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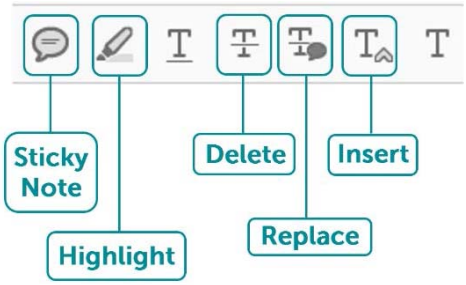

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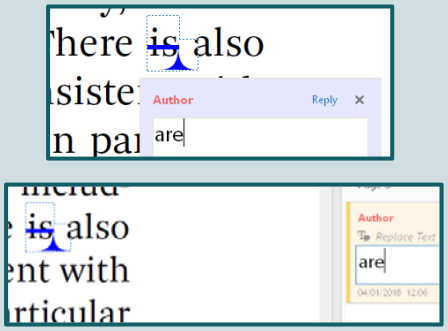

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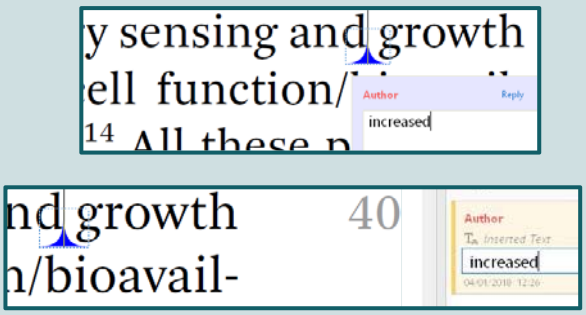

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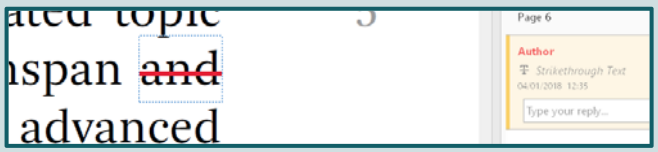

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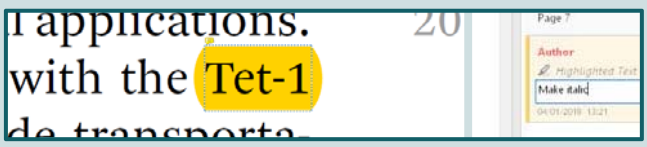

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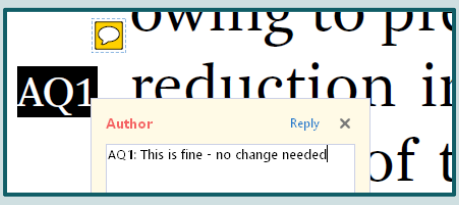

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Soft nanoparticles represent a unique class of nanomaterials that can be engineered to react and adapt distinctly in different biological milieus while modulating the presentation of biochemical and biophysical cues to neighbouring receptive cells. This flexibility has fuelled the development of soft nanoparticle-laden nanocomposite hydrogels that are increasingly sophisticated in stimuli-responsiveness and promising for satisfying a plethora of biomedical applications. Such hybrid platforms can be encoded with intelligent disease-discerning tools, smart adaptability under external triggers for bioactive cargo delivery or be engineered for manipulating biomechanical properties in different tissue microenvironments. In addition, they can be interfaced with biological components (*i.e.* enzymes, cell membranes) or specific substrates recognisable by biological machinery, yielding biomolecule-responsive systems that perceive changes in their surroundings and alter their therapeutic outputs accordingly. In essence, this chapter highlights the unique opportunities of soft nanoparticles to function as versatile building blocks for programming and modulating a large array of features in hydrogel-based platforms, thus extending their biofunctionality and applicability in tissue engineering and regenerative medicine practices.

CHAPTER 23

Stimuli-responsive Nanocomposite Hydrogels Incorporating Soft Nanoparticles for Biomedical Applications

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23.1 Introduction

In their native tissue microenvironments, cells are constantly influenced by a plethora of biochemical and biophysical cues that are dynamically orchestrated by the surrounding extracellular matrix components.[1] This supportive hydrogel-like bioarchitecture is inherently perceptive and adaptive in design, being capable of actuating biological responses towards maintaining homoeostasis.[2] Following this principle, over the last few decades, researchers have been pursuing the integration of such bioresponsive features within nano-to-macro sized biomaterial platforms (*i.e.* nanoparticles and hydrogels), in an attempt to mimic the hierarchic features, as well as the adaptive and intelligent behaviour of living tissues.[3–5] Engineering stimuli-responsive 3D bioarchitectures usually entails incorporating degradable linkages, dynamic covalent bonds or directing non-covalent/supramolecular

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interactions within the network of such systems.[6–8] Despite presenting valuable features such as stand-alone formulations, it has been increasingly demonstrated that the combination of nanoparticles and hydrogels can yield multifunctional hybrid platforms (*i.e.* nanocomposite hydrogels) that integrate the advantageous features of both biomaterial classes, often resulting in a combinatorial or synergistic gain of function for their envisioned biomedical application.[9,10]

Stimuli-responsive nanocomposite hydrogels have been engineered to respond to a wide array of endogenous signals (*i.e.* pH, temperature or certain biomolecules), that can be: (i) ubiquitously present throughout the human body, (ii) disease-associated, or (iii) tissue-specific, depending on the considered stimuli input. In contrast, other stimuli (*i.e.* light, magnetic fields or ultrasound) can be externally applied in a non-invasive manner to elicit remote-controlled localised responses in nanocomposite hydrogels following administration.[11] Moreover, certain types of stimuli (*i.e.* electric signals and mechanical forces) have been simultaneously exploited as endogenous triggers, taking advantage of bioelectrical intratissue communication or physical activity-induced mechanical loading, or as exogenous triggers in the form of on-demand electrical or mechanical stimulation inputs.[12] Due to such features, hybrid nanocomposite platforms provide unprecedented spatiotemporal control over biochemical cue presentation and can allow dynamic modulation of biomechanical properties for bioinstructing cell activity and phenotype.[13]

Nanocomposite hydrogels can be assembled by incorporating either soft organic nanoparticles materials (*e.g.* polymers, proteins, peptides or lipids) or solid inorganic nanoparticles (*e.g.* silicates, metal ions or rare earth elements), within the hydrophilic hydrogel network.[14] Inorganic-based nanocomposite hydrogels, namely magnetite or lanthanide-doped upconversion nanoparticles, are able to react to unique stimuli that are elusive to soft nanocarriers, such as sensing magnetic fields or converting incoming light from higher-to-lower wavelengths, respectively.[15] However, due to their mineral-based composition, expanding the range of stimuli available beyond their intrinsic ability is difficult, and this poor programmability can only be overcome to a limited extent through surface engineering. In contrast, soft nanoparticles are highly programmable due to their chemical versatility and ease of functionalisation and can therefore react to a wider array of biological inputs compared to their inorganic counterpart. Moreover, hybrid platforms embedded with soft nanomaterials benefit from their inherently biodegradable nature, thus avoiding serious concerns over poor biodegradability and body accumulation associated with certain inorganic nanomaterials.[16] Furthermore, inorganic nanoparticles lack suitable control over biomolecule transport and release, frequently resorting to surface adsorption for loading therapeutics and hence displaying sub-optimal controlled delivery capabilities. Conversely, soft nanocarriers can efficiently load bioactive molecules within their matrix and provide multimodal release profiles by tailoring the stimuli-responsive features in their backbones.

This sophisticated behaviour was further extended in recent hierarchic hydrogel platforms incorporating multiple soft nanocarriers that act as stimuli-converting components.[17,18] Such smart logic transducers are capable of relaying an initial stimuli input to a completely distinct stimuli as output, subsequently triggering a feedback cascade in dormant nanocomponents downstream. Leveraging this principle, multiple stimuli can be seamlessly interconnected to yield biomimetic hybrid platforms with self-regulating capabilities or amplifiable stimuli-sensitivity cascades tuned either to pathological or physiological input thresholds.

In this chapter, we describe current design iterations of dynamic hydrogel platforms containing embedded soft nanoparticles and specialised for biomedical purposes. Moreover, the interest in incorporating nanocarriers in such systems can be ascribed beyond their capacity to serve solely as bioactive cargo reservoirs, but also as components that can function as additional network crosslinking nodes or that can impart on-demand modulation of matrix mechanics (*i.e.* stiffening or softening) through their innate stimuli-responsive features. This focus is further complemented with a critical outlook on the implications and challenges of nanoparticle–hydrogel hybrid systems developed to date, as well as their potential role in benchmarking the design of next-generation nanocomposite hydrogel platforms. Overall, biofunctional nanocomposite hydrogels incorporating soft components are highly promising for numerous biomedical applications in light of their flexible design and multifaceted ability to respond under distinct biological scenarios.

