Supramolecular Hydrogels: Design Strategies and Contemporary Biomedical Applications

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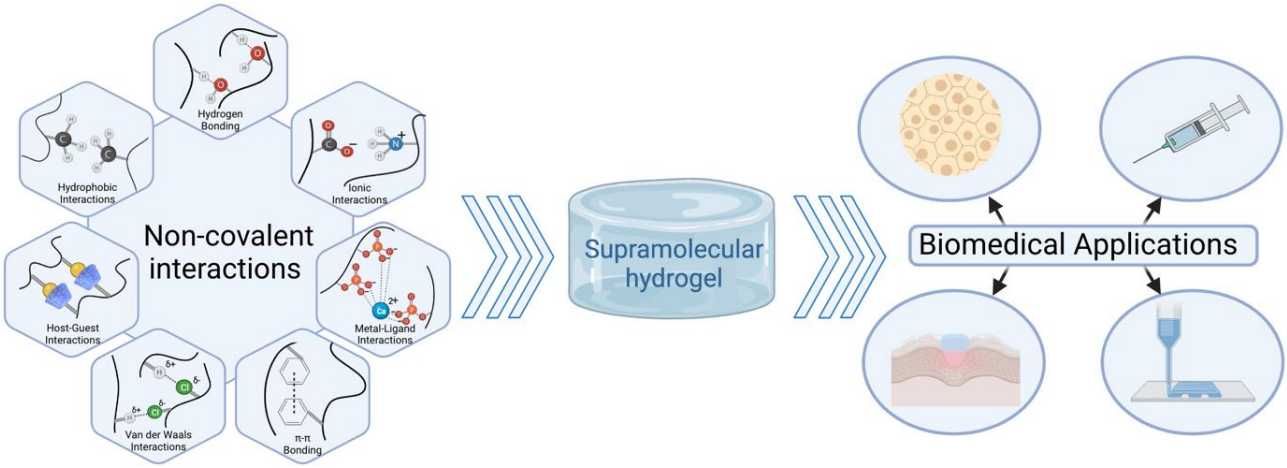
Abstract

Self-assembly of supramolecular hydrogels is driven by dynamic, non-covalent interactions between molecules. Considerable research effort has been exerted to fabricate and optimise supramolecular hydrogels that display shear-thinning, self-healing, and reversibility, in order to develop materials for biomedical applications. This review provides a detailed overview of the chemistry behind the dynamic physicochemical interactions that sustain hydrogel formation (hydrogen bonding, hydrophobic interactions, ionic interactions, metal-ligand coordination, and host-guest interactions). Novel design strategies and methodologies to create supramolecular hydrogels are highlighted, which offer promise for a wide range of applications, specifically drug delivery, wound healing, tissue engineering and 3D bioprinting. To conclude, future prospects are briefly discussed, and consideration given to the steps required to ultimately bring these biomaterials into clinical settings.

Keywords

biomedical applications, gels, molecular recognition, non-covalent interactions, self-assembly, supramolecular chemistry

Graphical Abstract



Biographies

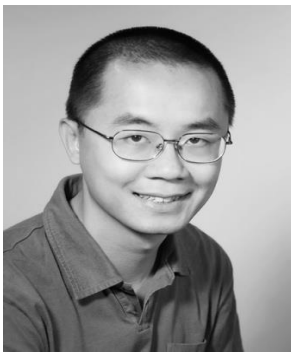
Jasmin Omar obtained her MPharm degree from King's College London (KCL) in 2018. She went on to complete her pre-registration year in hospital and qualified as a pharmacist in 2019. She is currently pursuing her PhD in pharmaceutical and material science at KCL and the Agency for Science, Technology and Research (A*STAR), Singapore. She is supervised by Dr. Cécile A. Dreiss and Prof. Xian Jun Loh and her research project focuses on temperature-responsive hydrogels as vitreous substitutes.

Daniel Ponsford completed his MChem with a Year in Industry at the University of York in 2018. During his industrial placement, he worked on various projects which aimed to develop next-generation latex binders for waterborne trim paints and coatings. As a PhD student, his current research investigates stimuli-responsive, multifunctional and supramolecular nanomaterials, and is jointly supervised by Dr. Tung Chun Lee (University College London) and Dr. Ming-Yong Han (A*STAR, Singapore).

Dr. Cécile Dreiss is a Reader in Soft Matter at King's College London. She graduated in Chemistry and Chemical Engineering from ENSIC (Nancy, France), completed her PhD at Imperial College, and then took up a postdoc position at the University of

Bristol. Her research focuses on understanding and exploiting self-assembly in colloidal, polymeric and biological systems, establishing relationships between properties on the macro-scale and morphology on the nanoscale and has a particular interest in hydrogels. She uses neutron scattering techniques extensively as well as rheology.

Dr. Tung-Chun Lee received his BSc in Chemistry from University of Hong Kong in 2005 and his PhD in Chemistry from University of Cambridge in 2012. He was a Post-doctoral Fellow at the Max Planck Institute for Intelligent Systems from 2011 to 2014. Currently he is an Associate Professor in Nanomaterials Chemistry at University College London (UCL), leading an interdisciplinary group of 16 researchers in exploring chemistry under nano-confinement for catalysis and developing novel plasmonic sensors for trace chemical detection. His research interests also include hybrid nanoparticles, nanofabrication, nanochemistry, supramolecular chemistry, active matter and transmission electron microscopy.



Prof Xian Jun Loh completed his basic and postgraduate studies at the National University of Singapore. A polymer chemist by training, he is the Executive Director at the Institute of Materials Research and Engineering (IMRE), A*STAR. He is also the current President and member of the Executive Committee of the Singapore National Institute of Chemistry. As a pioneer in the area of biodegradable thermogels, he is highly knowledgeable in developing these materials for various applications spanning biomedical, engineering, cosmetics, personal care and food.

1. Introduction

Hydrogels are three-dimensional networks, typically made of crosslinked hydrophilic polymers, block-copolymer micelles, colloids or peptides.[1] They swell in water without compromising their molecular structure, resulting in soft materials, which, because of their porous structure, mechanical properties, and high-water content, show similarities with biological tissues. This resemblance, in conjunction with hydrogels' ability to encapsulate and control the release of growth factors, cells and drugs, and support cell proliferation and migration, has catalysed an exponential growth of publications in the field over the last couple of decades, particularly in the development of novel biomedical materials. [2,3]

The structure of hydrogels relies either on chemical or physical crosslinking. Traditionally, hydrogels have been made by chemically crosslinking polymer chains *via* covalent bonds, creating a three-dimensional (3D) network which is capable of immobilising the surrounding solvent and sustaining its own weight. Although this generally results in a physically stable and mechanically strong hydrogel, the enduring nature of the crosslinks prevents injectability of the material and generally precludes imparting responsiveness to the material.[3,4] More recently, physically bound, or "supramolecular", hydrogels, have sparked tremendous research interest, due to the dynamic nature of the connections that offer considerable flexibility. This burgeoning interest is demonstrated by the number of publications featuring the term 'supramolecular hydrogels', which has increased more than 100 times from 2000 to 2021 according to Web of Science (Figure 1). [5]

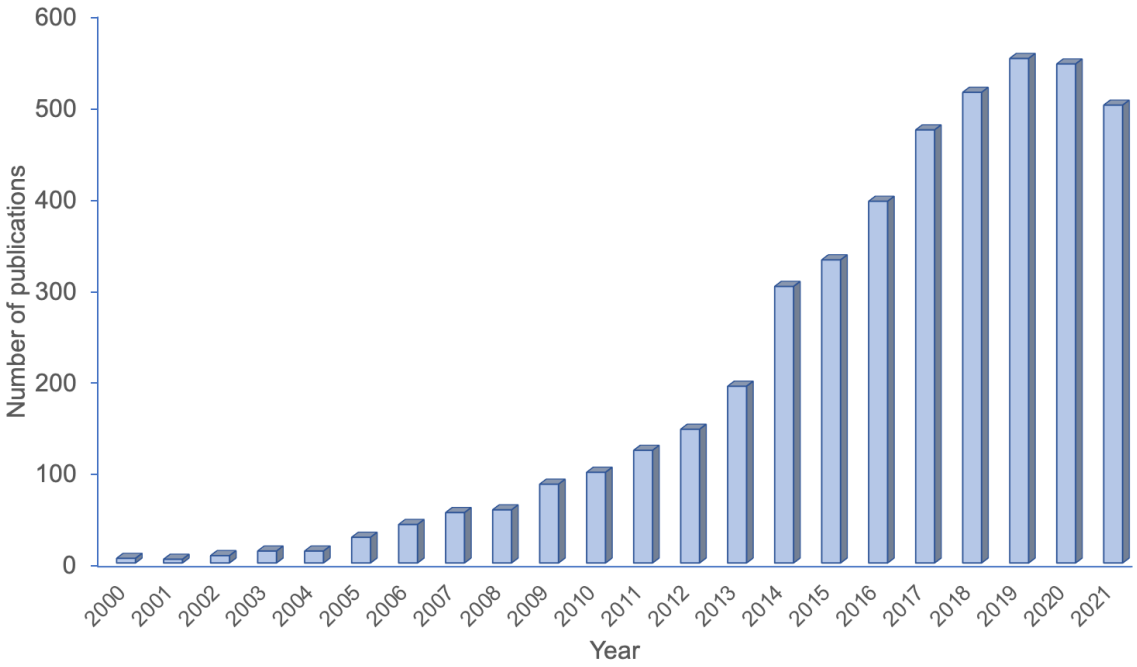


Figure 1: The number of publications featuring 'supramolecular hydrogels' from 2000-2021. Data extracted from web of science (accessed on 5th January 2022).

Supramolecular hydrogels are produced when gelator molecules (or macromolecules) spontaneously self-assemble to form a 3D solid-like network *via* dynamic intermolecular non-covalent bonds, notably: hydrogen bonding, hydrophobic interactions, van der Waals interactions, π-π bonding, electrostatic interactions, metal-ligand coordination and host-guest interactions[6], as illustrated in Figure 2. It is worth noting that these non-covalent interactions often occur synergistically to bring about gelation.[7] The strength and distance dependence of selected supramolecular interactions are presented in Table 1.

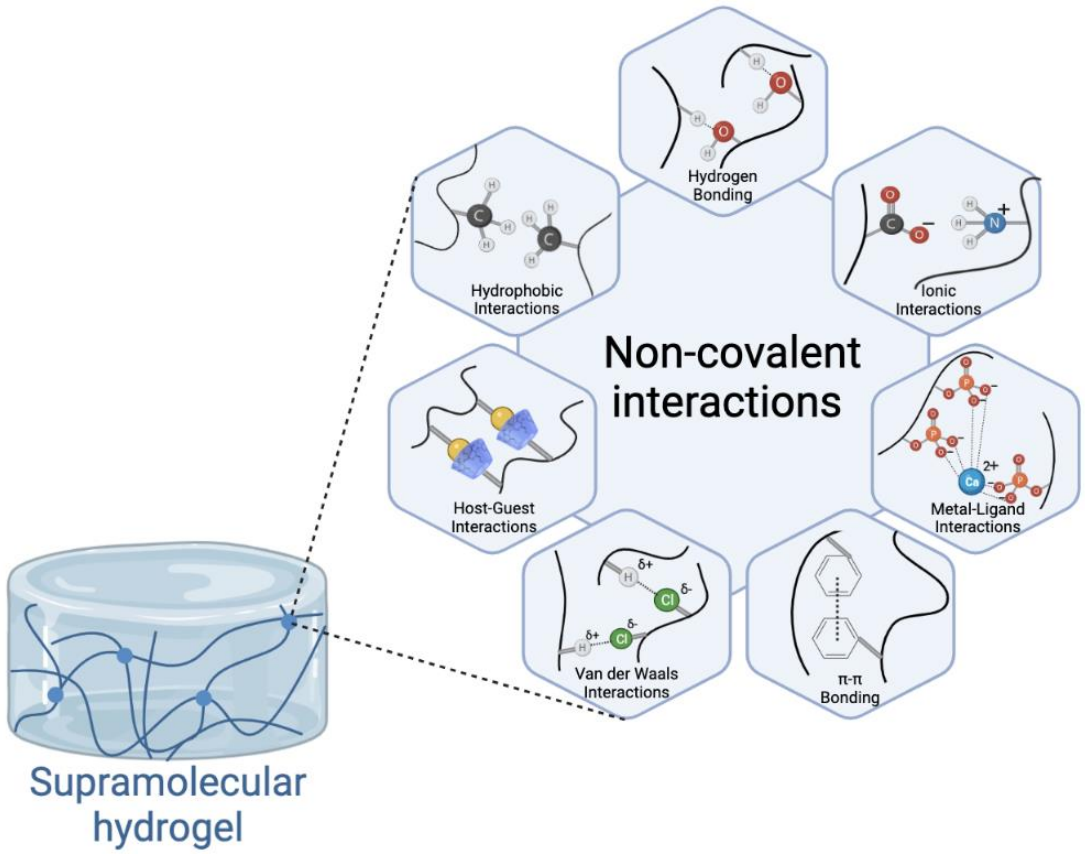


Figure 2: Non-covalent interactions used to form supramolecular hydrogels. Created win BioRender.com

