Expert Opinion

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Physical hydrogels with self-assembled nanostructures as drug delivery systems

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Introduction: As an essential complement to chemically crosslinked hydrogels, drug delivery systems based on physical hydrogels with self-assembled nanostructures are gaining increasing attention, owing to potential advantages of reduced toxicity, convenience of *in situ* gel formation, stimuli-responsiveness, reversible sol-gel transition, and improved drug loading and delivery profiles.

Areas covered: In this review, drug delivery systems based on physical hydrogels are discussed according to their self-assembled nanostructures, such as micelles, layer-by-layer constructs, supramolecular inclusion complexes, polyelectrolyte complexes and crystalline structures. The driving forces of the self-assembly include hydrophobic interaction, hydrogen bonding, electrostatic interaction, π–π stacking and weak van der Waals forces. Stimuli-responsive properties of physical hydrogels, including thermo- and pH-sensitivity, are considered with particular focus on self-assembled nanostructures.

Expert opinion: Fabricating self-assembled nanostructures in drug delivery hydrogels, via physical interactions between polymer–polymer and polymer–drug, requires accurately controlled macro- or small molecular architecture and a comprehensive knowledge of the physicochemical properties of the therapeutics. A variety of nanostructures within hydrogels, with which payloads may interact, provide useful means to stabilize the drug form and control its release kinetics.

Keywords: crosslinking, hydrogel, nanostructure, pH-sensitive, physical hydrogel, polyelectrolyte, self-assembly, thermo-sensitive

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1. Introduction

Hydrogels are crosslinked polymeric networks with high water content which can be prepared to possess a desired selection of morphological, biocompatible, biodegradable/non-degradable and mechanical properties depending on the composition and chemical structures of the building blocks used in their synthesis and the conditions selected for gel fabrication [1]. For decades, hydrogels have gained a great deal of attention due to their successful application as drug delivery systems (DDS), aiming to improve drug bioavailability at the desired site of therapy through controlled release [2-4]. This offers potential advantages over systemic administration, which may lead to short drug plasma circulation times through rapid renal clearance, together with adverse toxicity from non-target delivery of the drug. Hydrogel networks generally benefit from good biocompatibility due to their high water content, which mimics that of the tissues in the body. They may also provide protection of encapsulated biomacromolecular therapeutics, such as proteins or peptides, preventing loss of bioactivity and/or *in vivo* proteolytic degradation by use of mild gel preparation conditions [5,6]. Stimuli-sensitive hydrogels have been designed to deliver therapeutics in response to external stimuli, such as changes in temperature, pH,

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Article highlights.

- Physical hydrogels possess the advantages of simplified processing without the need to remove residues of toxic crosslinkers, reversible sol-gel transitions with high sensitivity under mild conditions and convenient incorporation of multiple properties to introduce heterogeneity for hosting hydrophobic drugs.
- One particularly useful family of physical hydrogels utilize micellar nanostructures to induce physical interaction between polymer chains and provide depots for drug interaction. They can be made responsive to various stimuli such as temperature and pH in order to trigger a desired behavior when in the body.
- In order to design specificity of interaction, control of polymer architecture, such as molecular mass, polydispersity and endgroup functionality are imperative. Controlled radical polymerization methods, such as atom transfer radical polymerization and reversible addition-fragmentation chain transfer polymerization, have shown to be particularly advantageous.
- Challenges faced by drug-loaded physical hydrogels are low drug stability accompanied with poorly controlled gel integrity and consequently rapid release of the payloads. In addition to the exploration of novel polymer structure and function, more in-depth understanding of the physicochemical properties of therapeutics and their interaction with polymeric moieties in hydrogels is desirable in the initial stages of hydrogel design.

This box summarizes key points contained in the article.

enzymes, salt concentration or small molecules, through volume change, volume phase-transition or sol-gel phase transition [4,7-8]. Among the potential applications in biomedicine and pharmaceuticals, temperature or pH-induced *in situ* gel formation provides a simple and safe method for the control of site-specific drug delivery. Thermo-responsive hydrophobic blocks such as poly(N-isopropylacrylamide) (PNIPAAm) are often copolymerized with other hydrophilic moieties to form amphiphiles with tailored gelation temperature and self-assembly properties for prolonged drug delivery [9,10].

Practically or clinically, the application of hydrogel DDS is often limited due to technical or inherent difficulties in gel preparation with drugs and their subsequent administration [11]. Chemically crosslinked hydrogels, for instance, require the complete removal of toxic crosslinkers, which is difficult to achieve *in vivo*. The high inherent water content of such systems thermodynamically prevents homogeneous loading of hydrophobic drugs and destabilizes entrapped drug causing recrystallization or solid phase separation from the hydrogels [11]. Another problem encountered by drug-loaded hydrogels is rapid drug elution from the carriers, which can limit the initial drug loading quantity in the hydrogel or cause high drug plasma concentrations in excess of the drug's toxic levels. In addition, irreversibly chemically crosslinked hydrogels may require surgical implantation and subsequent removal if they are not biodegradable. One approach has been to use reversible crosslinking systems. One such system is based on dithiol and its crosslinking can be *in vivo* [12,13]. Alternatively, natural crosslinking agents such as genipin, which reacts readily with molecules containing primary amine groups but has much lower toxicity than conventional crosslinking agents such as glutaraldehyde, can be used [14]. In order to address these general short-comings, more recently attention has turned to development of hydrogels with self-assembling nanostructures through physical interaction [5,15]. Physical hydrogels possess the advantages of simplified processing without the need of removing residues of toxic crosslinkers, reversible sol-gel transitions with high sensitivity under mild conditions and convenient incorporation of multiple properties to introduce heterogeneity for hosting hydrophobic drugs [16]. Hydrogels which are desired to be multifunctional, such as those containing drug combinations, or imaging agents in addition to therapeutics, have also been studied in order to expand the applications of the materials [17]. Much of this effort is based on exploiting different types of non-covalent interactions within hydrogels, which form organized nanodomains by self-assembled moieties of a variety of polymers to host therapeutic agents and regulate drug release kinetics [18]. This review summarizes recent developments in the preparation and application of hydrogel systems with self-assembled structures through non-covalent interaction between polymer components and loaded drugs or bioactive agents to achieve multifunctionality and controlled drug release.

2. Self-assembled structures in hydrogels via non-covalent interactions

The uniform dispersion of lipophilic pharmaceuticals in a hydrogel with high water content is often thermodynamically unstable, and the chemical potential along the drug concentration gradient drives drug recrystallization or redistribution inside or outside of the gel. One approach to overcoming this stability hurdle is to incorporate lipophilic moieties into the nanostructures with hydrophilic coronas to reduce the drug surface energy [11]. This may involve the use of groups chemically linked to the polymer backbone to form self-assembled nanostructures by mixing polymers directly or by environmental changes (e.g., temperature, pH or salt concentration) to finally reach or approach a state of thermodynamic equilibrium [19]. The physical interactions between the components of the polymer chains, which are the driving forces of the gelation process, mainly include hydrophobic interaction, hydrogen bonding, electrostatic interaction, π–π stacking and weak van der Waals forces [16]. The self-assembled complexes are associated with each other through either physical chain entanglement or chemical linkage to form gel networks, which have a direct impact on gel properties and integrity [20]. An alternative approach often adopted is to blend secondary delivery carriers into a hydrogel matrix to

form a so-called composite hydrogel [11], which may take advantage of the synergistic effects of both components for controlling drug release. The secondary delivery carriers can be in the form of nano/microgels, liposomes or micelles [21]. This strategy may avoid complex polymer modification chemistry and allow the use of well-characterized polymer systems for formulation into systems that may impart improved biocompatibility over the use of the nano-carriers alone; whilst the embedded particles inside the hydrogels effectively improve the drug release kinetics by reducing burst effects and controlling drug release [11]. This review focuses mainly on the former strategy of fabricating hydrogel structures to control drug delivery.

In chemically crosslinked hydrogels, the anchor points are usually 'volumeless' atoms, whereas the complex junctions in self-assembled hydrogels can provide a nano/microenvironment, different to the gel matrix phase, for drug encapsulation mediated by certain physical interactions, for example, hydrophobic interactions or hydrogen bonding [15]. Depending on the structure and polarity of the payload, the properties of the self-assembled complex could even be enhanced by the addition of drug molecules. A summary of self-assembled hydrogel structures based on non-covalent interactions is illustrated in **Figure 1**.

