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Recent trends on pH/thermo-responsive self-assembling hydrogels: from polyions to peptide-based polymeric gelators

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ABSTRACT: In this article, we highlight some recent developments on "smart" physical hydrogels achieved by self-assembling of block type macromolecules. More precisely we focus on two interesting types of gelators namely conventional ionic (or ionogenic) block copolymers and peptide-based polymers having as a common feature their responsiveness to pH and/or temperature which are the main triggers used for potential biomedical applications. Taking advantage of the immense skills of conventional block copolymer hydrogelators, namely macromolecular design, self-assembling mechanism, gel rheological properties, responsiveness to various triggers and innovative applications, the development of novel self-assembling gelators, integrating the new knowledge emerging from the peptide-based systems, opens new horizons towards bio-inspired technologies.

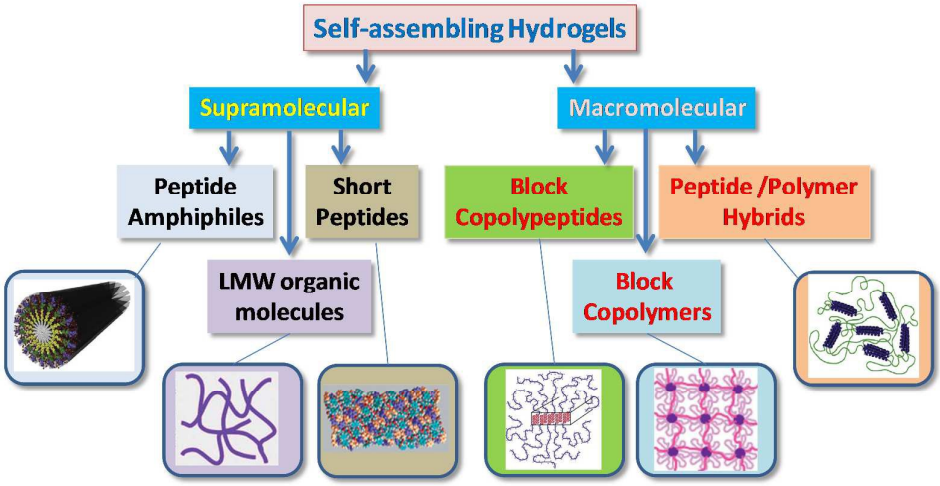
Introduction

Hydrogels are three-dimensional (3D) soft materials that consist of a matrix entrapping a high content of water (up to 95 w%). This remarkable feature makes them suitable for many applications especially in medicine where interactions with living tissues are needed. Hydrogels appear then to be good candidates for drug carriers as well as biomaterials for tissue engineering. [1] As long as polymeric matrices are considered, two main strategies for achieving these 3D network structures may be discriminated. The first one relies on the covalent bonding of hydrophilic polymer chains, leading to hydrogels referred as chemical networks. The major drawbacks of the chemical hydrogels, in terms of mechanical properties (i.e. low stiffness, poor non linear mechanical properties and no self-healing) have been remarkably circumvented during the last years by adding nanoparticles to the polymeric matrix (achieving the so-called soft nanocomposites) [2,3] or by considering double and/or interpenetrated

networks where one of them will self-sacrifice when the material is mechanically damaged.[4] Nevertheless, once a given geometric shape is achieved with the chemical hydrogels through a molding process, this shape is definitive and somehow irreversible.

The second approach for elaborating 3D polymeric matrices, considered the use of weak interactions namely hydrophobic, ionic, π–π staking, host/guest and so on for driving the self-assembly of tailor-made macromolecules in water. The resulting hydrogels are then referred as physically cross- linked. The use of reversible cross-links allows the design of "smart" materials that can self-adapt easily to their environment and more specifically when changes within this environment occur (in terms of pH, ionic strength, temperature, competitor, redox stimuli, shear...) [5] which make their processing much easier, e.g. injectable hydrogels. Such a design owes much to the development of the controlled polymerization methods and click chemistry techniques which allow the design of a large variety of macromolecular topologies in a more easy way.