Table 1. Selected supramolecular interactions employed to generate supramolecular gels. Adapted with permission. [8] Copyright *2019* WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim and copyright 2001, Neue Schweizerische Chemische Gesellschaft, Switzerland

Interaction	Strength / kJ mol^{-1}	Working distance
Hydrogen bond	4 – 120	Long range (~$1/d^2$)
π-π	0 – 50	Medium range ($1/d^3$, $1/d^6$)
Hydrophobic	Related to solvent-solvent interaction energy	-
Ion-ion	200 – 300	Medium range (1/d)
Ion-dipole	50 – 200	Short range ($1/d^2$ for fixed dipole, $1/d^4$ for freely rotating ion-dipole interaction)
Dipole-dipole	5 – 50	Short range ($1/d^3$ for fixed dipole, $1/d^6$ for freely rotating dipoles)
London dispersion or van der Waals forces	< 5 but variable depending on surface area	Very short range ($1/d^6$)

Noble prize laureates, Jean-Marie Lehn, Donald J. Cram and Charles J. Pedersen (1987) initiated the thriving field of supramolecular chemistry through the development of cryptates.[9] Supramolecular hydrogels were eventually developed with attributes such as self-healing (which increases material lifetime)[4,10,11], shear-thinning behaviour (which enables injection and 3D bioprinting)[12], responsiveness to physical stimuli[13], and reversibility of the sol-gel transition, making them ideal candidates for a multitude of biomedical applications.[7,14] This versatility has also allowed the application of supramolecular hydrogels in a wide range of other fields, including the food industry[15], agriculture[16] and adhesives.[17]

Ensuring that supramolecular hydrogels possess both the desired biomedical properties, and the required mechanical properties commonly presents a challenge to scientists. The formation of non-covalently crosslinked tough gels, that are also biocompatible and injectable, and can be made to response to specific stimuli, is of great scientific importance for biomedical applications. The aim of this review is to present the interactions that underlie the formation of supramolecular hydrogels, before exemplifying this versatility by highlighting a selection of recent state-of-the-art developments and their associated biomedical applications. The applications selected include drug delivery, tissue engineering, wound healing and 3D bioprinting (Figure 3).

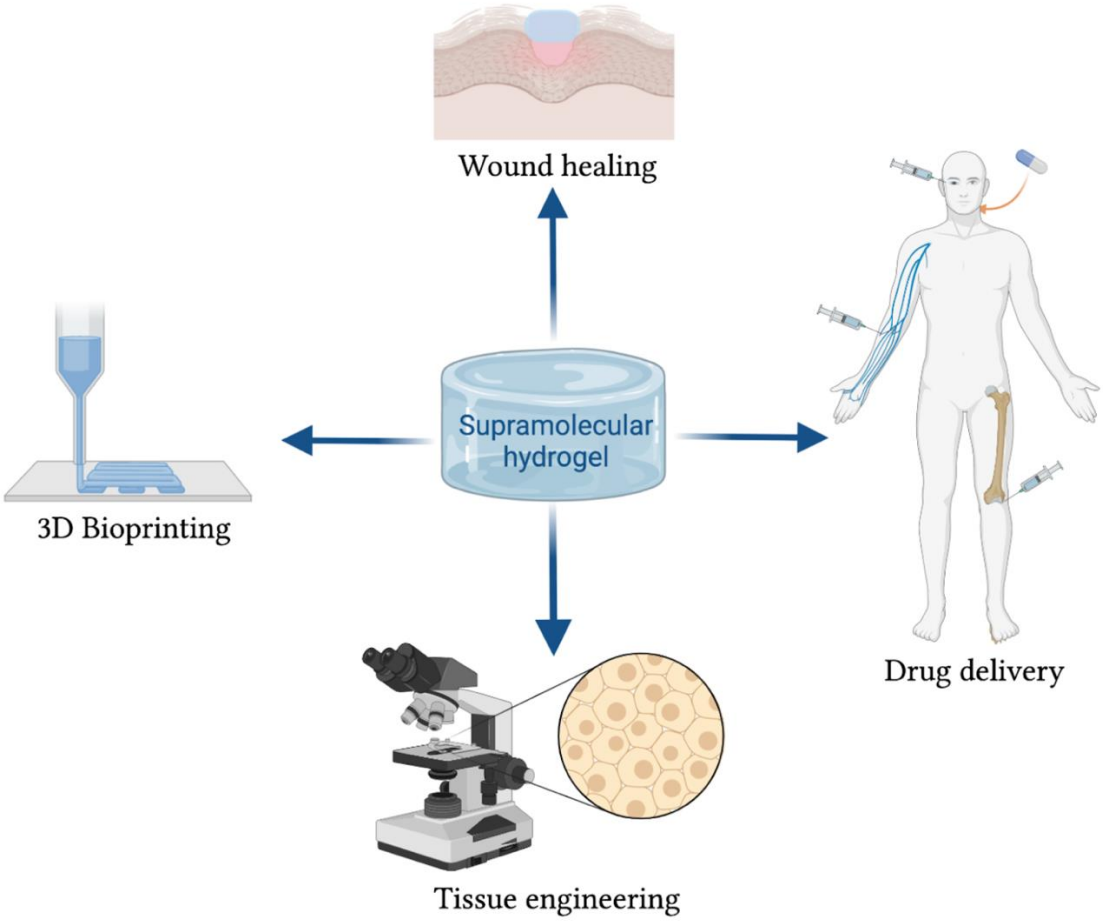


Figure 3: Biomedical applications of supramolecular hydrogels presented in this review. Created with BioRender.com

2. Supramolecular interactions and design considerations

Self-assembly and gelation processes for supramolecular hydrogels are commonly reliant on the simultaneous action of multiple, synergistic, non-covalent interactions. In this review, gels will be classified according to their most significant non-covalent interactions, for clarity and ease of discussion. Nonetheless, the high extent of overlap between different mechanisms of gelation (as presented here) should be appreciated.

a. Connections through hydrogen bonding

The hydrogen bond remains one of the most common and versatile physical bonding motifs which features in supramolecular hydrogels.[18] Although water molecules compete with gelators for hydrogen bonding sites, hydrogen bonds between electron-

rich acceptor atoms and electron-poor hydrogen (donor) atoms are still able to yield strong and functional hydrogels. A molecule relying purely on hydrogen bonding for self-assembly is susceptible to solvation rather than gel formation, hence most hydrogelators relying on hydrogen bonds for connections are amphiphilic[19–22]. The structural integrity of hydrogen bond-mediated hydrogels is heavily influenced by changes in pH (often a low pH is required for gel formation), which can hinder the use of these materials for applications including tissue engineering.[22]

The strength of hydrogen bonding can be enhanced in linear donor-acceptor arrays, such as the quadruple bonding present in 2-ureido-4-pyrimidinone (UPy) dimers, the pairing of nucleotide base pairs, and the lateral connection of β-strands to form antiparallel β-pleated sheets.[23–25] Varying the number, position or density of hydrogen bonding groups on a gelator offers a facile route to adapt the mechanical properties of a gel, and the extent of compatibility and interaction with biomolecules including sugars, proteins and other cellular structures.[24] A huge number of supramolecular hydrogelators based on hydrogen bonding interactions are known, including natural (e.g. DNA, polysaccharides and chitosan) and synthetic constructs. These hydrogelators have been engineered to create novel antimicrobial scaffolds[26], bioactive hydrogels which promote bone regeneration[27], and responsive drug delivery vehicles[24], amongst many other uses.[24,28]

Although the majority of supramolecular hydrogels exhibit some ability to repair themselves after being subjected to a form of stress (due to the reversible and dynamic nature of their physical bonds), only specific hydrogels are typically considered ‘self-healing’. Self-healing hydrogelators feature a much higher number of repeating motifs capable of reversible, multisite bonding.[29] Hydrogen-bonded hydrogels with the ability to self-heal include gelators based on ureidopyrimidinones (Figure 4), gallol and benzene-1,3,5-tricarboxamide.[4,30,31] Different self-healing hydrogels can be prepared separately and mixed to form a multi-gel scaffold. In this way, a single scaffold is able to coculture multiple cell types to differentiate and proliferate, as the hydrogel may have regions with different mechanical properties.[32,33]

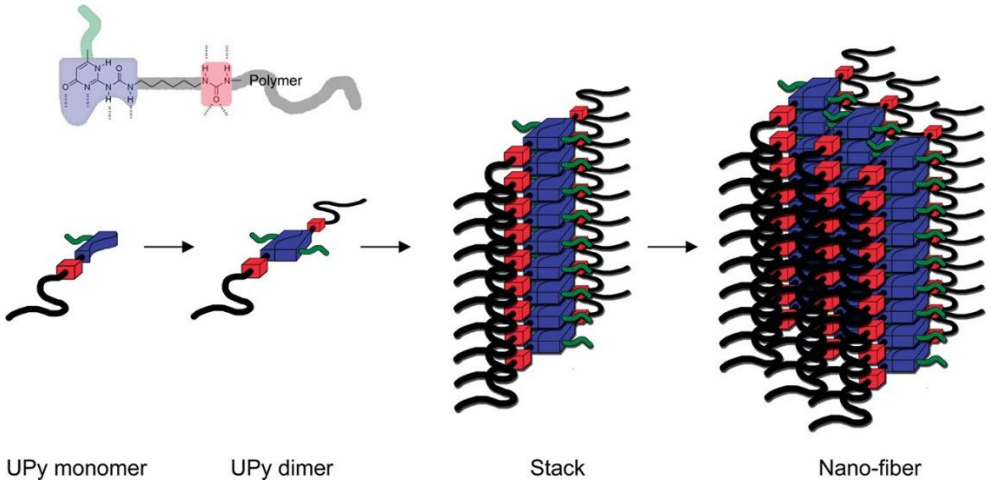


Figure 4: (A) Dimerisation of the ureidopyrimidinones (UPy) moieties by four-fold hydrogen bonding and stacking aided by additional urea functionalities. Adapted with permission.[34] Copyright 2011, American Chemical Society.

Self-healing hydrogels which are crosslinked by dynamic covalent bonds have also been synthesised for biomedical applications. Recent work has focused on chemistries including imine crosslinks[35], disulfide exchange[36], as well as traditional transesterification and acetal exchange reactions[37]. However, the reversibility of crosslinks typically relies on harsh stimuli (e.g. UV light[36], low pH[38] or high temperature[39] in these cases). As such, these hydrogels are suited to specific biomedical applications that replicate those conditions, such as cancer therapy, in which gelation can be triggered in the acidic microenvironment of a tumour. Furthermore, the formation and breakage of dynamic covalent bonds is slower than for other supramolecular interactions, and so the addition of a catalyst may be required.[4]

b. Network formation driven by hydrophobic interactions

The thermodynamic driving force for hydrophobic interactions is the minimisation of contact between hydrophobic moieties and water, which leads to a positive free energy change upon solvation.[29] This interaction leads to the organisation of nonpolar groups in a way that exposes as little surface area as possible to the surrounding aqueous environment. Typically, molecules which gel predominantly via hydrophobic interactions exhibit distinct hydrophobic and hydrophilic domains.[40,41] Within the supramolecular structures, the hydrophobic groups are buried within the innermost parts of the structure, whilst the hydrophilic polymer regions locate at the interface with

the surrounding aqueous medium.[17] The strength of these gels can be modulated by varying the number of hydrophobic groups in the polymer chain, or the relative amounts of hydrophobic and hydrophilic moieties[42]. Furthermore, changes in the extent of gelator hydration with temperature may also lead to phase transition events, such as in the case of thermogelling polymers.[43]

The mechanical properties of hydrogels formed mainly from hydrophobic interactions are typically weak, which reduces their applicability as load-bearing materials[41,44]. Moreover, the rheological properties of gels built on hydrophobic interactions are often impacted by other factors that affect the extent of gelator hydration, such as pH and the presence of kosmotropic or chaotropic agents. Changes in pH may protonate or deprotonate the polymer, whilst kosmo- or chaotropic agents act to strengthen (kosmotropes) or disrupt (chaotropes) interactions between water molecules surrounding the polymer, strengthening or weakening the hydrophobic effect, respectively[45]. Nonetheless, these polymers exhibit properties relevant to a number of biomedical applications, including drug delivery. [46,47]

Hydrophobic interactions leading to desolvation are at the origin of temperature-driven gelation in many systems, including thermogelling polymers, due to changes in hydrogen bonding interactions triggered by temperature changes. Thermogelling polymers undergo a sol-gel transition as temperature increases; for biomedical applications, physiological temperature is targeted. The transition temperature is termed the lower critical solution temperature (LCST), in contrast to the upper critical solution temperature (UCST), which is observed for systems that transition from gel to solution with an increase in temperature (sol-gel with a decrease in temperature)[48] (Figure 5). LCST behaviour is explained by hydrophobic polymer regions which become desolvated as the temperature increases, resulting in the formation of micelles or hydrophobic microdomains, which, by connecting to each other, trigger gelation.[49] This unique property has been harnessed for a number of biomedical applications, particularly those requiring injectable solutions that undergo gel formation in situ. Polymers with UCST behaviour are less studied for biomedical applications, due to the fact that the high temperatures required for their injection are prone to cause denaturation of peptides or protein cargo[50] and are also less practical to use. Thermogels have provided a platform for localised drug delivery and release,

and for targeted cell differentiation and tissue repair[51][52][53]. Poly(N-isopropylacrylamide) (PNIPAM) is the most well-studied thermogelling polymer that exhibits LCST behaviour, due to its LCST of approximately 32 °C and low cytotoxicity, though various other thermosensitive polymers with LCST are known.

