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Recent trends in peptide and protein-based hydrogels

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Hydrogels are classic examples of biomaterials that have found its niche in biomedical and allied fields. Here, we describe examples of peptide-based and protein-based hydrogels with a focus on smart gels that respond to various stimuli including temperature, pH, light, and ionic strength. With the recent advancements in computational modeling, it has been possible to predict as well as design peptide and protein sequences that can assemble into hydrogels with unique and improved properties. We briefly discuss coarse grained and atomistic simulations in designing peptides that can form hydrogels. In addition, we highlight the trends that will influence the future design and applications of hydrogels, with emphasis on bioadhesion, exosomes delivery, tissue and organoids engineering, and even intracellular production of gels.

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Introduction

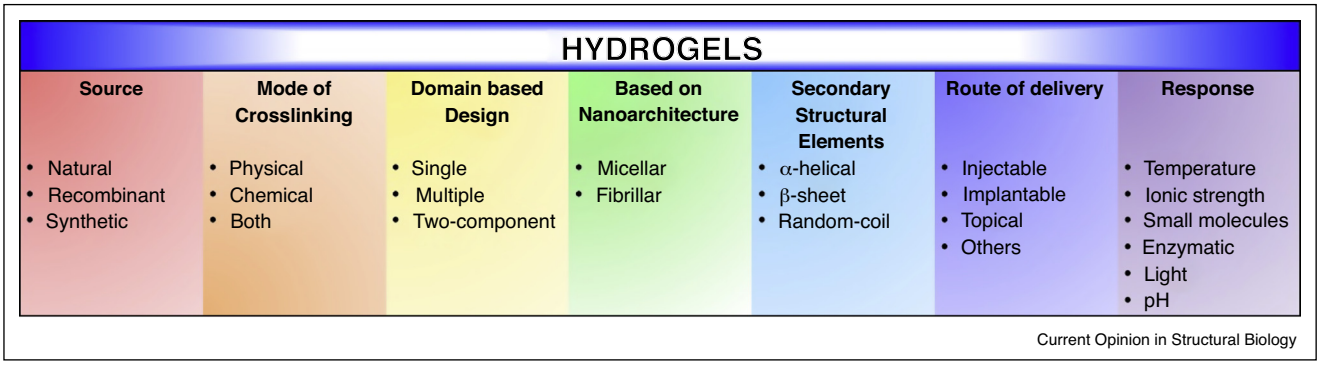
Hydrogels are attractive biomaterials comprised of three-dimensional (3D) networks of polymeric chains [1]. The 3D network, attributed to its porous microstructure, is formed either by physical or chemical crosslinking of synthetic, natural or hybrid polymers [1]. Although synthetic polymers exhibit superior mechanical properties, there is an emerging paradigm shift toward developing peptide-based and protein-based hydrogels, owing to their biocompatibility and tunable properties [1]. Peptide-based and protein-based hydrogels are a promising class of biomaterials as they can respond to external cues such as temperature, pH, as well as light and small molecules [2]. There are several ways to classify peptide-based and protein-based hydrogels (Scheme 1). Wang *et al.* have recently reviewed domain-based engineered hydrogels comprised of a single domain, multi-domain or two component hydrogel systems [3]. Other examples include physically and chemically crosslinked hydrogels and those bearing non-canonical amino acids and metal ions [3]. Hydrogels can also be classified based on the source of protein, secondary structural elements, methods of assembly, and routes of delivery [1]. Self-assembly of proteins into fibers and micelles also dictate the classification of hydrogels into fibrous or micellar based hydrogels, respectively [4•,5].

Of the various hydrogels reported in literature, stimuli-responsive hydrogels have a distinct advantage as they can precisely control assembly and disassembly as a function of an external trigger [3]. In the following section, we highlight some of the recent examples of stimuli-responsive hydrogels. The examples are chosen to represent the structural diversity present in peptide-based and protein-based hydrogels. In an effort to develop hydrogels with better mechanical and functional properties, *de novo* protein design has gained considerable interest. Significant advances in computational methods and synthetic biology have identified protein sequences that can mimic or even surpass the properties of natural proteins [6]. In this minireview, we present a brief overview of computational approaches used in the design of hydrogel forming peptides. Due to their versatility and modular nature, peptide-based and protein-based hydrogels have found wide-ranging applications in biomedical fields including tissue engineering, drug and gene delivery, as well as other fields, paving the way for emerging applications [2]. Here, we present some of the elegant applications of hydrogels and its future prospects.

