Bioresponsive hydrogels

We highlight recent developments in hydrogel materials with biological responsiveness built in. These 'smart' biomaterials change properties in response to selective biological recognition events. When exposed to a biological target (nutrient, growth factor, receptor, antibody, enzyme, or whole cell), molecular recognition events trigger changes in molecular interactions that translate into macroscopic responses, such as swelling/collapse or solution-to-gel transitions. The hydrogel transitions may be used directly as optical readouts for biosensing, linked to the release of actives for drug delivery, or instigate biochemical signaling events that control or direct cellular behavior. Accordingly, bioresponsive hydrogels have gained significant interest for application in diagnostics, drug delivery, and tissue regeneration/wound healing.

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Modern biomaterials are increasingly designed to interface with biological tissues in predefined ways. An important class of biomaterials are those that are highly hydrated – hydrogels. Hydrogels consist of elastic networks with interstitial spaces that contain as much as 90-99% w/w water[1,2]. They are prepared by chemical polymerization or by physical self-assembly of man-made or naturally occurring building blocks. Most commonly, these building blocks are macromolecules in a variety of architectures, including cross-linked polymers[2], entangled fibrillar networks[3,4], or colloidal assemblies[5-8]. Recently, small amphiphilic molecules have emerged as a new class of hydrogelators, forming supramolecular or molecular hydrogels[9-11].

Hydrogel materials are increasingly studied for applications in biological sensing, drug delivery, and tissue regeneration for a number of reasons:

- Hydrogels provide suitable semiwet, three-dimensional environments for molecular-level biological interactions[12-15];
- Many hydrogels provide inert surfaces that prevent nonspecific adsorption of proteins, a property known as antifouling;
- Biological molecules can be covalently incorporated into hydrogel structures using a range of well-established chemistries[14];
- Hydrogel mechanical properties are highly tunable, for example elasticity can be tailored by modifying cross-link densities; and
- Hydrogels can be designed to change properties (e.g. swelling/collapse or solution-to-gel transitions) in response to externally

applied triggers, such as temperature, ionic strength, solvent polarity, electric/magnetic field, light, or small (bio)molecules[16-21]. In this review, we focus on responsive hydrogel materials that have been designed to engage in a dialogue with their biological environment[22-25]. Material-to-biology communication, whereby a biological event occurs upon interaction with the material, can be achieved by introducing ligands that bind specific biomolecules into the material structure. These ligands provide instructions to control or direct biological interactions.

In tissue regeneration, biomaterials may be designed to contain precisely positioned bioactive ligands that instruct cell behavior. A well-known example is the cell-adhesive tripeptide, Arg-Gly-Asp (RGD). This peptide is derived from fibronectin, a component of the extracellular matrix (ECM) to which cells attach *in vivo*. Incorporation of RGD into hydrogel structures instructs many cell types to attach to the material via cell-surface proteins called integrins[26,27]. Another example is the laminin-derived peptide Ile-Lys-Val-Ala-Val (IKVAV), which can be incorporated into a hydrogel to trigger stem cell differentiation toward neuronal cells[28]. As well as peptides, polysaccharides may also be used as bioactive ligands to direct cell behavior. For example, Ranjangam *et al.*[29] have incorporated heparin into peptide amphiphile assemblies to control angiogenesis (new blood vessel formation). Biomaterials that contain biological instructions have been around since the 1980s and are often termed *bioactive* (Fig. 1, top).

A relatively new concept is the incorporation of *biology-to-material* interactions into hydrogels, whereby biomolecules or cells trigger macroscopic transitions[22,23,25,30]. These *bioresponsive* or *biointeractive* materials contain receptors for biomolecules that, when stimulated, cause localized or bulk changes in the material properties. This is of special interest in developing autonomous systems that can detect disease markers and respond to them to repair the diseased area[25].

Three types of stimuli for bioresponsive hydrogel systems can be distinguished. First, hydrogel materials can be modified to contain small biomolecules that selectively bind to biomacromolecules, including protein receptors or antibodies. Upon binding, a macroscopic transition follows (Fig. 1, system I). Second, systems may be modified with enzyme-sensitive substrates, such as short peptides (Fig. 1, system II). Here, the initial molecular recognition event is similar to that in the first category (enzyme protein binds to substrate), but it is followed by a chemical event involving the making or breaking of bonds within the enzyme-sensitive substrate. Since enzymes are highly selective, materials can be programmed to respond to a specific enzyme by incorporation of the specific substrate (or a substrate mimic). This concept is especially attractive because the distribution of enzymes can differ between healthy and diseased cells, between different cell types, and during cellular migration, differentiation, and cell division[31,32]. Third, systems may have biomacromolecules, such as enzymes, incorporated into their structures that recognize small biomolecules (Fig. 1, system III). Enzymatic conversion of these biomolecules into
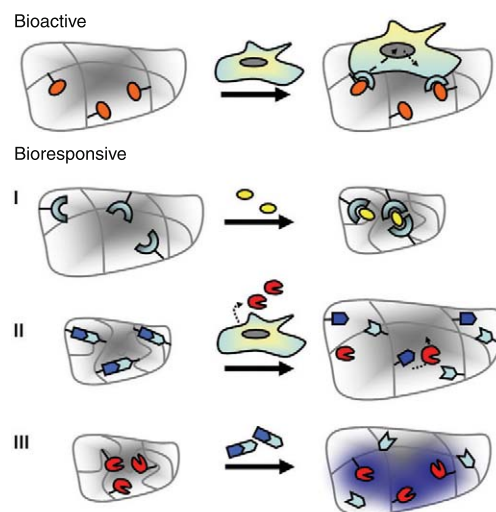