2.1 Hydrogels with micellar or microdomain nanostructures

2.1.1 Thermo-sensitive hydrogels

In situ gelation through the use of thermo-sensitive block copolymers is one of the most widely studied methods of fabricating hydrogels in biomedical applications [9,22], which provides a facile approach to controlled drug delivery by simple temperature change. Synthesized by grafting or sequential copolymerization with hydrophilic blocks, AB, ABA or BAB type copolymers can be prepared by using hydrophobic blocks balanced with hydrophilic chains. Commonly incorporated hydrophilic blocks include poly(ethylene oxide/glycol) (PEO/PEG), poly(2-methacryloyloxyethyl phosphorylcholine) (PMPC) [13] and polysaccharides [23]. When combined with hydrophobic blocks such as PNIPAAm, poly(propylene oxide) (PPO), poly(lactide-*co*-glycolic acid) (PLGA), poly (caprolactone) (PCL) and poly(vinyl ethers), block copolymers with thermo-sensitive properties may be formed (as shown in **Figure 2A**) [9,24-26]. Aqueous solutions of di- or triblock copolymers can undergo a phase transition from solution to gel by first transforming hydrophilic unimers to self-assembled micellar aggregates, when a lower critical solution temperature (LCST) is reached [9]. This corresponds to the switching of the thermo-sensitive blocks from hydrophilic to hydrophobic in nature because of the weakening and disruption of hydrogen bonding between water molecules and the polymeric moieties [26]. The gelation is through intermicelle association either by chain entanglement or direct chain linkage to form three-dimensional hydrogel networks as illustrated in **Figure 3**. The micelles *per se* are the physically crosslinked knots of the thermo-sensitive hydrogels. Therefore, intramicellar hydrophobic forces and interactions directly affect the strength of the hydrogels, which depend on the copolymer structure, ratio of hydrophobicity:hydrophilicity, chain length and copolymer concentration [27]. Thermal behavior of hydrogels, such as gelation temperature and gelation time, can be tuned by changing the composition of copolymers, the relative chain length and the ratio of hydrophilic:hydrophobic blocks to fulfill the requirements for practical application [9].

Hydrogel DDS, based on the release mechanism of drug diffusion and polymer erosion, often require biodegradable copolymers for their application *in vivo*. Polyester-type *in situ* gelling systems, such as PEG-poly(L-lactide)-PEG (PEG-PLLA-PEG) and PEG-poly(D,L-lactide-*co*-glycolide)-PEG (PEG-PLGA-PEG), have shown great potential in biomedical and pharmaceutical applications [9]. Other systems, including natural polymers, have also attracted significant attention [28]. A partially biodegradable temperature- and pH-responsive hydrogel, PNIPAAm/dextran-maleic acid, was synthesized by UV crosslinking and designed specifically as a drug carrier. The anticancer drug, doxorubicin, was used for studies in the correlation of gel structure and drug release [29]. By using atom transfer radical polymerization (ATRP), Armes and co-workers have reported the synthesis of a series of thermo-responsive and degradable ABA triblock copolymers, in which the outer A block is either PNIPAAm [12] or poly (2-hydroxypropyl methacrylate) [13], and the central B block is PMPC. These novel triblock copolymers form thermoreversible physical gels via bridging flower micelles through the hydrophilic PMPC block at critical gelation temperatures (**Figure 3B**). The disulfide bond-based PMPC corona and bridges are cleavable under reducing conditions, which results in the release of the trapped micelles by a rapid dissolution of the hydrogels. This strategy makes the polymer suitable for potential application in the biomedical field.

To address the issue of slow responsiveness exhibited by some thermo-sensitive hydrogels, novel two-level PNIPAAm hydrogels were recently reported which use PNIPAAm microgels as the crosslinker to form a bulk network by interconnecting microgels with linear chains through further polymerization of NIPAAm [30]. The resulting heterogeneous structure contains nano-sized gel phases with significantly improved thermo-sensitivity and response kinetics compared to homogeneous analogs. The novel structure imparts rapid shrinkage properties by creating a sufficient number of diffusion channels for water release. The compounds, Rhodamine B and ibuprofen, were used as model drugs to examine drug release and to demonstrate 'on-off' switching properties of the hydrogel.

2.1.2 pH-sensitive hydrogels

pH-sensitive hydrogels based on either weakly acidic or weakly basic polymers (polyelectrolytes) have been designed to possess precise responsiveness by exploiting the wide pH

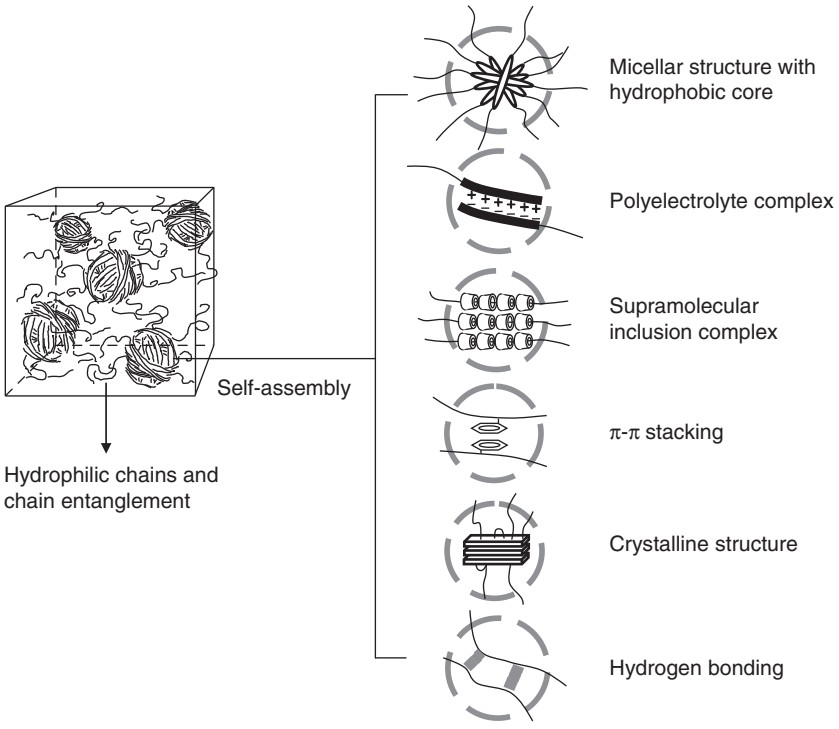

Figure 1. Schematic of self-assembled hydrogel network and interactions between polymeric components.

range of different sites of the human body in order to achieve *in situ* gelation or controlled drug delivery [31]. Polymers with carboxylic acid groups that have commonly been used include units of poly(acrylic acid) (PAA), poly(methacrylic acid) (PMAA), poly(L-glutamic acid) (PLG), poly(α, β-aspartic acid) and some natural polymers, for example, alginic acid and hyaluronic acid. Basic polymers containing amino groups that have been frequently studied include units of poly(2-(dimethylamino)ethyl methacrylate) (PDMA), poly (2-(diethylamino)ethyl methacrylate) [32], poly(2-(diisopropylamino)ethyl methacrylate) (PDPA) [33,34], poly(2-vinylpyridine) [35], poly(ethyleneimine) and chitosan (as shown in **Figure 2B**) [36]. At the pH above the pK_a of the acid or the protonated amino groups on these polyelectrolytes, deprotonation occurs along the polymer chain with variation of the degree of ionization, and finally leads to a phase separation and gelation of the polymer solution. The critical gelation concentration (CGC) and gel strength are influenced by the polymer molecular mass, hydrophobic/hydrophilic balance and hydrophobicity of the pH-responsive blocks in the copolymers [33,37]. Decreased CGC and increased gel strength are directly linked with the higher molecular mass and hydrophobicity of hydrophobic blocks.

A number of strategies for stabilizing the phase-separated polymers have been studied to obtain stable hydrogels. One of the most commonly adopted methods for fabrication of pH-sensitive hydrogels is the copolymerization of the polyacids/polybases with a hydrophilic constituent, such as PEG or PMPC, which form self-assembled micellar structures and may further aggregate to form hydrogel networks when the protonatable moiety becomes deionized (**Figure 3B or C**). Interestingly, it is possible to make materials that are responsive to multiple stimuli, by inclusion for instance, of different blocks that contain pH-responsive units such as those described in this section, in combination with thermosensitive units such as those described in Section 2.1.1. Such hydrogels with both pH and temperature-responsiveness have been prepared from cationic pentablock copolymers of PDMA-PEO-PPO-PEO-PDMA which exhibit pH and temperature dependent micellization [38], or by the modification of P(CL-co-lactide)-PEG-P(CL-co-lactide) triblock copolymers with oligomeric sulfamethazine end groups which undergo sol-gel transition under physiological conditions [39].