Stimuli-responsive hydrogels

Peptide-based and protein-based hydrogels that undergo sol-gel transition in response to external stimuli such as temperature, pH, and ionic strength are referred as stimuli-responsive or 'smart' hydrogels [7]. A great deal of effort has been devoted to developing thermoresponsive hydrogels as temperature can dynamically modulate protein properties, enabling hydrogels with spatiotemporal control [1]. A majority of thermoresponsive hydrogels are comprised of protein polymers that show sol-gel transition at a critical temperature. Hydrogels exhibiting upper critical solution

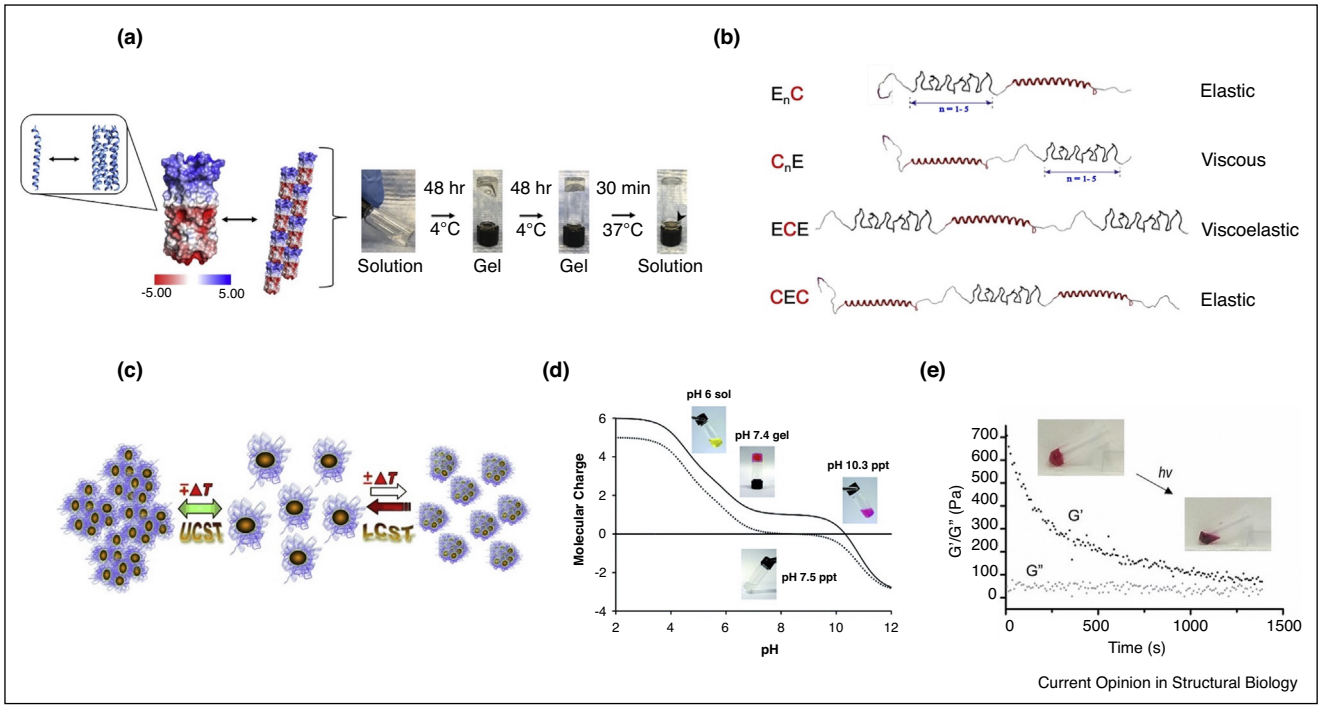
Scheme 1



Classification of peptide-based and protein-based hydrogels.

temperature (UCST)-behavior are miscible at higher temperatures and solidify when cooled below their UCST [7]. Recently, Hill *et al.* have reported a coiled-coil protein hydrogel based on a rationally designed Q protein (Figure 1a, Table 1) that exhibits UCST-type behavior [4•]. Q is an engineered variant of the coiled-coil domain of cartilage oligomeric matrix protein (COMPcc) that self-assembles into fibers [8]. These fibers can physically cross-link and form a hydrogel at low temperatures. When the gel is bound with small hydrophobic molecules, it is stable at physiological temperature for 17–18 days, imparting utility for drug delivery applications [4•].

Figure 1



Stimuli-responsive hydrogels. **(a)** Schematic representation of hierarchical assembly of Q protein hydrogel that assembles at 4°C and pH 8.0. Reproduced with permission [4•]. **(b)** Structure of diblock (EC and CE) and triblock polymers (ECE and CEC) with their characteristic mechanical properties. Reproduced with permission [22]. **(c)** Schematic representation of Rec-1 with both LCST and UCST behavior. Reproduced with permission [14]. **(d)** Gelation of AFD36 and AFD19 as a function of pH and molecular charge. Inset: photographs of sol, gel, and aggregate states for AFD36 (top) and aggregate state for AFD19 (bottom). Reproduced with permission [18]. **(e)** Gel-sol transition in $CarH_C$ hydrogels induced by light. The G' and G'' of the $CarH_C$ hydrogel were monitored at a fixed shear rate frequency of 1 rad/s and strain of 5%. Reproduced with permission [20•].