23.2 Design and Assembly of Soft Nanoparticle Functionalised Nanocomposite Hydrogels

As aforementioned, the herein described nanocomposite hydrogels arise from the formulation of 3D hydrogel networks embedded with soft nanoparticles or other nanostructures. Compared to inorganic nanoparticle functionalised nanocomposite hydrogels, the incorporation of soft nanoparticles is limited to assembly methodologies that require milder conditions, due to the inherent sensitivity/cross-reactivity in the case of employing lipidic, polymeric or protein-based building blocks. Moreover, *in situ* nanoparticle formation within pre-formed hydrogel templates is usually pursued with inorganic nanomaterials such as nucleating calcium nanoparticles or polymerisable polypyrrole nanorods.[19,20] Conversely, soft nanocomposite hydrogels manufactured with this approach are usually limited to self-assembling nanocarriers or other nanomaterials that can be assembled on-demand with external stimuli alterations (*i.e.* pH, temperature or light), but ultimately lead to poorly controlled nanoparticle size distributions and require the use of simpler soft nanoparticles with limited smart behaviour. The vast majority of soft nanoparticle–hydrogel networks are typically designed with pre-assembled nanomaterials, as this strategy

allows concerns over cross-reactivity during hydrogel crosslinking and nanoparticle formulation optimisation steps to be bypassed. This approach also enables the use of more sophisticated biomaterials, thus augmenting the multifunctionality and complexity of the resulting stimuli-responsive systems.

Briefly, the formation of such hybrid platforms could be achieved either: (i) independently of nanomaterial incorporation, where the latter act as network fillers with minimal nanoparticle–hydrogel interaction, or (ii) conditioned by their presence, where nanoparticles play a critical role in providing crosslinking nodes within the final hydrogel network. In the first strategy, hydrogel components are generally crosslinked in a solution containing pre-dispersed colloidal nanoparticles, resulting in their physical entrapment within the hybrid network. For this reason, the process of nanoparticle outwards extravasation to surrounding tissues following implantation occurs in a limited rate but it is not fully halted.[21] Nevertheless, this approach is widely explored for streamlined manufacturing of soft nanoparticle-based stimuli-responsive hybrid platforms, benefiting from ~~ease~~ of optimisation (*i.e.* focus on hydrogel formation and less constrains regarding nanoparticle/hydrogel ratios) and freedom to tailor nanoparticle content by simply manipulating colloidal suspensions concentration during crosslinking. This methodology is also attractive for generating cell-laden nanocomposite hydrogels and although high cell densities may impact the final hybrid network physicochemical or biomechanical properties, this effect is more prevalent in the second approach where cells can hinder nanoparticle crosslinking through internalisation or overall steric hindrance.

Alternatively, in the second main strategy, soft nanoparticles serve as crosslinking nodes within the hydrogel network. To do so, hydrogel components and nanoparticle surfaces are chemically engineered to present specific complementary moieties that can synergistically elicit network formation. The vast plethora of available functional groups reveals opportunities for nanomaterials to dynamically modulate hydrogels biomechanical properties from the nano- to the macroscale and influence assembly/disassembly states beyond their traditional bioinstructive molecular reservoir role. Besides, this particular methodology aims to improve the control over the presentation of bioactive cues loaded in nanoparticles or entrapped within the hydrogel matrix, by taking advantage of the correlation between nanoparticle content and network physicochemical properties, which subsequently impacts the bioactive molecule release profile. In such scenarios, increasing nanoparticle content (and therefore total drug load) simultaneously augments crosslinking density and offers more sustained release kinetics. In a recent report, acrylated nanomicelles were covalently embedded into poly(ethylene glycol) (PEG)-diacrylate hydrogel networks upon photocrosslinking, mechanically stabilising the hydrophobic domains at the micelle core which could be leveraged for templating nanocrystal formation of active pharmaceutical ingredients.[22] Interestingly, by using fenofibrate as

a model drug, the authors demonstrated that such hybrid platforms could be readily and efficiently loaded post-formation by soaking in a drug suspension, reaching up to 90% loading capacity and with 70 times improved drug solubility. Due to the permanent attachment of nanomicelles to the hydrogel network, this unique approach yielded replenishable bioactive reservoirs that can be efficiently refilled on-demand and function as long-term drug delivery depots. Based on such findings, researchers are currently focusing on transposing such technology to more relevant bioresponsive hydrogel networks that can rapidly and intuitively respond to physiological conditions.

Other than employing covalent linkages between nanoparticle and hydrogel components, non-covalent interactions are also highly promising for designing dynamic hybrid platforms. Researchers have recently developed injectable and self-healing viscoelastic nanocomposite hydrogels that can be placed between injured organs and surrounding tissues, functioning as dynamic barriers that allow these structures to move naturally while inhibiting adhesion formation.[23] This soft-based hybrid network is established from dynamic nanoparticle–polymer interactions among hydrophobically-functionalised hydroxypropyl methylcellulose and PEG–poly(lactic acid) (PLA) nanoparticles. This has been successfully employed as an effective post-operative pericardial adhesion barrier that can be easily sprayed into injured cardiac tissues due to the reversible and dynamic nanoparticle–hydrogel crosslinking. Interestingly, this system outperformed clinically available alternatives such as Seprafilm® and Interceed® in a rat model of severe pericardial adhesions. Alternatively, this kind of characteristic soft nanocomposite hydrogel system also serves as the platform for establishing a universal nanocarrier bioink, leveraging its ease of assembly, self-healing, shear-thinning and drug reservoir flexibility, all key features for additive manufacturing inks development.[24] This nanoparticle–hydrogel combination can be further engineered with secondary networks of different biomaterials (*i.e.* PEG-diacrylate, hyaluronic acid-methacrylate, gelatin-methacrylate, alginate, fibrinogen) that can react to distinct stimuli (*i.e.* light, ions or enzymes, respectively). Collectively, this work highlights the multiplexed biofunctionality of hydrogel nanocomposites for bioprinting living tissue constructs or for manufacturing platforms with controlled release of bioactive molecules.

In light of this, while covalently tethered nanoparticle–hydrogel networks prevent outward particle diffusion from the matrix, employing dynamic covalent bonds that readily respond to stimuli yields the advantages of assuring the colocalisation of nanoparticle depots, combined with the adaptability and smart behaviour of stimuli-responsive systems.[10] Moreover, due to their multivalent presentation of reactive handles, their incorporation can often amplify the bulk response threshold to incoming stimuli inputs. The downside to this approach stems from the increased complexity in optimising nanoparticle/hydrogel ratios to ensure network formation, a critical parameter which ultimately restricts the total

nanoparticle content that can be incorporated using this approach. This occurs because for a given concentration of hydrogel components, increasing nanoparticle content ultimately reaches a crosslinking density plateau, after which additional nanoparticle quantities have a detrimental effect on the network stability by reducing the number of effective crosslinks. Alternatively, this issue can be partially mitigated by employing a combination of the two different methodologies, *i.e.* incorporating two nanoparticles species, (a) one that is functionalised for providing crosslinking nodes, and (b) pristine nanocomponents that are physically embedded for the purposes of providing a reservoir of bioactive molecules at doses suitable to meet their therapeutic window. Interestingly, apart from operating as crosslinking agents, some soft nanoparticles can also act as crosslinking catalysts. Regarding this, some studies have reported the use of soft nanoparticles based on trimethylbenzoyl-diphenylphosphine oxide that can intrinsically respond to light stimuli and serve as an efficient photoinitiator in hydrogel crosslinking and during 3D bioprinting.[25] Although this particular instance represents an additional feature for expanding the versatility of the nanoparticle toolset, concerns regarding cell internalisation *versus* cytotoxicity, as well as long-term effect on cellular viability and bioactivity (due to radical species generation) are highly relevant and remain to be elucidated in future works.

23.3 Stimuli-responsive Nanocomposite Platforms for Bioactive Cargo Delivery

A flagship of soft nanoparticle-based nanocomposite hydrogels is their remarkably improved biomolecule loading and controlled release capacity over traditional pristine hydrogel platforms. The nanocarrier-laden nature of such networks sets opportunities for incorporating both hydrophilic and hydrophobic drugs, the latter which comprise nearly 70% of small molecules in commercial pipelines.[22] As highlighted above, due to their polymeric/protein-based backbone, soft nanoparticles can be encoded to react to several stimuli inputs, thus functioning as highly programmable building blocks capable of modulating or actuating a wide range of features in hybrid hydrogel matrices.[3] Therefore, stimuli-responsive hydrogel nanocomposites are being pursued as superior candidates in the context of improving biomolecules delivery, especially when compared to simplistic hydrophilic hydrogel matrices.

