004) Selective differentiation of neural progenitor cells by high-epitope density nanofibers. Science 303:1352–1355
54. Banwell EF, Abelardo ES, Adams DJ, Birchall MA, Corrigan A, Donald MA, Kirkland M, Serpell LC, Butler MF, Woolfson DN (2009) Rational design and application of responsive alpha-helical peptide hydrogels. Nat Mater 8:596–600
55. Kiyonaka S, Sada K, Yoshimura I, Shinkai S, Kato N, Hamachi I (2004) Semi-wet peptide/protein array using supramolecular hydrogel. Nat Mater 3(1):58–64
56. Lutolf M, Hubbell J (2005) Synthetic biomaterials as instructive extracellular microenvironments for morphogenesis in tissue engineering. Nat Biotechnol 23:47–55
57. Engler AJ, Sen S, Sweeney HL, Discher HL (2006) Matrix elasticity directs stem cell lineage specification. Cell 126:677–689
58. Ehrbar M, Rizzi SC, Schoenmakers RG, Miguel BS, Hubbell JA, Weber FE, Lutolf MP (2007) Biomolecular hydrogels formed and degraded via site-specific enzymatic reactions. Biomacromolecules 8:3000–3007
59. Ratner BD, Hoffman AS, Schoen FJ, Lemons JE (2004) Biomaterials science: introduction to materials in medicine, 2nd edn. Elsevier Academic Press, San Diego
60. Koutsopoulos S, Unsworth LD, Nagai Y, Zhang S (2009) Controlled release of functional proteins through designer self-assembling peptide nanofiber hydrogel scaffold. Proc Natl Acad Sci U S A 106:4623–4628
61. Chen L, Morris K, Laybourn A, Elias D, Hicks MR, Rodger A, Serpell L, Adams DJ (2009) Self-assembly mechanism for a naphthalene−dipeptide leading to hydrogelation. Langmuir 26:5232–5242
62. Soppimath K, Aminabhavi T, Dave A, Kumbar S, Rudzinski W (2002) Stimulus-responsive "smart" hydrogels as novel drug delivery systems. Drug Dev Ind Pharm 28:957–974
63. Walsh C (2001) Enabling the chemistry of life. Nature 409:226–231
64. Yang ZM, Liang GL, Guo ZH, Xu B (2007) Intracellular hydrogelation of small molecules inhibits bacterial growth. Angew Chem Int Ed 46:8216–8219
65. West JL, Hubbell JA (1999) Polymeric biomaterials with degradation sites for proteases involved in cell migration. Macromolecules 32:241–244
66. Reches M, Gazit E (2003) Casting metal nanowires within discrete self-assembled peptide nanotubes. Science 300:625–627
67. Ozbas B, Kretsinger J, Rajagopal K, Schneider JP, Pochan DJ (2004) Salt-triggered peptide folding and consequent self-assembly into hydrogels with tunable modulus. Macromolecules 37:7331–7337

68. Tang C, Smith AM, Collins RF, Ulijn RV, Saiani A (2009) FMOC-diphenylalanine self-assembly mechanism induces apparent pKa shifts. Langmuir 25:9447–9453
69. Hong H, Mai Y, Zhou Y, Yan D, Chen Y (2007) Synthesis and supramolecular self-assembly of thermosensitive amphiphilic star copolymers based on a hyperbranched polyether core. J Polym Sci A 46:668–681
70. Yang Z, Gu H, Fu D, Gao P, Lam JK, Xu B (2004) Enzymatic formation of supramolecular hydrogels. Adv Mater 16:1440–1444
71. Toledano S, Williams RJ, Jayawarna V, Ulijn RV (2006) Enzyme-triggered self-assembly of peptide hydrogels via reversed hydrolysis. J Am Chem Soc 128:1070–1071
72. Xu B (2009) Gels as functional nanomaterials for biology and medicine. Langmuir 25:8375–8377
73. Yang Z, Xu K, Guo Z, Guo Z, Xu B (2007) Intracellular enzymatic formation of nanofibers results in hydrogelation and regulated cell death. Adv Mater 19:3152–3156