Table 1

Amino acid sequences of peptides and proteins that assemble into hydrogels

Nomenclature	Sequence	Ref.
Q	MRGSHHHHHHGSIEGRVKEITFLKNTAPQMLRELQETNAALQDVREL	[4•]
MAX3	VKVKVK T KV^{D}PPTKVK T KVKV	[10]
MAX1	VKVKVK V KV^{D}PPTKVK V KVKV	[10]
Ac-VES3-RGDV	Ac-VEVSVSVEV^{D}PPTEVSVEVEVGGGGRGDV	[11]
EC	$MRGSH_6$GSKPIAASA-$\mathbf{E_5}$-LEGSELA$(AT)_6$AACG-**C**-LQA$(AT)_6$AVDLQPS	[12]
CE	$MRGSH_6$GSACELA$(AT)_6$AACG-**C**-LQA$(AT)_6$AVDKPIAASA-$\mathbf{E_5}$-LEGSGTGAKL	[12]
ECE	$MRGSH_6$GSKPIAASA-E_5-LEGSELA$(AT)_6$AACG-**C**-LQA$(AT)_6$AVDKPIAASA-$\mathbf{E_5}$-LEGSGTGAKLN	[12]
CEC	$MRGSH_6$GSACELA$(AT)_6$AAC-**C**-LQA$(AT)_6$AVDKPIAASA-$\mathbf{E_5}$-LEGSGT-**C**-LQALSI	[5]
E_5	$[(VPGVG)_2VPGFG(VPGVG)_2]_5VP$	[12]
C	GDLAPQMLRELQETNAALQDVRELLRQQVKEITFLKNTVMESDASG	[12]
ELP1-H1-25%	$[(VPGVG)_{15}G(A)_{25}]_4$	[13••]
ELP1-H2-25%	$[(VPGVG)_{15}GK(A)_{25}K]_4$	[13••]
ELP1-H3-25%	$[(VPGVG)_{15}GK(AAAA)_5K]_4$	[13••]
ELP1-H5-25%	$[(VPGVG)_{15}GD(A)_{25}K]_4$	[13••]
ELP1-H5-12.5%	$[(VPGVG)_{35}GD(A)_{25}K]_2$	[13••]
Rec-1 resilin	MHHHHHHPEP PVNSYLP PSDSYGAPGQSGP GGRPSDSYGAPGGGN GGRPSDSYGAPGQGQGQGQGQGGYAGK PSDSYGAPGGGNGN GGRPSSSYGAPGGGN GGRPSDTYGAPGGGN GGRPSDTYGAPGGGGNGN GGRPSSSYGAPGQGQGNGN GGRPSSSYGAPGSGN GGRPSDTYGAPGGGN GGRPSDTYGAPGGGNN GGRPSSSYGAPGGGN GGRPSDTYGAPGGGNGNGS GGRPSSSYGAPGQGQGGF GGRPSDSYGAPGQNQK PSDSYGAPGSGNGN GGRPSSSYGAPGSGP GGRPSDSYGPPASG	[14]
RZ10-RGD	M-MASMTGGQQMG-HHHHHH-DDDDK-LDHMRTLS -(AQTPSSKQFGAPAQTPSSQFGAP)-KWADRHGGMR-GGTVYAVTGRGDSPASSGGG-LE	[16•]
AFD19	Ac-LKELAKV LHELAKL VSEALHA-$CONH_2$	[17]
AFD36	Ac-LKELAKV LHELAKL VKEALHA-$CONH_2$	[18]
PEP-1	FALNLAKD	[19•]
SpyCatcher-ELP-$CarH_C$-ELP-SpyCatcher	MKGSSHHHHHHVDIPTTENLYFQGAMVDTLSGLSSEQGQSGDM TIEEDSATHIKFSKRDEDGKELAGATMELRDSSGKTISTWISDGQVKDFYL YPGKYTFVETAAPDGYEVATAITFTVNEQGQVTVNGKATKGDAHIDGPQ GIWGQLEGHGVGVPGVGVPGVGVPGEGVPGVGVPGVGVPGVGVP GVGVPGEGVPGVGVPGVGVPGVGVPGVGVPGEGVPGVGVPGVGEL PEDLGTGLLEALLRGDLAGAEALFRRGLRFWGPEGVLEHLLLPVLREVGEAW HRGEIGVAEEHLASTFLRARLQELLDLAGFPPGPPVLVTTPPGERHEIGAM LAAYHLRRKGVPALYLGPDTPLPDLRALARRLGAGAVVLSAVLSEPLRALP DGALKDLAPRVFLGGQGAGPEEARRLGAEYMEDLKGLAEALWLPRGPEKE AITSVPGVGVPGVGVPGEGVPGVGVPGVGVPGVGVPGVGVPGEGVPGVG VPGVGVPGVGVPGVGVPGEGVPGVGVPGVGVPGGLVDIPTTENLYFQGA MVDTLSGLSSEQGQSGDMTIEEDSATHIKFSKRDEDGKELAGATMELRDSS GKTISTWISDGQVKDFYLYPGKYTFVETAAPDGYEVATAITFTVNEQGQVT VNGKATKGDAHIDGPQGIWGQLEWKK	[20•]
SpyTag-ELP-$CarH_C$-ELP-SpyTag	MKGSSHHHHHHVDAHIVMVDAYKPTKLDGHGVGVPGVGVPGVGVPGEG VPGVGVPGVGVPGVGVPGVGVPGVGVPGEGVPGVGVPGVGVPGVGVPGVGVP GEGVPGVGVPGVGELPEDLGTGLLEALLRGDLAGAEALFRRGLRFWGPEGV LEHLLLPVLREVGEAWHRGEIGVAEEHLASTFLRARLQELLDLAGFPPG PPVLVTTPPGERHEIGAMLAAYHLRRKGVPALYLGPDTPLPDLRALARRL GAGAVVLSAVLSEPLRALPDGALKDLAPRVFLGGQGAGPEEARRLGAE YMEDLKGLAEALWLPRGPEKEAITSVPGVGVPGVGVPGEGVPGVGVPG VGVPGVGVPGVGVPGEGVPGVGVPGVGVPGVGVPGVGVPGEG VPGVGVPGVGVPGGLLDAHIVMVDAYKPTKLE WKK	[20•]
Peptide-1 (Troponin C)	H-VEQLTEEQKNEFKAAFDIFVLGA	[21]
Tripeptides	KYF KYY KFF KYW	[25]
LNK1	(Nal)K(Nal)KAKAK-V^{D}PPT-KAKAK(Nal)K(Nal)	[28]
ELPs	$(GVGVP)_3$ $(GVGVP)_6$ (GVGVP)(GKGVP)(GVGVP)	[30•]
SELP	$[(GVGVP)_4(GYGVP)(GVGVP)_3(GAGAGS)]_{14}$	[31]