The integration of stimuli-responsive elements within these hybrid platforms enables the design of sophisticated devices that can autonomously differentiate healthy from diseased states, by recognising specific stimuli that are key hallmarks dysregulated in certain pathologies or injuries.[3] Furthermore, by tailoring stimuli-sensitivity trigger thresholds to physiological levels, other intelligent hybrid systems can be developed that readily switch from dormant to therapeutically-active states upon

administration.[26,27] In addition, the following subsection will highlight the attractive features of nanocomposite hydrogels incorporating enzymes, which are inherently biomolecule-responsive entities, in the scope of attaining advanced biomimetic platforms with dynamic therapeutics presentation regulated by endogenous or exogenous biomolecules. Alternatively, hybrid systems engineered to react to exogenous, minimally invasive external stimuli, are also overviewed and showcased in light of their potential for operating as remote-controlled devices with on-demand bioactive cargo delivery for long-term disease management or timely presentation of biochemical cues for augmenting tissue regenerative processes.[15,28]

23.3.1 pH-responsive

Dysregulated pH gradients are hallmarks found in certain diseased states (*i.e.* tumours and inflammation) and represent one of the most widely explored stimuli in the scope of soft-based nanocomposite hydrogels with dynamic behaviour.[3] Moreover, besides its pathophysiological dysfunction, pH is characteristically altered in certain anatomical sites (*i.e.* along the gastrointestinal tract) and in intracellular endosomal compartments, enabling specialised biomedical applications for oral administration or specific intracellular delivery, respectively.[3] On the other hand, on-demand bioactive cargo delivery under a bioelectronic trigger has also been demonstrated by integrating pH-responsive systems with electrodes, thus coupling bioelectronic signalling to pH-responsiveness by leveraging current-triggered proton-release to elicit network disassembly.[29] Overall, pH-responsive hybrid platforms that dynamically alter their structural properties are highly attractive ~~for use~~ in triggering site-specific bioactive cargo delivery in such scenarios. This smart behaviour is typically achieved by either: (i) using pH-ionisable moieties, that shift their charge according to the pH of the surrounding environment based on their characteristic pK_a, or (ii) incorporating pH-cleavable dynamic covalent bonds with acid-labile sensitivity.

The two soft nanomaterials most commonly employed in the design of pH-responsive nanocomposite hydrogels are polymeric nanomicelles and cellulose nanocrystals. Earlier strategies took advantage of the intrinsic pH-sensitivity of hydrogel polymeric components combined with cellulose nanocrystals, arising from their pH-ionisable behaviour and swelling-induced accelerated bioactive cargo delivery.[30] Although promising for oral delivery applications, due to their non-covalent crosslinking, such constructs often lack appropriate mechanical robustness and present poorly controlled release profiles, which hinders further applicability. Over the years, the emerging strategy has been to engineer nanoparticles and biomaterials with dynamic covalent linkages, such as imine bonds, in order to simultaneously benefit from the structural robustness arising from covalent bonds as well as the adaptive features conferred from their intrinsic stimuli-responsive

behaviour.[31] Concerning this, researchers have combined quaternised chitosan with curcumin-loaded aldehyde-functionalised polymeric nanomicelles for developing injectable, tissue-adhesive, nanocomposite hydrogels suitable for joint skin wound healing applications.[32] Mixing the two components yields hydrogel nanocomposites – built from nanomicelle crosslinking with interbridging quaternised chitosan – that are pH-responsive due to the dynamic covalent crosslinking arising from Schiff base formation between the nanomicelle's exposed aldehyde moieties and chitosan amine groups. This hybrid platform showcased outstanding mechanical properties (*i.e.* repeated long-lasting stretchability and compressibility), self-healing behaviour and suitable tissue-adhesiveness on porcine skin. Apart from this, the inflamed wound environment provides the trigger to accelerate anti-inflammatory curcumin presentation under its characteristic acidic pH stimuli. Such nanocomposite hydrogels not only displayed enhanced haemostatic performance but also successfully accelerated *in vivo* wound healing closures compared to clinically used Tegaderm™ films in a full-thickness skin defect model. Similarly, another study described that nanocomposite hydrogels comprising hydrazide-pendant hyaluronic acid and benzaldehyde-functionalised Pluronic nanomicelles led to substantially improved wound closure rates over the standard-of-care Mepitel®, in an *in vivo* deep partial-thickness burn model.[33] Remarkably, owing to its quaternised chitosan backbone, the previous nanocomposite hydrogels presented remarkable antibacterial activity against *E. coli* and *S. aureus*, an attractive feature for further improving treatment of infected wounds.[32] Such hybrid platforms with encoded pH-responsiveness can also be promising for future application as platforms for localised cancer treatment, taking advantage the acidic microenvironment of tumours and the well-established anti-tumour/anti-metastatic activities of curcumin.

Alternatively, nanomicelles have been engineered to contain hydrophobic pendant moieties grafted in their polymeric backbone through a pH-sensitive Schiff base linkage, which endows hydrogels with pH-responsive disassembly. Moreover, this ligand was designed to coordinate selectively with divalent metal ions (Cu^{2+}, Co^{2+}, Ca^{2+}, Mn^{2+}, Pb^{2+} and Fe^{2+}) thus promoting ion-sensitive hydrogel assembly and crosslinking.[34] Due to their dynamic network, the obtained hydrogels were readily injectable and exhibited fast self-healing kinetics. Moreover, such multi-stimuli-responsive hydrogels could disassemble in acidic conditions or through exposure to chelating ligands (*i.e.* EDTA), as well as reversibly assemble under alkaline stimuli or ascorbic acid addition.

In a different approach, water-soluble glycol chitosan and aldehyde-functionalised polyurethane biodegradable nanoparticles were employed for manufacturing pH-responsive nanocomposite hydrogels.[35] Dynamic Schiff base linkages readily established between nanoparticles and biopolymer chains led to the production of self-healing hydrogels at room temperature and cryogels at −20 °C, respectively. Both these platforms display intrinsic injectability and cytocompatibility, while also showcasing low

immune response in rats at 14 days following *in vivo* administration. Moreover, as expected, due to its pH-responsive imine bond, the hydrogels could be rapidly degraded in acidic conditions, and this stimuli-triggered disassembly could be tuned by adjusting nanoparticle pH-sensitivity. In this context, the authors showed that the soft polymeric segments of nanoparticles can be precisely tailored to obtain biodegradable hydrogels/cryogels with distinct degradation rates. On a similar note, other researchers developed nanocomposite hydrogels comprised by aldehyde-terminated polymeric micelles (PEG–PLA copolymer backbone) crosslinked with amine-containing polyethyleneimine.[36] In particular, two distinct nanomicelle species possessing either (i) tightly packed, well-entangled inner cores, or (ii) a more loosely packed inner structure, that are characterised with slower and faster drug release kinetics, respectively, were developed. Interestingly, hydrogels produced from tightly packed micelles exhibited a higher storage modulus than hydrogels formed from loosely packed micelle nanostructures, showcasing that nanocarrier architecture influences the final mechanical properties of nanocomposite hydrogel platforms. Moreover, hybrid formulations containing both micelle species, each loaded with a fluorescent probe as the model drug (*i.e.* rhodamine B and auramine O), successfully achieved simultaneous release of the two compounds at independent rates. Such multimodal presentation of different biomolecule cocktails is extremely promising for potentiating tissue regeneration or improving disease attenuation and represents a flagship feature of nanocomposite hydrogels that is highly advantageous for tissue engineering and therapeutic applications. Following this rationale, the same group demonstrated that such concept can be further extended to other amine-containing polymers, such as chitosan, to manufacture wound dressing hybrid hydrogels with improved cytocompatibility and biodegradability.[37]

Besides its traditionally explored pH-responsiveness, dynamic crosslink equilibria among amine groups can be leveraged for designing bioresponsive nanocomposite hydrogels that dissolve on-demand upon making contact with amine-rich solutions.[38] This is particularly beneficial for achieving painless removal of hydrogel dressings applied for wound healing. In this context, researchers have designed nanocomposite hydrogels based on carboxymethyl chitosan and aldehyde-functionalised cellulose nanocrystals, with intrinsic injectable and rapid self-healing features.[38] Interestingly, it was demonstrated that spraying glycine solutions readily triggers hydrogel disassembly from burn wound sites *in vivo*, thus constituting a superior and painless alternative over surgical debridement of wound dressings. Another unique feature of cellulose nanocrystals is that these nanomaterials can react to shear forces and induce their alignment in the same direction.[39] Furthermore, recent works have also demonstrated that tannic acid can be leveraged for engineering nanocrystal surfaces with pendant pyrogallol moieties, which upon interacting with borax/poly(vinyl alcohol) networks can yield robust hydrogel nanocomposites with intrinsic self-healing, pH-responsive and glucose-sensing features.[40]

