*Review*

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# Biopolymer-based strategies in the design of smart medical devices and artificial organs

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**Abstract**

Advances in regenerative medicine and in modern biomedical therapies are fast evolving and set goals causing an upheaval in the field of materials science. This review discusses recent developments involving the use of biopolymers as smart materials, in terms of material properties and stimulus-responsive behavior, in the presence of environmental physico-chemical changes. An overview on the transformations that can be triggered in natural-based polymeric systems (sol–gel transition, polymer relaxation, cross-linking, and swelling) is presented, with specific focus on the benefits these materials can provide in biomedical applications.

**Keywords**

Biopolymers, smart materials, thermo-responsive biopolymers, pH-responsive biopolymers, chemical-responsive biopolymers, medical device design

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## Smart materials and biopolymers

Developments in artificial organs, medical devices, structures, and carriers for tissue engineering are increasingly supported by functional materials: these have the advantage of combining structural properties with a predetermined, favorable response to the environment.[1,2] Among such materials, stimulus-responsive materials have become a powerful design platform for a range of biomedical applications, from cardiovascular devices to drug delivery systems.[3–5]

Stimulus-responsive materials are functional materials in which macroscopic, reversible modifications in certain of their properties are triggered by small environmental variations.[3,6–10] The persistent interest in this class of materials is mainly due to the fact that many of the most important substances in living systems are macromolecules with structures and behaviors that respond to their surroundings[11] in an intelligent—or smart—way.

The bio-mimicking approach has thus become an effective strategy to target properties in the synthesis of new abiotic materials, by emulating smart behavior.[12–14] However, despite the immense progress that has been made, materials scientists are still far from matching nature's ability to engineer smart synthetic polymers, in terms of structure, versatility, and adaptability.[15] Furthermore, the biological origin offers several interesting features, including the possibility of enzymatic degradation, metabolic removal of by-products, or the presence of cell-instructive sequences.

A variety of stimulus-responsive materials can be found in nature, and different biopolymers exhibit smart behavior and show a significant change in one property upon an external trigger. An in-depth understanding of the mechanisms underlying their behavior provides the basis for mimicking their properties in synthetic systems and offers

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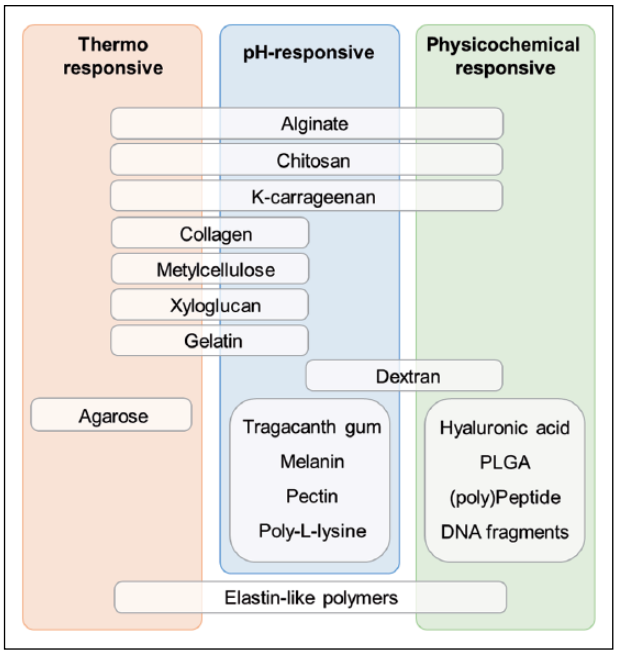


**Figure 1.** Activating stimuli and macroscopic response in biopolymers currently under investigation for biomedical applications.

a powerful tool for the development of advanced, more effective smart materials.[16]

The review examines the main classes of biopolymers employed as functional materials in the design of advanced medical solutions and artificial organs based on their smart responses and gives some representative examples to elucidate the advantages of their application; its scope is restricted to biopolymers whose smart response ability derives from their chemical structure (e.g. specific functionalities or sequences in polymeric backbone), since those whose smart behavior derives from grafting synthetic molecules were recently and comprehensively reviewed elsewhere. Indeed, an excellent recent review has already reported the state-of-the-art of possible modifications of biomacromolecules achieved by grafting synthetic stimulus-responsive macromolecules.[15] This class of smart hybrid materials is based on advanced synthesis routes, generally resulting in materials that combine well-controlled structures and multiple functionalities.

### *A useful classification*

Despite the ever-increasing use of adjectives associated with materials—smart, intelligent, adaptive—it is generally agreed that no clear, widely accepted definition of these terms exists.[17] A starting point toward a general definition might be the identification of smart materials as functional materials capable of (1) sensing a specific environmental stimulus, (2) responding in a predetermined way, and (3) returning to their original state when the stimulus is removed.[4] However, smart materials may also be defined as structural materials that inherently contain actuating, sensing, and controlling capabilities built into their microstructure.[18]

In this context, it is important to clarify that biopolymers inherently possess a strictly nonlinear response to external stimuli. The understanding of the mechanism of cooperative interactions involved in this response opened the floodgates to attempts at mimicking them in synthetic systems.[16] However, it is only under specific conditions that biopolymers can be efficiently and effectively used to design biomedical solutions, encompassing smart behavior. It is thus important to base the understanding of the use of biopolymers as smart materials upon a practical classification. Different approaches have been proposed, based on the class of material (alloy, polymer, ceramic),[19] its physical form,[20] the activating stimuli or modes of polymer response (thermal, electromagnetic, chemical),[20–22] the response to the stimulus (shape, permeability, elastic modulus modifications),[20,23] or even on the material's possible applications.[24]

The choice depends on background, field of application, and more, in general, on the aims of the review. However, vague boundaries, and the superimposition of properties and applications, make it particularly problematic to define categories and reach a general, comprehensive, and well-defined classification in this field. Materials-based classifications, in particular, do not alone suffice and require further classification. Moreover, they are rather an approximate classification method and are also affected by the limit of being, to some extent, more descriptive for material scientists.

Given the above considerations, the following paragraphs will present functional biopolymers for biomedical applications based on the activating stimulus, also giving selected examples of their potential use and the advantages deriving from it (Figure 1). Despite the drawback that the same material can in a number of cases respond to different stimuli, and that these can be combined to modulate their response, this classification appears to be the most practical approach when it comes to gathering information useful to support the development of novel medical devices.

## Thermo-responsive biopolymers

Thermo-responsive polymers can respond to an external gradient of temperature with a significant variation of some of their macroscopic properties (Figure 2): when the biopolymers' transition temperature is in the proximity of the envisaged application, they can be used as smart systems.[25,26] Thermo-responsive polymers have attracted particular attention in recent years because their properties can be tuned by modifying molecular parameters and their transformation processes can be optimized.[27]

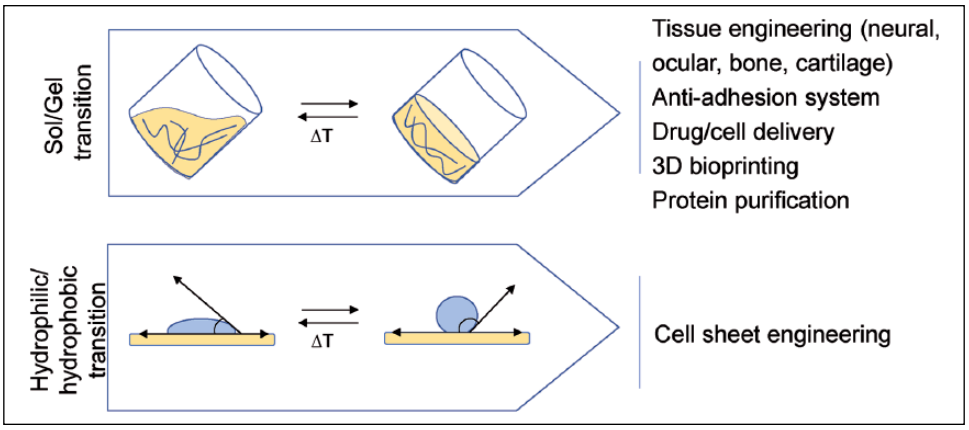


**Figure 2.** Examples of smart responses of natural polymers to temperature changes: sol–gel transition and hydrophilic/ hydrophobic transition.

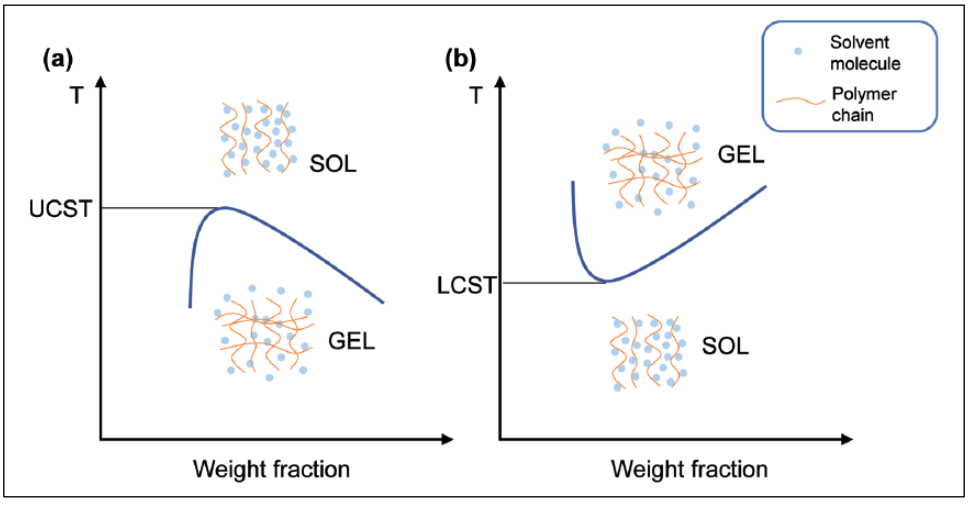


**Figure 3.** Thermo-responsive hydrogels: (a) UCST hydrogels undergo sol–gel transition as the temperature decreases and (b) LCST hydrogels undergo sol–gel transition as the temperature rises. The blue lines indicate the phase separation boundary, corresponding to the solution cloud point.