Table 1 (*Continued*)

Nomenclature	Sequence	Ref.
$(TM3)_3$-ELP_2	H_6-TM-RGD-ELP-TM-RGD-ELP-TM-RGD (TM3 (GRKYY), TM5-1 (GYKKYY) or TM5-2 (KKYYYKY) ELP = (VPGXG)n; *n* = 15, X = V/E at 4:1 ratio	[39]
$(ELY)_{16}$	M-MASMTGGQQMG-HHHHHH-DDDDK-LDGTL-(PGYGVPGKGVPGVGV)16-PVADRGMRLE	[33••]
ELP (patterned)	MASMTGGQQMG-HHHHHH-DDDDK-TVYAVTGRGDSPASSAA-$[(VPGIG)_2VPGKG(VPGIG)_2]_3VP$	[45]
Spider silk protein eADF4-(C16)	$(GSSAAAAAAAAASGPGGYG\ PENQGPSGPGGYGPGGP)_{16}$	[<span class="xps_reflinkin

Peptide-based and protein-based hydrogels can also exhibit lower critical solution temperature (LCST)-behavior. Such hydrogels are miscible at low temperatures and undergo gelation above their LCST [9]. The LCST-type hydrogels are often designed to self-assemble by varying the hydrophilic and hydrophobic content of the protein. For example, the MAX3 peptide (Table 1) comprised of alternating hydrophilic and hydrophobic residues, folds to form a ß-hairpin structure that self-assembles to form a hydrogel network [10]. MAX3 hydrogel exhibits an LCST at 60°C. The transition temperature of MAX3 can be tuned by varying the hydrophobic residues. Replacement of threonine with valine results in MAX1 peptide (Table 1), which exhibits a LCST of 25°C [10]. Within the past two decades, Schneider and Pochan groups have developed a series of MAX peptides that have potential applications in encapsulating and delivering small molecules, growth factors, DNA and cells [11]. More recently, Schneider and colleagues *et al.* have created anionic ß-hairpin peptides (Table 1) that form hydrogels under physiological conditions [11]. These hydrogels offer new opportunities in cell engraftment, especially in CAR-T cell immunotherapy.