Bioactive

Bioresponsive

I

II

III

Fig. 1 Bioactive hydrogel (top) and three different types of bioresponsive hydrogel (bottom) that change properties in response to (i) small molecules via receptor/ligand interactions; (ii) (cell-secreted) enzymes via cleavable linkers; and (iii) small molecules that are converted by immobilized enzymes. The macroscopic response (swelling/collapse of the hydrogel) is shown.

molecules with different physical properties (e.g. an acid or basic compound) then triggers hydrogel swelling or collapse.

Bioresponsive hydrogels for drug delivery

Much work on bioresponsive hydrogels for drug delivery relates to the release of insulin in response to raised blood sugar levels as a potential autonomous treatment of insulin-dependant diabetes[33-35]. In one approach, glucose oxidase molecules are immobilized onto a basic polymeric carrier[34]. Following the enzyme reaction that converts glucose to gluconic acid, thereby temporarily lowering the pH, the basic groups on the polymer are protonated, inducing swelling and enhancing the release profile of insulin (Table 1, entry 1)[33,34]. This system works as a feedback loop: upon release of insulin the sugar levels drop, resulting in a pH increase that stops the release of further insulin.

More recently, glucose oxidase has been employed in an oxidation-state responsive system based on polysulfide nanoparticles modified with biocompatible polymer chains known as pluronics (block copolymers of hydroxyl-terminated propylene and ethylene oxides). Enzyme action triggers the oxidation of sulfides in the presence of glucose, causing nanoparticle swelling and eventually the release of encapsulated molecules in a manner proportional to the extent of oxidation (Table 1, entry 2)[35]. This type of response is of importance in targeted drug delivery to inflammation sites and certain tumors where enhanced concentrations of oxidizing species are found[24,36].

An important aim in targeted drug delivery is to develop systems that carry a drug to the site where it is needed before releasing it. This approach[37], originally suggested in 1975, allows for maximum drug absorption at the disease site while simultaneously minimizing premature metabolism and excessive background levels of drugs that

Table 1 Recent developments in bioresponsive hydrogels for controlled release.

Entry	Hydrogel	Stimulus	Mode of release
1	Ethylene/vinyl acetate[29,41]	Glucose	Enhanced solubility
2	Polysulfide/pluronic[30]	Glucose	Oxidation induced swelling
3	Cyclohexane trisamide-based[42]	Temperature or pH, and enzyme	Gel to solution phase transition
4	4-hydroxymandelic acid based[43]	Enzyme	Enzyme-responsive linkers
5	Disulfide-based triblock copolymer[44]	Oligopeptide (glutathione)	Gel degradation
6	Poly(acrylamide)[45]	Enzyme	Gel dissolution
7	Peptide-based[46]	Enzyme	Matrix degradation
8	Dextran-graft-poly(NIPAm)[47]	Temperature and enzyme	Gel degradation
9	Poly(ethyleneglycol acrylamide)[48,49]	Enzyme	Controlled hydrogel swelling

may be toxic. Emerging therapies for site-specific release exploit the catalytic action of disease-specific enzymes to trigger drug release from polymeric prodrug carriers. Prodrugs are inactive precursors of drug molecules that are activated *in vivo*, usually through enzymatic hydrolysis. For example, a cancer-specific enzyme secreted by tumor cells can be used to trigger the release of a therapeutic agent to prevent or reduce metastasis (targeted chemotherapy). This objective may be achieved by immobilizing drug molecules linked to a polymeric backbone (such as polyethylene glycol, or PEG) via enzyme-cleavable linkers[30,38,39]. In addition, several strategies have been devised that use a range of stimuli-responsive molecular and polymeric carrier molecules[17,18,40]. In some systems, both of these concepts are combined, i.e. exploiting biological events as triggers to induce macroscopic transitions in hydrogel materials that release the payload.

A procedure for two-step mediated drug release has been developed[42]. Here, a drug mimic is incorporated into gel fibers where the enzyme cannot act because of limited access to enzyme-cleavable linkers within the fibrillar network. Upon application of temperature or pH, the fibers dissociate, greatly accelerating the rate of hydrolysis and consequent release of the drug mimic (Table 1, entry 3).

Lee *et al.*[43] have demonstrated a carrier-drug conjugate that is cleaved by penicillin G amidase (PGA) from *E. coli* cells containing the PGA gene. The release of the drug molecule and a fluorescent probe is observed when the carrier is incubated in *E. coli* containing the PGA gene. Release is not observed when the PGA gene is absent. The carrier lowers the levels of drug required to kill the bacteria in addition to providing a route for targeted combination therapy (Table 1, entry 4).