Thermo-responsive biopolymers are in large part hydrogels, in which gelation, degree of swelling, and water affinity can be guided by an external temperature change (Figure 2).[28] These hydrogels can be further divided into two main groups: (1) upper critical solution temperature (UCST) hydrogels and (2) lower critical solution temperature (LCST) hydrogels. UCST hydrogels are mainly composed of hydrophilic groups, and their ability to swell into a suitable solvent increases with temperature. Below a critical temperature (UCST), the polymer matrix undergoes a contraction and collapses (Figure 3(a)). As a consequence, they are in a gel state at temperatures below the UCST. Among natural polymers, gelatin and agarose belong to this group.[28–33]

Conversely, LCST-type hydrogels are composed of both hydrophilic and hydrophobic groups and also undergo temperature-dependent sol–gel transitions. When the temperature decreases below the LCST, the gel becomes a highly viscous liquid (Figure 3(b)). Typical biopolymers exhibiting an LCST are some cellulose derivatives[28–33] (Table 1).

## *Sol–gel transition*

*In situ–forming hydrogels.* Thermo-responsive hydrogels possessing a sol–gel transition are extensively proposed in the biomedical field as in situ–forming injectable gels (Table 2). These materials offer a fascinating alternative to the implantation of medical devices because they can be directly injected into the target site by a minimally invasive surgical approach. For this purpose, the transition temperature should be set so as to have a viscous liquid (sol) at room temperature, to allow mixing (with cells or macromolecules) and injection. When it reaches body

**Table 1.** Main natural-derived thermo-responsive polymers.

| Biopolymer | Hydrogel type |
|---|---|
| Methylcellulose (MC) | LCST |
| Chitosan-β-glycerophosphate | |
| Xyloglucan | |
| Matrigel | |
| Elastin-like polymers | |
| Gelatin | UCST |
| Collagen | |
| Agarose | |
| Kappa carrageenan | |

LCST: lower critical solution temperature; UCST: upper critical solution temperature. The smart biopolymers are classified for their driving stimulus: LCST or UCST evidence the transition between the sol and gel state.

temperature, the material turns to a gel, thus adapting to the shape of the defect and ensuring the gel's permanence at the implantation site.[28,29,33–36] In the case of polymeric blends, the same temperature restriction must be respected in order to set the gelation temperature close to 37 °C. The effect of a single polymer on the gelling temperature of the final blend can be inferred from Table 1. By choosing the appropriate polymer and concentration, the gelation temperature can be set as desired (e.g. 37 °C).

Methylcellulose (MC) is a water-soluble polymer derived from cellulose, obtained by the partial substitution of hydrophilic hydroxyl groups with hydrophobic methoxy groups.[116,117,124] MC is an LCST hydrogel, and its main advantage is its ability to form a gel at physiological temperature, whereas it is in a sol state at a lower temperature. MC sol–gel transition has been investigated in depth in order to develop MC-based hydrogels for tissue engineering and regenerative medicine applications (see section "CSE").

A number of parameters affect the swelling and degradation of MC-based hydrogels, primarily polymer concentration: higher swelling rates are generally observed for more concentrated hydrogels. For instance, 14% and 8% w/v MC-based hydrogels swell by up to 260%–300%,[47,118] while a 4% w/v MC-based hydrogel at most swells by 200%.[118,119] Moreover, more highly concentrated hydrogels exhibit an immediate weight loss after cell culture media are added, followed by a degradation profile, leveling off over a relatively long period (10–30 days). Conversely, less concentrated hydrogels display significant degradation and instability, with the loss of small portions of hydrogel coating from the test surface.

Due to these distinctive and favorable properties, MC has found applications in a wide range of regenerative applications. In particular, MC hydrogels have been investigated as in situ cells and drug carriers to promote tissue neo-formation. For example, in peripheral nerve regeneration, a 2% MC gel was found to be a suitable candidate for treating gap injuries in nerve guidance channels,[37] acting as a vehicle for growth and neurotrophic factors. Compared to other matrices examined (soluble collagen and laminin (LN)–containing extracellular matrix (ECM)-derived gel), MC gel was shown to be a superior matrix both in terms of effective nerve regeneration and flexibility in formulation. In the central nervous system, Tate et al.[38] selected MC as an eligible material for the development of an in situ–gelling hydrogel for treating brain injuries.

However, MC is a relatively non-bioactive hydrogel and displays limited protein adsorption and low cell adhesion.[38] For this reason, a number of attempts have been made to create a bioactive scaffold, by means of surface or bulk modification of MC with biological macromolecules. For neural tissue engineering, Stabenfeldt et al.[39] evaluated the effect of tethering LN, an ECM protein critical to neuronal cell activities, on either oxidized MC (OXMC) or non-oxidized MC. LN-functionalized oxidized MC (OXMC-LN) hydrogel was found to promote neuronal cell adhesion and supported higher levels of cell survival compared to MC, OXMC, or LN-functionalized MC (MC-LN). Interestingly, no significant difference was found between MC, OXMC, and OXMC-LN in terms of rheological parameters after samples were allowed to equilibrate at 37°C.

Similarly, Kim et al.[40] recently presented an injectable hydrogel constructed from adipose tissue–derived soluble extracellular matrix (sECM) and MC, for treating vocal fold paralysis. Introduction of biological cues into the hydrogel was found to have a positive effect, as the sECM/MC hydrogel enhanced vocal function in paralyzed vocal folds without undergoing early resorption and sustained vocal fold augmentation and symmetric vocal fold vibration in the rabbit larynx.

When MC is blended with hyaluronan (HA-MC), not only can the biocompatibility of the scaffold be enhanced but the thermo-responsive behavior can also be controlled.[46] Thermo-responsive hydrogel HA-MC blends have, for example, shown favorable results as injectable cell carriers in retinal degenerative disease. HA-MC was shown to be a promising vehicle for delivering retinal stem progenitor cells (RSPCs), since cell integration was observed in in vivo studies.[46] Thanks to their fast in situ gelation and tunable degradability, HA-MC blends have also been evaluated as potential drug delivery carrier material for spinal cord injuries.[42,43] By acting on the molecular weight of MC, the stability of blends can be varied over a relatively large range. The blend investigated in Gupta et al. (2% wt hyaluronan and 7% wt MC), for example, showed degradation in vivo within 4–7 days, while a similar formulation with higher molecular weight blend composed of HA and MC (HMW HA-MC) provided stability for more than 28 days in vitro.[48]

MC has recently been investigated as a cell carrier (8% w/v MC in 0.05 M $Na_2SO_4$) to seed cells in a porous polyurethane (PU) scaffold, to differentiate bone

**Table 2.** Biomedical applications of biopolymers activated by a thermal stimulus.

| Smart response | Biopolymer | Blend | Application | Reference |
|---|---|---|---|---|
| Sol–gel transition | MC | GFs | Nerve gap injuries | 37 |
| | | – | Brain injures treatment | 38 |
| | | LN | Neural tissue engineering | 39 |
| | | sECM | Vocal fold paralysis treatment | 40 |
| | | PEG, CMC, chitosan sulfate | Postsurgical anti-adhesion system | 41 |
| | | HA | Spinal cord injuries treatment | 42, 43 |
| | | – | 3D bioprinting | 44 |
| | | Alginate | 3D bioprinting | 45 |
| | | HA | Retinal degenerative disease treatment | 46 |
| | | PU scaffold | In vitro chondrogenesis of BMSCs | 47 |
| | HMW MC | HA, drugs | Spinal cord injuries treatment | 48 |
| | Hydroxypropyl cellulose (HPC) | Alginate | Controlled release of heparin | 49 |
| | Carboxymethyl cellulose (CMC) | Chitosan | Cell delivery (chondrocytes) | 50 |
| | Metolose® | – | Transdermal therapeutic system | 51 |
| | Chitosan | Glycerol phosphate salts | Cell delivery (chondrocytes) | 52 |
| | | Glycerol phosphate salts | Drug delivery | 53 |
| | | Glycerol phosphate salts, liposomes | Sustained drug delivery | 54 |
| | | GP | Cell delivery (rat BMSCs) | 55 |
| | | GP (+PEG) | Nasal drug delivery | 56 |
| | | GP, blood | Cartilage repair | 57, 58 |
| | | GP, demineralized bone matrix (DBM) | Bone tissue regeneration | 59 |
| | | GP, bioactive glass nanoparticles | Bone tissue regeneration | 60 |
| | | β-GP, collagen type I, bioactive glass | Bone tissue regeneration | 61 |
| | | β-GP, starch | ADSCs differentiation into chondrocyte-like cells | 62 |
| | | β-GP, HA, chondroitin-6-sulfate, collagen type II, gelatin, silk fibroin | Intervertebral disk regeneration | 63 |
| | | β-GP, gelatin | Nucleus pulposus regeneration | 64 |
| | | β-GP, poly-D-lysine (PDL) | Neural tissue engineering | 65 |
| | HTCC | PEG, α-β-GP | Nasal drug delivery system | 66 |
| | Hydroxybutyl chitosan (HBC) | – | Intervertebral disk regeneration | 67 |
| | Carboxymethyl-hexanoyl chitosan (CHC) | – | Corneal tissue regeneration | 68 |
| | Xyloglucan | – | Intraperitoneal drug delivery | 69 |
| | | – | Rectal drug delivery | 70 |
| | | – | Oral drug delivery | 71–73 |
| | | – | Ocular drug delivery | 74 |
| | | – | Percutaneous drug delivery | 75 |
| | | – | Nasal drug delivery | 76, 77 |
| | | Poly-D-lysine | Neural tissue engineering | 78, 79 |
| | Gelatin | MPEG-PDLLA | Drug delivery | 80 |
| | | Silk fibroin | Drug delivery | 81 |
| | | Dex-GMA | Drug delivery | 82–85 |
| | | Alginate | Control of porosity | 86, 87 |
| | | Agar | Drug delivery | 88 |