Another common strategy to generate LCST hydrogels is to combine blocks of similar or different self-assembling domains within a polymeric chain. Hydrogels based on elastin-like polypeptides (ELP) classically exhibit LCST behavior. These hydrogels have been extensively reviewed by several research groups [1,3]. Montclare and colleagues *et al.* have engineered a series of constructs combining short ELPs (E) with the coiled-coil domain of cartilage oligomeric matrix protein (C) [5,12]. The diblock and triblock polymers, EC and CEC (Table 1), exhibit elastic behavior while CE exists as a viscous solution (Figure 1b, Table 1) [5,12]. Interestingly, the triblock polymer, ECE displays properties that are in between EC and CE polymers (Table 1), exhibiting a viscoelastic behavior [12]. The elastic properties of CEC hydrogels are further modulated upon binding to curcumin, a small hydrophobic chemotherapeutic agent that confers structural stability to C domain [5]. Their studies demonstrate that length and order of the blocks can modulate self-assembly and mechanical properties. Analogous to EC, Chilkoti and coworkers have reported partially ordered polymers (POP) composed of ELP with polyalanine helices (Table 1) [13••]. These peptides are designed to exhibit LCST at physiologically relevant temperatures. The POP self-assemble to form porous, elastic networks with ELP1-H5 (Table 1) capable of creating stable depots, promoting vascularization and wound healing, when injected *in vivo*. These peptide-based hydrogels provide an attractive design strategy for producing injectable systems for regenerative medicine [13••].

Rec-1, a resilin-mimetic polymer enriched with repeats of (GGRPSDSYGAPGGGN), a motif based on the first exon of the *Drosophila* CG15920 gene, is one of the few examples that exhibit both UCST and LCST-type behaviors (Figure 1c, Table 1) [14]. Below 6°C and above 70°C, the UCST and LCST respectively, the protein forms a turbid hydrogel, whereas between these temperatures, Rec-1 exists as a transparent solution. Studies have focused on tuning the critical temperatures of resilin to more physiologically relevant temperatures by either combining it with other proteins or by utilizing resilin-like polymers derived from other species [15,16•]. Apart from thermoresponsiveness, resilin hydrogels have also been evaluated for their ability to assemble under different stimuli. Recently, Su *et al.* have developed redox-responsive hydrogels based on a resilin-like protein derived from *Anopheles gambiae* [16•]. The engineered protein, RZ10-RGD (Table 1), is crosslinked using a redox-responsive crosslinker, 3,3′-dithiobis(sulfosuccinimidyl propionate) (DTSSP) that rapidly degrades under reducing conditions. These gels show different drug release profiles under reducing and non-reducing environments, offering applications in targeted drug delivery and tissue engineering [16•].

pH-sensitive hydrogels are another widely studied system. Fletcher *et al.* have designed an α-helical peptide, AFD19 (Table 1) that is capable of forming a pH-sensitive coiled-coil fibril-based hydrogel [17]. These hydrogels are composed of fibers that self-assemble at pH 6. A single point variation of AFD19 results in AFD36 that gels at physiological salt and pH conditions (Figure 1d, Table 1) [18]. AFD36 hydrogels can support

fibroblast growth and can be employed as drug and cell carriers [18]. pH responsive hydrogels are, in particular, suited for tumor-targeted drug delivery applications as tumor tissues typically have lower pH relative to normal healthy tissues. Ghosh *et al.* have monitored the release of a water-soluble drug, calcein under two different pH conditions, using a hydrogel composed of PEP-1 peptide (Table 1), a variant of a natural ß-sheet forming galectin-1 protein [19•]. PEP-1 forms a stable hydrogel at pH 7.4 that disassembles at pH 5.5. pH triggered gel-to-sol transition enables faster release of encapsulated drug at pH 5.5 with minimal release observed at physiological pH [19•].

Other examples of stimuli-responsive protein-based hydrogels include light sensitive hydrogels comprised of ELP fused to adenosylcobalamin ($AdoB_{12}$)-dependent photoreceptor C-terminal adenosylcobalamin binding domain ($CarH_C$) [20•]. This domain undergoes oligomerization in dark and forms hydrogels (Figure 1e, Table 1) mediated by SpyTag-SpyCatcher chemistry. These hydrogels are capable of light-induced release and recovery of encapsulated cells and globular proteins [20•]. Another interesting example of stimuli-responsive hydrogel is reported by De-Leon Rodriguez *et al.*, who identified a fragment of human cardiac troponin C that exists as an α-helix in its native state but adopts a ß-sheet conformation when isolated from its parent sequence [21]. The fragment, referred as peptide-1 (Table 1), self-assembles to form ß-sheet fibrils. These fibrils undergo physical crosslinking to form hydrogels as a function of pH and ionic strength [21]. To further improve the properties of these hydrogels, efforts are being made to design protein polymers that respond to multiple stimuli. Such multi-responsive hydrogels are well suited for drug delivery applications and offer great promise in a variety of biomedical fields.