Physical hydrogels can be derived from the self-assembly of a vast variety of molecules and could be classified into two main categories namely supramolecular and macromolecular. The main characteristic of supramolecular based systems is that they form fibrous hydrogels from low molecular weight building molecules that can further be grouped to organic molecules, short peptides and peptide amphiphiles (Scheme 1). These families of hydrogelators have been reviewed recently [6-8] and will not be mentioned in the present article. Concerning the macromolecular-based



Scheme 1. Classification of self-assembling hydrogels

hydrogels, they are mainly constituted of block type high molecular weight molecules, bearing associative blocks serving to form the physical crosslinks. Further classification could be conventional block copolymers, copolypeptides and the hybrids of these two (Scheme 1). Macromolecular-based hydrogels demonstrate several advantages with respect to supramolecular one as they exhibit high molecular tunability namely macromolecular architecture, molecular size (molecular weight of the various segments), hydrophilic/hydrophobic balance (repeating unit composition) and multifunctionality (responsiveness) which allows designing "tailor" made hydrogelators with predictable network features and functionality.

In this article we highlight some recent developments on "smart" physical hydrogels, achieved by self-assembling of block type macromolecules. Provided that this area is quite broad, we choose focusing on two interesting types of gelators namely conventional ionic (or ionogenic) block copolymers and peptide-based polymers wishing to attract the interest and provoke interactions between the corresponding audiences of these scientific areas that could result to novel design potentials for gelators with superior properties. This choice was motivated from the fact that these two families emerge as a main common feature the ionic functionalities they bear and in turn the electrostatic interactions they develop, either intra-molecularly or inter-molecularly also with various molecules of the aqueous environment. Yet, the incorporation of ionic functions tunes the thermosensitivity of the gelators. Thus, all these interactions play crucial role in the self-assembly, network morphology and rheological properties of the hydrogelators as well as endowing them with responsiveness to pH, ionic strength and/or temperature which are the triggers that are mainly used in biomedical applications.

1) Polyelectrolyte-based hydrogels

The formation of hydrogels through the self-assembly of block copolymers based on polyelectrolyte moieties can proceed according to two main ways. The polyelectrolyte moieties can be part of the physical reticulation nodes through complexation with a polyelectrolyte of opposite charge (so-called coacervate hydrogels) or the polyelectrolyte can be coupled with hydrophobic block/units. When dispersed in water, the latter self-assemble within hydrophobic microdomains, that can act as physical reticulation nodes for the network, as long as two hydrophobic blocks at least are present per macromolecule. When diblock amphiphilic copolymers

are considered, the formation of hydrogels is due to the jamming of the polymeric self-assembled star-like micelles which occurs only at pretty high concentrations. Obviously combination of both types of crosslinks (hydrophobic and coacervates) has also been considered in the literature.[9]

1a) Coacervate and polyampholyte hydrogel

By mixing two triblock copolymers with a central block based on polyethyleneglycol (PEG) and cationic (ammonium or guanidinium) or anionic (sulfonate or carboxylate) end blocks in equal amount, Hunt et al. have obtained hydrogels whose mechanical properties range from viscoelastic to fully elastic by carefully choosing the cation/anion couple.[10] Stiffer hydrogels were formed when the strongest acidic and basic units were used, but were weakened when the electrostatic interactions were screened, by adding salt which even precluded the hydrogels formation at very high ionic strength.[11] As the polymer concentration increased, the structure of the hydrogels changed from disordered to Body-Centered-Cubic which induced an increase of their elastic modulus. Further concentration increase resulted in the formation of a hexagonal phase of cylinders and in a weakening of the hydrogels.[11,12] Such structural experimental results have been compared with theoretical calculations achieved with an embedded fluctuation model.[13] The fabrication process of coacervate hydrogels appears to be convenient, very fast and robust. However, from the structural point of view, it took a lot of time for the systems to reach equilibrium. This could be fastened by adding diblock copolymers.[14] Rather than using two triblocks, as mentioned previously, the formation of hydrogels based on coacervates of a triblock with either nanoparticles or homopolyelectrolyte of opposite charges has also been reported.[15,16]

The possibility of forming hydrogels with block copolyampholytes (that are macromolecules where both the cationic and anionic polyelectrolyte blocks are incorporated in the same polymer chain) have also been investigated.[17] A triblock with a central block based on quaternized poly(2-vinyl pyridine) (PQVP) and two end blocks made of polyacrylic acid (PAA) has been dispersed in water at different pH where electrostatic interactions between AA and QVP are likely to occur, but with a ratio of positive to negative charges that can be tuned by the pH (the number of cationic charges being constant). When increasing the charge asymmetry i.e. with

decreasing pH, the structure of the hydrogels varied from dense uncorrelated microgel to fractal clusters of core-shell aggregates and finally to a 3D network where the interpolyelectrolyte complexes acted as physical reticulation nodes. From the macroscopic point of view, the later cases appeared as self-supporting gel whereas in the other two cases viscous dispersions were obtained. Such a macromolecular topology is highly versatile since various types of gelators can originate from a single polymer precursor, the later being an amphiphilic triblock copolyelectrolyte, whose central block can be ionized in a permanent way or not, e.g. tertiary amine units can be quaternized or not.[18] Moreover, the acidolysis of the hydrophobic end-blocks of this precursor polymer leads to a polyacid, which can be complexed with a central block as illustrated before.