*(Continued)*

**Table 2.** (Continued)

| Smart response | Biopolymer | Blend | Application | Reference |
|---|---|---|---|---|
| | | – | 3D bioprinting: vascularization, cartilage TE, cell patterning, sacrificial material | 29, 89–100 |
| | Agarose | PLGA nanoparticles | Sustained drug delivery to spinal cord tissue | 101 |
| | | – | 3D bioprinting: bone tissue engineering, sacrificial material | 44, 89, 94, 97, 102, 103 |
| | Elastin-like polypeptides (ELPs) | – | Drug targeting via local hyperthermia | 104–108 |
| | | – | Protein purification | 109 |
| | | CaP | In vitro mineralization model | 110, 111 |
| | K-carrageenan | | Nanoparticles for controlled drug delivery | 112 |
| | Matrigel | Liposomes | Local delivery of antitumor drugs | 113 |
| | | – | 3D bioprinting: bone TE, liver TE | 44, 114, 115 |
| | Collagen | | 3D bioprinting: wound healing and cartilage TE | 89, 94, 140–145 |
| Hydrophilic/ hydrophobic transition | MC | – | Cell sheet engineering: HFF, ASC, L929 sheets | 116–119 |
| | | – | Myocardial tissue regeneration: MSC and hAFSC sheets fragmentation | 120–122 |
| | Xyloglucan hydrogel | RGD sequence | Cell sheet engineering: A375 cells | 123 |

MC: methylcellulose; LN: laminin; sECM: soluble extracellular matrix; PEG: poly(ethylene glycol); BMSCs: bone marrow–derived mesenchymal stem cells; HA: hyaluronan; 3D: three-dimensional; PU: polyurethane; HMW: high molecular weight; ADSCs: adipose-derived stem cells; HTCC: *N*-[(2-hydroxy-3-trimethylammonium) propyl] chitosan chloride; MPEG-PDLLA: monomethoxy poly(ethylene glycol)-poly(D,L-lactide); PLGA: poly(lactic-co-glycolic acid); CaP: calcium phosphate; HFF: human foreskin fibroblast; ASC: adipose stem cell; hAFSC: human amniotic fluid–derived stem cell.

marrow–derived mesenchymal stem cells (BMSCs). BMSCs were loaded into the MC solution, which was injected into the PU scaffold to allow MC sol–gel transition. A chondrogenetic effect was successfully achieved by means of mechanical conditioning of the cell-scaffold PU-MC, confirming that MC-based hydrogels are suitable materials for cartilage tissue repair.[47]

Together with MC, other cellulose derivatives are also being studied because of their thermo-responsive behavior. The thermo-sensitive gelation of aqueous hydrophobically modified MC (HM-MC) solutions was investigated by Lee.[125] for its applicability in the pharmaceutical and biomedical fields. Aqueous solutions of ethyl(hydroxyethyl) cellulose (EHEC) also exhibit thermo-responsive behavior, and by adding ionic surfactants (e.g. sodium dodecyl sulfate, SDS), in situ–forming structures can be obtained. EHEC/surfactant systems have been proposed for the delivery of local anesthetic agents to the periodontal pocket[126] and for nasal and ophthalmic drug delivery.[127,128]

The commercially available Metolose (R), a non-ionic cellulose ether, was considered for the formulation of a thermo-responsive drug delivery system for transdermal application. Drug release can be controlled by a change in temperature (i.e. body temperature), and by regulating the salt concentration, the LCST can be set close to the skin temperature.

Chitosan is an abundant natural polymer, obtained by the partial deacetylation of chitin under alkaline conditions or by enzymatic hydrolysis.[129] Chitosan as such is not a thermo-responsive polymer. However, Chenite et al.[52] reported the use of chitosan-β-glycerophosphate (C-GP) aqueous solutions as thermo-responsive and pH-dependent gelling systems. The phosphates of the GP salt neutralize the ammonium groups of chitosan, thus increasing hydrophobic and hydrogen bonds between the chitosan chains at high temperatures and forming a more cohesive gel than that occurs at lower temperatures. The C-GP sol–gel transition was demonstrated in vivo by dorsal subcutaneous injection in adult rats. Moreover, the same hydrogel was tested for the in vivo delivery of biologically active growth factors[52] and the in vitro delivery of drugs.[53] For low-molecular-weight hydrophilic compounds, release from the C-GP hydrogels was generally complete within 24 h; conversely, sustained delivery was achieved with the addition of liposomes to the C-GP solution.[54] The injectable C-GP systems were also shown to be promising materials for cartilage[57,58] and bone regeneration,[59,60,130] as they can be implanted in a minimally invasive manner; functional matrix deposition and adhesion were demonstrated for both tissue types.

Chitosan-based hydrogels have recently been exploited in different applications for nervous system regeneration.

C-GP was found to promote in vitro neural cell adhesion (fetal mouse cortical cells, FMCCs). Neural cell survival was improved with the covalent attachment of poly-D-lysine (PDL), via azidoaniline photocoupling, to support the use of C-GP as injectable scaffold for neural tissue engineering.[65] Chitosan hydrogels have also been studied as injectable carriers for the delivery of therapeutics in the treatment of degenerated intervertebral disk (IVD). Dang et al.[67] evaluated the conjugation of hydroxybutyl groups to chitosan, in order to make the polymer water soluble and thermo-responsive. The potential of the resulting hydroxybutyl chitosan (HBC) as an injectable matrix for the delivery of human mesenchymal stem cells (hMSCs) and, human lumbar disk cells (human annulus fibrosus cells (hAFCs) and human nucleus pulpous cells (hNPCs)) was assessed. Ghorbani et al.[63] studied a compound of C-GP, hyaluronic acid, chondroitin-6-sulfate, collagen type II, gelatin, and silk fibroin hydrogel, which they named NP hydrogel; it was tested as an injectable natural scaffold, similar to the ECM structure of IVD, and showed appropriate efficiency, suitable for IVD regeneration.

Modification of chitosan with synthetic polymers has also been explored as a strategy to prepare thermo-responsive materials.[66,131,132] Hydrogels consisting of *N*-[(2-hydroxy-3-trimethylammonium) propyl] chitosan chloride (HTCC), poly(ethylene glycol) (PEG), and α-β-glycerophosphate (α-β-GP) were investigated by Wu et al.[66] as a nasal drug delivery system. Compared with chitosan, HTCC shows better moisture retentiveness, antibacterial activity, mucoadhesivity, and permeability-enhancing properties.[131] Insulin was loaded into the hydrogel and its release in vivo was observed in a rat model, showing no apparent cytotoxicity and a decrease in the blood glucose concentration for 4–5 h after nasal administration.

Xyloglucan is a hemicellulose occurring in the primary cell wall of many higher plants; in particular, it can be obtained from the seeds of *Tamarindus indica*. Xyloglucan in solution shows thermo-responsive behavior after a cleavage of its galactose residues of above 35%.[29,33,34,123,133] Xyloglucan gels have been evaluated as sustained release carriers for the intraperitoneal administration of an antineoplastic antibiotic, mitomycin C (MMC). They displayed a broad concentration time profile in both peritoneal fluid and plasma versus a narrow peak and rapid disappearance when the drug was administered as solution.[69] In rabbits, rectal administration of indomethacin from xyloglucan gels also resulted in a broader absorption peak and longer residence time compared to delivery of the identical drug concentration in commercial suppositories.[70]

Thermo-reversible xyloglucan gels were also investigated as vehicles for the oral sustained delivery of indomethacin,[71] theophylline,[72] and paracetamol.[73] In all cases, the bioavailability of drugs from xyloglucan gels was higher than from control suspensions. More recently, xyloglucan formulations were also evaluated for the ocular delivery of pilocarpine,[74] for the percutaneous administration of non-steroidal anti-inflammatory drugs,[75] and as vehicles for nasal administration of drugs.[76]

For tissue engineering applications, PDL-functionalized xyloglucan was evaluated by Nisbet and colleagues[78,79] as a possible scaffold for neural cell growth and differentiation, with the advantage of being minimally invasive, thanks to its thermo-responsive nature.

Gelatin is probably the best-known biodegradable biopolymer with thermo-responsive properties. It is one of the UCST materials: below the UCST, gelatin aqueous solutions undergo sol–gel transition, as the protein coils begin to organize into triple helices, and progressively create a three-dimensional (3D) network structure.[134] For biomedical applications, however, the converse thermal behavior is desirable, thus gelatin is usually blended with other polymers to raise its thermal gelation to around 37°C.