In addition to the conjugate and prodrug methods where drug molecules are chemically incorporated into the hydrogel, there are examples of drug delivery via enzyme-triggered gel dissolution. Such systems deliver physically entrapped guest molecules, held freely within the carrier, and do not require chemical modification for targeted delivery. Using dissolving gels allows a variable volume of drug to be released that is no longer directly linked to enzyme catalysis. Instead, the pharmacokinetics of the system can be tuned by polymer design. Li *et al.*[44] have produced a biochemically and stimulus responsive triblock copolymer. The polymer forms a micellar, dithiol cross-linked *N*-isopropylacrylamide (NIPAm) gel at 37°C that can be degraded by glutathione via cleavage of a central disulfide bond (Fig. 2). Thus, the system offers the possibility of payload release from a loaded gel following glutathione-stimulated degradation (Table 1, entry 5).

Plunkett *et al.*[45] have developed a protocol to synthesize hydrogels with cross-links composed of different enzyme-cleavable peptides. Chymotrypsin hydrolyzes a Cys-Tyr-Lys-Cys tetrapeptide cross-link, causing degradation of the gel, but has no effect on a Cys-Ser-Lys-Cys cross-link (Table 1, entry 6). Law *et al.*[46] have reported nontoxic peptide-based matrices that degrade following enzyme reaction. The core peptide sequence consists of a protease-cleavable region flanked by two self-assembly motifs. Successful enzyme cleavage results in
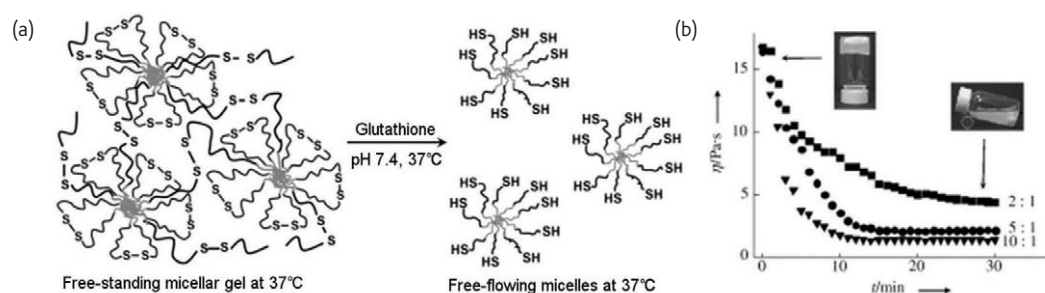

Fig. 2 (a) Biochemical degradation of a micellar, dithiol cross-linked NIPAm gel in the presence of glutathione. (b) Viscometry data showing the transition between these phases. (Adapted and reprinted with permission from[44]. © 2006 Wiley-VCH.)

drug release; however, the extent of cleavage is limited by the degree of crosslinker required to form a suitable gel (Table 1, entry 7).

Kumashiro et al.[47] have proposed a delivery mechanism based upon both a temperature range and enzyme activity. The group synthesized temperature-responsive hydrogels that only allow enzyme-triggered polymer degradation above a lower critical solution temperature and below a higher critical solution temperature. They anticipate that this technique will allow the release of drug molecules depending on both enzyme selectivity and changes in body temperature (Table 1, entry 8).

We have developed a nondissolving, enzyme-responsive hydrogel with physically entrapped guest molecules. Macromolecule release is determined by charge-induced hydrogel swelling, which is controlled enzymatically (Fig. 3, Table 1, entry 9). A cleavable peptide chain is modified to respond to a particular protease. Our studies detail the release of 40 kDa dextran and avidin from Asp-Ala-Ala-Arg modified gel particles following hydrolysis by thermolysin, a bacterial protease (Fig. 3b)[48]. Previously, we demonstrated that the accessibility of poly(ethyleneglycol acrylamide) particles can be controlled by varying the extent of charge present. Enzymatic hydrolysis to remove positive charge from the hydrogel results in structural collapse, reducing molecular accessibility[49]. This approach may have applications in the selective removal of (toxic) macromolecules in biological contexts.

Future work in this area is likely to focus on further optimization of the biocompatibility of polymer systems, their delivery (including intracellular delivery), and systems with multiple response modes.

Bioresponsive hydrogels for sensing

Biosensors are an obvious and increasingly important application of bioresponsive hydrogels. In these systems, a biological recognition event is coupled to a macroscopically observable change in hydrogel properties. Specifically in biosensing applications, it is convenient

that many hydrogels can be readily micro- or nanopatterned to allow the development of lab-on-a-chip devices. Hydrogel-based biosensor surfaces are frequently based on PEG, which prevents nonspecific adsorption of biomolecules. In cell-responsive sensors, this approach ensures that the response is governed by the surface chemistry rather than an adsorbed protein layer.

Holtz and Asher[50] have developed a hydrogel-based photonic crystal that acts as a glucose sensor for patients with *diabetes mellitus*. Glucose oxidase is attached to arrays of polystyrene nanospheres, which are then polymerized within a hydrogel matrix. The resulting material reversibly swells in the presence of glucose (Table 2, entries 1, 2), similar to the glucose-responsive systems described earlier. The swelling event increases the mean separation between the immobilized nanospheres, shifting the Bragg peak of diffracted light to longer wavelengths and producing a red-shift in the optical properties (i.e. a readily observed color change) of the polymer (Fig. 4). This system can be implanted as contact lenses or ocular inserts to detect small changes in blood glucose levels indirectly via tear fluid. In this modified system, boronic acid derivatives are attached to the array and polymerized within a network of polyacrylamide-PEG. Glucose binds to the derivatives, producing cross-links that shrink the hydrogel and cause a blue-shift. The patient is then able to determine their blood glucose levels via a color chart (Table 2, entry 1)[51-53].