Another combination of these weak polyelectrolytes in a P2VP-b-PAA-b-poly(n-butylmethacrylate) ($P2VP_{25}$-PAA_{576}-$PnBMA_{36}$) ABC triblock terpolymer topology, resulted to an interesting pH responsive gelator. [19,20] In particular, at low pH, PVP is ionized and the triblock self-assembled within aggregates whose cores consisted of PnBMA surrounded by PAA shell, and stabilized at the outer surface by a corona of ionized P2VP (core-shell-corona structure). At physiological pH, P2VP becomes neutral and thus hydrophobic while PAA is ionized driving the triblocks to self assemble forming flower-like aggregates whose cores consisted of PnBMA with P2VP at the outer surface, stabilized by PAA^- petals that could bridge adjacent cores at higher concentrations forming a 3D network. Thus a sol-gel transition was observed at pH 5 and at relatively low polymer concentration (ca 1 wt%) which renders this gelator suitable for creating an injectable hydrogel. Popescu et al. have taken the advantage of this pH-triggered hydrogelator to design a liposome/hydrogel soft nanocomposite as a controlled drug delivery system.[21] A suspension of the former aggregates at low pH has been mixed with liposomes and its pH has been raised, inducing the self-assembly of the ABC terpolymer which resulted at the macroscopic scale to the formation of a hydrogel hosting the liposomes (Fig. 1). Rheological characterization of the resulted nanocomposite gel showed that a nearly elastic soft solid was formed, with improved viscoelastic features with respect of the plain hydrogel. The liposomes remained intact within the gel matrix. Excellent control of the calcein release was achieved just by adjusting the gelator concentration, i.e. from 1wt% to 1.5wt% the drug release period was significantly prolonged from 14 to 32

days (Fig. 1). The cytotoxicity of such a soft nanocomposite is very low which makes it promising as drug release system.

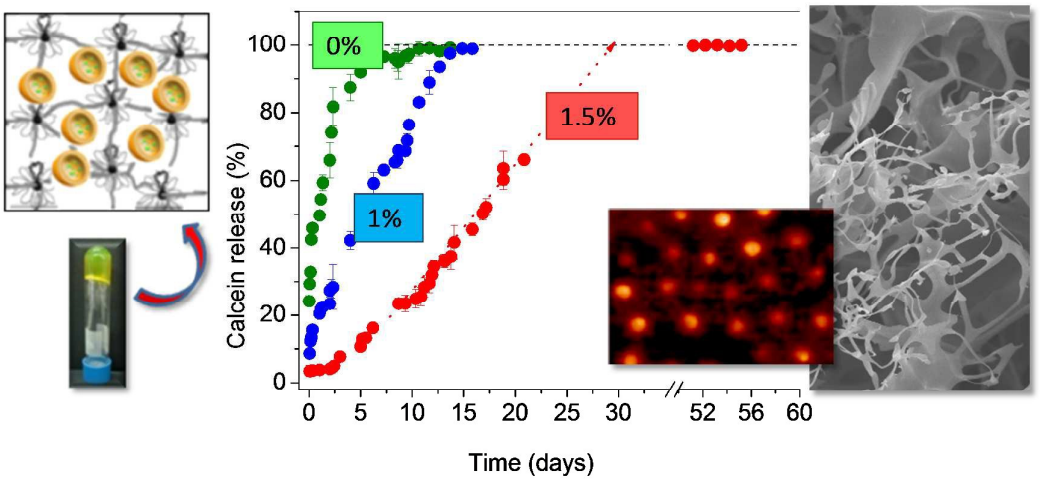


Figure 1. Sustained calcein release at physiological pH through the liposome/hydrogel formulation (left schematic representation) at different gelator concentration. In the right, SEM and AFM images of the involved hydrogel. Inspired from references 20 and 21.