Copolymers containing basic poly(tertiary amine methacrylate) have shown potential application as biomaterials for drug delivery because of their pH-sensitivity and low toxicity. An ABA triblock copolymer of PDPA-PMPC-PDPA synthesized by ATRP has been demonstrated to form physical gels from a 10 w/v % aqueous solution at neutral pH above the pK_a 6 of protonated PDPA [33,34]. The gelation is via the aggregation of self-assembled micelles with hydrophobic PDPA cores and hydrophilic PMPC coronas that, meanwhile, form bridges interlinking micelles as shown in **Figure 3B**. The release of a loaded hydrophobic model drug, dipyridamole, from the hydrogel was shown to be pH responsive, with slow release of dipyridamole at neutral pH and 37°C but

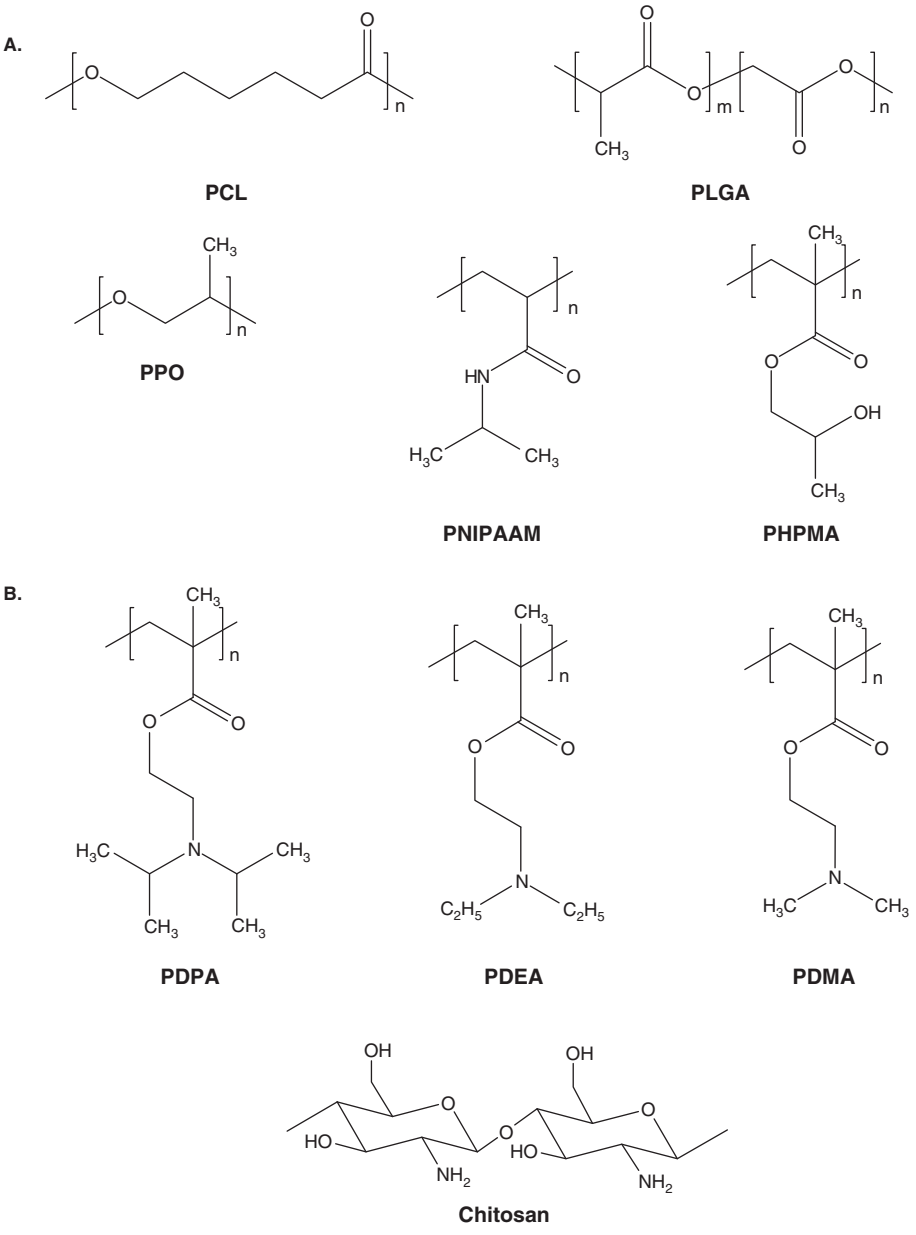

Figure 2. Chemical structures of blocks in (A) thermo- and **(B)** pH-responsive copolymers.

rapid drug release at pH 3, due to protonation of both the PDPA and drug followed by rapid gel dissolution.

In addition to the use of polyamines and poly(carboxylic acids) for pH sensitive hydrogels, recently Wu *et al.* reported a new method for fabricating *in situ* biodegradable hydrogels based on a pH-responsive thiol–disulfide exchange reaction dependent on the pK_a of the involved thiols, an excellent example of a system combining chemical crosslinking and physical self-assembly [40]. In the model system, core-shell structures were produced involving Michael addition polymerization between 1-(2-aminoethyl)piperazine and a double-molar *N*,*N*′-bis(acryloyl)cystamine (forming the core portion), followed by a second Michael reaction with α-amino-ω-methoxy-poly(ethylene glycol) that acts as the shell portion, stabilizing the hydrogel network (**Figure 4**). The amine-protonated polymer was water soluble in acidic conditions, whereas a hydrogel was formed when the polymer was basified due to the deprotonation of amines and the increased hydrophobicity of core. Activated thiolates converted from a few thiols at elevated pH (12) above their pK_a triggered thiol–disulfide exchange reactions, that is, core crosslinking, and led to the release of some of the PEG shell

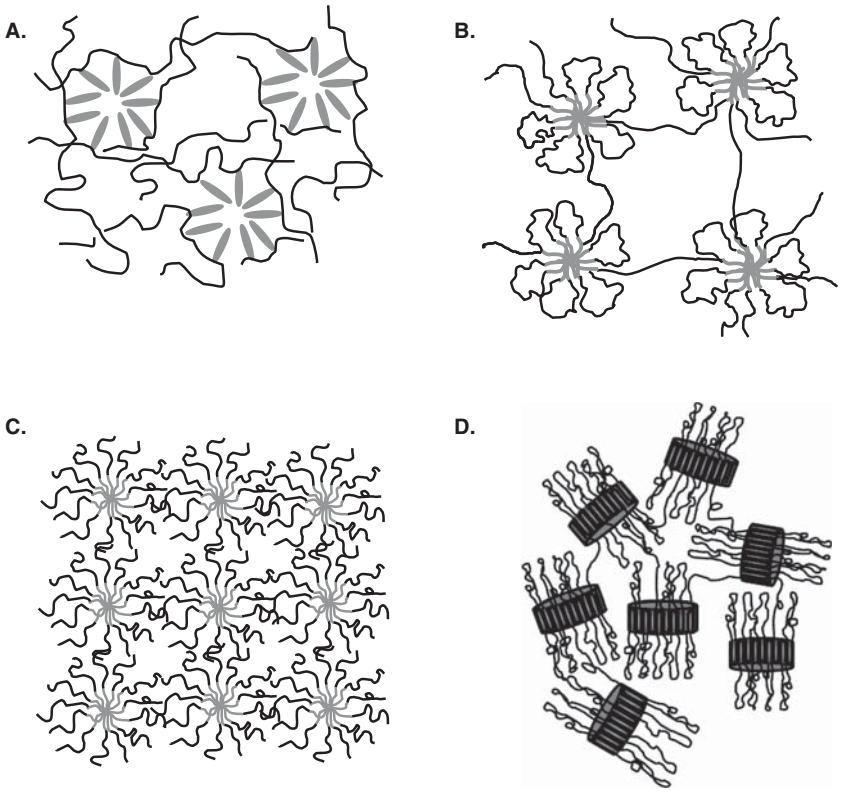

Figure 3. Schematic of hydrogel structures with micellar components. Grey blocks are hydrophobic, and black chains are hydrophilic. A. Example of hydrophobic aggregations through polymer chains modified with alkyl groups [53]. **B.** Thermo- or pH-sensitive ABA triblock copolymers formed flower micelles. **C.** Micelles formed by diblock copolymers [9]. **D.** Triblock copolymer formed lamellar micelles, e.g., PLLA-PEO-PLLA.

B. Adapted with permission from [33,34]. Copyright ©2003, American Chemical Society. **D.** Reprinted with permission from [108]. Copyright ©2008, American Chemical Society.

and formation of a more condensed core aggregate. The level of thiol-disulfide exchange and hence degree of core crosslinking could be controlled by tuning the pH of the environment allowing preparation of loose to compact hydrogels with the advantage of having no critical gel concentration associated with purely physically crosslinked systems. The hydrogel formation is activated, terminated, interrupted or re-activated by manipulation of pH, a process termed as 'living'. The loading and release of two model drugs, doxorubicin and paclitaxel, demonstrated that the drug release could be fine-tuned by this fine control of core crosslinking and the degradation of disulfide bonds.