Another sensor with an optical output signal uses microlenses made of poly(*N*-isopropylacrylamide-*co*-acrylic acid), or pNIPAm-*co*-AAc[54]. The pNIPAm-*co*-AAc microlenses are functionalized with biotin to detect avidin and antibiotin antibodies. Binding of these multivalent proteins to surface-bound biotin causes additional cross-links to form in the gel and increases the local refractive index of the hydrogel. The change in optical properties of the gels can be measured qualitatively: first by the appearance of 'dark rings' in the lenses and second by using the lenses to focus a square image; the higher the concentration of avidin or antibiotin, the larger the increase in refractive index and the more focused the image (Fig. 5; Table 2, entry 2).

Kim et al.[55] recently reported an example of a whole-cell sensing system using interactions between lymphocytes of the immune system.
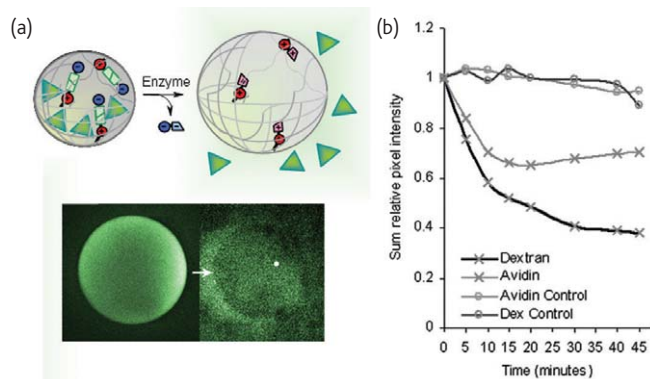

Fig. 3 (a) Schematic of enzyme-controlled hydrogel swelling (top). Creation of a positively charged gel results in increased bead swelling. The hydrogel is loaded by altering the pH and, after neutralization, payload release occurs following a specific enzyme reaction (bottom). (b) Charged (avidin) and neutral (dextran) macromolecule release profiles in the presence of a cleaving and a noncleaving control enzyme. (Adapted and reprinted with permission from[48]. © 2007 Wiley-VCH.)

Table 2 Recent developments in hydrogel-based biosensors.

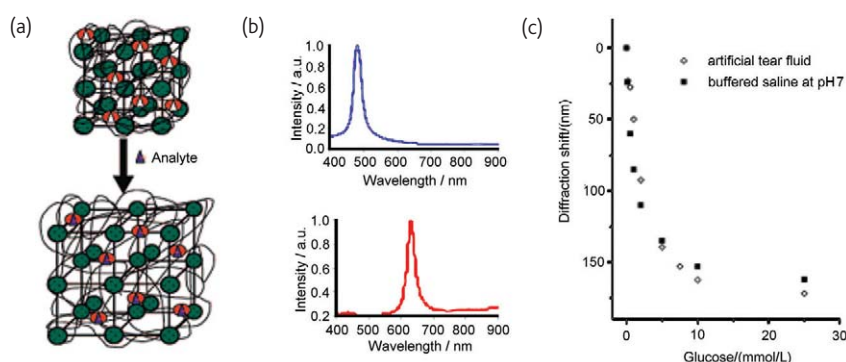| Entry	Stimulus	Hydrogel	Application	Output signal
1	Glucose[50-53]	PA-PEG	Glucose biosensor	Optical, color
2	Protein[54]	pNIPAm-coAAc	Avidin, antibiotin biosensor	Optical, focusing
3	Peptide[55]	PEG	Live cell biosensor	Biochemical, fluorescence
4	Enzyme[58]	Aromatic hydrogelator	β-lactamase	Gel-formation

Fig. 4 (a) Polystyrene particles arrayed within a three-dimensional hydrogel matrix. (b) Upon exposure to an analyte, the array changes in volume, causing a change in separation between the particles and a shift in the observed wavelength. (c) Diffraction shifts of up to ~170 nm are observed in response to changes in glucose concentration. (Reprinted with permission from[52]. © 2004 American Association for Clinical Chemistry.)

PEG hydrogel microwells are functionalized with antibodies that allow the specific immobilization of T-cells in a regular pattern at the surface. Antigen-capturing B-cells are overlaid on top, and act as receptors for target molecules. Upon addition of a solution containing a model peptide analyte, B-cells capture and process the peptide molecules, presenting them to neighboring T-cells. Receptors on the T-cells recognize the presented antigen, causing a biochemical pathway to be triggered. Such activation of T-cells can be detected by fluorescent monitoring of intracellular Ca levels (Fig. 6; Table 2, entry 3).

The use of small-molecule hydrogels for enzyme (inhibitor) sensing has been demonstrated by Yang et al.[10,56,57]. Hydrogel formation is exploited in the biological sensing of β-lactamases, bacterial enzymes that cause antibiotic inactivation in resistant bacterial strains (Table 2, entry 4). In this work, treating a nongelling, β-lactam-containing conjugate (β-lactam is a substrate for β-lactamases) with β-lactamase cleaves the scissile β-lactam amide bond, thereby releasing a potent hydrogelator. Gel formation is readily observed by the naked eye. This approach provides a low cost and easy to use method that could be used to screen for inhibitors of this class of enzymes, which holds promise for identification of next generation antibiotics[58].