Recently, P2VP and PAA blocks were also combined with thermo-sensitive poly(N-isopropylacrylamide) (PNIPAM) segments, displaying a lower critical solution temperature (LCST) at 32°C, to endow a heteroarm star terpolymer with dual pH and thermo responsiveness. The resulted complex macromolecular topology, namely PS_n(P2VP-*b*-PAA-*g*-PNIPAM) heteroarm star-graft quarterpolymer, exhibited rich self-assembly behavior controlled by temperature and pH. At relatively high concentrations (ca. 3 wt%) and temperatures above PNIPAM's LCST, a gel phase was revealed depending strongly on the solution pH and the grafting density along with the number of arms of the star-grafts. The gelation phenomenon was attributed to the formation of a transient 3D network arising from the extensive inter-star hydrophobic association which however, was influenced by the pH-controlled electrostatic interactions along the arms.[22]

Random polyampholytes can be used as potential stickers to design pH-triggered hydrogelators. According to this concept, a poly(2-(diethylamino)ethyl methacrylate-*co*-methacrylic acid)-*b*-(ethylene glycol methyl ether methacrylate)-*b*-poly(2-(diethylamino)ethyl methacrylate-*co*-methacrylic acid) (P(DEAEMA-*co*-MAA)-*b*-PEGMA-*b*-P(DEAEMA-*co*-MAA)) triblock terpolymer was synthesized and its solution properties were investigated.[23] In the vicinity of the isoelectric point

(*iep*) (pH-window) of the polyampholyte end-blocks, these blocks turn to hydrophobic stickers driving the triblocks to self-assemble forming flower-like micelles and at elevated concentrations a 3D micellar network. Thus by tuning the *iep*, through suitable choice of the monomers, the gel pH-window could be predicted at will. More interestingly, thanks to the thermo-sensitivity of the central PEGMA block the hydrogel exhibited a complex thermo-response. Finally, this triblock terpolymer showed satisfactory cytocompatibility, suggesting potential biomedical applications as drug delivery nanocarriers.

1b)Dual responsive hydrogels by combining ionic and LCST monomers

The amphiphilic nature of the ABA copolymer-gelators can either be permanent or tunable through an external trigger (e.g. temperature and pH). In the latter case double hydrophilic copolymers are involved with B hydrophilic block and A blocks exhibiting stimuli triggered hydrophobicity. [24,25] For instance, Lee et al. have considered a triblock based on PEG and N,N-diisopropyl ethalonamine glycidyl ether (DEGE), the latter being hydrophobic at high pH. Thus, by increasing pH, the block copolymers self-assembled in a 3D network, forming a pH-responsive and stealthy (thanks to PEG) hydrogels in relevant conditions for biological applications.[25]

Combining thermo-sensitive monomers, such as tertiary amines and/or oligo(ethylene glycol) methyl ether (meth-)acrylates with ionic moieties, to build random copolymer tunable stickers, multi-responsive gelling systems can also be designed with novel properties and potential applications. Triblock copolymers containing oligo(ethylene glycol) methyl ether methacrylate (OEG) as permanently hydrophilic central block, end-capped by diethylene glycol methyl ether methacrylate (DEG), randomly copolymerized with [2-(methacryloyloxy)ethyl] trimethylammonium chloride (TMA) cationic moieties, were prepared targeting salt and temperature responsive hydrogelators. This dual responsiveness relies on the control of the LCST of the end-blocks which depends both on the molar content of TMA charged moieties and the presence of salt (electrostatic screening effect). These copolymers displayed excellent hydrogel properties with salt- and temperature-dependent gel points. For instance, poly($DEG_{98}TMA_2$-*b*-OEG-*b*-$DEG_{98}TMA_2$) formed a hydrogel at 40°C in water and 26°C in 0.9 wt% NaCl aqueous solution.[26] By replacing the ionic moieties from strong to weak electrolytes, this kind of triblocks

can be endowed with pH sensitivity too. Examples have been reported by O'Lenick et al. using AA comonomer in the end-blocks (Fig.2). In this case, the pH-sensitivity was manifested in the acidic pH regime where the AA moieties being progressively deprotonated.[27,28]