Yang et al. developed a novel thermo-responsive hydrogel composed of gelatin (1%–10% w/v water solution) and monomethoxy poly(ethylene glycol)-poly(D,L-lactide) diblock polymer (MPEG-PDLLA) (15%–30% w/w, polymer/water), which undergoes thermal gelation at body temperature. The drug release kinetics of the hydrogel were assessed in vitro by incorporating gentamicin sulfate into the hydrogel matrix. At room temperature, 50% of the drug was released within 5 days, while at 37°C, the release profile was even slower. However, in the latter case, drug release was no longer detectable after 1 week because of degradation of the hydrogel matrix.[80]

Another approach toward a gelatin-based thermo-responsive hydrogel system was reported by Gil et al.[81] They formulated a mixed protein-based hydrogel by blending a gelatin solution (4% wt in distilled water) with a silk fibroin solution (4% wt in distilled water), varying the wt% of gelatin in the blend from 0% to 100%, and subsequently inducing β-crystallization of silk fibroin through exposure to methanol or methanol/water solutions. Swelling and protein release kinetics of gelatin/silk fibroin hydrogels were evaluated in vitro: these gels showed consistently not only higher swelling at body temperature than at 20°C but also greater mass loss caused by dissolution of gelatin in the aqueous solution.

Gelatin has also been blended with glycidyl methacrylated dextran (Dex-GMA) to obtain biodegradable hydrogels (20% w/w gelatin and 20% w/w dextran), in which drug release was controlled by sol–gel transition in response to temperature changes. Proteins (β-galactosidase, bovine serum albumin, bone morphogenetic proteins)[82–85] and drugs (5-fluorouracil)[82] were loaded into gelatin/Dex-GMA hydrogels, demonstrating the suitability of this blend for the development of drug delivery systems and for tissue engineering applications.

*3D bioprinting.* 3D bioprinting has emerged as a breakthrough approach for tissue engineering, thanks to the recent advances and availability of printing technologies. With the goal of combining additive manufacturing, cells, and biomaterials to fabricate complex structures that resemble original natural tissues and organs,[89,135] the choice of material used is clearly critical. Thermo-responsive hydrogels are particularly suitable for bioprinting applications, since rapid gelation can be exploited to obtain shape fidelity during the printing process.[89] Naturally derived thermo-responsive hydrogels studied to date for 3D bioprinting include ECM components (e.g. collagen), as well as other polymers of vegetable origin, such as agarose and cellulose.[136]

Gelatin has been intensively studied for 3D bioprinting applications, using different techniques (extrusion-based bioprinting (EBB), piezoelectric inkjet, and two-photon polymerization) at different concentrations (2%–20% w/v).[29,89–100] As gelatin-based hydrogels possess poor mechanical properties and are unstable under physiological conditions, various chemical and physical cross-linking methods have been investigated to increase their stability after printing.[94] Only a very small subset of the traditional methods used for gelatin cross-linking are applicable, obviously, if cell incorporation is involved.

Photopolymerizable gelatin hydrogels have been developed by chemical modification of gelatin with methacrylamide side groups (GelMA), and a cell-laden GelMA hydrogel was printed through EBB using a pneumatic dispenser equipped with a UV-light source.[93,137]

Lee et al. reported a 3D printing technique to create hydrogel scaffolds containing fluidic channels. A gelatin-based hydrogel was printed layer by layer, together with chemically cross-linkable collagen (sol phase at acidic pH and gel phase at neutral pH), to form a 3D hydrogel block. By increasing the temperature up to 37°C, the gelatin was selectively removed, leaving hollow channels inside the collagen scaffold.[138]

Agarose is a biopolymer with interesting properties for bioprinting processes. Agarose gelation occurs when the temperature reaches the UCST (UCST = 30°C–40°C). Above the UCST, agarose shows a random coil conformation in solution, while its structure changes to a double helix when cooled below the UCST. The UCST depends on the polymer concentration in solution, the molecular weight of the polymer, and its structure.[97] Agarose-based hydrogels have been printed by both pneumatic-based bioprinting and EBB, usually at low to medium concentrations (1%–5% w/v).[44,89,94,97,102,103] Similar to gelatin, agarose has been used as sacrificial material to create microchannels for engineered vascularized constructs.[103] It has also been investigated as potential candidate for 3D fiber deposition of cell-laden constructs for bone tissue engineering.[44] Moreover, agarose can be mixed with other hydrogels to confer or enhance the thermo-responsive properties of the blend.[139]

Together with gelatin, collagen type I is among the most studied natural polymers for 3D bioprinting. Collagen I macromolecules are usually dissolved in diluted acids, and when pH and temperature reach physiological values, they self-assemble to form a hydrogel. In particular, once the acidic solution is neutralized (pH = 7–7.4), collagen cross-links within 30/60 min at 37°C,[140] which makes it a suitable candidate for bioprinting applications.[135]

Collagen-based hydrogels have been printed with both inkjet and extrusion bioprinters, usually at low concentrations (0.1%–3% w/v).[89,94,140–145] However, they show poor mechanical properties,[146] and it is often necessary to blend them with other natural polymers, such as fibrin,[145] or with synthetic polymers.

Smith et al.[147] demonstrated the possibility to print collagen type I, loaded with bovine aortic endothelial cells (BAECs) via EBB. The neutralized solution of collagen was maintained at a low temperature during extrusion and heated up after completing the printing procedure, to allow full cross-linking of collagen within 30 min.

## Hydrophilic/hydrophobic transition

The specific behavior of thermo-responsive polymers can also be exploited to prepare surfaces capable of switching reversibly from hydrophilic to hydrophobic upon temperature changes. A general approach in this direction is based on grafting thermo-responsive polymers onto a surface in order to make it sensitive to temperature changes. The polymer chains are not able to aggregate or separate, as in sol–gel transition, since their degree of freedom is reduced. However, their conformation remains sensitive to temperature, and they swell or collapse on the surface, causing a change in the surface's affinity for water and a temperature-dependent interaction with solutes.[148] Surfaces with this thermal trigger property have engendered great interest in the field of tissue engineering, for the fabrication of continuous sheets of in vitro–cultured cells in cell sheet engineering (CSE).

*CSE.* In CSE, thermo-responsive hydrogels are used to retrieve cultured cells, without disrupting cell-to-cell bonds, and preserving the ECM secreted by the cells, which would be compromised by traditional enzymatic treatment. For this specific application, thermo-responsive polymers are designed to be hydrophobic at 37°C (Figure 4(a)), the ideal condition for cell seeding and adhesion, and hydrophilic at room temperature (Figure 4(c)), so that the cells can easily be detached from the substrate by lowering the temperature.[29,47,116–121,149,150]

Although the most popular solutions for this application are based on a synthetic polymer, poly(*N*-isopropylacrylamide) (PNIPAAm),[132,151] MC is also frequently used. Aqueous solutions of MC (1%–10% w/v) are generally used to prepare thermo-reactive surfaces. Chen et al.[119] first developed a method for harvesting contiguous cell

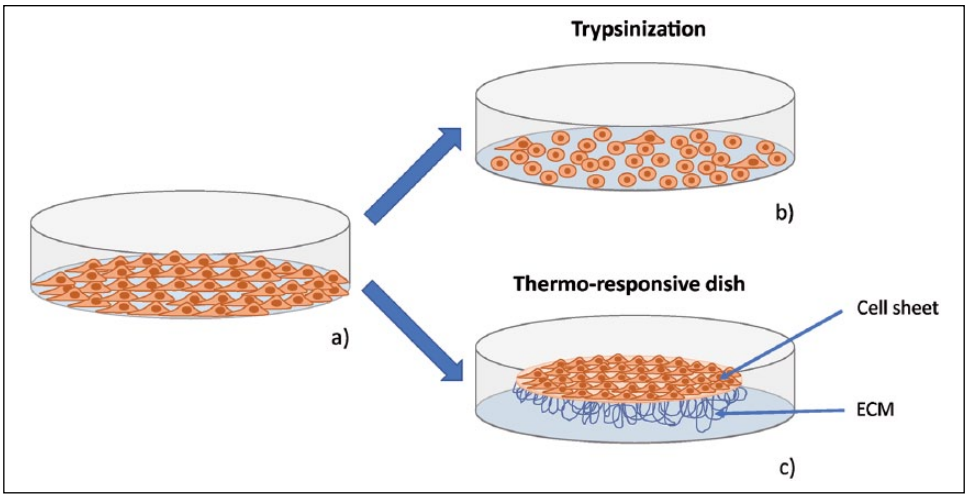


**Figure 4.** Cell sheet detachment from a thermo-responsive surface. (a) Cells adhere to a hydrophobic surface through membrane proteins and ECM, forming cell junctions. (b) Both membrane and ECM proteins are disrupted through enzymatic digestion, causing cellular detachment. (c) Cells cultured on a thermo-responsive surface can be harvested as a contiguous cell sheet, maintaining cell-to-cell junctions by lowering the temperature.

sheets, using tissue culture polystyrene (TCPS) dishes coated with MC-based thermo-responsive hydrogels. They were able to detach human foreskin fibroblast (HFF) sheets from these smart surfaces by simply lowering the environmental temperature (from 37°C to 20°C).