Bioresponsive hydrogels in tissue engineering

Tissue engineering aims to regenerate damaged or diseased tissues and organs[59]. The development of biomaterials that facilitate the mechanical and cellular regeneration of tissue is crucial to its success. Current strategies involve the production of porous scaffolds for cells to colonize. Ideal scaffolds are those that mimic the ECM that surrounds cells in their natural context. The current emphasis is on creating materials that are highly hydrated, nanofibrous, directional, of appropriate mechanical strength, and contain bioactive signals to direct cell behavior[23,25,34]. Here, we cover recent research on materials that respond to (cell-secreted) enzymes or are modified by enzymatic action, thereby mimicking the adaptive properties of natural ECMs.

ECM-mimicking hydrogel scaffolds that permit cell migration have been studied by Hubbell and coworkers[22,60]. The researchers use oligopeptides as cross-linkers in PEG-based hydrogels. The peptide sequences are cleavable by matrix metalloproteinases (MMPs) to form a gel into which cells can infiltrate. MMPs are a family of enzymes that have many roles including the breakdown of ECM molecules during tissue remodeling and disease. Therefore, the integration of MMP-cleavable sites is a logical approach toward ECM mimics. Human
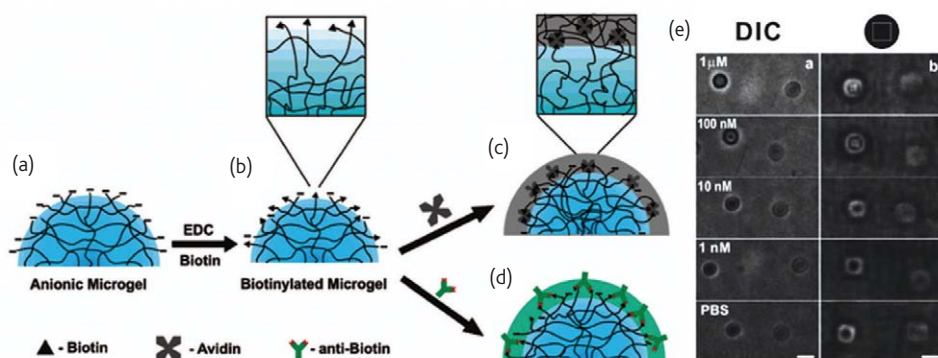

Fig. 5 (a) Synthesis of pNIPAm-co-AAc hydrogel microparticles by precipitation polymerization, and (b) their biotinylation. Binding of (c) avidin or (d) antibiotin causes additional cross-links to be formed in the hydrogel. (e) Formation of dark rings (left column) in different concentrations of avidin and the focusing of a square image (right column) in response to avidin binding. (Reprinted with permission from[54]. © 2005 American Chemical Society.)
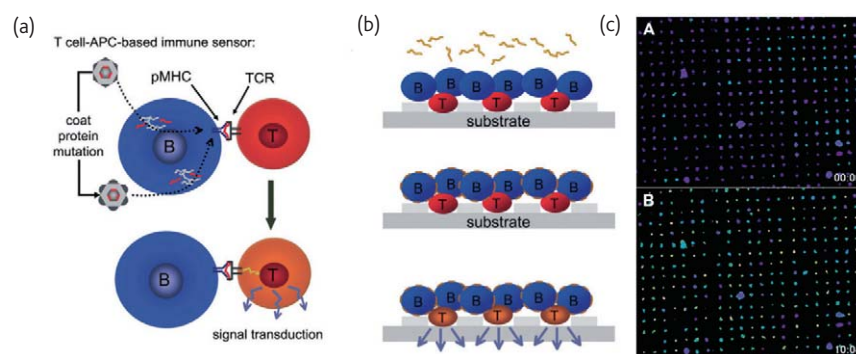
Fig. 6 (a) B-cells capture pathogens, such as the different viral particles shown, and present them as peptide-major histocompatibility complex (p-MHC) assemblies. T-cell receptors (TCRs) on T-lymphocytes recognize p-MHCs, causing a signaling pathway response. (b) This interaction is employed in a sensing system. (c) The T-cell signaling pathway can be detected by fluorescence. (A) A fluorescence micrograph of microwells immediately after pathogen addition. (B) The microwells after 10 min exposure to the pathogen. (Reprinted with permission from[55]. © 2006 Wiley-VCH.)

fibroblasts are encouraged to invade the hydrogel through integrin-binding domains (Arg-Gly-Asp-Ser-Pro) that are incorporated via PEG linkers. The fibroblasts then cause a local breakdown of the hydrogel cross-links via secreted MMPs (Table 3, entry 1). The potential for bone tissue engineering was tested by loading the gel with bone morphogenetic protein-2 (BMP-2), which is known to be involved in bone formation. An assessment of the degradation behavior of MMPs and the cell invasion of provisional matrices revealed that the healing response *in vivo* depends on the enzymatic sensitivity of the matrix.