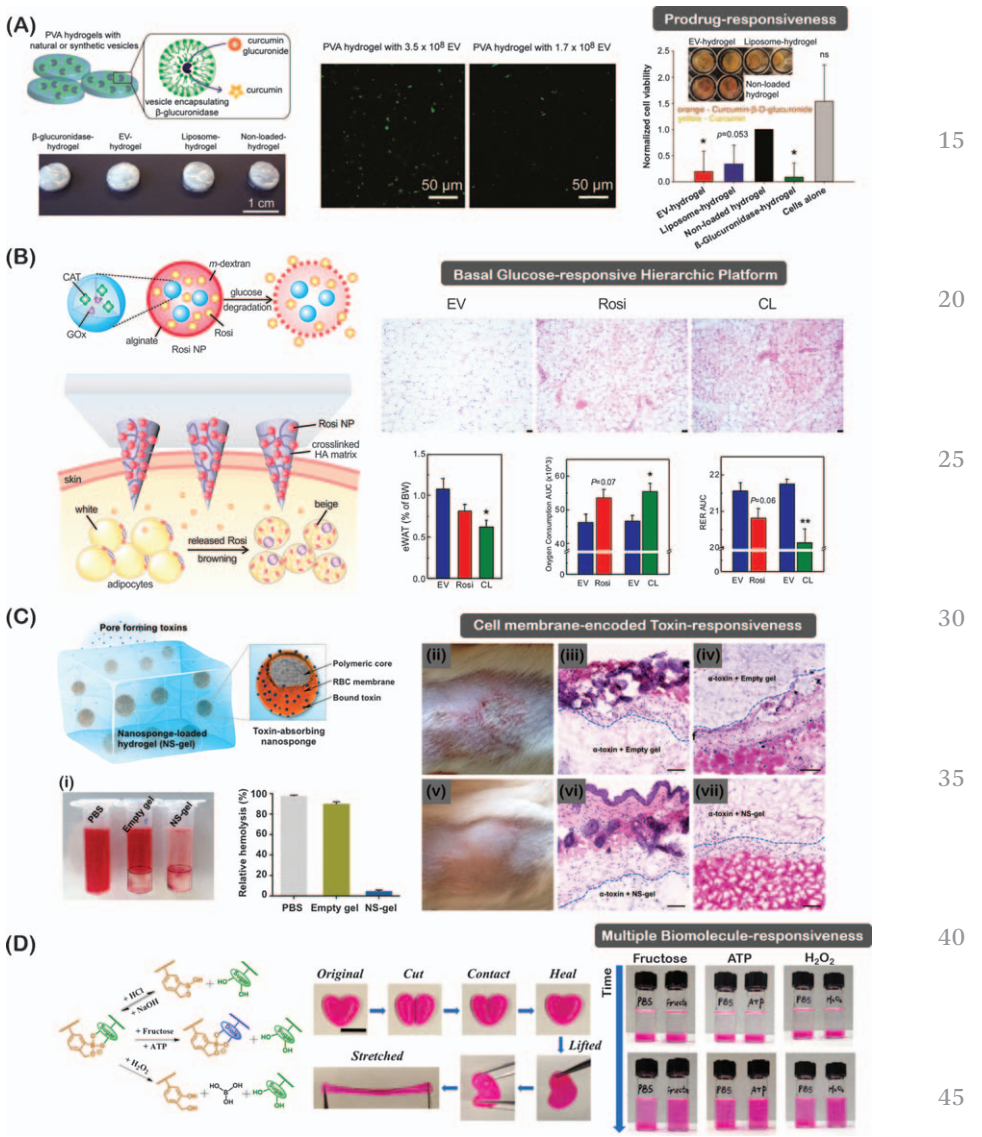
23.3.2 Biomolecule-responsive

Living tissues are characterised by their remarkable ability to dynamically adapt and respond to their surroundings, a process that is usually driven by complex ~~interwoven~~ biochemical pathways. Biomolecule-responsive designs are emerging as sophisticated systems with tissue-like intelligence since they mimic the intrinsic ability of native tissues in performing biomolecular recognition and adjusting their biological performance accordingly. Regarding this, two different approaches for driving the development of biomolecule-responsive systems have been explored so far. One strategy encompasses designing systems built from specific biomaterial components that tissues/cells recognise and can react to, namely enzyme substrates incorporated within hydrogel backbones able to be identified and processed by tissue circulating enzymes, and therefore the presence of the administered platform elicits a response from the body.[41] For instance, nanocomposite hydrogels comprised by gelatin nanoparticles (size: 300 nm), methacrylated collagen I and thiolated hyaluronic acid yielded self-supporting nanoparticle-laden bioinks that were leveraged for bioprinting large structures encompassing HepG2 organoids that efficiently supported their growth.[42] In recent reports, the incorporation of soft gelatin nanoparticles in autologous platelet-rich fibrin led to the generation of double network nanocomposite hydrogels with highly improved mechanical properties, thus enabling them to withstand bone mechanical loading while extending the release of bioactive growth factors.[43] Moreover, its material composition is entirely based on extracellular matrix-mimetic components, thus yielding enzymatically degradable constructs that can be continuously remodelled by living cells. In addition, nanocarrier content can be adjusted in such networks to tailor network porosity, thus allowing fine modulation of diffusion kinetics of nutrients or other biomolecules.[44]

In the second strategy, nanocomposite hydrogels are endowed with the biological recognition tools that allow them to dynamically react to biomolecules present in native tissues. Such biomolecule-identifying components can comprise: (i) enzymes hierarchically embedded within the hybrid network, (ii) cell membrane wrapping nanoparticles, or (iii) specific analyte-reporting chemical moieties attached to hydrogel backbones or exposed at nanoparticle surfaces. In this design, different biomolecular inputs (*i.e.* enzyme substrates, drugs, toxins, glucose or ATP) present in the body elicit a response (*i.e.* assembly, disassembly or architectural reconfiguration) from the administered platforms at thresholds which can be tailored and adjusted to meet either physio- or supraphysiological levels.

Living cells are extraordinary biofactories that perpetually sense and react to their environment with an encoded adaptiveness that is driven by a diversified enzyme toolset at their disposal. As aforementioned, advanced nanocomposite hydrogels can include the design of biomimetic nanocarriers carrying active enzymes to endow the platform with dynamic biomolecule-responsiveness. This unique stimuli-responsiveness was recently explored by embedding human mesenchymal stem cell-derived nanovesicles containing β-glucuronidase in

poly(vinyl alcohol)/PEG hydrogels, thus serving as locally controlled conversion depots of benign prodrugs to active anti-inflammatory compounds (Figure 23.1A).[45] The authors successfully demonstrated site-specific release of bioactive curcumin from its glucuronide precursor, as well as superior anti-inflammatory potential for repeated long-term incubation in macrophage inflammation models. Through its innate drug-responsive features, this design could allow for on-site bioactive drug activation following systemically administered non-toxic and inert prodrugs.