Hydrogel networks synthesized via controlled radical polymerization (CRP), such as ATRP and RAFT, have been reported [41,42] with the characteristics of well-defined polymer structure, narrow distribution in molecular mass and a wide variety of functional groups. More homogeneous and well-defined gel systems are obtained by use of ATRP compared to conventional free radical polymerization methods [43,44]. An additional attraction of the use of ATRP in hydrogel synthesis is its versatile initiating routes either from freely dispersed small/macro molecules in solution, such as the use of functional initiator-containing biodegradable disulfide crosslinking units [13,41], or on the surface of a hydrogel, such as growing PNIPAAm chains on the surface of dextran particles [45]. Moreover, ATRP is a particularly facile way to make polymers for the purposes of fabricating physical hydrogels. For instance, ABA type triblock copolymers containing biocompatible MPC blocks were synthesized from a small molecular initiator, capable of self-assembly into hydrogels with flower-like nanostructures under thermal or pH stimuli [12,33,34,41]. Macroinitiators of linear or multi-arm star PEG have also been used in the ATRP synthesis of thermogelating poly(2-(2-methoxyethoxy)ethyl methacrylate-*co*-oligo(ethylene glycol) methacrylate) (P(MEO_2MA-*co*-OEGMA)) [46]. Above the LCST, both chemical crosslinking from the PEG initiator and physical crosslinks from hydrophobic interactions between the P(MEO_2MA-*co*-OEGMA) units exist. Recently, a route of preparing physical hydrogels for the delivery of the anticancer drug cisplatin has been reported [47], in which ATRP-synthesized PEG-*b*-PAA forms micellar structures through complexation between the platinum(II) of the drug and carboxylic acid residues of the polymer. A subsequent supramolecular hydrogel was prepared

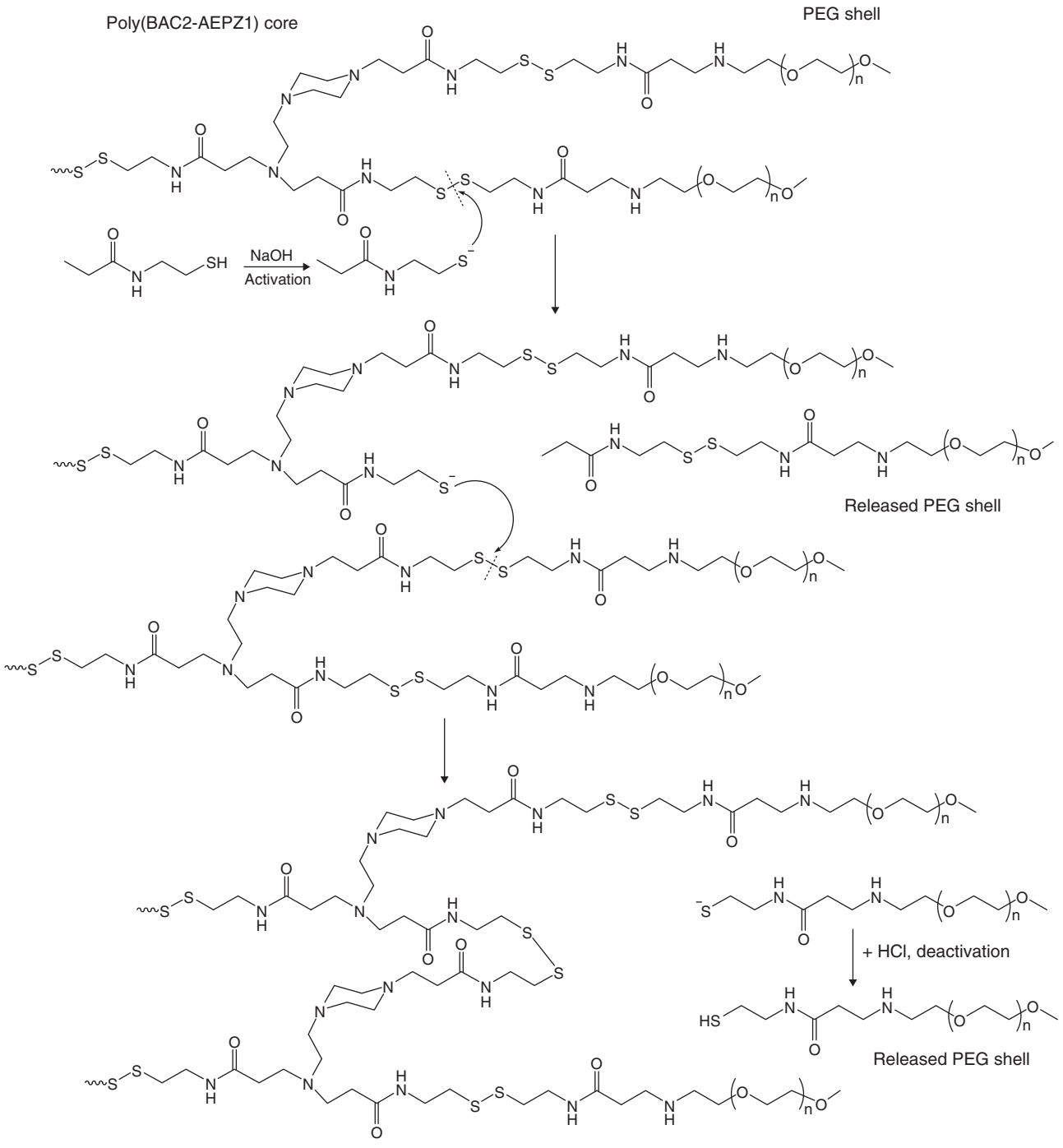

Figure 4. Illustration of thiol-disulfide exchange reaction causing the formation of crosslinked poly(BAC2-AEPZ1) cores and release of PEG shells.

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by the formation of inclusion complex of α-cyclodextrin (α-CD) and PEG chains (see Section 2.3). Therefore, the evolution of CRP techniques continues to provide numerous options for the synthesis and preparation of novel hydrogels by introducing novel functionality and properties useful for drug delivery applications.

2.1.3 Hydrogels with hydrophobic microdomains independent of stimuli

In addition to the stimuli-sensitive hydrogels with reversible hydrophilic–hydrophobic blocks, hydrogels with hydrophobic blocks, which are independent of stimuli, have also been described with an aim to increase gel strength and control

the release of hydrophobic drugs. Biphasic polymer hydrogels were first reported to possess zero- or near zero-order release kinetics of the model drugs tryptophan and theophylline [48]. The hydrophobic moiety methyl methacrylate (MMA) has been used in the modification of polyelectrolytes to form random, block or grafted copolymers. By grafting oligomers of methyl methacrylate (oMMA) onto PAA hydrogels, the swelling behavior and drug release profile of the hydrogel was changed remarkably due to the formation of hydrophobic domains of oMMA [49]. It has been demonstrated that the release of hydrophilic drugs, such as theophylline, is enhanced by the introduction of the hydrophobic domains that create large pores in the hydrophilic gel matrix. In contrast, hydrophobic drugs, such as propranolol hydrochloride and β-oestradiol, showed strong interaction with oMMA domains and consequently a remarkably decreased drug release profile.

A spontaneously forming physically crosslinked hydrogel composed of poly[MPC-*co*-methacrylic acid] and poly (MPC-*co*-n-butyl methacrylate) (BMA) has been extensively studied by Ishihara and co-workers [50-52]. The rationale for the design of this system was as an oral drug carrier for protection of polypeptide drugs such as insulin from acidic pH conditions (stomach) for subsequent release under neutral pH conditions (small intestine). Insulin was shown to interact with the aggregated hydrophobic domains of the butyl groups, which also hydrogen-bonds with the methacrylic acid blocks, under acid conditions. The hydrogel demonstrates pH-responsiveness because at neutral pH, hydrogel erosion occurs and this is caused by the ionization of the carboxyl group, which breaks the hydrogen-bonds with the BMA domains thereby releasing the hydrophobic insulin.