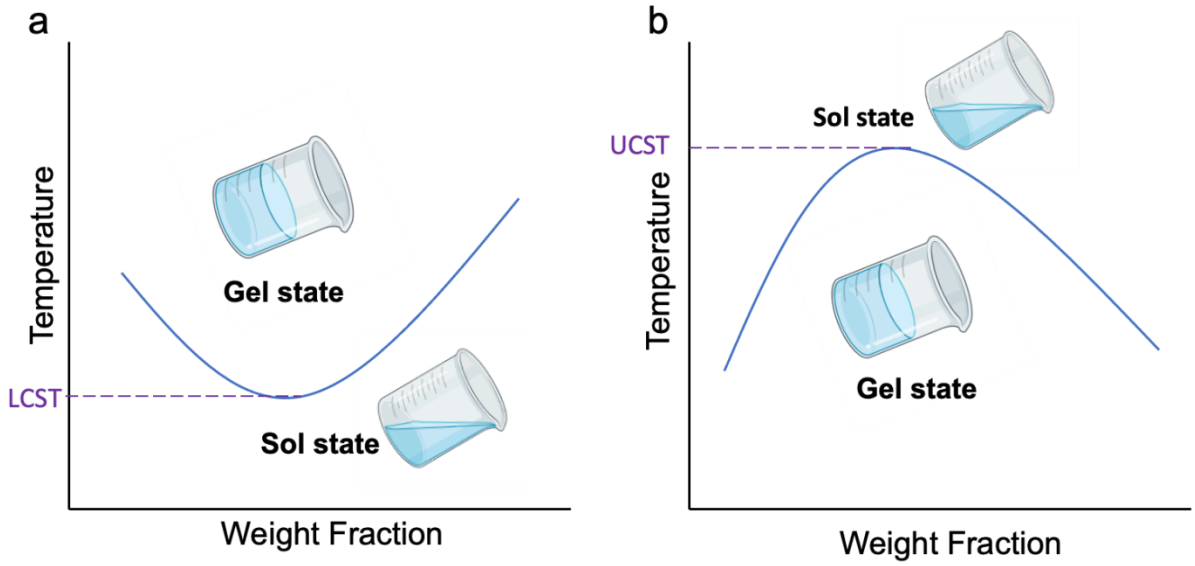


Figure 5: a) Sol-gel transition shown as the temperature increases at the lower critical solution temperature (LCST) b) Sol-gel transition shown as the temperature decreases at the upper critical solution temperature (UCST). Created with BioRender.com

i. Small gelators: bolaamphiphiles

In addition to polymeric gelators, hydrophobic interactions also contribute significantly to the gelation process of a number of small, amphiphilic molecules, which belong to the broad category of low molecular weight gelators (LMWGs).[54] Bolaamphiphiles (molecules with two hydrophilic head groups connected by a hydrophobic linker) can self-assemble to form fibres as a result of intermolecular π-π stacking interactions and hydrophobic associations between adjacent hydrocarbon chains.[55] The physicochemical and biological properties of bolaamphiphile-based gels are compatible with both *in vitro* and in vivo biomedical applications, including the modification of stem cell behaviour, and the sustained release of proteins.[55,56] The π-π stacking interactions which constitute part of the intermolecular attraction between these amphiphiles often involve triazole and nucleobase moieties. [29,57]

The hydrophobicity of bolaamphiphiles may be further tuned via fluorination of the linker region, as demonstrated by Latxague *et al*.[58] The low polarisability of fluorinated alkyl chains reduces the formation of instantaneous dipoles and subsequent attraction to H-alkyl chains. Fluorocarbons are hydrophobic yet lipophobic, whilst they remain able to self-assemble due to London cohesive forces between fluorinated hydrocarbon chains, when more than four CF_2 groups are present. [59]

ii. Small gelators: Peptides

The self-assembly of peptides is also partially controlled by interactions between hydrophobic amino acid residues, such as associative phenylalanine-phenylalanine (F-F) interactions, which are commonly observed in supramolecular peptide hydrogels.[60–62] In addition, enzymes can also be used as a stimulus to trigger the self-(dis)assembly in supramolecular hydrogels, and the inherent biocompatibility of peptide-based gels makes them ideal targets for enzymatic cleavage.[63] Moreover, the mild reaction conditions required for enzymatic reactions, as well as the homogeneity of the resulting hydrogels are additional advantages of peptide hydrogels.[64–66] Thermolysin has been shown to facilitate peptide coupling reactions to cause hydrogel formation via reverse hydrolysis, with the resulting hydrogels displaying an effective antimicrobial response.[67] The major advantage associated with reverse hydrolysis is that water is the only by-product of the reaction.

iii. Nanoparticles

Hydrophobic interactions between polymers and nanoparticles can also be leveraged to create polymer-nanoparticle (PNP) hydrogels or nanocomposite gels.[68] These gels rely on multivalent and dynamic interactions between nanoparticles and polymer chains, which act as non-covalent crosslinks sustaining the supramolecular network. Furthermore, the addition of nanoparticles can provide or enhance targeted in vivo release of drugs loaded into the gel.[69] For example, Wang and co-workers reported a gel comprising platinum nanoparticles embedded within modified polyethylene glycol dendrimers, which was shown to release therapeutic agents during photothermo-sensitive degradation of the gel under near-IR irradiation.[70] The non-invasive nature, and spatially and temporally controlled nature of this method of drug release is particularly advantageous.

Nanoparticles can be broadly assigned as “hard” or “soft” nanoparticles.[71] Soft nanoparticles are those with a compressive modulus similar to that of a natural hydrogel, whilst hard nanoparticles are much more resistant to compression. Hard nanoparticles are synthesised from materials including gold, silica, carbon nanotubes and polymeric nanoparticles (with a high glass transition temperature), whereas soft nanoparticles are primarily based on liposomes, polymeric micelles, dendrimers and nanogels.[72–74] While they generally present a highly ordered structure, intrinsic functionalities (such as localised surface plasmon resonance) and fair *ex* and in vivo stability - which are all desirable from a materials design perspective - hard inorganic nanoparticles can promote adverse inflammatory responses and become toxic when they accumulate in a region of the tissue.[75] As a result, soft nanoparticles which present the inert, insulating properties of organic structures, are more suited to applications requiring biocompatibility.[72]

A variety of nanoparticle types have proven to be effective in the creation of biocompatible and biodegradable PNP systems, including polyethylene glycol, polylactic acid and polylactic-co-glycolic acid.[76,77] PNP hydrogels are highly tunable viscoelastic materials, displaying useful shear-thinning and yield stress responses.[78] This enables facile clinical administration of these materials by spraying or injection directly into the required site, which is followed by the hydrogel regaining robust mechanical properties in the absence of high shear forces. As a result, the hydrogel is able to rapidly form a coating or drug depot after application.[79] Moreover, the diffusion of a cargo from such depots can be significantly retarded by formulating at a high solid weight percentage (up to 12 wt%), which decreases the effective mesh size of the gel network and hinders cargo diffusion.[80]

iv. Double network gels

Simple supramolecular hydrogel systems relying solely on hydrophobic interactions do not usually meet the mechanical strength requirements to perform well in applications requiring high mechanical strength and load-bearing capabilities, such as for bone regeneration. The complexity of biological supramolecular assemblies, such as actin filaments, is as yet unparalleled by synthetic materials, as their natural formation and disassembly is precisely regulated by a vast number of factors, including mechanical stress, biomolecules and pH.[81] This behaviour is essential for the

multifunctionality, autonomy and dynamic structural changes of living cells.[82] Both these mechanical strength and complexity limitations can be remedied via the introduction of another gelator, to form a double network gel. Double network gels with significant strength contributions from hydrophobic interactions have been reported to self-sort and respond orthogonally to separate stimuli, enabling the bidirectionally tunable release of a protein cargo embedded in the hydrogel.[82] A hydrogel with excellent mechanical strength and a hydrophobically associated interpenetrating network was synthesised by micellar copolymerisation of acrylamide and urethane methacrylate dextran.[83] The in situ mineralisation of hydroxyapatite further enhanced the mechanical and osteogenic properties of the hydrogel, affording a viable platform for bone repair and regeneration.

c. Synergistic hydrogen bonding and hydrophobic interactions: Peptides and LMWGs

Establishing the correct balance between hydrophobicity and hydrophilicity is essential to generate amphiphilic gelators that can form robust yet reversible gels. Hydrophobic interactions and hydrogen bonds are often seen to act synergistically to induce molecular self-assembly and are necessarily related, because hydrophobicity is a consequence of (a lack of) hydrogen bonding. The association of non-polar groups in adjacent molecules creates hydrophobic domains which exclude water molecules. This simultaneously reduces the competition for hydrogen bonding sites in the same gelator molecules, enabling maximisation of intermolecular hydrogen bond strength. Peptides and LMWGs are both examples of supramolecular hydrogelators that display this type of synergistic bonding. LMWGs are small molecules capable of self-assembly into sample-spanning nanostructures of very high aspect ratio, such as tapes, sheets, rods and fibrillar structures.[54] Peptides are often classified as LMWGs, and both are discussed here.

Peptides are often selected as building blocks for supramolecular hydrogels due to their inherent biocompatibility and biodegradability, and their ease of synthesis. Solid-phase synthesis techniques afford highly monodisperse peptide products at excellent yields[84,85], and also enable facile attachment of biological moieties to peptide gelators.[86] Depending on the primary structure of a given peptide, the secondary structure of derived self-assemblies may adopt β-sheet, α-helix, or β-hairpin

structures, mediated by hydrogen bonds.[87] These secondary structures subsequently assemble to form nanofibres, once more mediated by intermolecular hydrogen bonding and hydrophobic interactions.[88] The entanglement of the resulting nanofibres creates a 3D network. In some instances, imperfect hydrophobic collapse produces defects in gelator fibres, creating a nucleation point for other fibres which crosslink non-covalently, forming a gel.[88]

Owing to the amino acid blocks from which they are built, peptide-based hydrogels have similar properties to the extracellular matrix, thus allowing the adhesion and proliferation of cells.[89] Hence, supramolecular peptide hydrogels are naturally better suited to biomedical applications, such as tissue engineering and wound treatment, rather than those which require load-bearing capabilities, such as artificial bone or joint construction. However, conjugation or co-assembly with polymers provides a means to address this problem, whilst retaining the properties of a peptide hydrogel. Conjugation strategies require the modification of a peptide's primary structure to include functionalities (e.g. amines, carboxylic acids, thiols) for coupling reactions prior to, or after, synthesis.[90]

There are twenty naturally occurring, polymerisable amino acids found in eukaryotes. Consequently, there are a vast number of possible combinations for forming peptides. For tripeptides alone, 16,000 combinations are possible, including repetitions of symmetric sequences[91]. Moreover, it is possible to cyclise such peptide sequences, and to create branches within the sequence stemming from amino acids with pendant functional groups, increasing the number of potential peptide structures even further.[92] In light of this expansive number of combinations, developing effective strategies for the rational design of potential peptide hydrogelators is essential. To this end, previous studies have used strategies including amino acid substitution,[93] sequence variation[94] and chirality[95] to screen for promising self-assembling peptides.

The formation of anisotropic LMWG architectures relies upon the hierarchical self-assembly of gelator molecules via weak physical molecular interactions including London dispersion forces, hydrogen bonding, charge-transfer and coordinate bonds, dipole-dipole interactions, hydrophobic and solvophobic effects, and π-π stacking. Although each type of interaction plays a role in directing molecular self-assembly,

most are inherently weak in aqueous environments, and as such there exists a consensus regarding the importance of hydrophobic effects during the anisotropic aggregation of hydrogelators.[96,97]

As in the case of peptide gelators, hydrophobic interactions and hydrogen bonds positively reinforce each other to enhance the strength of directional gelator aggregation.[7] These intermolecular physical interactions introduce a preference for the formation of one-dimensional fibres over multi-dimensional crystallisation. Above a critical concentration, the fibres self-assemble through entanglements or physical crosslinks, yielding a sample-spanning network.[98] Fundamentally, gelation is governed by a delicate balance between molecular dissolution and crystallisation or precipitation[99], hence it remains challenging to predict *a priori* whether a given molecule has the capability to act as a hydrogelator.[100] As such, the derivatisation of pre-existing gelators has proven to be a particularly fruitful source of new hydrogelators.[101] The gelation process is also sensitive to processing methods, adding another layer of complexity to the problem of gelator prediction based on first principles.[54] In spite of this, the modification of LMWGs enables the precise tuning of gel properties, as minor molecular alterations or changes in conformation often translate to large physical changes at the macroscale.[102] As a result, LMWG gels have been investigated for applications such as intelligent drug release depots[103] or delivery vehicles[104,105] and molecular sensors.[106]

A large number of gelators exhibit at least one stereogenic centre, and chirality has been observed to play a significant role in gelation.[107] Mixtures of enantiomers are known to self-sort and form separate crystal structures where there is a preference over the formation of a racemic crystal.[107,108] Enantiomerically pure, chiral LMWGs commonly form helical fibres which are less prone to intractable crystallisation (and more stable) than the flat, tape-like aggregates formed by racemic samples. Cells have also been observed to adhere and proliferate differently in the presence of either enantiomer of a LMWG hydrogel.[109] The varying thermal stability of different crystal structures also offers another route to tailor the strength of the supramolecular network, and this is of particular relevance for uses of hydrogels under warmer, physiological conditions, such as tissue engineering.[110]

The majority of LMWGs that feature in biomedical applications are peptide-derived. However, the cost of peptide gels regularly eclipses that of the therapeutic agent with which they are being used.[111] As a result, there is growing interest in sugar- and nucleobase-derived supramolecular hydrogels. Hydrogels based on 1,3:2,4-dibenzylidenesorbitol have been investigated as cell growth supports.[112] and heparin release[113], and nucleobase gels have been observed to promote angiogenesis.[114] The formation of a typical LMWG hydrogel is depicted in Figure 6.