In a pioneering study, researchers developed self-regulated platforms with glucose-recognition capabilities for insulin delivery consisting of catalase (CAT) and glucose oxidase (GOx) enzyme nanocapsules (size: 12 nm) embedded in pH-responsive chitosan hydrogels loaded with human recombinant insulin.[17] The enzymes were engineered into nanogel-based assemblies to improve their stability and locally confine their action. In this pancreas-like hydrogel system, GOx converts glucose to gluconic acid and hydrogen peroxide, decreasing the pH of the system, while CAT regenerates undesired hydrogen peroxide back to oxygen and improves GOx activity. Thus, hyperglycaemic conditions lead to pH decreases, where the chitosan 3D network continuously swells and expands, thus triggering insulin release. Conversely, normoglycemic conditions shrink the hydrogel network which results in diminished insulin release. When implanted into diabetic mice, the hydrogel-based platforms (*i.e.* with enzyme nanogels + insulin loading) showed no fibrotic encapsulation and successfully prolonged the normoglycemic state, displaying higher insulin plasma levels and lower blood glucose levels compared with the control insulin-loaded platforms. However, the effectiveness of such pH-sensitive platforms can be compromised by slow responsiveness in buffered physiological environments. To tackle such issues, the same group has recently coupled the system's glucose-responsiveness to hypoxia-sensitive building blocks, achieving rapid *in vivo* pharmacokinetic responsiveness while maintaining ease of administration and biocompatibility.[46] This was achieved by developing glucose-responsive nanovesicles (size: 118 nm) loaded with GOx and insulin, resulting from the self-assembly of hypoxia-sensitive hyaluronic acid modified with pendant hydrophobic 2-nitroimidazole. When sensing high blood glucose levels,

Figure 23.1 Biomolecule-responsive nanocomposite hydrogels. (A) β-glucuronidase-loaded extracellular vesicles embedded in poly(vinyl alcohol) hydrogels for encoding *in situ* prodrug-responsiveness. Such hybrid hydrogels can convert incoming curcumin glucuronide inputs into bioactive curcumin, hence reducing inflammation *in vitro*. Adapted from ref. 45, https://doi.org/10.1002/adma.201706616, under the terms of the CC BY 4.0 license, https://creativecommons.org/licenses/by/4.0/. (B) Upon administration, basal glucose levels trigger the delivery of browning agents rosiglitazone (rosi) and CL 316243 for transforming white to brown-like adipose tissue in the scope of obesity treatment. *In vivo* performance of the platform with empty vehicle (EV), rosi and CL in mice. Adapted from ref. 48 with permission from American Chemical Society, Copyright 2017. (C) Hybrid platforms endowed with toxin recognition using cell membrane-coated nanoparticles, that can efficiently bind α-toxin *in vitro* (i) and perform *in vivo* neutralisation as observed in empty hydrogels (ii–iv) *versus* nanosponge-loaded hydrogels (v–vii). Adapted from ref. 49 with permission from John Wiley & Sons, Copyright 2015, WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim. (D) Self-healing hybrid platforms programmed with multiple biomolecule-responsiveness (*i.e.* fructose, ATP and H_2O_2) due to dynamic benzoxaborole crosslinking. Adapted from ref. 51 with permission from American Chemical Society. Copyright 2019.

local oxygen is rapidly consumed during GOx-mediated glucose oxidation, producing a local hypoxic environment. This hydrophobic moiety readily switches to a hydrophilic state in hypoxic conditions, triggering the self-destruction of the loaded nanovesicles embedded in a photocrosslinkable hyaluronic acid hydrogel network. Administration of this hydrogel nanocomposite system in type 1 diabetes mouse models demonstrated efficient avoidance of hyper- and hypoglycaemia during glucose challenge, particularly *versus* free insulin treatment. Collectively, these studies showcase the tremendous potential and advantageous features of integrating hydrogel platforms with intelligent soft nanocarriers, yielding autonomous and sophisticated self-regulating biomedical devices.

As aforementioned, it is possible to tune the threshold sensitivity levels in biomolecule-responsive platforms, which is extremely useful for expanding their biomedical applicability. For instance, instead of relying on pathophysiological glucose levels to trigger a therapeutic response, the system can be adjusted to recognise physiological concentrations (4-fold lower), thus enabling on-implantation responsiveness. Following this rationale, the formerly described glucose-responsive system was leveraged for engineering physiologically-responsive self-dissolving hierarchic hydrogel platforms for efficient melanoma therapy.[47] In this approach, the GOx/CAT enzymatic system and immunomodulatory anti-programmed death-1 (aPD1) antibody were loaded in pH-responsive acetylated dextran nanoparticles (size: 250 nm), which in turn were embedded in photocrosslinkable hyaluronic acid hydrogel networks. In this design, enzymes transduce basal blood glucose levels (100 mg dL^{-1}) into a local pH decrease, autonomously signalling for the self-destruction of the pH-responsive nanoparticles carrying the aPD1 antibodies and facilitating their sustained release. Remarkably, *in vivo* administration of this hydrogel-based patch in a mouse melanoma model showed that a single administration inhibited tumour growth and improved survival rates over intratumor injection of free antibody. Moreover, this system can be greatly improved by co-delivery with other immunomodulators (*i.e.* anti-CTLA-4), showcasing efficient melanoma control with long-term disease-free survival in 70% of treated mice. The success of this nanocomposite hydrogel platform has since been extended to other applications, namely clinical treatment of obesity and its comorbidities such as type-2 diabetes (Figure 23.1B).[48] Regarding this, the same group employed this platform for local delivery of browning agent rosiglitazone, capable of triggering the transformation of white adipose tissue to brown-like adipose tissue.[48] *In vivo* studies in diet-induced obese mice demonstrated that such brown remodelling led to a systemic increase in energy expenditure, fatty acid oxidation, significant body weight control and improved insulin sensitivity. Through its localised therapeutic delivery, this platform also alleviates safety concerns regarding the systemic administration of browning agents, thus establishing its promising biomedical potential.

Nanoparticle surfaces can also be bioengineered with cellular membranes, for instance those from red blood cells, thus inheriting their capacity to

efficiently sense and bind toxins.[49] Following this rationale, researchers designed smart biomimetic detoxification platforms in the form of toxin-responsive nanocomposite hydrogels (Figure 23.1C).[49] To achieve this, red blood cell membranes were wrapped onto polymeric poly(lactic-*co*-glycolic acid) (PLGA) nanoparticles, yielding nanosponges with acquired toxin-sensitive behaviour, followed by loading into hydrogels. Using a methicillin-resistant *Staphylococcus aureus* subcutaneous model, the administration of nanosponge-hydrogel hybrids showed remarkable anti-virulence therapeutic efficacy, demonstrated by significantly reduced skin lesion development. This advanced hybrid platform can harness the toxin-sensitive features of red blood cells and allow efficient treatment of localised bacterial infections through broad detoxification biomimetic action and without resorting to antibiotic delivery. Alternatively, nanosponge-laden photocrosslinkable PEG hydrogels were employed for 3D bioprinting patient-specific architectures with built-in open channels that allow directional body fluid permeation for further enhancing toxin-sensitivity.[50] Collectively, these examples demonstrate that integrating hydrogel systems with cell membrane-engineered nanoparticles is expected to yield advanced platforms that inherit the bioresponsive and cell-communicatory features of their underlying biological building blocks.

On the topic of analyte-sensing chemical moieties, researchers have recently designed elegant nanocomposite hydrogels with encoded multi-responsiveness, namely glucose, pH, hydrogen peroxide and ATP (Figure 23.1D).[51] This system combined temperature-responsive nanogels (size: 213 nm) bearing galactose residues at their surface with interbridging benzoxaborole-containing polymer chains. Benzoxaboroles readily establish dynamic covalent adducts between neighbouring galactose moieties, thus rapidly producing self-healing and mouldable hydrogel networks. Moreover, due to boronate esters intrinsic responsiveness (*i.e.* pH, sugar, ROS and ATP), nanocomposite hydrogels also inherit such multi-responsive features, showcasing great potential to be explored as intelligent drug depots with biomolecule-responsive release features.

23.3.3 Thermo-responsive

As a mild and ubiquitous stimulus, temperature has been widely explored for manipulating the properties of thermo-responsive hybrid platforms, particularly in the scope of attaining injectable hydrogels, on modulating drug delivery profiles, establishing sophisticated shape memory actuating systems, as well as providing sacrificial materials for bioprinting endeavours. A vast range of biomaterials have been disclosed with thermo-responsiveness, from methylcellulose derivatives, poly(*N*-isopropylacrylamide), poly(2-oxazoline), poloxamers, up to genetically engineered proteins.[52,53] More specifically, their thermo-sensitivity stems from their intrinsic ability to abruptly undergo phase/volume transitions in response to temperature changes on their upper and lower critical solution temperature (UCST and LCST, respectively).[54] Due to this

behaviour, hydrogel networks that exhibit LCST are formed upon heating, whereas networks with UCST are produced upon cooling. Human homoeostatic body temperature at around 37 °C can be conveniently exploited for developing nanocomposite hydrogels that can recognise intra-body heat upon administration. By adjusting their UCST/LCST threshold parameters, it is possible to achieve thermo-responsive platforms with distinct outcomes upon implantation.