Computational driven design of hydrogel forming peptides and proteins

Employing computational methods to design hydrogel forming peptides and proteins through simulations has attracted considerable attention in recent years. Computer simulations can be divided into two major types of coarse grained (CG) [23] and all-atom [24] models. Coarse grained models treat a group of atoms as a single 'bead' in order to reduce the complexity of the system [23]. In simulations involving a large number of biomolecules, for example, peptide and protein self-assembly and hydrogel formation, these simplified representations have the advantage of higher computational efficiency compared to atomistic models. In contrast, atomistic simulations represent each atom in the system individually and provide more accurate explanations of how small variations in the sequence affect the self-assembly and resulting structure of peptides or proteins.

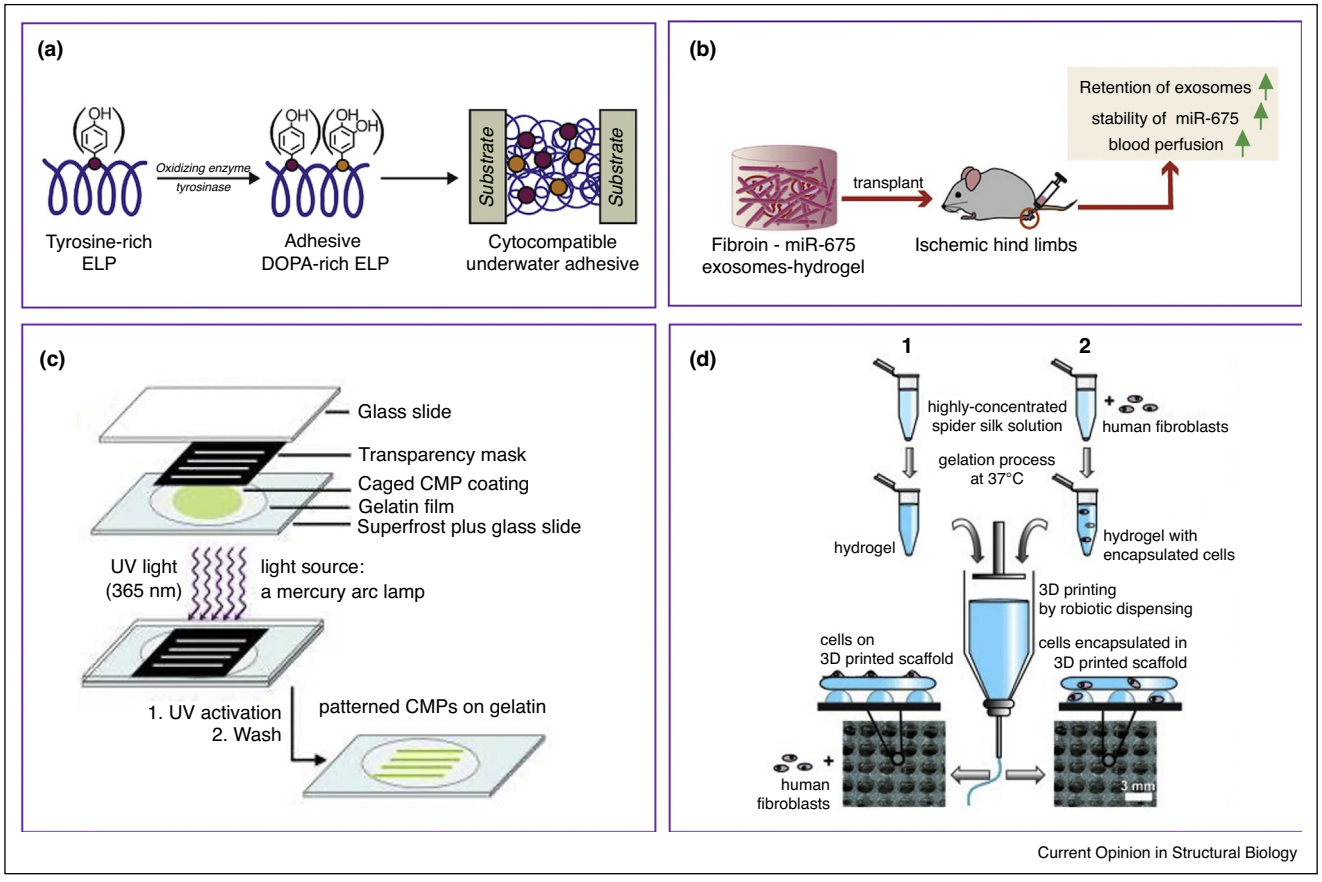
Many computational studies on hydrogels focus on the design of sequences capable of forming gels. Using CG models, Tuttle and colleagues *et al.* have performed a systematic search of all the possible combinations of dipeptide and tripeptide sequences and discovered four new tripeptides (KYF, KYY, KFF and KYW) (Table 1) that form hydrogels [25,26]. Another study by Moreira *et al.* has employed Martini force field to design systems that form hydrogels by co-assembly of di-peptides and tri-peptides. CG simulations reveal that aromatic amino acids promote co-assembly of tripeptide-dipeptides [27••].