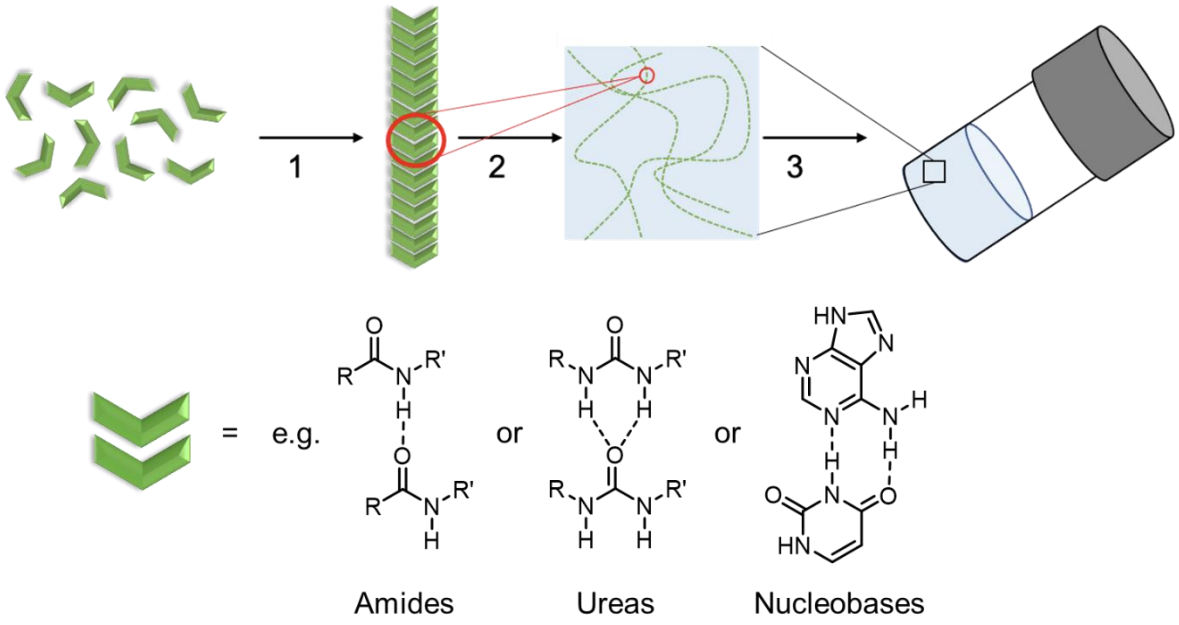


Figure 6: The formation of a supramolecular polymer in water from the self-assembly of LMWGs, creating a gel (shown above right)

d. Crosslinking via ionic interactions and metal coordination

Ionic (Coulombic) interactions between charged species (e.g. protonated amines, carboxylates, phosphates) are typically used to fabricate supramolecular hydrogels because of the rapid kinetics of complexation reactions[4,17,115] and the sensitivity of ionic bonds to a wide range of stimuli, including pH, temperature and the presence of other ions.[116] Ionic interactions are relatively long-range compared to other non-covalent interactions and are not directional, unlike hydrogen bonds. In addition, the strength of ionic interactions (50 – 200 kJ mol^{-1}) greatly exceeds that of other non-covalent interactions such as hydrogen bonds (5 – 65 kJ mol^{-1}).[117] It is generally

understood that the viscoelastic nature of these materials allows temporal changes in gel structure to relieve forces exerted on cells, so that cell processes such as shape changes and proliferation are not hampered, allowing the gel to more accurately simulate biological tissues, or to mechanically signal adherent cells.[118] However, attractive (or repulsive) forces between charged species in ionic crosslinked hydrogels are highly susceptible to screening by other charged moieties in the vicinity, such as the salts present in biological environments, and the dielectric constant of the solvent.[29]

Although electrostatic interactions are non-directional, the addition of metal ions can be used to impart a preference for the geometry of ligand coordination.[119] The recognition of the vital biological importance of metal-ligand interactions has contributed to the growing wealth of research into the development of biomimetic hydrogels. For example, the self-healing and adhesion characteristics of byssal threads (filaments secreted by molluscs for surface attachment) are reliant on the coordination of histidine and catechol to transition metals found in mussel habitats.[120] Within polymeric materials, the thermodynamic stability of metal-ligand complexes usually adheres to the Irving-Williams series, for a given metal ion.[121] Furthermore, the preference for a metal to bind ions of a particular valency can be exploited to alter the mechanical properties of a hydrogel. In the case of polyanionic alginate, complexation with trivalent metal cations has been observed to induce the formation of more physically robust gels compared to divalent metal cation complexation.[115] Alginate-based gels have also been used for the uniform microencapsulation of single cells, which promoted cell differentiation.[122] In this study, calcium carbonate nanoparticles were adsorbed to cells, which enabled calcium-mediated crosslinking of the alginate gel applied later. Hydrogel microcapsules have also been successfully engineered for drug transport and release, mediated by ionic interactions. Lilienthal et al. developed nucleic acid-based polyacrylamide gels with controllable stiffness arising from K^+/18-crown-6 ether quadruplex formation, capable of cofactor-dependent (Mg^{2+}, Zn^{2+}) drug delivery.[123,124]

Despite the tunability and responsiveness of complexation reactions for both ionic and metal coordination interactions, their dynamic nature can also lead to processing difficulties. Challenges frequently arise when attempts are made to directly crosslink

a solution polymer to form a hydrogel, as the strong ionic interactions often cause coacervation rather than the formation of a sample-spanning network[125]. In the same vein, mixing polyionic and polycationic solutions commonly results in inhomogeneous precipitation due to the formation of strong interfacial polyion complexes, which prevents further reaction.[126] Traditionally, this restriction limited the use of these materials to thin films formed by layered reactions.[127,128] This synthetic challenge has recently been addressed by strategies including in situ polymerisation of an anionic monomer in the presence of a pre-formed cationic polymer[115], and the creation of tannic acid-functionalised water soluble polymers (polyvinylpyrrolidone, polyethylene glycol, polystyrenesulfonic acid, polydimethyldiallylammonium chloride) which undergo a sol-gel transition upon the addition of Fe^{3+} ions[129] (Figure 7).

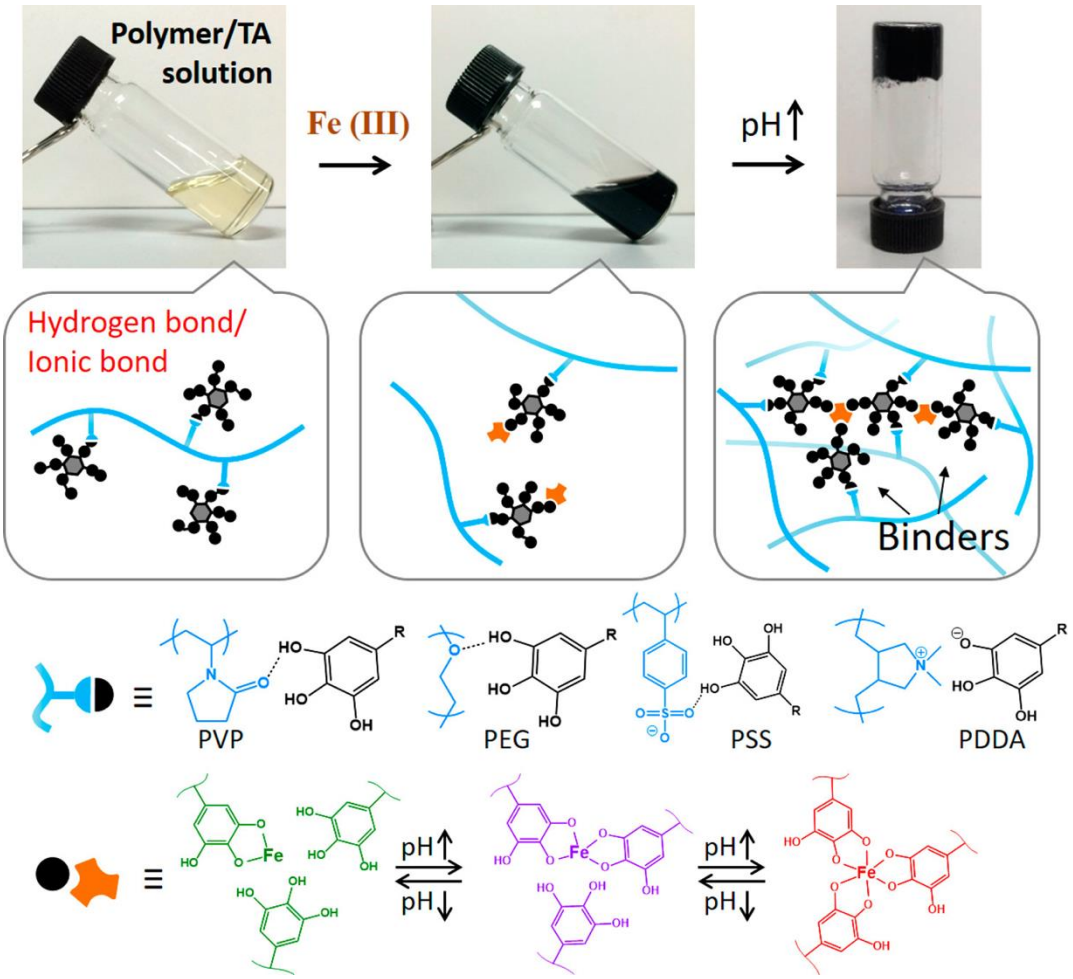


Figure 7: Schematic demonstrating the formation of a pH-responsive supramolecular hydrogel based on (polyvinylpyrrolidone (PVP), poly(ethylene glycol) (PEG), poly(sodium 4-styrenesulfonate) (PSS), and poly(dimethyldiallylammonium chloride) (PDDA) with tannic

acid and Fe^{3+}. Both hydrogen bonding and metal-ligand interactions were present in this system. Reproduced with permission.[129] Copyright 2017, American Chemical Society.

e. Crosslinking for host-guest inclusion complexes

Supramolecular inclusion complexes between 'host' and 'guest' molecules are another type of dynamic and reversible interaction that can be exploited to generate a network, provided the polymer displays the appropriate guest and/or host moieties. In comparison with other non-covalent interactions, such as hydrophobic interactions and electrostatic interactions, the fixed, directional nature of host-guest bonding, and the well-defined stoichiometry of the bonding, enable more reliably structured supramolecular hydrogels to be synthesised.[130] Two of the most commonly used hosts to make physical hydrogels are cyclodextrins (CDs) and cucurbiturils (CBs). Both are families of macrocyclic homologues featuring a hydrophobic cavity between two hydrophilic outer portals.[17,131] In each case, host-guest complex formation is thermodynamically driven by a combination of hydrogen bonding, electrostatic interactions, π-π stacking and the hydrophobic effect.[132] Along with their structural rigidity, the incremental sizes of the homologues in the CD and CB families allows effective encapsulation of guests of a variety of shapes and sizes. The cavity sizes of α-, β- and γ-CD sizes are approximately equivalent to those of CB 6, 7 and 8 respectively, where the integer denotes the number of repeating glycoluril units in the macrocycle (Figure 8).