One strategy is to design *in situ* forming hydrogels that can be injected or sprayed as precursor solutions, which then recognise the body's characteristic temperature increase and thus autonomously self-assemble into free-standing hydrogel constructs.[55] With this behaviour, hydrogels are rendered as complex tissue defect fillers by jellifying in the necessary 3D geometrical configuration, while subsequently conveying sustained passive release of loaded bioactive cargo to its surroundings. For instance, researchers have devised thermo-responsive hydrogels based on polypeptide poly(L-alanine-*co*-L-phenylalanine)–(PEG)–poly(L-alanine-*co*-L-phenylalanine) copolymers that displayed heat-induced gel transition at body temperatures arising from the hydrophobic collapse of the soft nanomicelles (*ca.* 95 nm) architecture into 3D macroscale hydrogel constructs.[56] This platform readily loaded bone marrow-derived mesenchymal stem cells and successfully regenerated cartilage in an *in vivo* rabbit osteochondral cartilage defect model. In this case, the embedded living cells functioned as biofactories constantly releasing tissue-specific bioactive cocktails to their surroundings. As a result, it induced cartilage formation with improved biomechanical properties (about two-fold that of the control) and extracellular matrix composition (*i.e.* glycosaminoglycans and collagen type II), as well as fostering chondrogenic formation and maintaining a chondrocyte phenotype over time. Although this particular study does not exploit pre-incorporated biomolecules within the network, it illustrates that nanoparticles with intrinsic stimuli-responsiveness can impart cell-laden hydrogel networks with dynamic adaptability under stimuli. Alternatively, researchers have recently developed temperature-responsive Pluronic® F127 hydrogels embedded with soft PLGA nanoparticles previously loaded with platelet lysates as the bioinstructive cargo.[57] Platelet lysates are comprised of multiple bioactive growth factors, and this multifactorial composition is suitable for accelerating the wound healing process. Owing to this bioactivity and its thermo-assembling behaviour, this platform could be easily sprayed to injured sites, adapting to the wound shape as a hydrogel and subsequently accelerating the wound closure rate in *in vivo* full-thickness wound models. However, in certain biomedical applications, enclosing cells or multifactorial bioactive cocktails can be detrimental for maintaining a specific cellular phenotype.[58] Alternatively, *in situ* forming nanocomposite hydrogels encompassing temperature-responsive polymers with embedded solid drug nanocrystals also constitute an attractive biomedical platform with high biomolecule loading content.[59] Although extremely promising and widely explored in numerous studies, such strategies are limited by the initial diffusion/clearance of precursor solution before the hydrogel crosslinking

process is concluded, which potentially reduces the amount of bioactive cargo that is retained in the matrix and that can ultimately be released to the administration site.

Conversely, thermo-responsive hybrid systems with UCST can be designed to pre-assemble outside the body (*i.e.* under room temperature conditions, *ca.* 25 °C) as mechanically-robust bulk hydrogels, that upon implantation and recognising the internal body temperature, slowly self-disassemble at the administration site, thus providing localised bioactive cargo delivery in a controlled mode. These reversible assembly/disassembly states are also usually employed to efficiently load therapeutics and/or living cells within these 3D structures by simply manipulating temperature conditions during the manufacturing process. Alternatively, by tuning the threshold sensitivity to supra-physiological temperature levels, it is possible to develop bulk hydrogels that are mechanically stable and retain their payload even after implantation, but readily react (*i.e.* disassembly) to externally applied mild heating (*i.e.* 42 °C). In this regard, thermo-responsive lysolipid liposomes that experience enhanced bilayer membrane permeability around their melting temperature (*i.e.* 41 to 43 °C).[60,61] These soft nanocarriers can efficiently load different drugs (*i.e.* doxorubicin and deferoxamine) and be incorporated within injectable chitosan/β-glycerophosphate hydrogels for distinct applications, namely chemotherapy and pro-angiogenesis, respectively. In the former approach, the remote-controlled activation mechanism yielded latent drug reservoirs that are inert while inactive and can be rapidly converted to chemotherapeutic-eluting platforms at determined timepoints.[60] In the latter, researchers demonstrated both sequential multimodal delivery of multiple biomolecules (*i.e.* hepatocyte growth factor and deferoxamine), as well as delayed presentation of proangiogenic cues in a user-defined manner in order to investigate optimal delivery regimens in augmenting angiogenesis processes.[61]

Besides opening the possibility to produce therapeutic devices with switchable on/off behaviour and on-demand bioactive cargo delivery in a minimally invasive mode, this approach also benefits from the ability to mature cells in their final 3D microenvironment before implantation, which is difficult to achieve with previous strategies without resorting to harvesting from other cell culture platforms. Furthermore, by embedding cold temperature-responsive nanocapsules within pancreatic β cell-laden alginate hydrogels, researchers have recently proposed an alternative cryopreservation method for improving current islet transplantation strategies.[62] Such nanocapsules are loaded with natural trehalose as the sole cryoprotectant and can readily undergo a hydrophobic-to-hydrophilic transition at temperatures below 10 °C, thus releasing trehalose inside β cells during the freezing process. Consequently, this cold-responsive cryoprotectant intracellular delivery vastly improves cell viability after freeze-thawing over traditional cryopreservation methods. Furthermore, upon *in vivo* transplantation, such cryopreserved cell-laden hydrogels attained equally effective reduction in blood glucose levels compared to freshly-prepared cell-laden hydrogels, illustrating its potential for serving as a ready-to-use biomedical

platform, namely with significantly reduced logistics and benefiting from streamlined manufacturing, storage and implementation processes for advanced cell therapies.

23.3.4 Light-responsive

Light represents an attractive external stimulus that is uniquely suitable for endowing biomedical platforms with improved spatiotemporal control over biomaterial behaviour, as well as enabling dynamic modulation of its bioactive cargo delivery.[63] Hydrogels with photoresponsive functionalities encompass systems that can be: (i) light-assembled on-demand *via* photocrosslinking, (ii) elicited to disassemble in a spatiotemporally controlled manner *via* photodegradable linkages, or (iii) showcase multiple interconnected stimuli by leveraging soft nanoparticles as stimuli transducing units, capable of converting initial light irradiation to locally dissipated heat. Moreover, unlike thermo-responsive systems that can only be tuned regarding their temperature sensitivity threshold, light stimuli can be adjusted on-demand in terms of intensity and wavelength inputs, which can be exploited for triggering differential responses in light-responsive systems.[64] This wavelength-specific reactivity further expands the range of available configuration states that can be attained over traditional binary on/off nanocomposite hydrogels, thus opening new avenues for designing remote-controlled platforms that respond to the same input (*i.e.* light), but display independent orthogonal activation pathways that can be programmed for providing distinct outputs.

Across the plethora of soft nanomaterials employed for developing light-responsive systems, endogenous components such as self-assembled biliverdin and melanin nanoparticles are some of the most widely pursued for encoding light-responsiveness within hydrogel networks. For instance, nanoparticles based on the biological bile pigment biliverdin are intrinsically bioactive (*i.e.* anti-oxidant, anti-inflammatory and anti-mutagenic) and exhibit photothermal behaviour with intense NIR absorption capabilities.[65,66] Photothermal nanomaterials can convert incoming light inputs into local temperature outputs and are therefore frequently integrated within thermo-responsive hydrogel networks which can take advantage of the generated heat to produce further downstream outputs in a cascaded manner. Such hierarchical nanocomposite hydrogels with interconnected light-temperature stimuli have demonstrated significant impact in photothermal ~~chemo~~therapy applications. Melanin nanoparticles can also operate as photothermal agents to enable light-induced actuation of temperature-responsive hydrogel architectures and on-demand bioactive cargo delivery.[67] Researchers have combined cuttlefish-derived melanin nanoparticles with PLGA–PEG–PLA triblock polymer networks, which display temperature-responsive transitions, to obtain photoreconfigurable hydrogels. Hydrogels experienced considerable softening, from an initial storage modulus of 7.3 kPa to 5.6 kPa after 1 minute of UV light irradiation ($\lambda = 365$ nm).