Sathaye *et al.* have performed all-atom molecular dynamics simulations to elucidate the effects of non-canonical amino acids on assembly and gelation properties of MAX1 fibrils [28]. LNK1 (Table 1) is obtained from MAX1 peptide by substituting the non-turn valines by 2-naphthylalanine (Nal) and alanine residues. The simulations show that Nal and alanine sidechains form a 'lock and key' hydrophobic core packing, which assists in stabilization of the self-assembled LNK1 structure. Higher stability of LNK1 restricts the fibril from branching, resulting in weaker hydrogels compared to MAX1 gels [28]. In addition, Miller *et al.* have investigated the network morphology of MAX1 hydrogels through atomistic simulations, indicating that MAX1 peptide prefers the ß-hairpin conformation, rather than the amyloid-like beta-arch structure [29]. The simulations also demonstrate that mechanical rigidity of MAX1 hydrogel is due to short persistence length of the fibrils.

Hydrogels based on ELPs have been computationally designed by Buehler and coworkers [30•]. Employing all-atom molecular dynamics, they have investigated the effects of ionic concentration and mutation on ELPs (Table 1). Tarakanova *et al.* have demonstrated that ions promote intra-peptide hydrogen bonds by disrupting the hydrogen-bonded network of water molecules in the nearest hydration shell of the ELP. This phenomenon facilitates the folding of the ELP with increasing temperatures, resulting in higher stability of the peptide. The study also reveals that a single point mutation of valine to lysine at the seventh position of the peptide increases the solvent accessible surface area, which prevents a structural collapse at higher temperatures and renders the protein unstable [30•].

In another study by the same group, mechanical functions of a silk-elastin-like peptide (SELP) (Table 1), capable of forming hydrogels, have been probed by atomistic simulations [31]. At a single molecule scale, SELP experiences a structural collapse at temperatures higher than its transition temperature. The computational results were supported experimentally. These results were also applicable at the macroscale, where hydrogel undergoes shrinkage phenomenon with an increase in temperature [31].

Figure 2



(a) Schematic illustration of ELP-based underwater adhesive. Reproduced with permission [33••]. **(b)** Schematic showing the effect of implanting silk fibroin hydrogel bearing miR-675, a microRNA packaged within the exosomes. Reproduced with permission [34•]. **(c)** Photopatterning of gelatin hydrogels with caged CMPs. Reproduced with permission [35]. **(d)** Schematic illustration of 3D-printed silk hydrogels. The cells can either be cultured on the scaffolds or encapsulated during printing. Reproduced with permission [36].

Atomistic simulations provide insights on stability of the sequences that are known to self-assemble, whereas coarse grained simulations have been generally used to predict the peptide and protein self-assembly behavior. Therefore, a combined approach of utilizing coarse grained models to design new candidates, experimentally validating such peptides and proteins and using all-atom simulations to fine-tune the structures would lead to new biomaterials with improved properties. Such materials-by-design approaches have produced significant results and are becoming more common in the field of biomaterials [30•,31,32].

Novel applications of peptide-based and protein-based hydrogels

Hydrogels are widely used for delivery of cells and a range of therapeutics. The abovementioned examples of hydrogels have potential applications in providing sustained or targeted therapeutic delivery, rendering them excellent candidates as drug delivery vehicles, cell carriers and tissue engineering substrates [4•,5,11,12,20•]. New hydrogels with improved properties and functions are continuously being developed that extend their use in a range of applications. Here, we describe some of the recent trends that we believe will influence the future design and applications of hydrogels.

Hydrogels with bioadhesive properties have high potential in biomedical applications, serving as tissue adhesives, hemostatic agents or tissue sealants. Inspired by the adhesion mechanism of marine mussel foot protein (Mfp), several groups are developing hydrogels that can adhere to wet surfaces [37]. Mfps are rich in catechol-bearing non-natural amino acid, 3,4-dihydroxyphenyl-L-alanine (DOPA), which can undergo oxidative self-polymerization, giving them a high adhesive strength [38]. Several strategies have been utilized to introduce DOPA within the protein polymers. A common strategy is to employ tyrosinase, an enzyme capable of converting tyrosine residues to DOPA [39]. Sun and colleagues *et al.* have designed three protein polymers, $(TM3)_3$-ELP_2, $(TM5\text{-}1)_3$-ELP_2 and $(TM5\text{-}2)_3$-ELP_2 (Table 1), comprising of short peptide fragments

derived either from Mfp-3 or Mfp-5, two repeats of ELP and cell adhesive RGD motifs [39]. Upon oxidation with tyrosinase, these protein polymers form hydrogels at room temperature that exhibit good adhesiveness and cytocompatibility [39]. Although studies have suggested a vital role of Mfps in governing the mechanical properties of hydrogels [39], these motifs are not required for wet adhesion. Brennan *et al.* have utilized tyrosinase-modified ELPs (Table 1) to design smart underwater adhesives (Figure 2a). These hydrogels demonstrate better adhesion strength when compared with a commercially available fibrin sealant [33••].