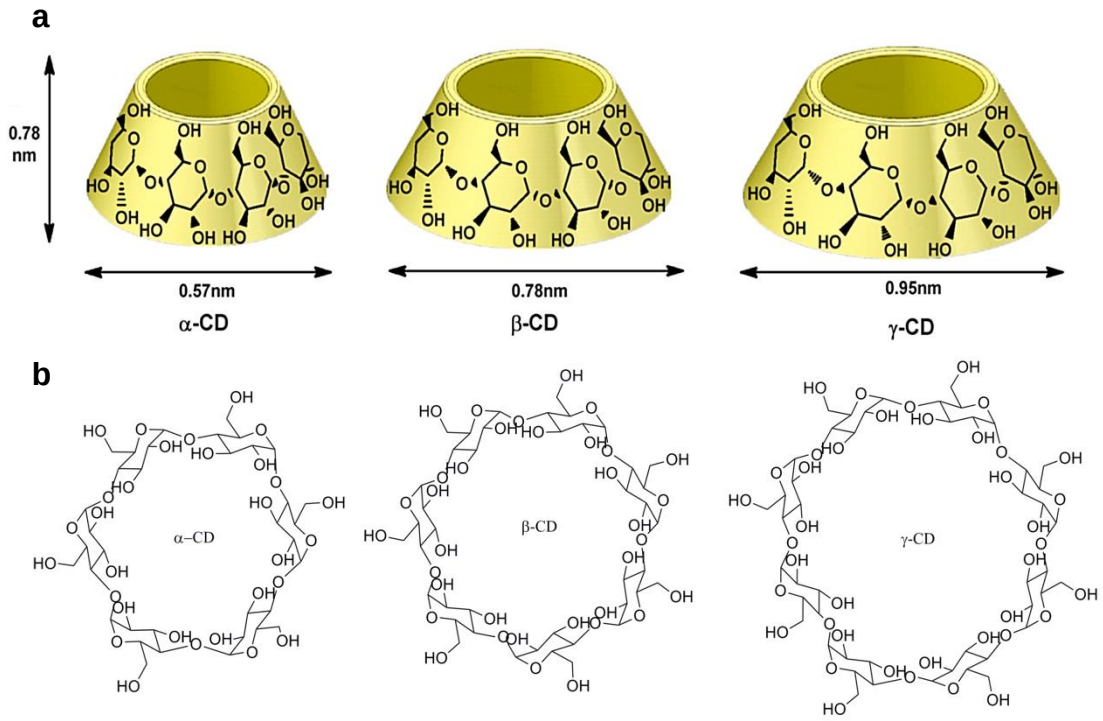


Figure 8: a) Morphological and b) skeletal chemical structures of cyclodextrin α-, β- and γ, having 6, 7 and 8 glucose repeating units respectively. Adapted with permission. [133,134] Copyright 2016, Published by Elsevier B.V and Copyright 2013, Licensee IntechOpen.

Due to the high specificity of molecular recognition by CDs and CBs, changes in guest properties, or environmental changes, can trigger complex formation or dissociation, enabling the creation of stimuli-responsive, or “smart” hydrogels. For example, the *trans* to *cis* isomerisation of azobenzene – a possible guest molecule of β-CD - triggered by UV irradiation induces complex dissociation, which can be reversed by visible light irradiation.[135] The *cis* isomer displays a markedly lower preference for encapsulation by CD, and if these are used as junctions for the supramolecular network, these junctions are broken, and a solution phase is formed. This interaction has been exploited by Burdick et al. to create hyaluronic acid-based hydrogels (Figure 15) with tunable mechanical properties and crosslink density, which were able to modulate the release of a bovine serum albumin-fluorescein conjugate.[136] In addition to light, CD-guest complex formation mediated by pH (e.g. β-CD-benzimidazole[137], β-CD-3-(trimethylsilyl)propionic acid[138]), redox reactions (e.g. CD-ferrocene[139]) or metal ions (β-CD-bipyridine and Fe^{+} or Cu^{2+}) have been studied. A number of studies have also reported supramolecular hydrogels based on the complexation between CDs and cholesterol, an essential cell membrane component.[140,141] Elastic networks based on the interaction between cholesterol-functionalised polyethylene glycol and

β-CD have been synthesised by van de Manakker and coworkers.[142] These hydrogels displayed storage moduli of up to 500 kPa; gels exhibiting storage moduli between 0.1 - 1.0 MPa can be considered 'tough' hydrogels. Gels with excellent self-healing properties and cytocompatibility, formed from poly(l-glutamic acid functionalised with cholesterol and β-CD), have also been reported as potential materials for tissue engineering applications.[143,144] Supramolecular hydrogels based on the formation of CD-polymer complexes, where the CDs thread onto the polymer chain (Figure 9), so-called poly(pseudo)rotaxanes, have also been widely studied.[145]

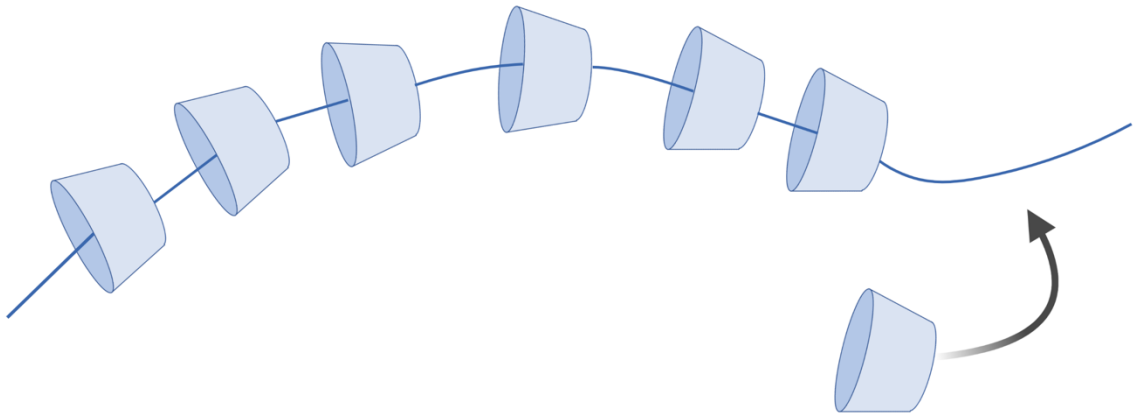


Figure 9: Simple graphical representation showing the cyclodextrins (truncated cones) threading onto the polyethylene glycol (PEG) chain (navy line).

The grafting of CBs to polymer chains presents more of a challenge than with CDs, as the urea-based structure of these macrocycles is prohibitive to functionalisation. On the other hand, the binding constants between CBs and their guests tend to be significantly higher than those obtained with CDs.[146] Furthermore, the electron-rich carbonyl groups that fringe CB portals facilitate the strong binding of cationic guests, or noble metals.[147–149] As a result, the most strongly bound guests for CBs are those comprised of a hydrophilic spacer between two cationic groups. CB6 and CB7 are excellent hosts for aliphatic and aromatic guests, respectively. Somewhat uniquely, CB8 is able to accommodate two guests simultaneously, forming either homo- or heteroternary complexes.[150] The strongest heteroternary binding occurs when the first and second guests are electron-deficient and electron-rich respectively.[151]

The interaction between CB6 and aliphatic diamines has been harnessed to tether various functionalities into polysaccharide gels.[152,153] In addition, the dynamic crosslinking of hyaluronic acid and other polysaccharide hydrogels by ternary CB8 complexes has enabled stimuli-responsive tuning of drug release and rheological properties, and the creation of novel supermolecules, such as supramolecular peptide amphiphile assemblies.[154–159] Highly fatigue- and fracture-resistant materials have also been created by physically crosslinking covalently bound polymers with CB8. This provides a means to reinforce the strong mechanical properties of the polymer and enhance self-healing and energy dissipation characteristics, which are desirable for biomimetic materials designed to replace cartilage or muscle.[160] Moreover, the inherent affinities of CBs towards biomolecules such as amino acids and cellulosic materials, have been employed to synthesise supramolecular hydrogels of a highly viscoelastic nature, which have potential applications in tissue engineering.[37,161,162] Although the applications of CDs and CBs host-guest chemistry to the design of hydrogels are numerous and are being developed for biomedical applications, there are other examples of hosts that can be used for similar purposes, such as pillararenes. [132]

3. Biomedical applications

This section highlights research that has been conducted on supramolecular hydrogels for different biomedical applications in recent years (2018-2021). These studies present novel solutions to address the current limitations of supramolecular hydrogels, by fabricating and optimising hydrogels that exploit the physical interactions detailed in the previous section. Examples include improving the mechanical properties such as robustness and strength, ensuring that gelation occurs at the desired site of action, and enhancing other properties of the physically crosslinked hydrogel that make it suitable for its intended use.

a. Drug delivery

Increasing efforts have been made to develop supramolecular hydrogels for drug delivery applications that can control the release rate of drugs to the targeted site, as well as increase their bioavailability. Drugs are released from supramolecular hydrogels via various routes, which include diffusion or erosion. [17,163] This release is primarily dependent on the ratio between the drug's hydrodynamic diameter and the

hydrogel mesh size.[164] Figure 10a and b presents a diffusion-controlled system, where the drugs are either encased in a reservoir core or throughout the entire hydrogel matrix. In each case, the drug is released in the same way, by travelling from a region of higher to lower concentration in the biological environment (down the concentration gradient). The reservoir system releases the drug at a constant rate over time, whereas the initial drug release in the matrix system releases at a rate proportional to time$^{0.5}$.[165] A swelling-controlled system is presented in Figure 10c. The biological fluid, mainly consisting of water, penetrates the system to swell the hydrogel and subsequently releases the drug. There are several engineered mechanisms for the drug release at the site of action as a result of chemical or physical erosion. Generally, with highly hydrophobic hydrogels, the penetration of water and enzymes is limited, leading to a slower surface erosion. (Figure 10di and dii) Hydrophilic hydrogels are highly susceptible to the hydrolytic and at times enzymatic - degradation, where the bulk of the hydrogel erodes at a steady rate (Figure 10diii). [2,164]

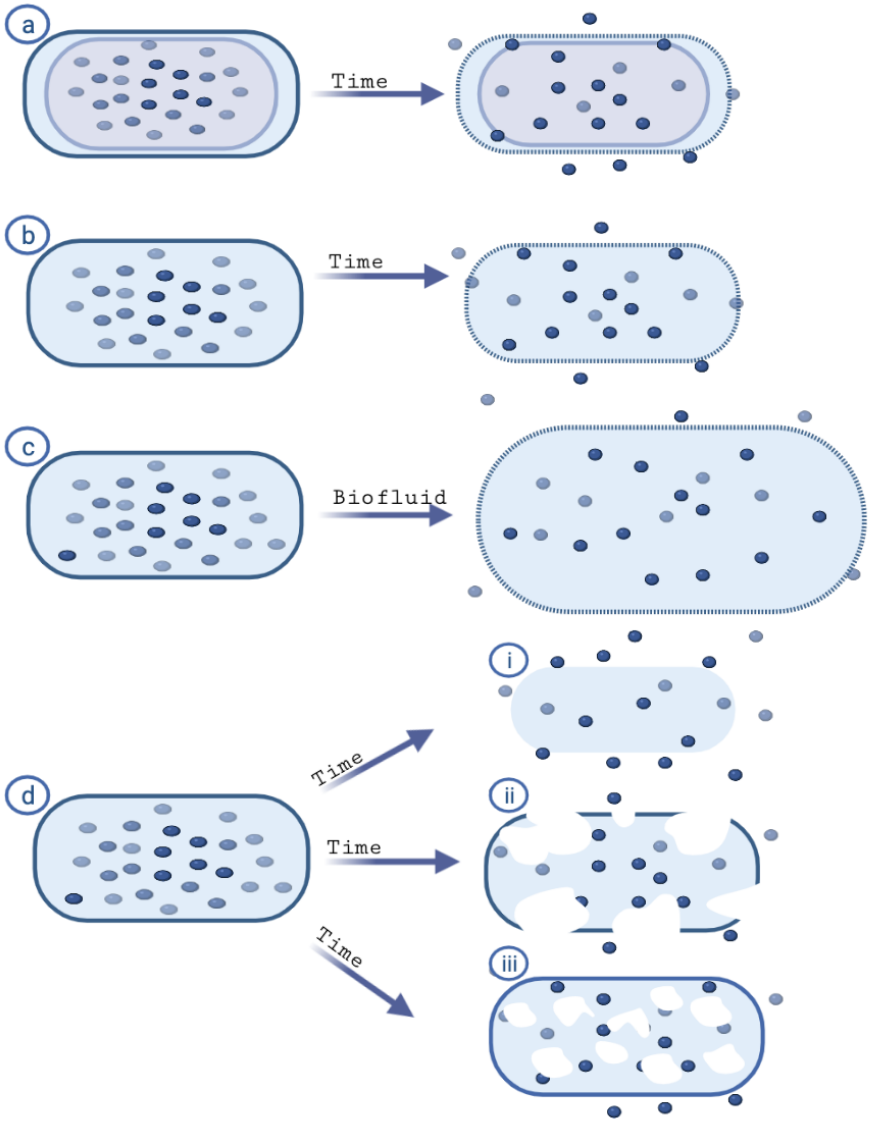


Figure 10: Drug release mechanisms from a supramolecular hydrogel: a) diffusion-controlled reservoir b) diffusion-controlled matrix c) swelling-controlled d) erosion-controlled i) homogenous surface ii) heterogeneous surface iii) bulk erosion. Created with BioRender.com

The unique crosslinking interaction between α-CD and PEG chains (host-guest interactions) has been investigated as a means to alter the viscoelastic behaviour of a hydrogel by forming a so-called 'polyrotaxane'. This occurs as the α-CD threads into the PEG chains as shown in Figure 9 (section 2e), and further interact between them through hydrogen bonds, creating junctions between the threaded polymer chains, and thus either increasing the viscoelasticity of the system or leading to gel formation.[166][167] Poudel and his group utilised this principle to form a supramolecular hydrogel comprising α-CD and PEG-b-PLA (Figure 11).[168] The structural integrity of

this gel relied on both weak associative (hydrophobic) interactions between the PEG-PLA chains of neighbouring micelles and host-guest interactions between PEG chains and α-CD. A dramatic decrease in gelation time was observed as the concentration of α-CD was raised from 7% w/v (92.6 min) to 11% w/v (6.3 min). The hydrogel robustness and viscosity increased concurrently, confirming that the mechanical properties could be tuned with varying concentrations of α-CD. The presence of the host-guest crosslinks reduced the hydrogel degradation rate, leading to a slower release of doxycycline (a hydrophilic, tetracycline compound) by. Additionally, high *in vitro* biocompatibility and cancer cell inhibition efficacy were reported, indicating the future potential of this hydrogel for tumour treatment applications.