Recently, some efforts have been made to systematically investigate the thermo-responsive behavior of MC-based hydrogels, in order to evaluate their potential in the field of CSE.[116–118] An increase in polymer concentration was found to promote polymer–polymer interactions at lower temperatures, causing a decrease in the sol–gel transition temperature (LCST). The dissociation of salts in MC aqueous solutions produces ions, which act on the interactions between polymer macromolecules and water molecules, shifting the LCST of MC-based hydrogels either to lower (salting-out ions) or to higher (salting-in ions) temperatures than a purely aqueous MC solution. Typical LCSTs for MC-based hydrogels are 20°C–70°C, depending on polymer concentration, molecular weight, and salt concentration.[116–119] Selected MC-based hydrogels were tested in vitro to study the possibility of obtaining cell sheets from coated TCPS dishes by decreasing the temperature. Contiguous cell sheets were obtained both with adipose stem cells (ASCs)[118] and with murine fibroblasts (L929).[116,117]

Myocardial tissue regeneration is one of the most advanced fields of research involving CSE; both in vitro and in vivo applications have been investigated. In particular, a promising approach to in vivo myocardial tissue regeneration consists of injecting cell sheets directly to the injury site. To support cardiac wound healing, fragmented cell sheets, obtained by detachment from MC-based surfaces, are injected locally.[120–122] Compared to dissociated cells, in fragmented cell sheets, the intercellular junctions and endogenous ECM are preserved; this helps to retain the cell phenotype and affords effective attachment of cell sheets to the damaged tissue. Both mesenchymal stem cells (MSCs)[120,121] and human amniotic fluid–derived stem cells (hAFSCs)[122] have been employed in myocardial tissue regeneration, providing an adequate delivery vehicle for retention of the transplanted cells at the damaged area.

Xyloglucan has been also investigated for cell sheet applications. In particular, Silva et al.[123] proposed xyloglucan culture films, chemically modified with the tripeptide sequence Arg-Gly-Asp (RGD), in order to obtain surfaces that promote adhesion and proliferation, while also permitting temperature-assisted cell detachment. This promising approach provides an alternative to better known thermo-responsive cell culture surfaces (i.e. PNIPAAm and MC) for the harvesting of cells sensitive to proteolytic treatment.

## *Perspective and future developments on thermo-responsive biopolymers*

In conclusion, thermo-responsive hydrogels, transformed upon temperature change from a polymer solution in water suspension to a gel, are a class of biomaterials of great interest for biomedical scientists, playing a potential key role in the field of drug delivery. Specifically, in situ–gelling hydrogels are promising vehicles for the local delivery of drugs in a minimally invasive manner, also providing sustained release for localized treatments. Such systems have been investigated in depth in connection with specific regenerative applications because of their remarkable advantage of being injectable at the site of the defect, irrespective of its shape and geometry. Despite these advantages, though, the poor mechanical properties of natural thermo-responsive hydrogels may limit their use to certain areas of the biomedical field. The development of

**Table 3.** Biomedical applications of biopolymers that evidence smart response to a pH stimulus.

| Smart response | Biopolymer | Blend | Application | Reference |
|---|---|---|---|---|
| (De)swelling | Carrageenan | – | Drug delivery | 152 |
| | | Chitosan | Drug delivery | 153 |
| | | Cellulose | Drug delivery | 154, 155 |
| | Chitosan | – | Other | 156, 157 |
| | | Sodium caseinate or bovine serum albumin | Delivery | 158 |
| | | Hydroxyethyl cellulose and polyol | Drug delivery | 159 |
| | | Heparin | Anticancer drug delivery | 160 |
| | | Dimethylmaleic acid and urocanic acid | Anticancer drug delivery | 161 |
| | | PEG | Anticancer drug delivery | 162 |
| | | Folate-modified chitosan | Anticancer drug delivery | 163 |
| | | PEGDA | Anticancer drug delivery | 164 |
| | | 6-*O*-dodecyl-chitosan carbamate | Gene delivery | 165 |
| | | *N,O*-carboxymethyl chitosan and alginate | Drug delivery | 166 |
| | | Pyrophosphate and tripolyphosphate | Drug delivery | 167 |
| | Alginate | Chitosan | Drug delivery | 168 |
| | | PVA | Drug delivery | 169 |
| | | Chitosan and pectin | Drug delivery | 170 |
| | Collagen | – | Drug delivery | 171 |
| | Carboxymethyl cellulose | – | Bioengineering applications | 172 |
| | | PVA | Drug delivery | 173, 174 |
| | | Acrylic acid/PVP | Delivery | 175 |
| | Bacterial cellulose | Acrylic acid | Drug delivery | 176 |
| | Dextran | – | Drug delivery | 177, 178 |
| | Tragacanth gum | – | Drug delivery | 179 |
| | Poly-L-lysine | Hyaluronic acid | Biomaterials applications | 180 |
| | Pectin | – | Drug delivery | 181 |
| Sol–gel transition | Alginate | Pectin | Drug delivery | 182 |
| | Chitosan | Palmitoyl groups | Injectable reservoir | 183 |
| | | – | Biomedical application | 184 |
| | | Gelatin type B and nanosilver | Biomedical application | 185 |
| | | – | Neo-vascularization | 186 |
| | Hydroxypropyl methylcellulose | Silanol groups | Biomedical application | 187 |
| Polymer relaxation | Melanin | – | Drug delivery | 188 |
| | Gelatin | – | Anticancer drug delivery | 189 |
| | Alginate | Gelatin | Drug delivery | 190 |

PEG: poly(ethylene glycol); PEGDA: dibenzaldehyde-terminated poly(ethylene glycol); PVA: polyvinyl alcohol; PVP: polyvinylpyrrolidone.

injectable, mechanically strong materials is thus still an open problem, requiring researchers' attention and efforts.

## pH-responsive biopolymers

A wide extensively studied smart behavior that natural polymers may exhibit is their response to pH changes; pH-responsive materials are of particular interest owing to the differences in pH that usually exist or that may occur in the body as a consequence of physiological or pathological events.

For example, there is a wide variation in pH along the gastrointestinal tract, which has a strongly acidic environment (pH 1–2) in the stomach and is alkaline in the intestine. pH-sensitive systems can thus be used to avoid drug release in the stomach and extend the drug's efficacy to the intestine. pH-sensitive hydrogels have also been proposed for cancer drug targeting due to the fact that a significant acidic environment has been observed in tumor tissues (pH range 5–6) compared to healthy tissues (pH 7.4). pH-responsive hydrogels can thus efficiently release the drug at the acidic target site, minimizing the amount of drug released elsewhere.

A number of different natural polymers that undergo changes in physical properties (Table 3) in response to

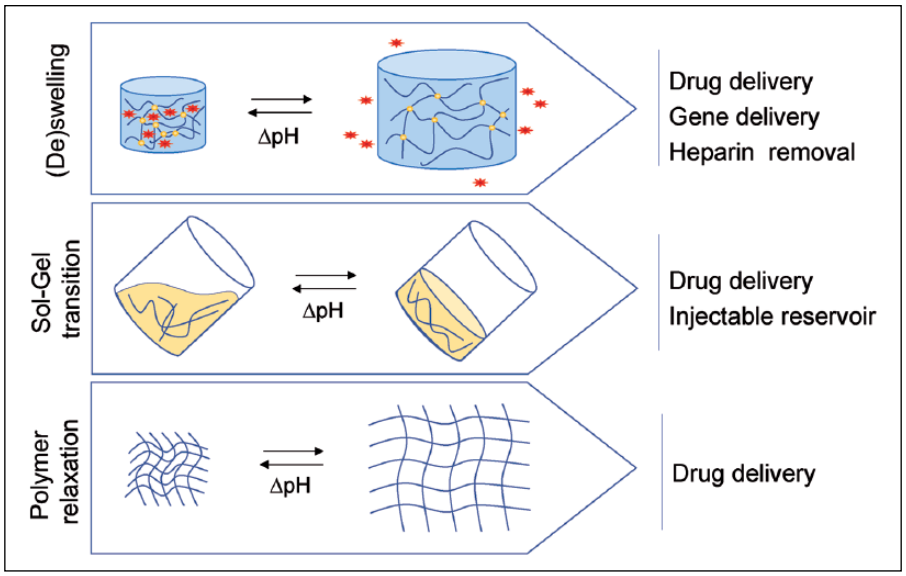


**Figure 5.** Examples of smart responses of natural polymers under pH variation. Swelling/de-swelling: the hydrogel can retain or release substances (drug or cells), depending on pH value; sol–gel transition: the hydrogel can be in a sol or a gel state when a change in pH occurs; polymer relaxation: the cross-linked macromolecular structure of the material can shrink or relax at different pH values.

environmental pH have been evaluated; most have been developed in the form of hydrogels (injectable hydrogels, microcapsules, nanogels). pH-responsive materials have thus found applications mainly in the development of controlled-release systems. A change of pH triggers a change in the ionization level of the polymer's functional groups and acts on the state of the hydrogel, thus controlling the uptake and release of particles or biomolecules. However, other applications, including tissue engineering and regenerative medicine, have also been developed.

The general mechanism underlying pH responsiveness is linked to the ionization of polymer chain pendant groups, which undergoes an abrupt change close to the $pK_a$. This induces a rearrangement of the polymer network that, at the macroscopic level, leads to biopolymer self-assembly (e.g. micelles or gels), modification of the swelling ratio, or sol–gel transition (Figure 5).