Raeber *et al.*[61] (Table 3, entry 2) subsequently tested the suitability of two proteolytically degradable PEG hydrogels as ECM mimics that allow regulated cell migration in three dimensions (Fig. 7). In one system, the PEG hydrogel is chemically cross-linked with an MMP-sensitive sequence (M-PEG). In the second, a plasmin-sensitive sequence (P-PEG) is used. Both sequences have been previously found to allow proteolytic remodeling of bone defects. The three-dimensional migration patterns and cell morphology of human fibroblasts in M-PEG and P-PEG have noticeable differences, with cell migration

only observed in M-PEG. To regulate the MMP function of the PEG hydrogels, two new hydrogels were made, one containing an MMP inhibitor and the other an MMP stimulator linked to the PEG hydrogel. The former results in complete suppression of cell migration, while the latter results in a significant increase in cell migration. These results indicate that migration in M-PEG gels is highly sensitive to MMP modulation. The ability of a gel to respond to a single class of enzyme represents an effective communication between cells and the matrices.

Kim *et al.*[62] have created an injectable hydrogel of pNIPAm-*co*-AAc to mimic the ECM (Table 3, entry 3). These hydrogels are prepared by cross-linking an MMP-13/collagenase-3-degradable peptide sequence and NIPAm in the presence of Arg-Gly-Asp-modified poly(AAc). The proteolytic degradation and cell adhesion properties of this hydrogel were studied using rat calvarial osteoblasts. Collagenase was found to degrade the hydrogel, with the rate dependent on the concentration of collagenase in relation to the poly(AAc) chain. Migration of osteoblasts is observed in hydrogels both with and without the Arg-Gly-Asp peptide. However, greater migration is seen in those hydrogels

Table 3 Enzyme-responsive gels for tissue regeneration and culture.

| Entry	Material	Stimulus	Cell type
1	Oligopeptides Ac-CGYGRGDSPG[60]	Metalloproteinase (MMP)	Human fibroblasts
2	Polyethylene glycol (PEG)[61]	MMP	Dermal fibroblasts
3	pNIPAm-Co-AAc[62]	MMP-13	Rat calvarial osteoblasts (RCOs)
4	Gelatin/gellan[65]	TGase	Fibroblasts NIH T3 cells
5	Gelatin[66]	mTGase	Retinal tissue
6	Three-dimensional fibrin hydrogels modified with $\alpha_V\beta_3$ receptor[68]	TGase	Human umbilical cells
7	$CH_3(CH_2)_{14}CO$-GTAGLIGQRGDS[69]	MMP-2	Dental pulp cells
8	Nap-FFGEY[70]	(i) kinase (ii) phosphatase	HeLa cells
9	Polyurethane/polycaprolactone/PEG[73]	Elastase	Endothelial cells
10	Phosphoester/PEG[72]	Alkaline phosphatase	Mesenchymal stem cells
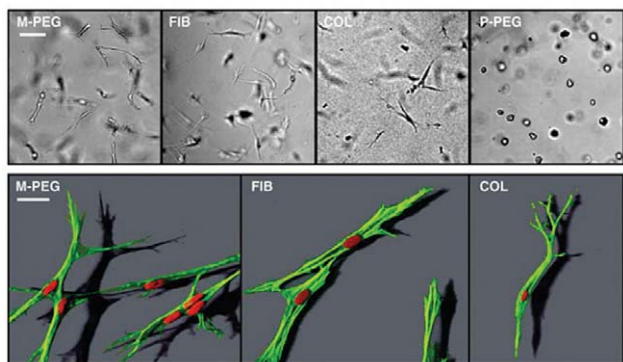
Fig. 7 M-PEG gels allow spindle-shaped cell morphologies similar to natural fibrin (FIB) and collagen (COL) gels, whereas P-PEG gels inhibit cell spreading. (Adapted and reprinted with permission from[61]. © 2005 Biophysical Society.)

that contain Arg-Gly-Asp. There is also an increase in cell migration in MMP-degradable hydrogels compared with nondegradable gels, indicating the advantage of bioresponsive hydrogels.

An alternative to man-made polymers is to use natural proteins or their by-products. One example is gelatin, which is commonly used in biomedical applications. Gelatin is a hydrolyzed form of collagen and has been suggested for tissue engineering scaffolds and wound dressings. However, cross-linking is essential for these purposes to ensure sufficient mechanical strength[63]. Transglutaminases (TGases) are a family of enzymes that catalyze the formation of isopeptide bonds between the γ-carboxamide of Gln and a free amine group (often from Lys), thereby cross-linking peptide chains[64-67]. TGase has been used as a cross-linking agent for gelatin, making it suitable for tissue-engineering applications, such as ophthalmic adhesives and scaffolds for tissue reconstruction (Table 3, entries 4 and 5).

Recently, Chen et al.[66] produced a biomimetic adhesive based on TGase-catalyzed cross-linking of gelatin. This adhesive mimics part of the blood coagulation mechanism, the factor XIIIa-mediated cross-linking of fibrin. This approach improves retinal reattachment during

eye surgery when compared with currently used materials, such as silicone oil. The group also demonstrated that the strength of the bond formed is comparable to that of other soft-tissue adhesives. This is clearly shown in Fig. 8, where a cotton swab coated in the adhesive is able to pick up bovine retinal tissue. A study into the use of gelatin for tissue-engineering scaffolds has been undertaken by Bertoni et al.[65] (Table 3, entry 4). To improve the long-term stability of this system, gelatin is integrated into a polysaccharide, gellan. The fibroblast response to the gelatin/gellan hydrogel is better than gelatin alone.