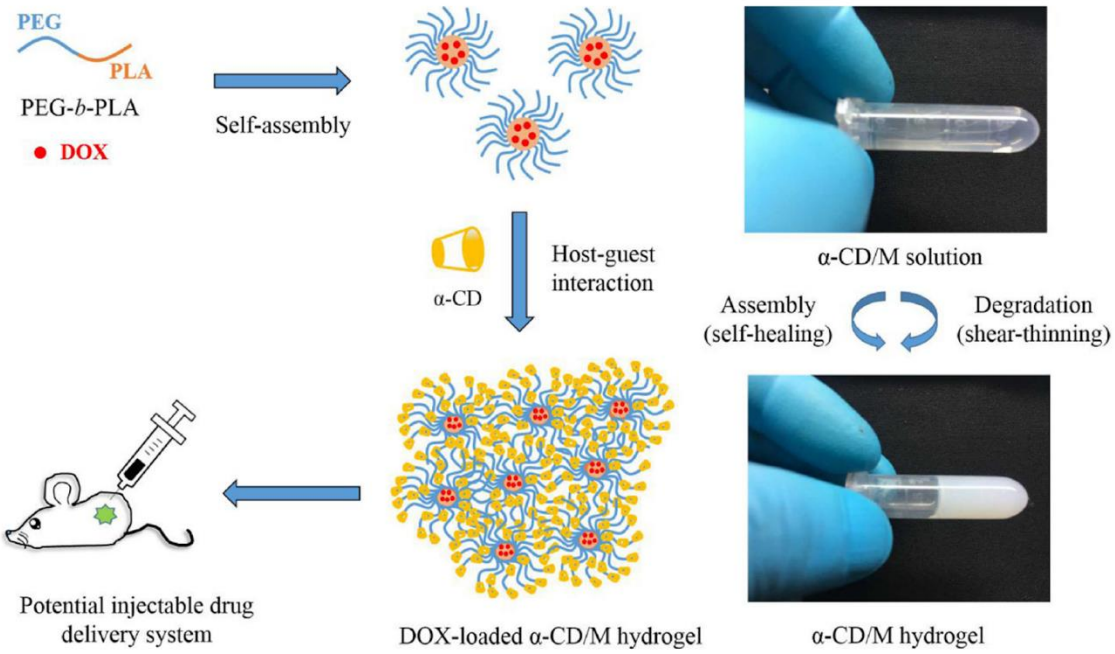


Figure 11: Schematic of the self-assembly of Polyethylene glycol (PEG) and polylactic acid (PLA) polymers into micelles (solution state). PEG-b-PLA micelles (M) form a hydrogel as cyclodextrin (CD) is added (gel state), through host-guest interactions. Doxycycline (DOX) is loaded into the hydrogel for drug delivery applications such as tumour treatment. Reproduced with permission. [168] Copyright 2018, Elsevier Ltd.

α-CD/PEG-b-PLA micelles (α-CD/M) were

This dynamic CD-PEG interaction was explored further by Lorenzo-Veiga et al., who proposed the use of poly(pseudo)rotaxanes to increase the solubility of poorly soluble drugs.[169] Pluronic, a triblock copolymer composed of polyethylene glycol and

polypropylene glycol monomers was combined with Soluplus (polyvinyl caprolactam-polyvinyl acetate-polyethylene glycol), α-CD and natamycin, to treat fungal keratitis, a disease affecting the cornea, which can lead to blindness. This supramolecular hydrogel offered advantageous properties compared to individual components, such as an increase in the elastic (G') and viscous (G'') moduli by an order of magnitude compared to the Pluronic used, P103. The threading of CDs onto the Pluronic chains decreased the hydrophilicity of the polymer, resulting in an increased solubilisation of natamycin into the polymer micelles. Functionalising PEG in this way could be a facile and effective strategy to increase the loading of poorly soluble drugs within PEG-based micellar hydrogels.

Zhang et al. reported a biomaterial consisting of chitosan (CS), hyaluronic acid (HA) and sodium glycerophosphate (GP) that display pH sensitivity and temperature responsiveness.[170] Previous work had focused on the chemical modification of CS to enhance gelation properties and mechanical strength.[171] However, these systems were not able to respond to pH changes under acidic conditions, which is a key determinant for tumour site-specific drug administration. The CS-HA-GP gel studied by Zhang provided 90% doxorubicin release *in vitro* at acidic pH (4.00) (with 1% w/v HA) compared to less than 30% at pH 6.86.[170] The electrostatic repulsion between the protonated amine group (NH_3^+) on the CS chains enables drug release through a diffusion mechanism at low pH, whilst the H-bonds between the carboxyl groups in HA and the amine groups on CS impedes burst release, providing a pH-sensitive system with sustained release behaviour. This hydrogen bonding interaction also enhances the mechanical strength and reduces the gelation temperature without affecting the injectability (Figure 12). Other studies have aimed to harness this dual responsive behaviour for other cancer treatments and have reported promising results for targeted and long-term therapeutic effects.[172,173]

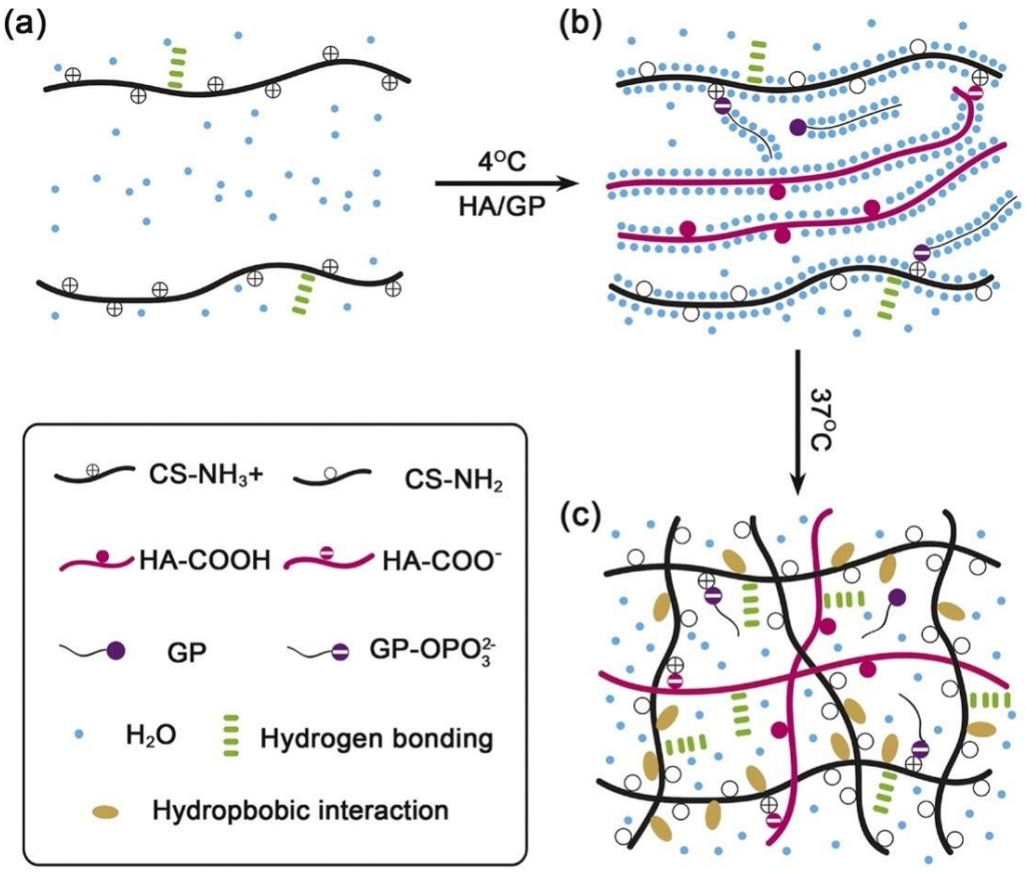


Figure 12: Gelation schematic of chitosan (CS), hyaluronic acid (HA) and sodium glycerophosphate (GP) CS-HA-GP hydrogel. a) electrostatic repulsion on the CS chains (b) addition of HA and GP neutralises the CS chains, reducing the repulsion and bringing the chains closer (c) as the temperature is increased to 37°C, electrostatic interactions between (NH_3^+) and HA/GP induce hydrogen and hydrophobic interactions, forming a hydrogel. Reproduced with permission. [170] Copyright 2018, Elsevier Ltd.

Lee and co-workers have formulated a supramolecular hydrogel which not only enabled controlled drug release at the site of action, but also extended the short half-life of proteins, a common problem encountered when using proteins therapeutically.[174] This was achieved by conjugating human serum albumin (HSA) to urate oxidase (a therapeutic protein that converts insoluble uric acid to soluble allantoin, reducing the level of uric acid in the blood, for the treatments of certain conditions such as gout) and incorporating a poly(ethylene glycol)-poly(β-amino ester urethane)- albumin-binding peptide complex (PEG-PAEU-ABP), shown in Figure 13. Gelation occurred under physiological conditions (37 °C, pH 7.4) predominantly via hydrophobic interactions, yielding a gel with a viscosity of 50 kPa·s. The authors

reported a slightly faster degradation rate in comparison to the control that had no ABP, owing to the hydrophilicity of ABP, which allows for easier penetration of cells and biofluids into the gel matrix. Furthermore, the serum half-life of urate oxidase-HSA was 96.3 hours, an 88-fold increase compared to urate oxidase alone. These results highlight how the interaction between HSA and ABP in this supramolecular hydrogel can drastically influence the release of specific proteins.

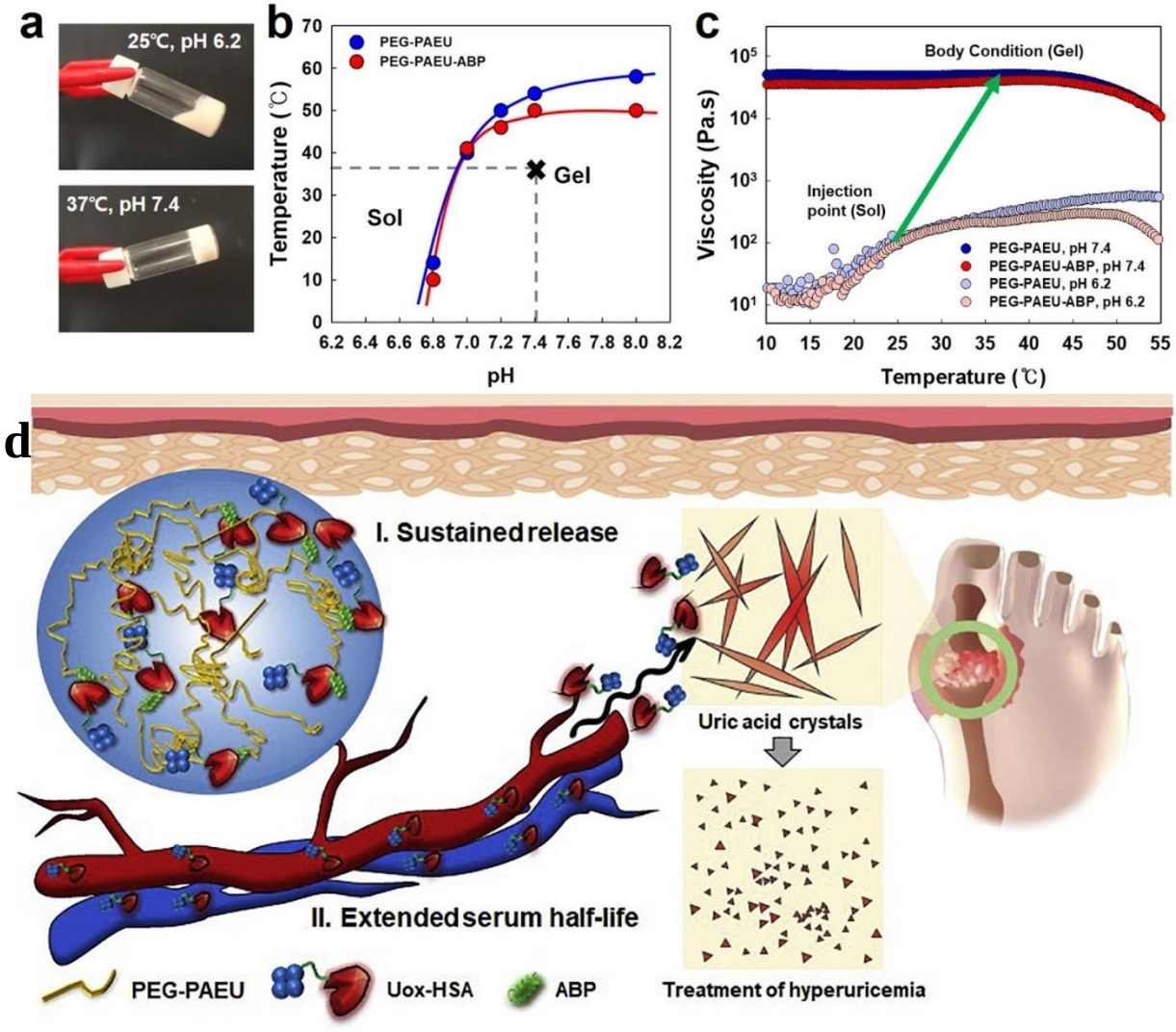


Figure 13: (a) sol-gel transition of the poly(ethylene glycol)-poly(β-amino ester urethane)-albumin-binding peptide (PEG-PAEU-ABP) hydrogel (b) sol-gel phase diagram with and without ABP at 20 wt% as a function of temperature and pH (c) Gel viscosity as a function of temperature with and without ABP at pH 6.2 and 7.4 (d) graphical representation of the formation of PEG-PAEU hydrogels with urate oxidase- human serum albumin (Uox-HSA), which solubilise, thus lower the concentrations of uric acid in the blood, resulting in hyperuricemia treatment. Adapted with permission.[174] Copyright 2020, Elsevier B.V.