In recent years, an unprecedented amount of scientific work has been published on the use of exosomes in therapeutic applications. Exosomes are small extracellular vesicles that participate in cell to cell communication. They have an immense therapeutic potential and since they can be readily packed with functional proteins, nucleic acids, lipids and small molecules, they serve as excellent delivery vehicles [40]. One of the major challenges in delivering exosomes is its rapid clearance from target organ [41], thus there is a critical need to develop formulation strategies that can increase the retention time of exosomes at the desired organ. Peptide-based and protein-based hydrogels are increasingly being used to prolong the exosome retention [34•,42]. A notable example includes the use of silk fibroin hydrogel for sustained delivery of microRNA (miRNA) packaged into stem cell-derived exosomes in preventing vascular aging (Figure 2b) [34•]. The use of protein-based hydrogels for exosome delivery is anticipated to witness a growth in the years to come.

Another interesting application of protein-based hydrogels includes their production within the living cells. Nakamura *et al.* have developed a non-invasive strategy, referred as iPOLYMER (intracellular production of ligand yielded multivalent enhancers) composed of two proteins, FK506 binding protein (FKBP) and the FKBP-rapamycin binding protein (FRB), which undergo dimerization and gelation upon addition of rapamycin [43••]. This approach has been tested *in silico* by Monte Carlo simulations and confirmed experimentally by tracking the expression and rapamycin-induced gelation of fluorescent labeled FKBP and FRB proteins within the cells. The intracellular hydrogel mimics RNA granules and functions as molecular sieves that allow protein molecules to pass through but prevent diffusion of large vesicles [43••]. The use of this approach in future for high-throughput production and characterization of hydrogels is promising, eliminating the need to purify proteins at early stages of development.

Hydrogels are appealing scaffold materials in tissue engineering applications and have been used to emulate the extracellular matrix (ECM). In order to mimic the dynamic 3D microenvironment of ECM, techniques such as molding and photoactivated patterning are being used to engineer protein-based hydrogels [44]. Paul *et al.* have used poly(dimethylsioxane) molds to create micro-sized and nano-sized wavy patterns on ELP (Table 1) hydrogels [45]. These hydrogels can support and orient stem cells along their patterns. On the other hand, Li *et al.* have developed a strategy to create 2D-photopatterns and 3D-photopatterns of collagen mimetic peptides (CMPs) on gelatin hydrogels (Figure 2c) [35]. This method utilizes nitrobenzyl bearing caged CMPs, which cannot bind to gelatin due to steric hindrance. However, upon photoactivation, nitrobenzyl group is cleaved, triggering triple helix hybridization of CMPs with gelatin chains. These patterned hydrogels have promising applications in ocular drug delivery and tissue replacement therapies. Another area gaining traction is fabricating ECM-mimicking scaffolds using 3D printing [46]. Hydrogels based on recombinant spider silk proteins (Table 1) have been used as bioinks to print scaffolds (Figure 2d) that can maintain cell viability of human fibroblasts [36]. Apart from ECM, there is a growing interest in using hydrogels for developing organoids or organs-on-a-chip system [47]. Although peptide-based and protein-based hydrogels may suffer from poor mechanical properties and long-term instability issues, novel sequences can be designed that offer promising opportunities in organoids formation.

Conclusions and future prospective

Hydrogels have revolutionized the field of biomaterials, with peptide-based and protein-based systems emerging as versatile platforms for a wide range of applications. Here we have presented a brief overview of stimuli-responsive hydrogels, described the role of computational modeling in designing peptides and proteins that can undergo gelation, and discussed the latest trends in the use of hydrogels. There are still many new avenues being explored in the field, for example: the development of hydrogels for oral delivery of proteins and peptides [48]; the use of novel chemical and enzymatic approaches to design hydrogels [49]; and 4D patterning and bioprinting of hydrogels [50]. New strategies that combine proteins with synthetic polymers are also on the rise; such hybrid systems can provide the desired mechanical rigidity to hydrogels, making them ideal for many biomedical applications.

While substantial progress has been made in the field of peptide-based and protein-based hydrogels, there is still a long way to fully realize the clinical potential of these systems. There is a need to design hydrogels with predictable and tunable properties and reduce the inherent high costs of protein production. Advanced technologies that can improve scalability, and together with new computational methods, will enable the generation of future hydrogels that can move beyond the clinical trial phase toward approval by the Food and Drug

Administration, resulting in successful clinical translation of peptide-based and protein-based hydrogels.

Conflict of interest statement

The authors declare no conflict of interest.

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- • of special interest
- •• of outstanding interest

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