Natural polymers have been of persistent interest in the study of the relationship between hydrogel functionality and heterogeneity by structure modifications. As illustrated in **Figure 3A**, amphiphilic alginate derivatives modified with covalently bonded dodecyl/octadecyl chains have been described that possess increased elastic and viscous moduli, and an effective gel mesh size (mean spacing between crosslinking nodes of a network), due to the hydrophobic microdomains introduced by alkyl groups in the alginate hydrophilic phase [53,54]. Chemically-modified chitosan has also been physically crosslinked via hydrophobic interactions to improve the compatibility between these hydrogels and hydrophobic drugs. Grafted with D,L-lactic acid and/or glycolic acid, chitosan hydrogels were prepared by physical crosslinking via hydrophobic side chain aggregation and intermolecular interactions through hydrogen bonds between the side and main chains [55]. Glycol chitosan was modified with palmitoyl groups to increase the crosslinking and the interaction between the hydrogel and model drugs [56,57]. Chitosan derivatives with carboxymethyl-hexanoyl or carboxymethyl-palmitoyl groups showed more pronounced pH sensitivity and reduced the burst release of the hydrophobic drug ibuprofen, with increased degree of carboxymethyl substitution or hydrophobic chain length [58]. Another version of such pH-responsive hydrogels based on N-palmitoyl-conjugated chitosan (NPCS) was developed with a fast gelation rate and a narrow pH range of phase transition at 1% w/v of concentration and physiological conditions [59]. TEM and small angle X-ray scattering revealed at pH 3 the existence of rod-like entities with the dispersion of maximum protonated amine groups on the chitosan and the limited association of the hydrophobic palmitoyl groups. Above pH 7.4, the viscous NPCS liquid transitioned into a hydrogel with a sponge-like structure, which was composed of nanodomains produced by further phase separation, due to the increased hydrophobic interaction from micelle-like structures formed from palmitoyl aggregation and deprotonated amine groups in the chitosan.

2.2 Composite hydrogels with embedded micellar structures

The strategy of embedding prefabricated microspheres, micelles or vesicles into hydrogel networks has proved an effective means to overcome the difficulties of loading lipophilic/lyophilic pharmaceuticals into hydrophilic polymer gels. For example, nanoparticles and microparticles from liposomes, surfactants [60-62], nanogels [63,64] and CD–drug inclusion complexes [65] have been dispersed into polymer solutions in order to prepare composite hydrogels. Compared to the hydrogel systems with intrinsic hydrophobic/micellar domains, which often require certain levels of chain modifications such as synthetic blocking or grafting, composite hydrogels with embedded micellar structures are more convenient and versatile, allowing preparation of wide-ranging hydrogel systems based on synthetic or natural polymers and a myriad of micelle-forming amphiphilic copolymers. The core-shell structures of micelles further stabilize the hydrophobic drug within the hydrogels by decreasing their surface energy in the hydrophilic environment. For instance, a nanoscale drug-entrapment approach using chitosan hydrogels blended with drug-loaded Pluronic micelles has been reported [21]. Sirolimus, a hydrophobic anti-proliferative/immunosuppresive drug, was first entrapped into the hydrophobic core of the Pluronic L121 micelles by dropping a tetrahydrofuran (THF) solution of L121 and drug mixture into water, followed by the evaporation of the THF. The resultant micelle solution was blended with the chitosan solution, followed by cast-drying and fabrication of a self-expandable drug-eluting stent. Both *in vitro* and *in vivo* tests showed the micelle-embedded hydrogel possessed high drug loading efficiency could significantly extend the duration of sirolimus release without an initial burst, therefore, reducing the side effects such as delayed endothelial healing and leading to decreased in-stent restenosis. A dual-DDS based on composite hydrogels of polyvinyl alcohol (PVA) or chitosan/PVA with PLG-poly (propylene oxide)-PLG (GPG) micelles has been reported [66]. Doxorubicin-encapsulated GPG micelles were prepared using a dialysis method, followed by mixing with aspirin-loaded PVA or chitosan/PVA hydrogel. The resulting GPG micelles

are pH- and thermo-sensitive, and chitosan is pH-sensitive. Therefore, this composite hydrogel/micelle system demonstrated distinctive drug release profiles with pH and temperature responsiveness.

2.3 Polyelectrolyte hydrogels with electrostatic interaction

Hydrogel network formation via electrostatic interactions either between ionic molecules and polyelectrolytes (unpaired) or between polyelectrolytes (paired) has been used in the development of systems for a wide range of biomedical and pharmaceutical applications. Many different synthetic and natural polymers and biomacromolecules have been used in the studies of polyelectrolyte hydrogels, such as PAA, chitosan, alginate, gelatin and albumin to name a few. The hydrogels prepared possess not only the biocompatibility of the aforementioned polymers, but also the collective unique properties of each component. The inherent charge characteristics of polyelectrolytes provide a convenient mechanism for the *in situ* fabrication of hydrogels in the aqueous phase, with minimum involvement of chemical crosslinking, reactive agents and organic solvents. A further advantage of polyelectrolyte systems is that the strong and reversible physical interaction between polyelectrolytes and oppositely charged drugs makes polyelectrolytes suitable carriers for charged hydrophilic drugs, peptides or protein biomacromolecules; whilst the integrity and activity of the peptides and proteins remain largely protected [67]. The release mechanism of polyelectrolyte complexes can involve ion-exchange with salts from solution, environmental pH change causing protonation/deprotonation of drugs or polyelectrolyte moieties around their pK_a values, or gel dissolution/degradation. **Figure 5** shows the summary of polyeletrolyte structures and interactions between polymers and payloads.

Ionic and polyelectrolyte complexes are amongst the most studied hydrogel systems crosslinked by charge–charge interactions. Ionic complexes involve the interaction between small molecules or metal ions and the polyelectrolytes, for instance, divalent cations (Ca^{2+}, Ba^{2+} or Sr^{2+}) with alginates, sulfates or orthophosphates with chitosans. Interaction between cationic/anionic drugs and anionic/cationic polymers have also been studied, as illustrated for example by the complex formation of salicylic acid with the amino groups in chitosan films [68,69]. Cationic drugs, such as doxorubicin hydrochloride and the active lactone form of irinotecan hydrochloride, have been loaded into PVA hydrogel microspheres modified with anionic 2-acrylamido-2-methylpropane sulfonate via ionic complexation and used in the treatment of liver cancer by chemoembolization [70-72]. The microspheres exhibited decreased volume and increased Young's modulus with increasing drug loadings. Doxorubicin-loaded hydrogel microspheres also showed a much slower drug release profile in comparison to the faster release of irinotecan from these loaded microspheres. The electrostatic interaction between drug and polymer accompanied by π–π stacking interaction between the planar aromatic anthracycline ring systems of the doxorubicin molecules results in a much denser hydrogel network structure, significantly decreased gel volume and slower drug release due to stronger interactive forces [73]. In comparison to doxorubicin loading in these microspheres, the weaker hydrophobic interaction between irinotecan molecules and their increased water solubility due to the bipiperidino side chain leads to a quicker drug elution by an ion-exchange mechanism.

Polyelectrolyte complexes are formed by the coacervation of two opposite charged polyelectrolytes. Some typical polycations include chitosan, polyethylenimine, poly-L-lysine, poly-L-arginine (Arg), PDMA, poly(*N*-vinylpyrrolidone) (PVP) and polyallylamine. Typical polyanions include alginic acid/alginate, hyaluronic acid/hyaluronate, heparin, xanthan gum, dextran sulfate, PAA, poly(glutamic acid), DNA, gelatin and albumin. Based on the electrostatic interaction between two polyelectrolytes with opposite charges, core-shell structured nano or microparticles have been obtained by the addition of alginate solution into chitosan solution under high shear conditions [74,75]. The pH, concentration, ionic strength, charge density, molecular mass, sequence and mixing method for the polyelectrolytes are important factors in preparing stable and mechanically strong hydrogels under physiological conditions.

The fabrication of polyelectrolyte hydrogels modified with hydrophobic moieties has been designed to improve the loading of hydrogels with lipophilic drugs and subsequently modulate their drug release kinetics. Alkyl chain-substituted alginate was prepared to fabricate physical hydrogels based on hydrophobic or dual hydrophobic/ionic interactions [54]. In a recently reported study, cationic poly(DMA)-grafted β-cyclodextrin (β-CD) synthesized by ATRP has been demonstrated to form complex microgels with the di- and triblock polyanions, MePEG-*b*-PMAA and PMAA-*b*-PEG-*b*-PMAA, in phosphate buffer (pH 7.4) [76]. A model drug, naproxen, was loaded into the hydrophobic cavity of β-CD by forming a host–guest inclusion complex and showed sustained release after an initial burst effect.