74. Hirst AR, Roy S, Arora M, Das AK, Hodson N, Murray P, Marshall S, Javid N, Sefcik J, Boekhoven J, van Esch JH, Santabarbara S, Hunt NT, Ulijn RV (2010) Biocatalytic induction of supramolecular order. Nat Chem 2:1089–1094
75. Williams RJ, Smith AM, Collins R, Hodson N, Das AK, Ulijn RV (2008) Enzyme-assisted self-assembly under thermodynamic control. Nat Nanotechnol 4:19–24
76. Das AK, Hirst AR, Ulijn RV (2009) Evolving nanomaterials using enzyme-driven dynamic peptide libraries (eDPL). Faraday Discuss 143:293–303
77. Sadownik JW, Ulijn RV (2010) Locking an oxidation-sensitive dynamic peptide system in the gel state. Chem Commun 46:3481–3483
78. Ryan DM, Nilsson BL (2012) Self-assembled amino acids and dipeptides as noncovalent hydrogels for tissue engineering. Polym Chem 3:18–33
79. Adams DJ, Topham PD (2010) Peptide conjugate hydrogelators. Soft Matter 6:3707–3721
80. Yang Z, Liang G, Xu B (2008) Enzymatic hydrogelation of small molecules. Acc Chem Res 41:315–326
81. Collier JH, Messersmith PB (2003) Enzymatic modification of self-assembled peptide structures with tissue transglutaminase. Bioconjug Chem 14:748–755
82. Winkler S, Wilson D, Kaplan D (2000) Controlling beta-sheet assembly in genetically engineered silk by enzymatic phosphorylation/dephosphorylation. Biochemistry 39:12739–12746
83. Hirst AR, Coates IA, Boucheteau TR, Miravet JF, Escuder B, Castelletto V, Hamley IW, Smith DK (2008) Low-molecular-weight gelators: elucidating the principles of gelation based on gelator solubility and a cooperative self-assembly model. J Am Chem Soc 130:9113–9121
84. Adams DJ, Butler MF, Frith WJ, Kirkland M, Mullen L, Sanderson P (2009) A new method for maintaining homogeneity during liquid–hydrogel transitions using low molecular weight hydrogelators. Soft Matter 5:1856–1862
85. Sadownik JW, Leckie J, Ulijn RV (2011) Micelle to fibre biocatalytic supramolecular transformation of an aromatic peptide amphiphile. Chem Commun 47:728–730
86. Yang Z, Ho P-L, Liang G, Chow KH, Wang Q, Cao Y, Guo Z, Xu B (2007) J Am Chem Soc 129:266–267
87. Roy S, Ulijn RV (2010) Advances in polymer science. In: ARA P, Heise A (eds) Enzymatic polymerisation, vol 237. Springer, Berlin, pp 127–143
88. Thornton K, Smith A, Merry CLR, Ulijn RV (2009) Controlling stiffness in nanostructured hydrogels produced by enzymatic dephosphorylation. Biochem Soc Trans 37:660–664
89. Prabaharan M, Mano JF (2006) Stimuli-responsive hydrogels based on polysaccharides incorporated with thermo-responsive polymers as novel biomaterials. Macromol Biosci 6:991–1008
90. Santos SD, Chandravarkar A, Mandal B, Mimna R, Murat K, Saucede L, Tella P, Tuchscherer G, Mutter M (2005) Switch-peptides: controlling self-assembly of amyloid beta-derived peptides in vitro by consecutive triggering of acyl migrations. J Am Chem Soc 127(34):11888–11889
91. Yanlian Y, Ulung K, Xiumei W, Horii A, Yokoi H, Shuguang Z (2009) Designer self-assembling peptide nanomaterials. Nanotechnol Today 4:193–210

92. Ehrbar M, Rizzi SC, Schoenmakers RG, San Miguel B, Hubbell JA, Weber FE, Lutolf MP (2007) Biomolecular hydrogels formed and degraded via site-specific enzymatic reactions. Biomacromolecules 8:3000–3007
93. Corbett PT, Leclaire J, Vial L, West KR, Wietor J-L, Sanders JKM, Otto S (2006) Dynamic combinatorial chemistry. Chem Rev 106(9):3652–3711