b. Tissue engineering

Tissue engineering, also known as 'tissue regeneration', is a branch of medicine which utilises biological materials such as cells and highly porous biomaterial scaffolds to restore, maintain and enhance the function of damaged tissues. [175][17] For the reasons stated in the introduction, supramolecular hydrogels are attractive materials to fabricate tissue constructs or scaffolds to encapsulate drugs and biomolecules, allow cells to infiltrate the site of action and regulate routine cellular functions. [4,176] Recent successes within the field include the restoration of retinal tissue and vitreous humour [177] and the re-establishment of blood flow to the heart following myocardial infarction. [178] Perhaps surprisingly, given their soft nature, these gels have also been shown to promote the growth of hard tissues, including bones and even enamel, the hardest substance in the human body. [179] Supramolecular hydrogels present some key properties that make them suitable candidates for tissue scaffolds[180]: responsiveness (physiological triggers, such as enzymes, can alter the materials properties); tunability (mediation of the strength of interactions between molecular recognition motifs, and of the mechanical strength of the polymer); flexibility (deformation with retention of mechanical properties), biomimicry (preventing the rejection of the material by the host, and activating cell receptors to induce cellular processes) and modularity and specificity (various selective functionalities can be built into the polymer, enabling a multitude of biological activities).

Skin is a multifaceted organ, the largest in the body, and comprises multiple differentiated tissue layers. The development of biomimetic skin-like material has proven challenging due to the skin's versatile sensitivity, mechanical strength to resist external variations, and stretchability. Lei and Wu have attempted to recreate the tissue of the skin using polyelectrolyte copolymers, acrylic acid and 3-dimethyl (methacryloyloxyethyl) ammonium propane sulfonate (PAA-co-DMAPS).[181] A wide array of non-covalent interactions were used, notably hydrogen, ionic and hydrophobic. The polyelectrolyte supramolecular hydrogel was fabricated into a thin skin layer for a prosthetic hand, demonstrating remarkable shape reformation and excellent compressive modulus (27.6 kPa), comparable to natural skin. The ions present in the hydrogel enabled ionic conductivity, which helped mimic multiple stimuli-receptors in the skin, sensing strain and temperature for example. This platform could

carve the way for supramolecular hydrogels to be formulated into biomimetic skin with advantageous properties.

A common premise for research related to supramolecular hydrogels in tissue engineering is to enhance the mechanical stiffness and strength of the biomaterial. Wang et al. have synthesised a series of ultra-stiff supramolecular hydrogels of poly(methacrylamide-co-methacrylic acid) (P(MAAm-co-MAAc)) based on hydrogen bonds for artificial cartilages and other tissue regeneration applications (Figure 14).[182] A dense network was produced, with the movement of the H-bond donor and acceptor groups restricted by encasing them between carboxylic acid and amide groups. Exceptional mechanical properties were exhibited with elastic moduli of 2.3-217.3 MPa, substantially greater than previously reported data (0.01-1 MPa). [183,184] Furthermore, the tensile breaking strain and fracture energy were similarly high, with values of 200—620% and 2.9−23.5 kJ/m^2, respectively.

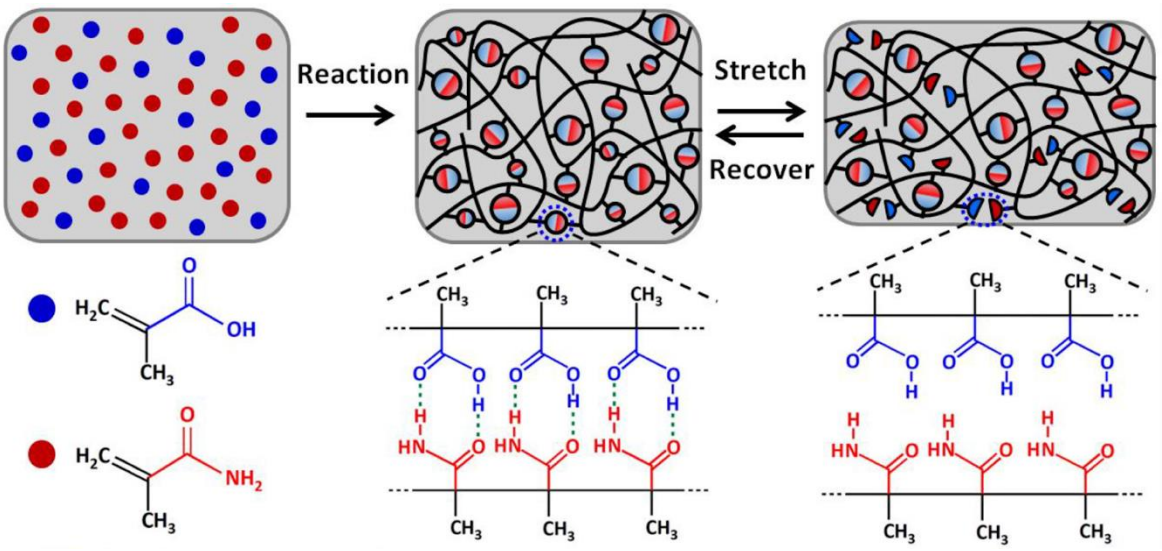


Figure 14: Formation of poly(methacrylamide-co-methacrylic acid) (P(MAAm-co-MAAc)) ultra-stiff hydrogels via hydrogen bonds. Reproduced from [182]. Copyright 2019, American Chemical Society.

Whilst the use of soft scaffolds has been more widely applied to soft tissue, the ability of a supramolecular hydrogel to promote hard tissue growth, such as bone, is a highly sought after property, and has been more extensively researched in recent years. [185]

In 2020, Kim et al. synthesised an alginate-based temperature responsive hydrogel, by modifying alginate with a triblock copolymer of PEG-*b*-poly(ε-caprolactone-*co*-lactide) and poly(ε-caprolactone-*co*-lactide) (PCLA) and *O*-phosphorylethanolamine. [186]The polymer self-assembled into flower-like micelles upon reaching physiological temperature, as the hydrophobicity increased, forming a gel. Additionally, the phosphate functional groups (from calcium phosphate in the simulated body fluid) were found to form a complex with calcium ions, accelerating the growth of hydroxyapatite crystals, resulting in in situ biomineralization. Hydrogel degradation in the murine study (male Sprague Dawley rats) occurred over an 8-week period, as 22.5 wt.% hydrogel solution was injected, allowing sufficient time for new bone to grow, with no signs of haemorrhages or necrosis.

An emerging approach, linking both tissue engineering and drug delivery is known as drug-induced regeneration. Cheng et al used 1,4-dihydrophenonthrolin-4-one-3-carboxylic acid (DPCA) synergistically as a therapeutic agent as well as a structural component of the hydrogel.[187] This was coupled with PEG for the regeneration of soft tissues of the ear, which self-assembled into long nanofiber structures, and further entangled all via hydrophobic interactions. To determine the self-healing and recoverability of the supramolecular hydrogel, a high strain of 200% was applied to break the network, shortly followed by a low strain of 1%, where the recovery was recorded within seconds. Due to the weak hydrophobic interactions, either shear stress or an increase in temperature can lead to a break in the network and give rise to a sol-gel transition. Once eroded, the DPCA is released as a therapeutic agent in a burst-controlled manner and then gradually over a 12-day period, leading to hypoxia inducible factor-1α stabilisation, maintaining oxygen homeostasis and tissue regeneration. This promising drug-induced concept requires further work to be able to regenerate different tissues in the body and broaden the application prospects.

c. Wound healing

Any damage in the skin tissue resulting from direct trauma or certain medical conditions (e.g eczema) is defined as a ‘wound’. Wounds are classified according to the depth of the injury or their severity. [2] In order to support wound recovery, it is essential to consider the biological healing process. Wound healing is a dynamic and highly complex biological process which is regulated by numerous mediators such as

cytokines, epidermal growth factors and mononuclear blood cells.[188,189] Hence, formulating hydrogels for wound dressing is a particularly challenging endeavour.[190] The 'ideal' hydrogel-based wound dressing should be capable of protecting the wound from physical damage and bacterial growth, have efficient gas permeability to accelerate the growth of epithelial cells, absorb exudate from the wound, maintain a moist environment and be simple to apply and remove. [190,191]. Bioactive moieties can also be incorporated to actively support the wound healing process.

Exploiting the use of epidermal growth factors (EGF) to accelerate wound healing has recently been investigated by Zhao et al.[189] First, a photo-responsive supramolecular hydrogel was fabricated by combining β-cyclodextrins (CD) grafted onto hyaluronic acid (HA) chains with azobenzene (Azo) also conjugated to HA chains. Azo was considered for its photoisomerization property, and β-CD to produce a series of host-guest interactions. HA is a common mediator found in wound healing; it is a natural linear anionic polysaccharide composed of d-*N*-acetylglucosamine and d-glucuronic acid, present in the extracellular matrix.[14] These gels could be effectively loaded with EGF (Figure 15a). The hydrogel exhibited a stiff (*G'* ~155Pa) to gel-like (*G'* ~144 Pa) transition when exposed to UV radiation as well as a 2-3-fold increase in EGF release ratio, which could be controlled by switching between visible and UV radiation exposure. These smart hydrogel dressings demonstrated high biocompatibility, with ~90% viability and excellent healing efficiency. The final wound closure was 96% when applied to a $1 \times 1\ cm^2$ full thickness wound on rats, compared to 78% for non-treated wounds, proving the hydrogel as an effective means to deliver EGF to accelerate the healing process.

The use of HA has also been reported by Shi et al.[192] In this study, hyaluronic acid was modified by coupling with bisphosphonate (BP) groups and adding an antibacterial entity, silver ions (Ag+), to form metal-ligand coordination bonds, (Figure 15b) simultaneously improving the mechanical properties of the gel whilst also targeting bacteria at the wound site. These novel supramolecular hydrogels demonstrated an *in vitro* cell viability greater than 90% with an in vivo wound reduction rate from 77.8 ± 3.7% to 48.2 ± 3.7% compared to 68.6 ± 9.4% to 58.5 ± 4.0% in the control (no treatment) group within 3 days.