Structures with layered or multilayered membranes via polyelectrolyte complexion have recently been of great interest in the fabrication of drug delivery carriers, biosensors and microreactors either in particulate forms or on flat surfaces with the advantage of modulating permeability of payloads [77,78]. The multilayer structures can be prepared by repeated coatings of alternately-charged polyelectrolytes on a substrate. A layer-by-layer (LbL) approach was also demonstrated in the self-assembly of multilayered PVA-chitosan films mediated by borax. The neutral PVA was physically crosslinked by the interaction between the hydroxyl groups and borax via hydrogen-bonding and was converted to carry negative charges for coacervation with chitosan [79]. Instead of modification with hydrophobic motifs, polyelectrolyte hydrogels such as chitosan formed in alcohol water solution have been demonstrated to assemble into 'onion-like'

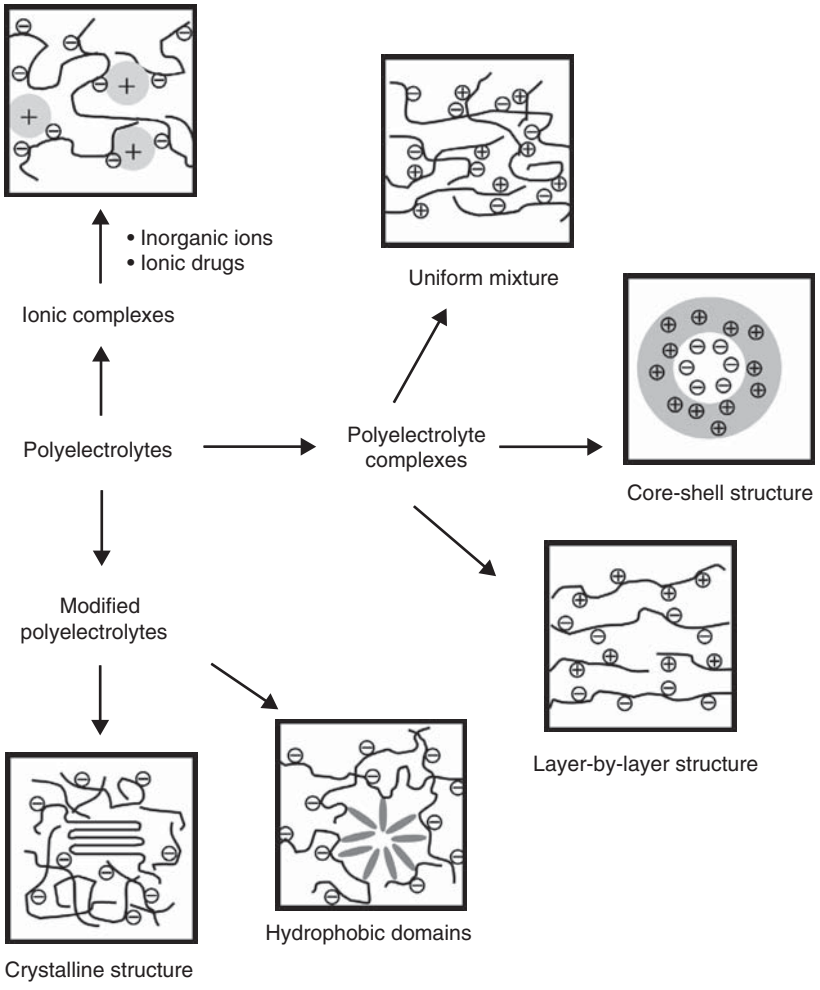

Figure 5. Physical interactions between components of hydrogels involving polyelectrolyte complexes.

multilayered structures through repeated neutralization above the pK_a 6.3 of the chitosan amine with NaOH solution followed by water washing [80]. This method could also be applied to the alcohol hydrogel of alginate, an anionic polyelectrolyte, by fabricating multilayered assembly structures of ionically-complexed hydrogels in a $CaCl_2$ bath. Hydrogels with multilayer structures from natural polyelectrolytes have been shown to have potential as new platforms for the study of cell proliferation in tissue engineering, cell interaction and communication [81], and controlled delivery of multiple drug payloads in a pulsed manner [36].

2.4 Supramolecular hydrogels containing CD inclusion complex

CD inclusion complexes of therapeutic agents have been widely used in pharmaceutical formulations for improving drug solubility and bioavailability. Since the 1990s, *in situ* gelation of novel supramolecular hydrogels based on inclusion complexes between CD and various copolymers have shown similar promise in biomedical and pharmaceutical applications [82]. For a gelation process involving α-CD, the hydrogel fabrication starts with the penetration of linear PEO into the hydrophobic cavity of α-CD to form an inclusion complex [83]. The necklace-like supramolecular structures further self-assemble to form crystalline domains which are not water soluble and function as physical crosslinking points for the gel network. Whereas, β-CD has been demonstrated as a host to accommodate PEO-*b*-PPO-*b*-PEO triblock copolymers (Pluronics) and form the inclusion complex via selective interaction between the hydrophobic cavity of β-CD and the PPO blocks, not PEO blocks [84]. The inclusion complex aggregated to form an ordered supramolecular crystal structure to which the tethered PEO blocks were partially crystallized to prevent the inclusion complex from de-threading [84]. The subtle structural difference between α- and β-CDs and their interaction with PEO and PPO blocks provides an interesting potential approach to tune physical hydrogels.

Due to its reversible thixotropic property, the α-CD/PEO supramolecular hydrogels could be delivered by syringe injection and regain their viscosity gradually. To overcome

rapid drug release from α-CD/PEO hydrogels, strategies for the incorporation of more hydrophobic moieties along the PEO polymer chains have been adopted. Block copolymers including PEO-PPO-PEO [85], PEO-poly((R)-3-hydroxybutyrate)-PEO [86], and PEG-PCL-PEG or PEO-PCL [82,87] have been developed to increase physical crosslinking and drug retention by the aggregation and micellization of hydrophobic blocks.

A reverse micellar hydrogel system from the diblock copolymer of PLG-PEO and α-CD is a novel system for pH-triggered fabrication of hydrogels (as described in Section 2.1.2) which further includes supramolecular inclusion complexes and hydrogen bonding [88]. By using PLG_{10}-*b*-PEO copolymer, micro phase separation between PLG and PEO in neutral pH water caused by hydrogen bonding amongst PLG chains led to the self-assembly of micelles with PLG as core and PEO as corona. The subsequent addition of α-CD induced PEO/α-CD inclusion complexation and formation of a physically crosslinked hydrogel. When PLG_{35}-*b*-PEO was used, reverse micelles were formed with a PEO–α-CD supramolecular inclusion complex as the micelle core and anionic PLG as the corona in pH 8 water, followed by the gelation of the micelles through hydrogen bonding between the PLG groups at lower pH 5. The average size of normal and reverse micelles was 33 nm and 210 nm in solution by dynamic light scattering measurement, respectively. Drug loading and release experiments showed that the anticancer drug, doxorubicin, could be loaded into the hydrogels, and the release lasted up to 45 days in the case of the reversed micellar hydrogel. In addition to PEO and PPO, other polymers containing hydrophobic moieties have been used to form inclusion complexes with CDs for hydrogel preparation [89,90]. An organic solvent free method for preparing nanogels based on host–guest interactions was reported [90], which was through the self-assembly between hydrophobic lauryl groups on dextran and polymerized β-CDs with apolar concavities. ^{1}H NMR demonstrated that the organic compound, benzophenon, was loaded into the cavities of β-CD with the coexistence of alkyl chain–β-CD complex.

2.5 Hydrogels with crystallites as crosslinkers

Systems in which crystalline structures serve as physical crosslinks in hydrogels have been regularly reported for drug delivery and tissue engineering applications [91,92], because of the increased mechanical strength of the hydrogels, compared to other physical crosslinks [93,94]. PVA hydrogel is a type of biomaterial with non-toxic, non-carcinogenic, biocompatible and bioadhesive properties and is used in a wide range of applications, including soft contact lenses, biomembranes in artificial kidneys and artificial articular cartilage. Many simple, effective and benign fabrication processes for the physical crosslinking of such hydrogels include slow-drying, annealing, aging, or freezing and thawing [95]. An aqueous PVA solution which has undergone repeated freezing and thawing can form hydrogels which are crosslinked by PVA crystalline regions composed of layers of folded PVA sequences mediated by van der Waals forces and through the interaction of hydroxyl groups [93]. Small angle neutron scattering and ultra-small angle neutron scattering experiments further indicate the existence of bicontinuous phases, that is, polymer rich regions with dimensions of the order of 1 – 2 μm and water rich regions [96]. The average radius of PVA crystallites in the polymer rich regions were shown to be ~ 45 Å. The degree of crystallinity of PVA affects the physical crosslinking and the strength of its hydrogels. Thus, drug release can be adjusted by increasing the number of freeze/thaw cycles and freezing time of the PVA solutions [97]. The factors influencing the degree of crystallinity include the molecular mass and stereoregularity of the PVA, and the addition of different salt solutions. With its relatively simple chemical structure and processing methods, contact lenses prepared from PVA physical hydrogels showed enhanced mechanical strength and reduced protein adsorption. Composite hydrogels of PVA/NaCl [98] and PVA-NaOH/PVA-PAA films [99] with increased degree of crystallization were reported, and the anti-asthmatic drug, theophylline, was loaded and shown to have a Fickian-type release. The interaction between the PVA-PAA hydrogel and aspirin loaded into the hydrogel can have a significant effect on the crystalline structure of PVA and decrease the overall mechanical strength of the hydrogel [100]. Similarly, double-stranded DNA has been loaded into PVA and PVA/NaBr composite hydrogels fabricated by three cycles of the freezing-thawing method, and increased hydrogel swelling was observed which was attributed to the interruption of the crystalline structure by the DNA and salt present in the PVA [101].