94. Rowan SJ, Cantrill SJ, Cousins GRL, Sanders JKM, Stoddart JF (2002) Dynamic covalent chemistry. Angew Chem Int Ed 41:898–952
95. Vegners R, Shestakova I, Kalvinsh I, Ezzell RM, Janmey PA (1995) Use of a gel-forming dipeptide derivative as a carrier for antigen presentation. J Pept Sci 1:371–378
96. Zhang Y, Gu H, Yang Z, Xu B (2003) Supramolecular hydrogels respond to ligand−receptor interaction. J Am Chem Soc 125(45):13680–13681
97. Hughes M, Frederix PWJM, Raeburn J, Birchall LS, Sadownik J, Coomer FC, Lin I-H, Cussen EJ, Hunt NT, Tuttle T, Webb SJ, Adams DJ, Ulijn RV (2012) Sequence/structure relationships in aromatic dipeptide hydrogels formed under thermodynamic control by enzyme-assisted self-assembly. Soft Matter 8:5595–5602
98. Hughes M, Xu H, Frederix PWJM, Smith AM, Hunt NT, Tuttle T, Kinloch IA, Ulijn RV (2011) Biocatalytic self-assembly of 2D peptide-based nanostructures. Soft Matter 7 (21):10032–10038
99. Hughes M, Birchall LS, Zuberi K, Aitkin LA, Debnath S, Javid N, Ulijn RV (2012) Differential supramolecular organisation of fmoc-dipeptides with hydrophilic terminal amino acid residues by biocatalytic self-assembly. Soft Matter 8:11565–11574
100. Jayawarna V, Richardson SM, Hirst AR, Hodson NW, Saiani A, Gough JE, Ulijn RV (2009) Introducing chemical functionality in FMOC-peptide gels for cell culture. Acta Biomater 5 (3):934–943
101. Ruoslahti E (1996) RGD and other recognition sequences for integrins. Annu Rev Cell Dev Biol 12:697–715
102. Hughes M, Debnath S, Knapp CW, Ulijn RV (2013) Antimicrobial properties of enzymatically triggered self-assembling aromatic peptide amphiphiles. Biomater Sci 1:1138–1142
103. Brake JM, Daschner MK, Luk Y-Y, Abbott NL (2003) Biomolecular interactions at phospholipid-decorated surfaces of liquid crystals. Science 302:2094–2097
104. Lin IH, Birchall LS, Hodson N, Ulijn RV, Webb SJ (2013) Interfacing biodegradable molecular hydrogels with liquid crystals. Soft Matter 9:1188–1193
105. Gao Y, Kuang Y, Guo Z-F, Guo Z, Krauss IJ, Xu B (2009) Enzyme-instructed molecular self-assembly confers nanofibers and a supramolecular hydrogel of taxol derivative. J Am Chem Soc 131(38):13576–13577
106. Williams RJ, Hall TE, Glattauer V, White J, Pasic PJ, Sorensen AB, Waddington L, McLean KM, Currie PD, Hartley PG (2011) The in vivo performance of an enzyme-assisted self-assembled peptide/protein hydrogel. Biomaterials 32:5304–5310
107. Andrieu J, Kotman N, Maier M, Mailänder V, Strauss WSL, Weiss CK, Landfester K (2012) Live monitoring of cargo release from peptide-based hybrid nanocapsules induced by enzyme cleavage. Macromol Rapid Commun 33(3):248–253
108. Fuchs AV, Kotman N, Andrieu J, Mailander V, Weiss CK, Landfester K (2013) Enzyme cleavable nanoparticles from peptide based triblock copolymers. Nanoscale 5(11):4829–4839
109. Baier G, Cavallaro A, Vasilev K, Mailänder V, Musyanovych A, Landfester K (2013) Enzyme responsive hyaluronic acid nanocapsules containing polyhexanide and their exposure to bacteria to prevent infection. Biomacromolecules 14(4):1103–1112
110. Lin C-C (2015) Recent advances in crosslinking chemistry of biomimetic poly(ethylene glycol) hydrogels. RSC Adv 5:39844–39853
111. Dalby MJ, Gadegaard N, Tare R, Andar A, Riehle MO, Herzyk P, Wilkinson CD, Oreffo RO (2007) The control of human mesenchymal cell differentiation using nanoscale symmetry and disorder. Nat Mater 6:997–1003