In generating new tissue, blood vessels must be formed to provide nutrients and dispose of metabolic waste, so the integration of growth factors to regulate new blood vessel formation (angiogenesis) is pivotal for cell survival. Recently, Hall and Hubbell[68] designed a three-dimensional fibrin hydrogel scaffold that acts as a depot/release system for growth factors to aid in angiogenesis (Table 3, entry 6). The fibrin matrices are modified by covalently adding receptor binding sites for the cell survival integrin $\alpha_v\beta_3$. The receptor also contains an N-terminal recognition site for a TGase. The mechanical stability of the fiber structure depends on the receptor concentration, which also activates NFκB, a signaling molecule that aids cell survival. In vivo analysis of angiogenesis reveals that a denser capillary network forms in receptor-stimulated matrices than native fibrin networks. Co-stimulation with additional growth factors gives similar results to stimulating with either the receptor or the growth factors alone.

One method of combining the advantages of synthetic and natural systems is the use of peptide amphiphiles (PAs)[4]. Jun et al.[69] described a strategy to regenerate dental tissues using PA molecules that form rigid, cell-responsive fibrous nanostructures and incorporate biological epitopes (small parts of macromolecules that are recognized by antibodies of the immune system) as the peptide part of the molecule (Table 3, entry 7). The material is designed to be adhesive to dental pulp cells and contain MMP-cleavage sites. At neutral pH, the net
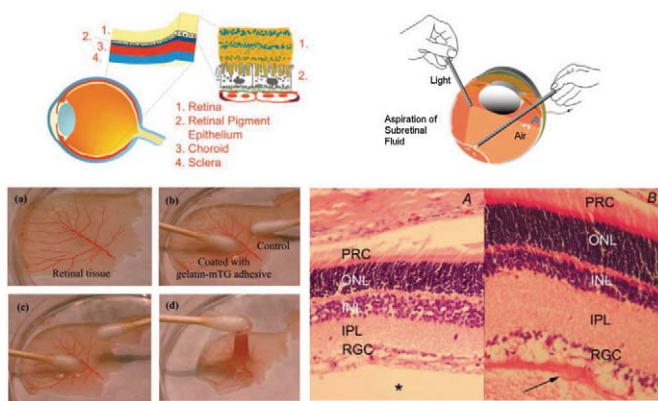

Fig. 8 (Top) Schematic of eye and retinal reattachment surgery. (Bottom left) Ability of a gelatin-microbial transglutaminase-coated swab to lift retinal tissue. (Bottom right) Rat retina 14 days after injection of gelatin solution (A), showing normal histology without damage or cell loss. Rat retina 14 days after injection of gelatine + microbial transglutaminase (B). The arrow shows acellular material that represents adhesive deposition or fibrin formation. (Adapted and reprinted with permission from[66]. © 2006 Wiley-VCH.)

negative charge on the peptide prevents assembly. Gel formation is triggered by the addition of Ca^{2+} ions, which results in nanostructured cylindrical micelles. At high concentrations, physical cross-linking occurs. The gels are broken up by adding type IV collagenase, an MMP that cleaves the peptide and causes the nanostructure to become deformed and more permissive. Pulp cells suspended in a solution prior to gelation are found to form dense colonies throughout the gel.

Enzymatic sol-to-gel transitions have been demonstrated by Yang *et al.*[70] using napthyl-pentapeptide, Nap-Phe-Phe-Gly-Glu-Tyr. The process reversibly controls self-assembly for *in vivo* hydrogelation, a strategy useful for precise control of cell delivery at the molecular level. To regulate the assembly, a pair of enzymes with complementary and opposite activities are used: a kinase, which catalyzes the adenosine triphosphate (ATP)-driven phosphorylation of Tyr residues; and a phosphatase, which catalyzes removal of phosphate groups. Tyrosines that carry phosphate groups are negatively charged and prevent self-assembly by electrostatic repulsion. Dephosphorylation of the tyrosine residues by phosphatase triggers hydrogel formation. This system is therefore highly dynamic and, with both enzymes present, the level of ATP determines the gelation state of the system (Table 3, entry 8).

We recently demonstrated the use of a protease to produce amphiphilic peptide hydrogelators that spontaneously assemble into nanofibrous gel structures[71,72]. This process, termed enzyme-assisted self-assembly, forms gelling peptides from nongelling precursors in a controlled manner. Since the enzyme reactions proceed under thermodynamic control, this method is thought to result in fewer defects in the resulting self-assembled structures, thereby inherently giving rise to homogeneous gels[10]. The approach may be used in triggered gel-formation in three-dimensional cell culture experiments, as demonstrated for similar nonenzymatically gelled systems[72].