Hydrogels with superior mechanical performance required by many biomedical applications have remained a challenge, and numerous efforts have been made to address this issue [102]. Chemically crosslinked double-network hydrogels have been synthesized and demonstrated excellent fracture strength and wear resistance [103]. Recently, novel double-network hydrogels of PVA/PEG have been fabricated, through a simple freezing and thawing process, to possess a remarkably high mechanical strength, which can sustain compressive pressure up to several megapascals [104]. Differential scanning calorimetry results demonstrated the existence of multicrystallization of both PVA and PEG in the hydrogel. A structural model was proposed to describe the high mechanical strength of PVA/PEG hydrogels in which PVA-rich phases form microcrystallites as the network backbone of the hydrogel. Between the cavities or voids of the backbone, dilute PEG phases partially crystallize and effectively absorb the crack energy by viscous dissipation and the deformation of PEG chains.

Stereoregulated crystallization in hydrogels introduced by block copolymers of PLLA has been of great interest due to the significant increase in gel mechanical properties, in addition to their attractive biodegradability, which lends itself to many applications in tissue regeneration and drug delivery

devices [16]. Due to the hydrophobicity of PLLA, the polymer is frequently copolymerized with hydrophilic blocks to increase its solubility and biocompatibility. Injectable hydrogels of PEG-PLLA-PEG triblock copolymers with thermosensitivity were first reported by Kim and co-workers [105]. In addition to the hydrophobic interactions of the PLLA in this system, as discussed in Section 2.1.1, subsequent studies have revealed that the increase in hydrophobicity, molecular mass and crystallinity of the hydrophobic blocks in di- and triblock copolymers consisting of PEO and polyesters could increase the gel-sol transition temperature at given concentration and decrease critical gel concentration of the polymers [106]. Chemically changing the copolymer sequence to PLLA-PEO-PLLA, Tew and co-workers found that the physically associated hydrogels exhibited significantly higher elastic modulus [107]. Small angle neutron scattering studies revealed that in PLLA-PEO-PLLA hydrogels, the network was associated through 'lamellar micelles' (**Figure 3D**), in which the L-lactide blocks formed a crystalline lamellae structure with PEO chains aligned on their surface [108]. Whilst adjusting the stereoregularity of PLLA to PRLA (racemic mixture of D- and L-lactide), the PRLA-PEO-PRLA hydrogels were formed via the self-assembly of spherical micelles with amorphous PLA domains as core and PEO as corona and bridges between micelles. It was found that the model drug, sulindac, released from polymers with crystalline PLA blocks much faster than those with amorphous PLA blocks because of the polymer–drug interactions [109].

2.6 Hydrogels via hydrogen-bonding interactions

Hydrogen bonding can play a crucial role in the fabrication of polymer hydrogels, especially in systems involving poly (carboxylic acids) [110,111], polysaccharides [11], peptides and proteins [4,112]. Mediated by the hydrogen-bonding interactions, interpolymer complexes (IPCs) between hydrogen donors and acceptors can be formed in either aggregation or physically crosslinked gel networks. As secondary interactions, although hydrogen bonding from a single pair hydrogen donor-acceptor is weak, the interactions in IPCs are collectively significant to produce self-organized structures and hydrogels. The factors that can influence the hydrogen bonds include water dilution, shear disturbance, pH change and temperature. Sometimes, other physical or chemical crosslinking moieties are introduced to strengthen gels. Hydrogels formed primarily via hydrogen bonding have been used as injectable forms by taking advantage of the weak interactions under shear. Hydrogels made from the IPC of poly(carboxylic acid) and hydroxypropylmethylcellulose are pH-sensitive and can be used as *in situ* gelling systems [113]. Meanwhile, the use of hydrogen bonding to fabricate self-assembled nanostructures has attracted much attention for drug delivery and biomedical applications. Sukhishvili and co-workers reported that assembled LbL structures via hydrogen bonding between PVP and PMAA followed by chemical crosslinking have been prepared as thin films that could encapsulate and release dyes and proteins mediated through charge interactions at different pH [114]. They also reported the studies of thermoresponsive LbL hybrid films with alternating PNIPAAm/PMAA and PVP/PMAA strata [115]. The higher stacks could be released after the elimination of lower stacks of PNIPAAm/PMAA triggered by a temperature decrease to 10°C at a pH range between 5.2 and 5.8, which could have potential applications in tissue engineering. Hydrogen bonding can introduce self-assembly of di/triblock copolymers to form micellar or vesicle nanostructures [116], whereas stable hydrogels of synthetic polymers built on hydrogen bonding often need the incorporation of other interactions, such as electrostatic interactions, hydrophobic interactions, van der Waals forces, π–π stacking interactions and supramolecular inclusion complexes. In an example of fabrication of small molecular hydrogels, Karp and co-workers reported that the analgesic and antipyretic drug acetaminophen was transformed into amphiphilic prodrugs through esterification reaction with carboxylic acids [117]. The prodrug hydrogelators could self-assemble in hydrogels which were enhanced by hydrogen-bonding, π–π stacking and van der Waals interactions. A second model hydrophobic drug, curcumin, was encapsulated into the hydrogels and showed a high concentration of encapsulation due to its localization within the hydrophobic pockets of the gels. Enzyme-triggered gel degradation and drug release were demonstrated at physiological conditions. This novel hydrogel with low toxicity, excellent cytocompatibility from existing drugs and known metabolic pathway shows a promising strategy of fabricating drug delivery hydrogels.

2.7 Self-assembling peptide hydrogels

Since the early 1990s, hydrogel fabrication mediated by the self-assembly of peptides or proteins has been extensively investigated, providing methods for precise control of gel structure and properties via tailor-made peptides or proteins [112,118-120]. Peptide systems have been reported to be non-toxic and cytocompatible and on biodegradation can produce easily metabolized amino acids [121,122]. Peptide hydrogels are generally described as non-immunogenic but recent work in vaccine development has allowed the nanostructures to act as immune adjuvants with the introduction of an epitope to the end of self-assembling peptides [123]. Ease of use is an important consideration and systems which gel in response to physiological stimuli or have demonstrated the ability to shear thin are desired for injectable delivery [124]. Peptide hydrogel scaffolds can mimic many characteristics of native extracellular matrices providing support for cell culture and act as valuable tools in biological research [16,125-126]. In addition to cell encapsulation, peptide hydrogels can be used to encapsulate and release other therapeutic compounds. Compounds can be loaded into pre-formed gels or mixed in during the self-assembly process without detrimental effects to their structure [127]. β-Sheets and coiled coils are naturally occurring peptide motifs which when used in hydrogel design

can encourage greater biorecognition. Interactions between hydrogen bonding-mediated β-sheets and hydrophobic and electrostatic interactions between α-helices, with the presence of complementary groups on peptides, are the driving forces of self-assembly [112].

The EAK and RADA family of peptides designed by Hauser and Zhang contain alternating hydrophilic and hydrophobic amino acids and self-assemble to form stable β-sheets. The original EAK peptides contained lysine (positively charged) and glutamate (negatively charged) amino-acid residues whereas the RADA peptides have residues of arginine (Arg) (positively charged) and aspartate (Asp) (negatively charged) [128]. The β-sheets self-assemble into nanofibers which further organize into hydrogel scaffolds. The hydrophilic side of the β-sheet allows the gels to have water contents of > 99% [128]. The release kinetics of various proteins from acetyl-(Arg-Ala-Asp-Ala)$_4$-CONH$_2$, that is, [Ac-(RADA)$_4$-CONH$_2$] peptide scaffolds has been reported [129]. Release was controlled by diffusion through the pores of the hydrogel network and was dependent on protein size, with larger proteins demonstrating slower release. Release could be further restricted using higher peptide concentrations to form the gels, consequently increasing the density of nanofibers in the network and reducing pore size. The authors predict complete release of the larger proteins would occur with biodegradation of the hydrogel. A noteworthy result was that the proteins, lysozyme, trypsin inhibitor and IgG, were shown to retain their functionality on release [129]. The release studies were performed at pH 7.4 and the RADA peptide used carried only a slight negative charge. It is suggested that tailored design of the peptide, to carry a net charge, may reduce protein release from the hydrogels by increasing interaction with the charged surfaces. This was demonstrated in a previous study by Nagai *et al.* who acidified the RADA hydrogel and observed release of small molecules with varying charge densities [130]. Nanofibrous hydrogels can also be formed from short self-assembling peptides such as the fluorenyl methoxycarbonyl protected dipeptide [131,132] or tetrapeptide systems [133]. The hydrogels contain anti-parallel β-sheets which exist through π–π stacking interactions of the aromatic amino-acid residues stabilized by hydrogen bonding. These hydrogels have also demonstrated controlled drug delivery.