Such biologically-derived nanoparticles may be exploited to confer photoreconfigurability to other hydrogel systems designed with temperature-responsive materials, as well as harness its potential for attaining pulsatile bioactive release. Beyond extracting biogenic melanin nanoparticles, researchers have developed polydopamine nanomaterials inspired by melanin biosynthesis and the adhesive proteins in mussels, which have broadened their versatility in biomedical endeavours.[68] By inheriting features from its natural counterpart, such melanin-like nanoparticles are attractive for developing multi-stimuli-responsive nanocomposite hydrogels for combinatorial application of photothermal and ~~multidrug~~ chemotherapy.[69] This platform was based on a polydopamine nanoparticle/poly(acrylamide-*co*-*N*-isopropylacrylamide) crosslinked network and leverages the intrinsic photothermal capacity of such mussel-inspired nanoparticles to convert incoming NIR light to locally dissipated heat, which damages nearby cancer cells *via* hyperthermia. Consequently, the generated heat also softens the thermo-responsive polymeric network, thus facilitating the release of loaded doxorubicin. Besides the traditionally employed doxorubicin, polydopamine nanoparticles incorporated within PEG-based hydrogels can also efficiently deliver ethyl-10-hydroxycamptothecin in a pulsatile manner under intermittent NIR pulses and ultimately augment *in vivo* tumour suppression in mice.[18]

More recently, on the topic of wound healing, researchers disclosed dual temperature-NIR light-responsive nanocomposite hydrogels assembled from the combination of polydopamine nanoparticles, a poly(acrylamide-*co*-*N*-isopropylacrylamide) hydrogel network and graphene aerogel.[70] In particular, this system combined suitable tissue adhesiveness endowed by polydopamine nanoparticles with electrical conductivity conferred by graphene doping, the latter of which was leveraged for providing real-time monitoring of drug release (*i.e.* doxorubicin or epidermal growth factor). Upon NIR light irradiation, the accompanying shrinkage of the hydrogel leads to changes in conductivity that can be successfully correlated with release rates and predict drug concentration. Besides the on-demand controlled pulsatile drug release capacity, hybrid hydrogels loaded with epidermal growth factor markedly improved wound regeneration in rats bearing full-skin defects, namely by enhancing mature collagen fibre deposition and vastly accelerating wound closure rates compared to control groups. Such platforms with on-demand bioinstructiveness and encoded with real-time monitoring features are extremely promising for materialising sophisticated regenerative medicine platforms moving forward.

Besides employing characteristic photoabsorbing nanomaterials, photothermal molecules (*i.e.* indocyanine green and derivatives) can be loaded in nanocarriers to endow them with photothermal properties, thus further expanding the range of available nanomaterials that can function as NIR light-to-heat stimuli transducers. ~~By using~~ nanocarriers that can simultaneously hold bioactive cargo and photothermal agents within their matrix, ~~this strategy is advantageous~~ for further improving bioactive

cargo delivery compared to surface adsorption of biomolecules in pre-assembled polydopamine nanoparticles. For instance, self-crosslinkable cytosine-phosphate-guanine oligodeoxynucleotide-based nanoparticles were combined with indocyanine dye IR-820 in PEG-based hydrogels to extend its immunostimulatory effect and potentially act synergistically with chemotherapeutics delivery triggered by deep tissue penetrating NIR irradiation.[71] Due to their intrinsic theranostic features, the self-fluorescent immunoadjuvant nanocomposite hydrogels achieved *in vivo* imaging-guided combined photothermal-immunotherapy in mice.

Alternatively, the heat energy converted from initial light inputs in nanoparticle transducers can serve as a stimulus to impart actuating behaviours in soft hydrogel-based machines.[72] This has enabled the development of switchable hydrogel valves, artificial muscles or constructs with finger-like bending, as well as the design of soft robots with phototropism and omnidirectional self-orienting capabilities.[73–75] Recently, light-induced dehydration triggered by polydopamine nanoparticles embedded in alginate-based bioinks has enabled the manufacturing of shape-morphing cellularised constructs.[76] In more advanced endeavours, nanocomposite hydrogel materials are being designed for 4D printing biomimetic gradient structures with selective and programmed dynamic/flexural features characteristic of living marine organisms.[77] Nevertheless, most examples reported to date have relied on the use of inorganic nanomaterials (*i.e.* typically gold or iron oxide nanoparticles), which lack appropriate biomolecule loading capabilities. This paradigm potentially provides tremendous opportunities for further coupling mechanical locomotion with light-triggered bioactive cargo delivery in such systems. As a result, in the near future, researchers will undoubtedly pay attention to incorporating soft nanoparticles in hydrogel-based actuators, where they can simultaneously serve as programmable motion-generating units as well as dynamic biomolecule reservoirs.

23.4 Stimuli-responsive Biomechanical Modulation – Stiffening and Softening Platforms

Living cells not only inhabit and thrive in an interwoven biochemical communication permeating tissues, but are also constantly perceptive to and influenced by biophysical cues ubiquitously present in their extracellular matrix environments.[78] In fact, besides the great importance of biochemical cues, mechanobiology studies emphasise that our bodies constitute a dynamic biomechanical landscape that heavily impacts cell activity, fate and functionality throughout life.[79,80] For instance, several biological events are associated with progressive stiffening, namely liver cirrhosis, cardiac fibrosis and tumour progression, or characterised with intermittent softening, as in the case of organ development or tissue enzymatic degradation.[81] As a result, progress in the development of more advanced tissue engineering platforms has revealed that harnessing control over biomechanical

properties (*i.e.* stiffness), in the form of local or time-controlled changes is vital for guiding cells biomolecular output profiles and ultimately contribute to enhanced biomimetic performance.[79,81] Moreover, the development of biomaterial platforms embedded with gradient stiffnesses has also demonstrated cell durotaxis-mediated migration, which can partially reflect the heterogeneous rigidity in biological systems, especially considering that mechanical properties can drastically vary in an inter- and intra-tissue manner.[82,83] However, beyond concepts of endowing biodegradability to tissue engineering scaffolds, most traditional hydrogel platforms developed so far are still static in design and heavily contrast with the dynamic extracellular matrix mechanics found *in vivo*.[84]

Whereas static platforms provide constant biomechanical cues that can be set initially to meet specific values, in dynamic platforms the biophysical landscape can either be (i) biomechanically modulated either passively or on-demand in an unidirectionally progressive manner, or can be (ii) reversibly cycled between distinct biomechanical binary states (*i.e.* stiffer and softer).[81] The ability to dynamically tune the biophysical properties (*e.g.* stiffness, porosity, anisotropy, viscoelasticity) either globally or locally in such platforms is extremely attractive for developing biologically-relevant 3D models, augment microtissue biofunctionality and to further expand the current understanding and impact of mechanobiology in cell behaviour and fate. Current strategies for imparting stiffening or softening behaviour typically rely mostly on post-assembly crosslinking strengthening or unwinding, which is achieved by spatially modulating network interactions through swelling-induced expansion/shrinkage, configurational changes arising from the collapse/extension of hydrophobic domains or chemically promoted bond formation/dissociation.

Seminal studies have explored reversible and on-demand stiffening and softening of advanced hydrogel platforms, driven by stimuli-responsiveness in engineered protein building-blocks/synthetic matrices[85,86] or inspired by myosin motors,[87] but research into mechano-modulable nanocomposite hydrogels has only recently began to unfold. The hierarchical aspect of hybrid platforms offers unprecedented design flexibility and greatly expands the control range over biomechanical parameters by allowing manipulation of nanostructure–hydrogel interactions at the nanoscale, an interplay that is observed in certain organisms. For instance, some sea cucumber species are capable of rapid and reversible stiffening of their inner dermis by a factor of 10 (from 5 to 50 MPa) from relaxed (soft) to threatened (stiff) states.[88] Researchers inspired by this dynamic biological material have devised chemoresponsive nanocomposite hydrogels comprised by cellulose nanocrystals embedded in polymeric matrices based on ethylene oxide-epichlorohydrin and poly(vinyl alcohol).[89,90] The authors found that exposure to emulated physiological conditions elicits a large stiffness reduction (from 800 to 20 MPa), due to competitive hydrogen bonding subsequently introduced in the network.[89] Moreover, by manipulating thermal transition temperatures of the base biomaterials, the authors could further expand the range of modulus

changes in physiological conditions. Recently, nanocomposite hydrogels were obtained by embedding cellulose nanocrystals engineered with temperature-responsive polymers in a poly(vinyl acetate) matrix (Figure 23.2A).[91] Such hybrid platforms underwent significant stiffening upon exposure to warm water (from 1 to 350 MPa), due to the collapse of nanocrystal-grafted thermo-responsive polymers and augmenting stress transfer throughout the network, similar to how echinoderms control stress transfer among adjacent collagen fibrils through transient interactions.