## *Sol–gel transition*

*Hydrogel formation.* In a variety of natural-based materials, the physiological pH induces a sol–gel transition, resulting in rapid hydrogelation. However, to exploit this trigger mechanism effectively and adjust the gelation pH, it is usually necessary to modify or blend the materials. Among biopolymers, chitosan is a pH-sensitive material, but it can be further modified to enhance its pH sensitivity.[183] A convenient synthetic approach can modify the chitosan backbone through the addition of palmitoyl groups, to prepare an injectable reservoir system for minimally invasive tissue engineering applications. The introduction of alternating charges, chitosan-protonated amine groups, and hydrophobic side chains of palmitoyl groups can regulate the sol–gel transition and narrow the hydrogel formation pH window (pH 6.5–7.0). This approach can lead to a nanophase-separated sponge morphology with good in vivo cohesion of the hydrogel at the location of subcutaneous injection.[183]

Mixing is also an effective strategy. Alginate in combination with pectin can produce a pH-sensitive hydrogel that dissolves as pH increases. By adjusting the alginate/pectin ratio, it is possible to control the temperature and pH of the gel–sol transition and the release of bioactive molecules. Guo and Kaletunç developed a mathematical model to characterize the dissolution kinetics of this hydrogel. It emerged that hydrogel particles were stable at low pH, while a zero-order kinetics model characterized their dissolution at pH 5.0 and 7.0.[182]

The opposite type of pH dependency was obtained in hydroxypropyl MC (HPMC), by grafting silane groups along the HPMC chains. In an alkaline environment (pH 12), this product remains in gel form, as the grafted silanes are in their ionic form. When the pH decreases (i.e. in physiological conditions), the silanes interact and the gel becomes cross-linked, leading to a self-hardening process due to condensation of the silanes.[187] Investigation of this property has shown this material to be interesting, particularly for articular cartilage tissue regeneration.[191]

*Electrophoretic deposition.* Sensitivity toward pH variation can be extremely beneficial also for processing specific materials. The pH-dependent solubility of chitosan, for

instance, provides a convenient mechanism for its processing under mild conditions. This property can be exploited in wet spinning or electrophoretic deposition (EPD). Wet spinning of chitosan fibers comprises extruding the viscous chitosan solution in a dilute acid in a coagulation bath. Below pH 6, the free amino groups are readily protonated (pKa value of 6.3) and the molecules become soluble.[192] Chitosan fibers containing different phosphate contents were successfully prepared in baths having different pH through ionotropic cross-linking. The mechanism of chitosan fiber formation was found to be strongly influenced by pH variations, leading to different physico-chemical properties. A lower pH favored a high degree of cross-linking, causing the polymer network to freeze and resulting in low crystallinity. This also reduced the thermal stability of the modified chitosan fibers.[193] Similarly, the EPD process is a controllable method of assembling materials, by exploiting their physico-chemical properties under an electric field.[194] The EPD process can produce uniform deposits/coatings with highly uniform microstructure, adequate thickness, and a porous structure, using chitosan as smart biopolymer.[195] As mentioned above, the presence of the amino groups means that the charged state and properties of chitosan alter substantially with the pH. At low pH, these amines become protonated and positively charged, making chitosan a water-soluble cationic polyelectrolyte. As the pH increases above 6, chitosan amines become deprotonated and the polymer loses its charge, becoming insoluble. The soluble–insoluble transition occurs at its pKa value, at pH between 6 and 6.5. During the EPD process, the protonated amino groups of chitosan lose their charge in the high pH region on the cathode surface:[196] close to the electrode surface, the pH is above chitosan pKa 6.3–6.5 and the chitosan amino groups are deprotonated. An insoluble deposit thus forms on the electrode surface.

The use of chitosan in combination with EPD fabrication technique has been widely investigated and finds applications in coatings for orthopedic implants[197] or to enhance neo-vascularization in diseased tissue.[186] For example, Wang et al.[185] used a deposition mixture of chitosan, gelatin type B, and nanosilver for the EPD process. On applying a voltage (2.5 V) from a DC (direct current) power supply, the cathodic reaction created a localized increase in pH adjacent to the cathode surface, which induced chitosan to undergo sol–gel transition. Furthermore, thanks to complexation with gelatin molecules, it was possible to co-deposit both molecules on the electrode surface.[185,198] A study by Altomare et al.[184] also showed that the composition of electrophoretic bath, in terms of anion and pH, enables different structures with different degrees of porosity to be deposited. Moreover, our research group further demonstrated that by coupling EPD techniques with positive replica approach, hierarchical structures can be conveniently designed to support scaffolds neovascularization.[186]

### *Swelling/de-swelling*

The majority of pH-responsive smart biopolymers show a swelling/de-swelling transition in response to pH change that can be used as targeted delivery mechanism. Carrageenan has been investigated as a potential material for drug delivery since it possesses this type of behavior. This natural linear polysaccharide, extracted from red seaweed, is usually functionalized by addition of carboxyl groups through a carboxymethylation process, so as to implement site-specific targeted release of encapsulated macromolecules.[154] Ionization of the carboxyl groups changes in response to the pH, causing a relaxation of the hydrogel network structure, from which the drug is released via swelling.[152]

The combination of carrageenan with chitosan generates a pH-sensitive system, in which drug release in an alkaline environment is regulated by electrostatic interactions between the sulfate groups of carrageenan and the amino groups of chitosan.[153]

Chitosan is frequently used in combination with other molecules as a system for encapsulation[199] and pH-modulated drug delivery.[159,166] Kurukji et al. reported the fabrication of electrostatic sub-micron complexes made of chitosan and a protein (sodium casein or bovine serum albumin) for the delivery of active compounds in response to pH changes. The presence of chitosan in protein–chitosan complexes enhances the mechanism of active binding compared to protein alone. Thanks to its encapsulation effectiveness and pH-triggered release, this material combination has been proposed not only for pharmaceutical applications but also for designing food and agrochemical formulations.[158]

Several studies have also reported upon the use of chitosan-based nanoparticles for the controlled release of doxorubicin, one of the most widely used chemotherapeutic agents. These nanoparticle systems entail functionalizing chitosan with different polymers, such as heparin or dimethylmaleic acid/urocanic acid; the pH sensitivity of chitosan accelerates drug release in an acidic environment due to protonation of the chitosan amine groups and subsequent nanoparticle swelling.[160,161]

Analogous behavior is reported for alginate-based hydrogels in the form of nanoparticles. In a study by Maity et al., core–shell nanoparticles made of chitosan and alginate were developed for oral administration of antidiabetic drugs. In simulated intestinal fluid, repulsion between carboxylate ions on alginate shell supported penetration of the solvent into the chitosan core and gave slow, sustained release of the encapsulated agent. Interestingly, in vivo studies demonstrated the nanoparticles' non-toxicity and their greater efficacy in lowering blood glucose levels, in comparison with free drug oral administration.[168]

Smart polymers with reversible swelling properties can be used not only to deliver biomolecules but also to remove them, in suitable conditions. Zazakowny et al. aimed to develop

pH-sensitive polymeric devices to remove heparin from the bloodstream after it has exerted its anticoagulant effect. Genipin-cross-linked chitosan microspheres were found suitable for this application due to chitosan's swelling properties. Chitosan chains undergo protonation at low pH, leading to inter-chain repulsion and consequent water absorption into the gel. The results showed that, at pH 7.4, which is characteristic of the blood, heparin absorption was slower than it was at the low pH of water; functionalization with glycidyltrimethylammonium chloride (GTMAC), which imparted a positive charge to chitosan molecules including at higher pH values, increased the rate and efficiency of heparin binding.[156]

Chitosan also has properties of great interest in gene delivery therapy, where it has been used to obtain nanometric pH-sensitive micelles that are able to form complexes with pDNA. In this system, the pH responsiveness of chitosan enables the release of DNA in the acidic endosomes/lysosomes environment to be controlled readily.[165]

### *Polymer relaxation*

In addition to sol–gel transition and swelling/de-swelling mechanisms, in some cases, a pH change can induce conformational rearrangement in the structure of natural polymers, which can be exploited for drug delivery. This occurs in gelatin and melanin.

Gelatin-capped silica nanoparticles have been developed as an anticancer drug delivery system using a pH-triggered mechanism. At neutral pH, a gelatin capping layer grafted onto mesoporous silica particles effectively prohibits drug release. However, in an acidic environment, the electrostatic repulsion between gelatin and the mesoporous silica core triggers uncapping and the subsequent release of the encapsulated drug.[189]

A study by Araújo et al.[188] reported similar behavior: rearrangement of the melanin structure in response to pH variations was exploited to produce drug delivery nanocarriers. Melanin is composed of two different monomers, whose carboxyl groups play a fundamental role in the final polymer conformation. At physiological pH (i.e. 7.4), drug release is promoted when repulsion between polymer chains occurs upon deprotonation of the carboxyl groups, which become negatively charged. Conversely, at lower pH, the carboxyl groups are protonated, minimizing the electrostatic effect and allowing drug retention in the internal structure of the nanoparticles.

### *Perspective and future developments on pH-responsive biopolymers*

pH-responsive polymers possess fascinating properties that make them optimal candidates for designing smart devices for biomedical applications and in particular for developing carriers for drug delivery. Interestingly, the ionization level of polymer pendant groups is susceptible to variations in environmental pH and can be used as a trigger mechanism for the release of different molecules. The challenge in pH-sensitive hydrogel design is to control this mechanism, so as to obtain versatile and site-specific drug carriers. An in-depth knowledge of materials chemistry is mandatory in order to define the most suitable release mechanism under a specific physiological or pathological pH transition. Of the available polymers, natural pH-responsive polymers will be those most preferred in biotechnological applications, thanks to their response to biological conditions and favorable interactions with the biological environments concerned.

## Physico-chemical stimulus-responsive biopolymers

In addition to temperature and pH, other physical or chemical stimuli can be used as triggers for tuning macroscopic modifications in biopolymers. Systems responding to chemical stimuli mainly show changes in their rheological or physical properties.