As with other hydrogel systems those based on peptides can be designed to respond to external stimuli. MAX peptides described by Branco *et al.* respond to solutions of high ionic strength folding into amphiphilic β-hairpins due to the effect of charge screening of the selected residues [127]. Lateral assembly of the hairpins is driven by van der Waals interactions and hydrogen bonding whereas as a result of hydrophobic interactions a bilayer is formed through facial assembly [134]. The hairpins self-assemble to form nanofibrils which produce hydrogel networks through entanglement and interfibril crosslinks [127]. In addition to increasing peptide concentration, smaller mesh sizes can be produced by reducing the number of residues to be screened, increasing the rate of self-assembly [124]. The release of model macromolecules was dependent on electrostatic interactions, mesh size of the MAX hydrogels and size of the macromolecule [127].

There are numerous reports of peptide amphiphiles being used for controlled release [118,135-136]. Peptide amphiphiles are lipid-like structures, where the peptide sequences have hydrophobic tails, composed of alkyl chains or non-polar amino acids, and charged head groups. Self-assembly is a balance of electrostatic repulsion with the attractive forces of hydrogen bonding and hydrophobic interactions [137]. With modification of the amphiphile tail or head group, self-assembly into cylindrical nanofibers, formed from β-sheets, can be altered by pH, redox conditions or use of divalent ions [138]. The amphiphiles are commonly studied for intracellular delivery as with careful design bioactive peptides can be presented on the outside of an assembled nanostructure for recognition by cells [136,139]. Hydrogel networks formed from entangled amphiphile nanofibers, at concentrations of 1% by weight, can form scaffolds in an artificial extracellular matrix [137]. Attachment of bioactive epitopes to the peptide amphiphiles have been used to promote neural regeneration, hard tissue replacement and angiogenesis [137]. Self-assembly of a peptide amphiphile with a heparin binding domain is initiated as the heparin binds, screening the amphiphile's charge, forming nanofibers with heparin presented on their surface. When incorporated into hydrogel scaffolds with PLA, VEGF and fibroblast growth factor 2 were able to bind to the heparin. Delivery of these angiogenic growth factors from the scaffolds was shown to increase vascularization in islet isograft models and increase cell viability [140,141].

In addition to β-sheets, another type of recognition motif, coiled coils are formed when two or more α-helices assemble together, mediated through hydrophobic and electrostatic interactions of the seven repeating residues of the primary peptide structure [4]. Coiled coils are often used in the formation of block copolymers either as polypeptides or with synthetic polymers forming hybrid hydrogels [112]. Triblock copolymers with two coiled coils either side of a random block self-assemble through hydrophobic interactions of the coils [142]. Hydrogels are formed due to intermolecular association of the coiled coils. However, there are situations where intermolecular association can be difficult and higher peptide concentrations are required to form hydrogels, for example, with increased chain length the blocks associate with themselves forming intramolecular loops [142,143]. Conjugation of the coiled coils to polymers such as PEG is possible with only minimal impact on the secondary structure of the peptides [144,145]. Triblock peptide-PEG-peptide hydrogels are stabilized by coiled coil dimers and tetramers [144]. Coiled coil peptides with binding affinities for biological molecules such as heparin, when conjugated with PEG, can self-assemble to form hydrogels which release growth factors [145].

3. Conclusions

Recent progress in the creation of physical hydrogels with self-assembled nanostructures based on non-covalent interactions has provided many novel hydrogel systems as drug delivery carriers with improved toxicity, handling, drug release profiles and patient compliance. By exploiting physical interactions, varieties of hydrogel nanoscale structures have been designed via self-assembly, involving both polymeric moieties and therapeutic agents, to achieve stable drug existence in the gel whilst maintaining gel integrity in a controlled manner. These carefully fabricated hydrogels possess many advantages, such as low toxicity compared to chemically crosslinked gels, stimuli-responsiveness, simplified gel preparation under mild conditions, protection of delicate payloads such as protein or peptide drugs, ease of administration, and improved drug–hydrogel compatibility and drug release profiles. The physical hydrogels can be used as *in situ* gelling systems for direct injection, capsules, nano/microparticulates or coatings for drug delivery. Although significant challenges such as gel integrity and fast drug elution remain, physical hydrogels with precisely controlled structures in the nanoscale continue to offer great potential in biomedical and biomaterial applications.

4. Expert opinion

Physical hydrogels have marked advantages compared to chemically crosslinked hydrogels as aforementioned due to the aspects of low toxicity, ease in fabrication and handling, and reversible sol-gel transition and so on. However, technically the major challenges that physical hydrogels face in their practical applications is the difficulty in creating a microenvironment inside the gels for the stable storage of drugs thus resulting in rapid drug release, which could be exacerbated when the gel integrity decreases with time. For a controlled DDS, physical hydrogels are required with the properties of fast stimuli-responsiveness, reversible sol-gel transition, predictable release kinetics and preferably injectable drug formulations. To achieve these targets and solve these issues, it not only requires polymers and hydrogels with new structures and functions, but also a deeper understanding about the interactions between the polymers and their cargoes.

In the last decade, there has been excellent progress in building hydrogel nanostructures with block copolymers synthesized by living radical polymerization (ATRP and RAFT, for instance) and other techniques for the delivery of therapeutics. Meanwhile, approaches using molecular imprinting, small-molecule hydrogels and peptide hydrogels, which prioritized the role of payloads and provided accurately controlled nano/microstructure and polymer–drug interactions, have shown great progress in the drug delivery field. Accordingly, the two current approaches adapted in the design of hydrogel systems for drug delivery, that is, polymer→hydrogel→drug loading and drug template→polymer→hydrogel, both point to the significant role to be played by the precise nanostructures self-assembled via polymer–polymer and polymer–drug interactions. This requires macromolecules synthesized with narrowly distributed molecular mass, and controlled block or graft polymeric architecture. In this respect, synthetic techniques, notably living radical polymerization and click chemistry, have provided abundant materials and tools for linking functional groups and gel fabrication. Physical characterization methods such as X-ray/neutron scattering and imaging measurements from AFM, SEM and TEM are powerful in ascertaining structures of polymer and drug self-assembly at the nanoscale. Meanwhile, information from the physicochemical properties of drug candidates, such as structural factors, charge distribution, hydrophobicity/hydrophilicity, solubility, pK_a, chemistry and self-aggregation, should be fully evaluated and incorporated into the gel design principles. The drug–polymer interaction induced by loaded therapeutics could disturb the distribution of the local nanostructure, thus collectively affecting the gel properties.

Finally, the eventual *in vivo* environment into which the hydrogel system is to be placed will have a crucial effect on the drug and hydrogel and needs to be evaluated in detail in order to design a DDS with optimum release properties. Multiple factors must be considered, ranging from the requirements necessary for hydrogel delivery, such as their rheology during drug-gel administration within the body, the gel integrity over time and the eventual fate of the components of the hydrogel including their clearance pathway. Indeed, it is now well-recognized that some polymers, such as the Pluronic block copolymers that have been used in many drug delivery applications and once thought to be simply inert carriers, can have distinct biological activity in themselves. The intracellular depletion of ATP and inhibition of ATPase activity of drug efflux proteins exhibited by P85 is one such unexpected phenomenon that demonstrates the need for a thorough evaluation of the effects of nanostructured materials on cellular activity [146]. Biological systems themselves possess complex nanostructures and thus it should not be too surprising that certain block copolymer systems can be incorporated into cellular membranes and alter the microviscosity and permeability. Indeed, for the physical hydrogel systems with self-assembled nanostructures of the future, this may become an important part of their design and critical to their overall performance *in vivo*.

Declaration of interest

The authors declare no conflict of interest. The authors are employees of Biocompatibles UK Ltd.

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