Alternatively, researchers have developed soft nanogel-actuated hybrid platforms with reversible stiffening and softening capabilities inspired by native skeletal muscle contraction and relaxation under the influence of motor neuron inputs (Figure 23.2B).[92] By embedding thermo-responsive

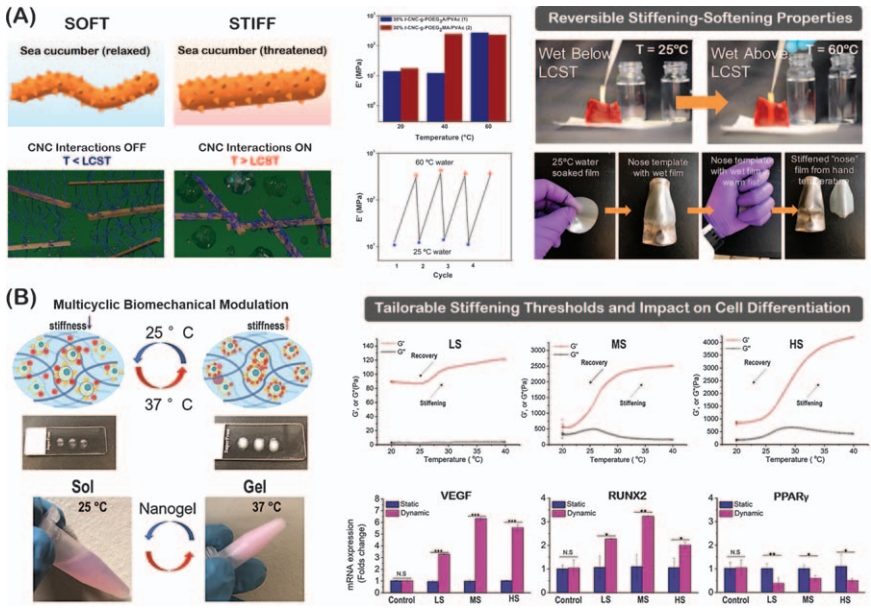


Figure 23.2 Nanocomposite hydrogels displaying on-demand biomechanical-modulation (*i.e.* softening/stiffening). (A) Sea cucumber in the relaxed (soft) and threatened (stiff) state. Thermo-sensitive polymers are grafted onto cellulose nanocrystals (CNC) embedded in hydrogels, which when exposed to water above the LCST, lead to polymer chain collapse, turning on CNC interactions and stiffening the network. Upon cooling down, chains extend and sterically hinder the reinforcing effect. Adapted from ref. 91, https://pubs.acs.org/doi/10.1021/acscentsci.7b00215, with permission from American Chemical Society, Copyright 2017.[91] Further permission requests related to the material excerpted should be directed to the ACS. (B) Dynamic nanogel-actuated modulation of stem cell spheroid microniches with tailorable stiffness thresholds and multicyclic cell biomechanical differentiation. Adapted from ref. 92 with permission from John Wiley & Sons, Copyright 2019, WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

poly(*N*-isopropylacrylamide-*co*-hydroxyethyl methacrylate) nanogels within photocrosslinkable gelatin methacrylate hydrogels, the authors developed stiffness-cyclable hydrogels that can convert temperature into reversible mechanical deformations, thus serving as 3D dynamic cell culture platforms specialised in probing the role of mechanosensitive transcriptional regulators in cell behaviour. Interestingly, the nanogel-strengthening effect was increasingly augmented for stiffer initial hydrogel meshes. Concerning this, gelatin methacrylate concentration could be increased while maintaining nanogel content, producing formulations with distinct thermo-responsive (from 25 to 40 °C) stiffening ranges: 80–120 Pa, 500–2500 Pa and 800–4000 Pa, for increasing gelatin concentrations (1.5 to 3.5 w/v %), respectively. Such biomechanically-modulated platforms could encapsulate human mesenchymal stem cell spheroids and readily stimulate their micro-niche with reversible mechanical loading cycles, which ultimately favoured osteogenesis over adipogenesis due to shifts in the expression and distribution of mechanosensitive regulators.

As experienced in several soft tissues (*i.e.* skin, blood vessels, cornea or lung parenchyma), mechanical forces can also drive their stiffening in a reversible manner, which serves both for maintaining tissue integrity, while protecting cells from large deformations and establishing long range mechanotransduction between them. ~~Herein, instead~~ of relying on conventional stimuli explored for reversible stiffening–softening (*i.e.* pH, temperature or light), in biological systems mechanical cues simultaneously function as inputs and outputs, where an initial mechanical loading triggers stiffening of the network.[93] Therefore, inspired by this mechanism, researchers are attempting to recreate such mechano-responsive features in hydrogel platforms. Recently, strain-induced stiffening hydrogel nanocomposites were developed by leveraging supramolecular protein nanocages for establishing multivalent sacrificial crosslinks within a polymeric network.[94] The authors crosslinked azide-functionalised polyisocyanide polymers with dibenzocyclooctyne (DBCO)-modified virus-mimetic capsids that exhibited pH-responsive self-assembly. At high mechanical stresses, nanocages are pulled apart, yielding smaller subunits bearing unreacted DBCO groups at their surfaces, which then participate in a secondary crosslinking with free azide moieties. This results in higher crosslinking density, leading to 3-fold modulus increase after deformation. Therefore, in this case, mechanical cues served both as input triggers and the output in the form of modulated mechanical properties. Furthermore, due to the recognised drug delivery ability of virus particles/capsids, such biomimetic nanocomposite hydrogels with encoded strain-responsiveness can operate as sophisticated and intelligent controlled release devices. Overall, one of the current gaps between stiffening tissues and hydrogel-based platforms is that owing to their fibre-based architectures, biological matrices display various degrees of anisotropy whereas conventional hydrogels are typically isotropic materials.[87] This means that when muscles stiffen, they contract in one direction, whereas in these hydrogels this effect takes place in all directions simultaneously. Among other biomaterial processing methodologies, this

limitation could be overcome in nanocomposite hydrogels by embedding high-aspect ratio nanomaterials (*i.e.* soft cellulose nanocrystals or inorganic LAPONITE® and metal-based nanorods) that can rotate and reorient themselves along the stretching direction, thus shaping and reorienting the 3D architecture of the hydrogels while encoding strain-stiffening behaviours.[95]

23.5 Conclusions

Living tissues are characterised by their ability to constantly adapt and respond dynamically to their surroundings. Harnessing such adaptability in tissue-mimetic 3D platforms, such as nanocomposite hydrogels, is undoubtedly valuable for improving the overall biomedical performance across several therapeutic modalities (*i.e.* drug delivery and cell-based therapies). Soft-based nanomaterials incorporated in hydrogel matrices establish hierarchical architectures that enable precise tuning of nanoparticle–hydrogel interactions and bulk system configuration changes, as well as facilitate the encoding of multimodal bioactive cargo delivery ~~that be~~ independently controlled under distinct biological stimuli or multi-cascade feedback programmes. The ability to convert incoming stimuli into multiple cascaded signals in such advanced platforms expands the biochemical/biophysical control over hydrogel- or tissue-resident cell populations, while seamlessly providing bidirectional biocommunication among cellular and acellular components. Furthermore, the nature of the system's stimuli-responsiveness can either enable on-demand tuning of hydrogel–nanoparticle configuration states (*i.e.* through adaptability to exogenous cues) or allow the development of intelligent physiologically-monitoring platforms that react to specific alterations within the human body (*i.e.* by sensing and reacting to endogenous cues). Likewise, hybrid platforms that display re-programmable biophysical landscapes are also rapidly emerging ~~with increasing interest~~ and aid in bridging the gap between our fundamental understanding and functional capabilities of biological systems.

Besides attaining organ-like 3D constructs, the complexity of the biofunctionality conundrum represents the ultimate compromise that researchers will have to face in the design of effective and translational administrable hybrid platforms. As showcased in this chapter, the complete programmability of soft nanoparticles greatly extends the range of biofunctionality and complexity that can be attained in current designs. Ideally, this flexibility will provide valuable opportunities for developing biofunctional constructs with complexity optimally maintained at a minimum. In addition, advances in biomaterial design and processing are highly warranted due to the elusiveness of attaining certain stimuli-responsiveness in soft nanocomposite hydrogels that are currently restricted to inorganic nanomaterials (*i.e.* magnetic fields, upconversion features). Judging by the tremendous progress witnessed by the scientific community over recent decades in revolutionising multifunctional platforms, this field will continue to establish new benchmarks and seek additional sophisticated features that could bring us even closer to native tissue biofunctionality.

In the foreseeable future, the programmability of soft nanomaterials or synthetic biology nanocompartments will undoubtedly push the narrative of soft nanocomposite hydrogels even further out of the proof-of-concept realm and ultimately into realistic and functional biomedical platforms uniquely specialised in tissue engineering and regenerative medicine endeavours.

Abbreviations

PEG	poly(ethylene glycol)
PLA	poly(lactic acid)
CAT	catalase
GOx	glucose oxidase
aPD1	anti-programmed death-1
PLGA	poly(lactic-*co*-glycolic acid)
(UCST and LCST, respectively)	upper and lower critical solution temperature
DBCO	dibenzocyclooctyne
rosi	rosiglitazone
EV	empty vehicle
CNC	cellulose nanocrystals

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