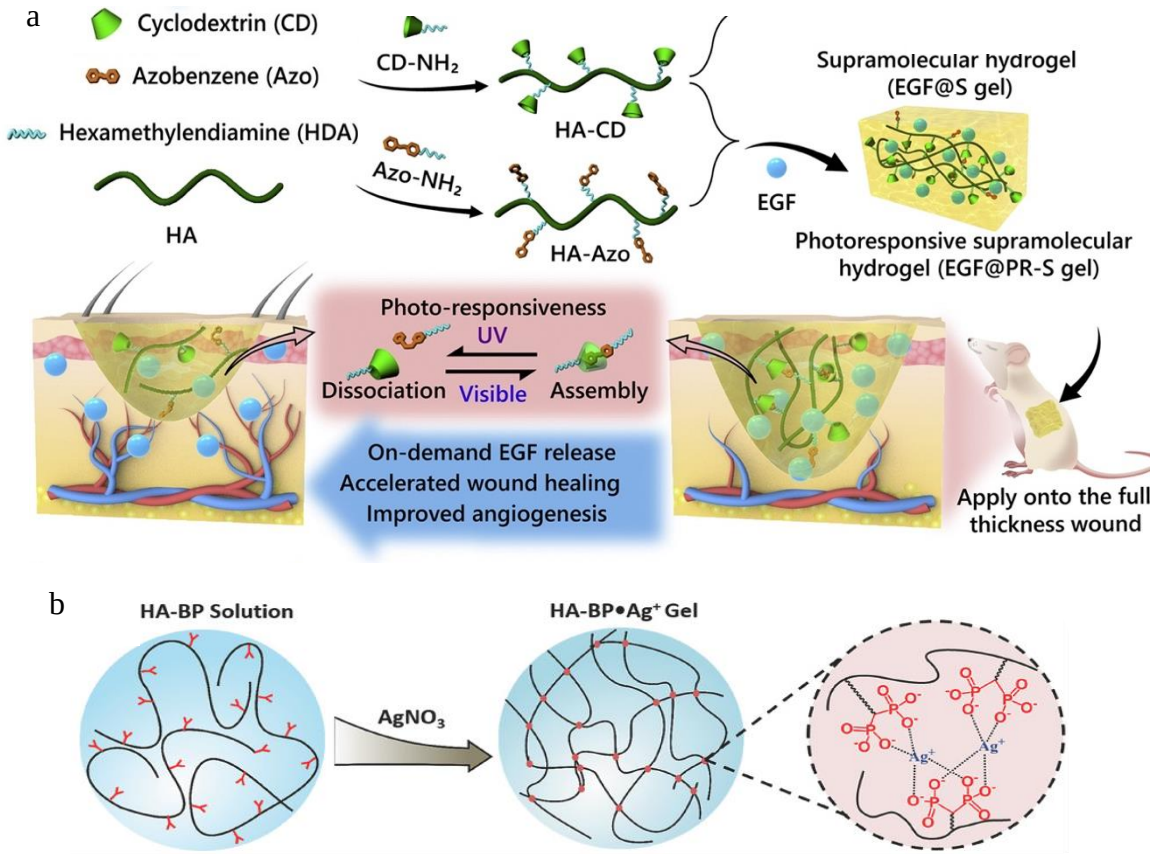


Figure 15: Representative methods used to modify hyaluronic acid (HA) to form supramolecular hydrogels for wound healing: a) formation of a photoresponsive supramolecular hydrogel mediated by host-guest complexes, cyclodextrin (CD) and azobenzene (Azo) conjugated to HA chains. Epidermal growth factors (EGF) are added to the hydrogel and as they are released in the wound, they accelerate wound healing and improve angiogenesis. Adapted with permission. [189] Copyright 2020, Elsevier B.V. b) formation of HA-bisphosphonate (HA-BP) hydrogel mediated by Ag+ metal-ligand coordination. Adapted with permission.[192] Copyright 2017, WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

A recent article published by Amato et al. also reported a modification of hyaluronic acid. By adding a polylysine, novel physically bound nanogels were formed.[193] Nanogels present a greater surface area to volume ratio, and increased potential interactions within in vivo components, in comparison to their bulk counterparts. [194] Berberine was incorporated within the hyaluronic acid-polylysine nanogel for its anti-inflammatory and antioxidant properties, as well as its ability to effectively load into the hydrogel. Within 24 hours of the nanogel particles being introduced into solution, 100% of berberine was released. Relaxation of the polymer chains caused the nanogel particles to swell, promoting diffusion-mediated drug release. Using an *in vitro* wound

healing assay, it was reported that the empty HA-ε-polylysine nanogel reduced the wound gap size compared to both the non-treated fibroblasts and berberine-loaded hydrogels. The wound gap was sealed entirely at 48 hours with the HA-ε-polylysine nanogel, in comparison to 52 hours for both the non-treated fibroblasts and berberine loaded hydrogels. Despite the beneficial therapeutic properties of berberine, its inclusion was observed to slow the healing process and wound gap closure, and thus potential alternatives to berberine are likely to feature in subsequent investigations utilising this effective nanogel platform.

d. 3D printing

A revolutionary technique which has taken the world by a storm is 3D printing, and more recently 4D printing. The superior 4D printing technique has an added benefit that includes the responsivity to external stimuli, allowing the printed structures to change their morphology, property or function.[195] Rapid progress is being made in the field of biomaterials as polymers and gels are being 3D & 4D printed for various biomedical applications, predominantly tissue regeneration. The shear-thinning, self-healing and biocompatible nature of physical hydrogels makes them attractive candidates for extrusion-based printing to create complex replicas of natural tissues.[196] During the 3D printing process, a layer-by-layer hydrogel-based ink is continuously deposited to form a stable structure.[131,197] Crosslinking of the biopolymer chains, forming self-assembled 3D structures, either takes place during the printing procedure or thereafter.[196] Research has largely focused on covalently crosslinked hydrogels due their high mechanical strength. However, as mentioned earlier, these chemical hydrogels are limited by their irreversibility, lack of shear-thinning properties, and toxicity induced from crosslinking agents. While supramolecular gels constitute an attractive alternative, more extensive research is still required in this area. A couple of examples of recent studies are presented below.

Liu and co-workers have developed a complex supramolecular hydrogel suitable for 3D printing.[117] First, hydrogen bonds were linked to the four petrin rings found in folate (a naturally occurring small molecule) to form a tetramer, which layered on top of each other via π- π stacking. Then, zinc ions (Zn^{2+}) were added, resulting in metal-ligand coordination, forming an excellent mechanically strong hydrogel to be injected then printed into various 3D structures (Figure 16). The mechanical strength is

demonstrated with a 10^5 -fold increase in G', as the molar ratio of folate:Zn^{2+} was raised from 1 to 2. The authors also reported a rapid reversible recovery rate of less than 30 seconds after shear was applied. Since both folate and zinc play vital roles in the body, are available naturally and through supplements, the biocompatibility, as expected, was very high with more than 98% cell viability. This hierarchical self-assembled hydrogel using small molecules can act as a gateway to develop other supramolecular hydrogels with multiple physical interactions for 3D printing.

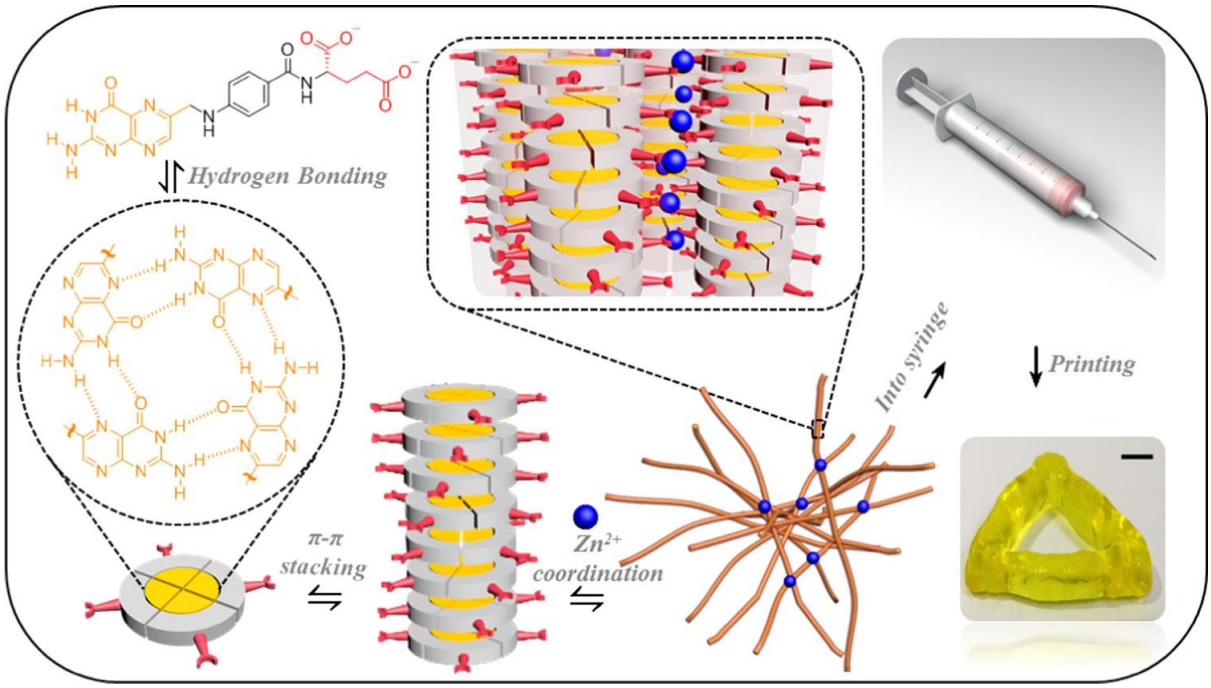


Figure 16: Schematic of the hierarchical self-assembly of folate/Zn^{2+} supramolecular hydrogel for 3D printing employing the use of hydrogen bonding, π- π stacking and metal coordination. Reproduced with permission.[117] Copyright 2018, American Chemical Society.

A recent article by Xu et al. adopted a different strategy as the group used an existing synthetic polymer, poly (N-acryloyl glycinamide) (PNAGA) hydrogel for 3D printing of meniscus scaffolds.[198] Previously, achieving the ideal balance between mechanical strength and printability properties was problematic. Therefore, this work incorporated concentrated NAGA monomers in-between the pre-formed thermoreversible PNAGA network to form a self-strengthening and self-thickening biomaterial for 3D printing ink. This is shown by the impressive compressive strength of 7.17 MPa and modulus of 0.9 MPa. Furthermore, in vivo results proved that the 3D printed hydrogel protected

the cartilage from degeneration and restored the movement of rabbits 4 weeks post-surgery. It also remained intact for the entirety of the experiment.

A new concept which has the potential to be applied to the biomedical field are emulgel-based inks with inherent biocompatibility. These inks are fabricated by the combination of Pickering emulsions and supramolecular hydrogels (based on PEG and α-CD), to afford materials with enhanced rheological properties and structural integrity. [199]

Studies such as those mentioned above can motivate the development and optimisation of supramolecular hydrogels and open new avenues for 3D printing. This can ultimately act as a tool to develop personalised medicine and tissue constructs.

e. Other biomedical applications

Contact lenses are part of a growing industry which help improve patients' quality of life and are becoming more prominently worn to correct vision, with over 175 million users worldwide.[200] During the early years of the 21st century, ophthalmic drug delivery using contact lenses were primarily being explored. [201]More recently there have been notable advancements using hydrogel-based soft contact lenses for sustained drug release[202], which are made via spin casting, lathe cutting or cast molding.[2] However, these are mostly covalently bonded hydrogels.[203] These hydrogels contact lenses were previously limited by their oxygen permeability, which could lead to hypoxia-induced complications,[204] such as limbal redness neovascularization and corneal swelling,[205] which is now being addressed. In addition to high oxygen permeability, soft contact lenses are developed to be transparent, stable with good mechanical properties, comfortable, not irritate the eye and safe for daily use. Furthermore, the drug loading capacity and release has been explored by various techniques, such as controlling the hydrophilic:hydrophobic balance of the polymeric constituents as well as incorporation of colloidal and ligand structures.[2] Future work is necessary to utilise the existing knowledge from covalently bonded hydrogel-based lenses and applying it to non-covalently bound supramolecular hydrogels.

Other biomedical applications which require more extensive research while utilising supramolecular hydrogels, include but are not limited to biosensors and bioanalytical detection techniques. [206]

4. Conclusion and future perspectives

This review provides an overview of the new generation of supramolecular hydrogels, highlighting new design principles and contrasting them with traditional polymer hydrogels. It is clear that the versatile, programmable and functionalisable nature of physical hydrogels offers a multitude of potential pathways in which to improve on the features of conventionally crosslinked gels. As the understanding behind specific gelator-gelator interactions continues to improve, the advent of rational, *a priori* hydrogelator design strategies becomes increasingly important (though it seems this concept is still some way from realisation). In general, much of the existing literature related to supramolecular hydrogels has focused on the use of fewer types of non-covalent interactions to modulate gel properties, presenting many opportunities for current and future research to make the most out of combining a larger number of supramolecular interactions to develop more specific and optimised hydrogels with finely tailored properties. Designing supramolecular hydrogels with excellent rheological properties, such as mechanical strength tailored to specific tissues or adaptative – as tissues regrow -, shear-thinning to enable non-invasive procedures, and self-healing behaviour appears to be the commonly reported goals in recent studies. This is owing to the significant lack of robust supramolecular hydrogels (in comparison to covalently bound hydrogels) which can be tailored to healthcare applications.

Moving forward, we envision the requirement for continued and stronger interdisciplinary collaborations between scientists to create ever higher-performing and more realistic biomimetic materials. Future research in tissue engineering should include additional biocompatibility and in vivo animal studies to monitor the long-term immune response. This, coupled with new advances in stimuli responsiveness (e.g. adapting to multiple biological and mechanical stimuli) will aid in more closely mimicking human tissue and its dynamic nature. In drug delivery, optimisation of drug loading capacity and finite control of hydrogel degradation to release drugs in a programmed manner is necessary. Supramolecular hydrogels for wound healing

should enable the incorporation of multiple growth factors and mediators to expedite the healing process, in addition to expanding the generation of 'smart' hydrogel dressings. Major advances are required for supramolecular hydrogels in 3D and 4D bioprinting and contact lenses, which can reconcile the requirements of mechanical strength and either injectability or reducing rigidity and increasing oxygen permeation, respectively. A promising future direction appears likely to be the application of molecular dynamics (MD) simulations to predict the molecular assembly of newly developed hydrogels, in order to better understand and harness structure-function relationships. Undeniably, the huge increase of research interest in the field is certain to yield rapid advancements to create more viable biomaterials. Taking this into account, several challenges (e.g. large scale and homogeneous production) are still associated with these biomaterials, which should be addressed before reaching clinical approval. Supramolecular hydrogels offer an incredibly versatile platform to engineer functional materials. Hence, with sufficient knowledge and optimisation of specific molecular interactions, researchers are primed to realise their potential as next-generation materials for biomedical applications.

Conflict of interest

The authors declare no conflict of interests.

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