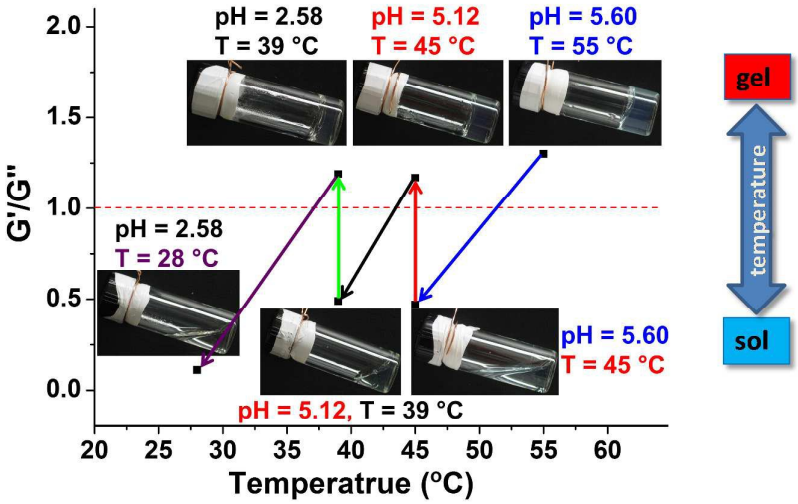


Figure 2. pH tunable thermo-responsiveness of a hydrogel formed by self-assembly of a ABA triblock copolymer composed of poly(ethylene oxide) (PEO) end-capped by poly(methoxydi(ethylene glycol) methacrylate-*co*-methacrylic acid) pH/thermo-sensitive outer blocks. Reprinted with permission from *Langmuir*, 26, 8787-8796 (ref 27) Copyright 2010, American Chemical Society.

Using the same concept (i.e. random copolymer stickers incorporating ionic moieties) CO_2-switchable triblock copolymer hydrogelators were prepared. The presence of CO_2 in the aqueous media induces protonation or deprotonation of tertiary amines or acrylic acid respectively, equivalent with the addition of protons or hydroxyl ions (pH control), affecting thus the LCST of the end-blocks.[29] Therefore, depending on the ionic nature of the adding moieties in the thermosensitive end-blocks, gel-to-sol or the reverse sol-to-gel transition can be induced by passing CO_2 or an inert gas respectively without using acids or bases. This type of hydrogels was used to show CO_2-induced protein controlled release as potential biomedical application.

Random copolymers bearing ionic moieties can also be used as the central block of a responsive gelator. Combining two PNIPAM end-blocks with a central one based on AA and O-phoshoethalonamine, Lin et al. have designed thermoresponsive

hydrogels under physiological conditions displaying potential as scaffold for bone tissue engineering.[30] The partial phosphorilation of the PAA central block was dictated from two reasons. Firstly, to avoid strong interpolymer complexation (H-bonding) between PNIPAM and PAA which leads to precipitation and secondly, to provide higher affinity for calcium ions which will benefit the biomineralization process.

P(NIPAM-co-AA) blocks incorporated to an ABC triblock copolymer has also be used as the thermo-sensitive sticker to induce gelation at the desired temperature regime. As shown, the gelation temperature can be controlled by the mole fraction and the degree of ionization (pH dependent) of the AA moieties in the P(NIPAM-co-AA) block.[31] In general, the ABC terpolymer topology exhibits additional potential to design multi-responsive gelators since the outer A and C blocks can incorporate different functionalities.

Henn et al. have applied the same concept with ABA triblocks involving low amount of tertiary amine based monomer in the thermosensitive end-blocks.[32] The B block was PEO and the outer A blocks were random copolymers comprising three monomers, two providing thermosensitivity [methoxy and ethoxy di(ethylene glycol) methacrylate] and the third one pH-sensitivity using 5 mol % of tertiary amines (N,N di-R-aminoethyl methacrylate where R = ethyl, isopropyl, n-butyl) of different pK_α values. These triblocks exhibited thermo-induced sol-gel transitions affected by pH which was manifested by a $T_{sol\text{-}gel}$ versus pH sigmoidal curve shape. The $T_{sol\text{-}gel}$ decreased with increasing pH due to the deprotonation of the tertiary amine groups. Interestingly, the sigmoidal curves shifted to higher pH values with increasing pK_α (by replacing tertiary amine monomers) and at a given pH, the $T_{sol\text{-}gel}$ decreased with the hydrophobicity of the tertiary amines.