A family of enzyme-responsive thermoplastic elastomers containing polyurethane, polycaprolactone, and PEG has been developed for soft-tissue-engineering applications[73]. These polyurethanes have enzymatic remodeling abilities via incorporation of an Ala-Ala-Lys tripeptide that is selectively hydrolyzed by elastases. To enhance cell adhesion, the peptide Arg-Gly-Asp-Ser is coupled to the hydrogel. Degradation of the hydrogels is significantly increased by addition of elastase, and endothelial cell adhesion is greater than a plastic tissue culture control (Table 3, entry 9).

A photopolymerized phosphoester-PEG hydrogel has shown potential application in bone tissue engineering[74]. The hydrogel is hydrolytically degradable, and the degradation rate increases in the presence of a bone-specific enzyme, alkaline phosphatase (Fig. 9; Table 3, entry 10). Goat mesenchymal stem cells secrete bone-specific markers, such as osteocalcin and osteonectin, and show alkaline phosphatase activity. Gene expression of key markers is promoted without addition of growth factors, compared with pure PEG-based gels. Calcium deposition in the hydrogels, i.e. mineralization, is also observed.
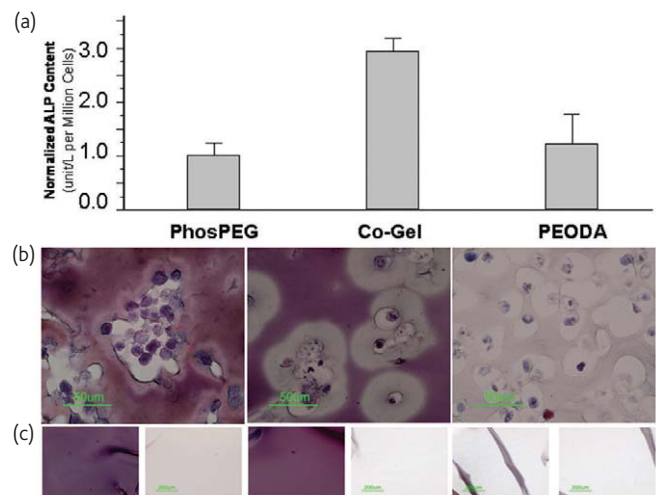

Fig. 9 (a) Normalized alkaline phosphatase (ALP) content in goat mesenchymal stem-cell-encapsulated hydrogels after three weeks of culture in osteogenic medium. Final histological phenotype stained with Masson's trichrome for cellular hydrogels (b) and acellular hydrogels (c). Acellular controls cultivated in the presence of ALP at 50 units/l in osteogenic medium for three weeks are also shown in (c). The three hydrogels are (left) 20% (w/v) Phos-PEG gels; (middle) 10% Phos-PEG, 10% PEODA co-gel; and (right) 10% PEODA gels. (Adapted and reprinted with permission from[71]. © 2005 Mary Ann Liebert Publishers.)

Since proteases play essential roles in a large number of biological processes, including wound healing and cell differentiation, it can be expected that enzyme responsiveness will remain an important concept in the future. The use of enzymes to trigger self-assembly may provide a useful means of generating cell matrices *in situ*, with new approaches being developed to explore a range of enzymes for this purpose[70,72].

Conclusion and outlook

The design of bioresponsive materials is a relatively new area but it has already demonstrated a range of successful systems for the detection of biological compounds, delivery of actives in response to disease-specific biomolecules, and on-demand presentation of bioactive ligands to direct cell behavior and repair tissue. Key areas of future research are likely to focus on the understanding and exploitation of molecular communication methods found in cell biology for biomaterials design. The ultimate aim is to design materials that direct (stem) cell behavior in wound healing and tissue regeneration. In order to aid discovery, it is likely that further miniaturization will produce hydrogel microarrays for massively parallel screening and optimization arrays. These setups will help elucidate the space-time requirements of ligand presentation, including the use of multiple ligands to induce complex responses.

Enzyme-triggered presentation of ligands will hold the key to precise control of cell fate, with ligands organized with nanoscale resolution, as observed in natural ECMs[23]. This level of control can, in principle, be achieved by self-assembly, and future biomaterials can be expected to be increasingly constructed using bottom-up approaches. Currently, self-assembly is limited to fairly simple structures, usually

composed of one or two different building blocks. Making more complex structures is difficult because molecular building blocks may get kinetically trapped. Future approaches will focus on overcoming kinetic entrapment, which may involve enzyme-assisted assembly approaches that are thermodynamically controlled, and the assembly of structures one molecule at a time[10,72].

Few of the systems described here are truly dynamic. As with all spontaneous processes, under a given set of conditions (pH, temperature, pressure) only one direction is thermodynamically favored. Nature overcomes this limitation by combining thermodynamic and kinetically controlled reactions to obtain dynamic systems. Indeed, by combining ATP-dependant kinases and phosphatases, dynamic systems have already been produced[10,32].

One major limitation of new materials like these is that the barriers to use in medical applications are significant. Especially when polymeric materials are used, it is essential to determine what happens to all the ligands/components once inside the body. Use of small-molecule hydrogels may provide a significant advantage, as their breakdown profiles are more predictable[11]. In any case, all new hydrogel materials will require extensive testing to gain approval in products, which means that it is unlikely that any of these materials will be found in the clinic in the near future. However, many of the concepts described here are likely to be incorporated in *ex vivo* applications, including three-dimensional cell culture, wound dressings, and diagnostic devices, and will increasingly touch our everyday lives. mt

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