Chemical stimulus response occurs, thanks to the presence of specific ions or chemical species in the surrounding environment, which selectively interact with biopolymer macromolecules and may induce a variety of responses, including self-assembly,[200] sol–gel transition,[201–205] hydrogel cross-linking or folding of protein backbones,[206] or may allow the release of molecules/cells after induced swelling or degradation.[207,208] Together with chemically induced modifications, some external physical stimuli can also cause in situ modifications of the material (Figure 6).[209,210]

Among natural polymers, different kinds of polysaccharides can be used as smart systems activated by physical or chemical stimulus (Table 4). Gellan gum, carrageenans, and alginates, for example, have been extensively investigated[211,212] as hydrogel-based devices. In addition, protein-based materials can be developed as smart systems responding to chemically induced transformations, which may trigger different folding modalities in the structure of the material.[213]

Other mechanisms related to specific chemical interactions, such as the protein–protein recognition occurring naturally in living cells, offer useful tools to investigate functional mechanisms in living cells.[223,224]

### *Sol–gel transition*

Natural polysaccharides are excellent materials from which to develop in situ–gelling systems based on material–ion interactions since they interact with monovalent or divalent cations. Of the polysaccharides, alginate has been most widely investigated.[226] Alginate chains consist of two different monomers, (1-4)-linked β-D-mannuronate

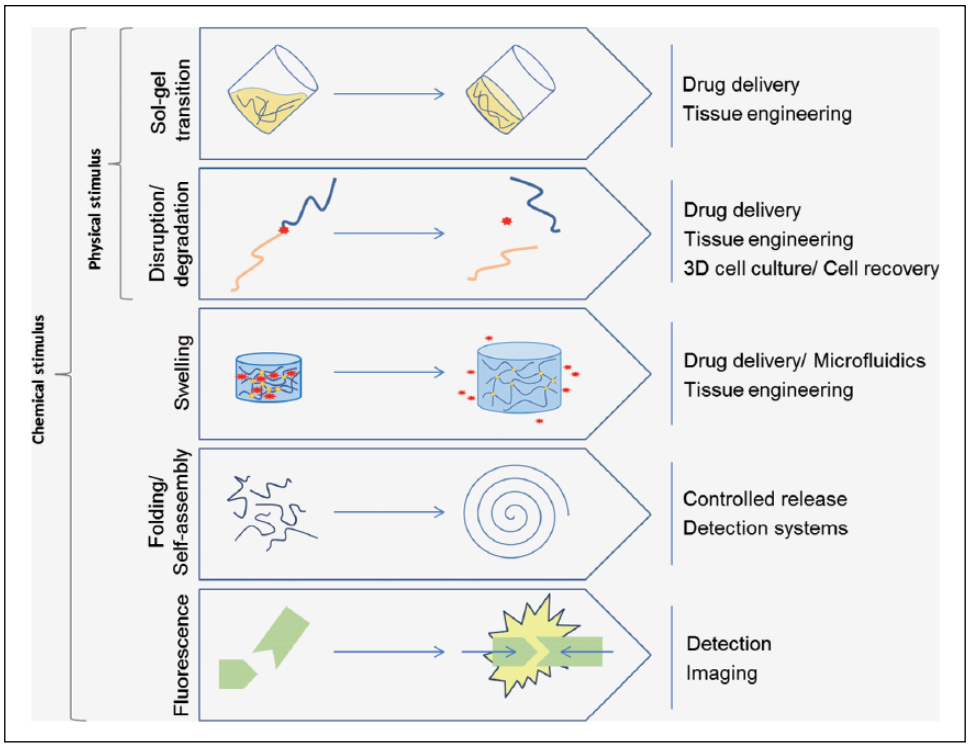


**Figure 6.** Examples of smart responses to physical or chemical stimuli in biopolymers: shape recovery, gelation, macromolecule disruption, swelling, fluorescence.

**Table 4.** Biomedical applications of biopolymers with a smart behavior activated by a physico-chemical driving force.

| Smart response | Biopolymer | Blend | Application | Reference |
|---|---|---|---|---|
| Sol–gel transition | Kappa carrageenan | Gellan gum | Ocular safety | 201 |
| | | Methylcellulose | Ophthalmic drug delivery system | 202 |
| | Alginate | Gelrite | Ocular safety | 157 |
| | | Hydroxypropyl methyl cellulose | Ophthalmic drug delivery system | 205 |
| | | – | Ophthalmic drug delivery system | 204 |
| | | Aminocaproic acid | Drug delivery | 175 |
| | Dextran | Tyramine | Drug delivery/tissue engineering | 214 |
| | Hyaluronic acid | Tyramine | Drug delivery/tissue engineering | 215 |
| | Modified chitosan (chitosan-graft-glycolic acid) | – | Tissue engineering | 179 |
| Swelling | Modified chitosan (*N*-succinyl-chitosan) | Aldehyde hyaluronic acid | Tissue engineering | 207 |
| | Modified calmodulin (calcium-binding protein) | 3-[2-(trifluoromethyl)-10H-phenothiazin-10-yl]propan-1-amine | Drug delivery/microfluidic | 178 |
| | Poly(L-glutamic acid) | Phloretic acid | 3D cell culture and recovery/ tissue engineering | 208 |
| Degradation and release | Poly(L-glutamic acid) | Phloretic acid | 3D cell culture and recovery/ tissue engineering | 208 |
| | Alginate | – | Drug delivery | 210 |
| | Dextran | | Drug delivery | 209 |
| | Liposome | Cholesterol graft copolymer | Cancer therapy | 216 |
| | L-aspartic acid polyester nanoassemblies | | Cancer therapy | 217 |
| | Peptide dendrimers | PEG | Drug delivery | 218 |

**Table 4.** (Continued)

| Smart response | Biopolymer | Blend | Application | Reference |
|---|---|---|---|---|
| Self-assembly/ folding | DNA fragments | Cationic gelatin | Drug delivery | 219 |
| | Peptide dendrimers | PEG | Cancer therapy | 220 |
| | Peptide dendrimers | PEG | Cancer therapy | 200 |
| | Peptide-hyaluronan hybrid hydrogel | – | Controlled release | 206 |
| | Polypeptides | Gold nanoparticles | Detection system | 221 |
| | Oleosin | | Biomedical application | 213 |
| | Silkelastin-like block copolymers | – | Biomedical application | 222 |
| Molecular complementation | Protein sequences | | Detection/imaging in live cancer cells | 223, 224 |
| Shape memory | α-keratin fibers | – | Generic | 225 |

PEG: poly(ethylene glycol).

(M) and its C-5 epimer α-L-guluronate (G) residues, covalently linked together. Alginate's biocompatibility, and its structural similarity to extracellular matrices of living tissues, enables it to be employed in many biomedical applications (e.g. wound healing, delivery of bioactive agents as drugs or proteins). Alginate undergoes sol–gel transition in the presence of ionic cross-linking agents, especially divalent cations (e.g. $Ca^{2+}$). Divalent cations are believed to bind more effectively to the guluronate blocks of the alginate chains, which offer a higher degree of coordination of divalent ions. The formation of junctions between guluronate blocks of adjacent polymer chains leads to the "egg-box model" of cross-linking.[226,227]

Other relevant materials of the polysaccharide family are gellan gum, a natural polymer derived from bacteria, and the carrageenans, sulfated galactans derived from red algae. In both cases, formation of helices and their corresponding aggregation are thought to be the two mechanisms leading to gel formation.[228,229] Chemical characteristics of the starting material may affect gel properties and formation. For instance, in the case of alginate, in which ion coordination is exploited by the G-residues, the material's gelation time can be modulated by acting on the proportion of M and G-residues, which may alternate in different sequences or blocks in the chains. Furthermore, different types of gels can be obtained under suitable conditions: weak elastic gels are obtained using native gellan gum, while stiff and brittle gels are formed when gellan gum undergoes deacylation.[227] Conversely, the gelation properties can be modified depending on the presence of specific cations, as, for instance, divalent cations, which promote the formation of thermally stable junction zones in gellan gum helix aggregation, whereas monovalent cations mainly trigger kappa carrageenan ionic gelation.[230]

The choice of a specific hydrogel depends on its intrinsic properties and on the intended therapeutic use. For instance, the interaction between cations present in tears (i.e. $Na^+$, $K^+$, $Mg^{2+}$, and $Ca^{2+}$) and the above-mentioned polysaccharides (alginate, gellan gum, and carrageenans) provides a promising alternative for the development of ion-sensitive systems for ophthalmic formulations.[201–205] The results of these studies pointed up the possibility of easier and decreased drug administration, resulting in better patient acceptance. Ion-sensitive in situ ocular gels, based on gellan gum and kappa carrageenan, were proposed by Fernández-Ferreiro et al.[201] The coupling of two different reactive systems was thought to be relevant for patients who show alterations in ionic composition of tears due to systemic or ocular diseases. In addition, kappa carrageenan is particularly valued in this field, thanks to the presence of sulfonic acid groups in the polymer chain that can increase interactions with the mucosal tissues, enabling adhesion to the target surface. This is a fundamental requirement for drug delivery systems that offer optimal drug concentration at the site of action, bypassing rapid drug clearance.[202–205] In this connection, the preparation of binary systems, such as carrageenan and MC, was studied to investigate the mixture's viscoelastic properties.[202] The resulting formulations were proposed for transscleral delivery of macromolecules to manage diseases of the posterior eye. Furthermore, thanks to the in situ gel-forming capability of an alginate-based formulation, prolonged delivery of a pressure-reducing agent (pilocarpine nitrate) was reported by Cohen et al.,[204] demonstrating long residence times in the eye and an extended drug effect.

In situ gelation may be triggered by external stimuli (i.e. ultra-sonication), as reported by Chejara et al.[231] in the synthesis of alginate-based amide conjugate. The presence of amide and acid functional groups at a particular ratio (1:0.5 alginate to aminocaproic acid) facilitated gel formation through inter-molecular hydrogen interactions. The resulting gel showed thermal responsive and thixotropic behavior.

An extremely promising approach for in situ formation of hydrogels is based on enzyme-catalyzed cross-linking reactions. In addition to drug release, hydrogels injected

at the defect site have recently received much attention in tissue engineering.[214,215] Injectable hydrogels may be formed in situ via enzymatic cross-linking, under physiological conditions. This is not only a minimally invasive procedure but it also offers an extremely mild condition for the incorporation of cells and very sensitive bioactive molecules.

### *Swelling or disruption*

Polysaccharide-based hydrogels can also be combined appropriately with selected molecules (e.g. enzymes), often via chemical linkage to the polymer backbone, in order to obtain biochemical-responsive systems that recognize and respond to specific biological events, releasing molecules or cells through material swelling[232,233] or degradation.[207,208] The hydrogel swelling is generally attributed to changes in non-covalent interactions within the polymer network, while its degradation generally occurs when enzymes that possess exceptional bio-recognition capabilities cleave chemical bonds[217,218] or by disruption of ionic interactions.

Since the sensing capability is particularly necessary in insulin delivery systems, various efforts have been made to develop self-regulated insulin release devices in which insulin can be released in response to the blood glucose concentration. Tan et al.[207] proposed a glucose-responsive system based on immobilization of glucose oxidase and catalase enzymes within pH-sensitive hydrogels. The immobilized enzymes trigger glucose conversion, causing pH variation in the microenvironment and consequently inducing insulin release upon hydrogel swelling.

Molecular release can also be achieved by disruption of the hydrogel itself. Xu et al.[208] investigated an injectable and biomolecule-responsive disulfide-containing polypeptide hydrogel, which undergoes degradation in the presence of glutathione as reducing agent. Depending on the concentrations of glutathione and the polymer, the degradation time can be prolonged or shortened, thanks to cleavage of the disulfide bond.

In situ drug release upon hydrogel degradation can also be triggered by external stimuli. For example, on-demand ultrasound-triggered drug delivery can be achieved from calcium alginate hydrogels.[209] Taking advantage of ultrasound treatment, Huebsch et al.[210] demonstrated accelerated drug release from an alginate-based hydrogel. Ultrasound was reported not only to damage the alginate permanently but also to provide release of small molecules, proteins, and condensed oligonucleotides by disrupting calcium cross-links in the material. The presence of $Ca^{2+}$ in physiological fluids means that a reversible cross-linking mechanism occurs upon removal of the stimulus. Using this approach, the release of a chemotherapeutic agent was tested, showing improved release of the molecule in the presence of ultrasound.

As mentioned above, chemical cleavage of specific molecules can also be achieved in a target area, thanks to the presence of enzymes. Therapeutic strategies can be based on enzyme recognition in the external biological environment, based on the assumption that particular enzymes are overexpressed in altered conditions, as in the presence of tumors.[234] This approach offers the possibility of setting up an interactive dialogue between the material and the biological environment. Responding to physiological conditions, glycyl phenylalanyl leucyl glycine tetrapeptide (GFLG) was conjugated to an antitumor agent that was then released in the tumor cellular environment. Tumor cells specifically contain secreted cathepsin B, a lysosomal cysteine protease capable of cleaving the GFLG sequence; this gives faster drug release in cancer cells than in healthy ones.[218] Similar concepts have been implemented in different natural systems that respond to cellular/biochemical stimuli;[216] examples are given in dedicated reviews.[235,236]

Controlled release can also be achieved by environment-responsive polymer–drug conjugates, thanks to their high stability in the bloodstream and their potential for selective drug release in tumor tissues. Peptide dendrimers were recently explored as candidate drug carriers for cancer therapy, owing to their precisely controllable size, low polydispersity, and multi-modifiable surface functionality.[219,220] However, structures less than 10 nm in size may rapidly be cleared by the kidneys, while high-generation dendrimers (namely, those that undergo more rounds of reactions resulting in bigger dimensions) may cause cytotoxicity[237] and PEGylated structures are often designed to improve blood circulation times and reduce side effects.[200]

### *Peptide folding/self-assembly and molecular complementation*

Assembly is a process that is ubiquitous in nature, being particularly evident in the folding of peptides and proteins. Mimicking this assembly process offers interesting opportunities in the context of drug delivery but still remains a challenge.[238]

Specifically, tailored protein–metal ion interactions that occur widely in nature can be used to control the self-assembly of complex supramolecular structures. Furthermore, peptide chains can be specifically designed, and appropriate conformational changes can be induced, as a result of metal ion coordination. Selegård et al.[206] investigated the folding-driven self-assembly of a hyaluronan hybrid hydrogel, conjugating a peptide to the hyaluronic acid backbone. Investigation of the secondary structure of the hyaluronic acid–conjugated peptides revealed a random coil structure, in the absence of $Zn^{2+}$ ions, and an α-helical conformation in their presence.

Engineering the surfactant protein backbone is an alternative route to controlling the self-assembly of the

structure. When 65% of the hydrophobic domain is removed from oleosin (sunflower protein), the helical secondary structure is abolished; addition of five glycines into the hydrophobic block creates a random coil triblock surfactant protein. These variants are reported to self-assemble into spherical micelles in solution above a critical concentration.[213] The concentration at which assemblies form depends on the secondary structure of the protein. This finding might lead to structure-driven assembly at controlled concentrations.

Molecular engineering has also produced useful tools to identify structures and sequences of stimulus-responsive proteins and enabled them to be produced recombinantly.[239] Block copolymers containing repeating sequences from silk and elastin have been synthesized using genetic engineering techniques, inserting glutamic acid or valine substitutions at strategic positions.[5,222] Such substitutions made it possible to control sensitivity to pH, temperature, and ionic strength precisely and to demonstrate reversible transition of the polymer.

The design and expression of artificial proteins that are programmed to form covalent molecular networks may offer important advantages in the field of biomaterials engineering. In this context, it is interesting to consider the bimolecular fluorescence complementation phenomenon, based on the complementary reconstitution of a functional fluorescent protein from its split non-fluorescent fragments. Via this approach, fundamental studies on real-time interactions between proteins can be carried out.[223] A very recent paper reported the development, characterization, and application of bimolecular fluorescence complementation assays based on a reversibly photoswitchable fluorescent protein, to detect and visualize protein–protein interactions in living cells at super-high-resolution, by combining it with specific detection techniques.[224] Extensive and detailed reviews have been dedicated to this procedure.[240,241]

### *Perspective and future developments on physical- and chemical-responsive biopolymers*

In principle, the family of physical- and chemical-triggered biopolymers offers unlimited possibilities for designing new therapeutic and clinical strategies. Namely, polymeric backbones and peptide chains can be used as source material to shape devices that interact with highly specific targets. Since the response is often induced by selective molecule recognition mechanisms, therapeutic benefits can be optimally restricted to the area involved, maximizing their activity and further excluding the risk of side effects. In brief, this approach offers increased versatility in comparison with other stimulus-responsive polymers (i.e. thermal and pH).

Conversely, because the stimulus response is so specifically triggered, an in-depth knowledge of materials chemistry, the biological environment, and the biochemical mechanisms involved, and additionally of the medical background, is absolutely necessary to define the chemical approach. Thus, the more selective is the tool, the greater is the initial effort for engineering the solution: integration among different disciplines becomes mandatory when dealing with such complex systems.

## Concluding remarks: are smart materials the basis of a revolution in biology and biomaterials studies?

Undoubtedly, smart materials are gaining increasing importance in the fields of biomaterials and medical device design: they have played a major role in introducing a new approach to materials in medicine, based on bio-mimicking and property tailoring. New materials with specific and critical properties have been developed, by applying modern design methods of materials science to biomaterials science. In this contest, the several advantages offered by natural polymers with regard to availability, versatility, adaptability, and compatibility are rarely achieved by synthetic systems. Above all, biology offers a multitude of different structures and materials that have evolved to interact easily through specific mechanisms in living organisms. Moreover, natural-based systems represent an extremely powerful tool, considering, for example, the highly selective chemical interactions governed by enzymes, or the possibility to control cellular behavior through cell-instructive sequences. All the extraordinary features of smart materials are further enhanced when natural structures are used, since mild environmental conditions, consistent with those of biological systems, are sufficient to trigger a specific response.

The next step toward more effectively designing natural-based devices, and overcoming the variability that necessarily occurs in those materials, is to gain a better understanding of the mechanisms regulating the smart response and to gain a deeper knowledge of structure–property relationships. The development of multi-responsive materials goes hand in hand with this learning process. The design of smart systems that can respond to numerous physiological signals (i.e. temperature, pH, chemicals) may allow disease-specific treatments to be developed. Although in its infancy, this cutting-edge technology looks extremely effective in mimicking the body's physiological regulation mechanisms.

In conclusion, as extensively discussed in this review, biopolymers can be used effectively to address a plethora of medical problems, by proposing solutions based on the technological advantages their smart behavior can offer. This class of materials has thus the potential, in the near future, to solve some of the critical problems still open in medicine, and to introduce groundbreaking medical procedures, thus improving health care, healing processes, and patients' quality of life.

Has this novel era already begun?

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