Rather than considering linear chains, Schmalz et al. have applied the same kind of strategy with star-block copolymers, whose arms consisted of diblocks based on poly(di(ethylene glycol) methyl ether methacrylate) (PDEGMA) and poly(2-(dimethylamino)ethyl methacrylate) (PDEAEMA).[33] In solution, upon increasing temperature, PDEGMA located at the outer surface of the star collapsed, driving self-assembly into flower-like aggregates that bridged at high concentrations. At the same time, depending on pH, the PDEAEMA inner blocks contracted also upon increasing temperature which stiffened the hydrogels. The resulting hydrogels are then formed upon heating and their mechanical properties depend on the pH. [34-36]

Different groups have also considered the use of a poly(amino urethane) (PAU) group combined with linear or star-like PEG.[32-37] At low pH and temperature triblocks and stars were well dispersed in solution, but underwent self-assembly under physiological condition due to the neutralization of PAU. The cytotoxicity of these hydrogels has been measured as well as their in *vivo* degradation profile. The same kind of pH sensitive block has been combined with a triblock based on PEG and two hydrophobic block of poly(ε-caprolactone-lactide) generating biodegradable hydrogels.[38]

The use of permanently hydrophobic blocks for elaborating hydrogels through self-assembled amphiphilic copolyelectrolytes has also been considered for obtaining 3D networks based on the so-called telechelic polyelectrolytes. [9] Henderson et al. have yet considered a poly(methacrylic acid) (PMAA) middle block end capped at both ends with poly(methyl methacrylate) (PMMA), the latter forming glassy reticulation nodes. The network was further crosslinked through ionic interactions between PMAA and divalent cations achieving thus tough hydrogels.[39] Swann et al. have used the same hydrophobic end-blocks but combined with a PDMAEMA central block and have reported their swelling properties in the presence of various monovalent sodium salts and anions from the Hofmeister series.[40] It should be noted that in both cases, the self-assemblies are frozen which means that the hydrophobic block cannot self-extract from the reticulation nodes. This could be accounted for the fact that the escape of the hydrophobic block from an hydrophobic microdomain is controlled by its size and its surface tension with respect to water.[41] This is the reason why most of the telechelic polyelectrolytes do not display any dynamics of exchange even when hydrophobic block with low glass transition temperature (such as poly(nButylAcrylate) (PnBA) Tg=-50°C) was used. In order to circumvent such a drawback, very short hydrophobic block must be considered. This has been implemented by Ward et al. [42] who have synthesized a series of telechelic polyelectrolytes with a central block based on 2-(dimethylamino)ethyl methacrylate (PDMAEMA) and hydrophobic end-blocks consisting of poly(n-butyl methacrylate) (PnBMA) with very low polymerization degrees (from 2 to 15). Moreover, the propensity to form hydrogels has been related not only to the composition of the triblocks but also to the asymmetry (i.e. difference in size) of their hydrophobic blocks and to the length of the ester pendant bear by the methacrylate monomer.[43] The

same group has combined the same blocks with a methoxy(poly(ethylene glycol methacrylate) one, the hydrophobic block being trapped between the hydrophilic ones. Thermosensitive hydrogels based on jammed micelles of these triblocks were then obtained at a critical temperature than can be tuned through the nBMA content. Another way for obtaining hydrogels at equilibrium with telechelic polyelectrolyte is to consider solvents mixture rather than only water.[44,45]

1c. Hydrogels from copolyelectrolytes: tunning the hydrophobicity of stickers

The hydrophobic character of the hydrophobic block may also be tempered by incorporating hydrophilic subunits within it, as documented by Charbonneau et al. who have considered amphiphilic triblock copolymers based on a PAA central block capped by P(AA-co-nBA) end blocks, the microstructure of which were statistical.[46,47] When dispersed in water at low concentration, these triblock copolyelectrolytes self-assembled within flower-like micelles. Upon increasing polymer concentration above a critical concentration, extensive micelle bridging led to the formation of a 3D spanning network (hydrogel).[48] This percolation concentration depended on the ionization degree (α) of AA units which also influenced strongly the exchange dynamics of the tempered hydrophobic end-blocks as evidenced by rheological measurements. The hydrogels displayed viscoelastic properties and while their elastic modulus did not depend on α but only on the polymer concentration, their terminal relaxation time (τ) can be increased over 11 orders of magnitude by decreasing α from 0.9 down to 0.2. This result can be explained by the fact that decreasing α is equivalent to an increase of the hydrophobic character of the end-blocks which slows down or even freezes their exchange dynamics (Fig 3, top). Such a feature was fully reversible which means that a frozen gel can display fasten exchange dynamics by increasing α and can be further refrozen by decreasing α. When increasing the amount of AA units, incorporated within the end-blocks, Shedge et al [49] have shown that the increase of the relaxation time occurs at lower α values keeping constant all the other structural parameters and that it could be explained by taking into consideration the sole ionization of the AA units embedded in the hydrophobic blocks (Fig 3, bottom).[50]

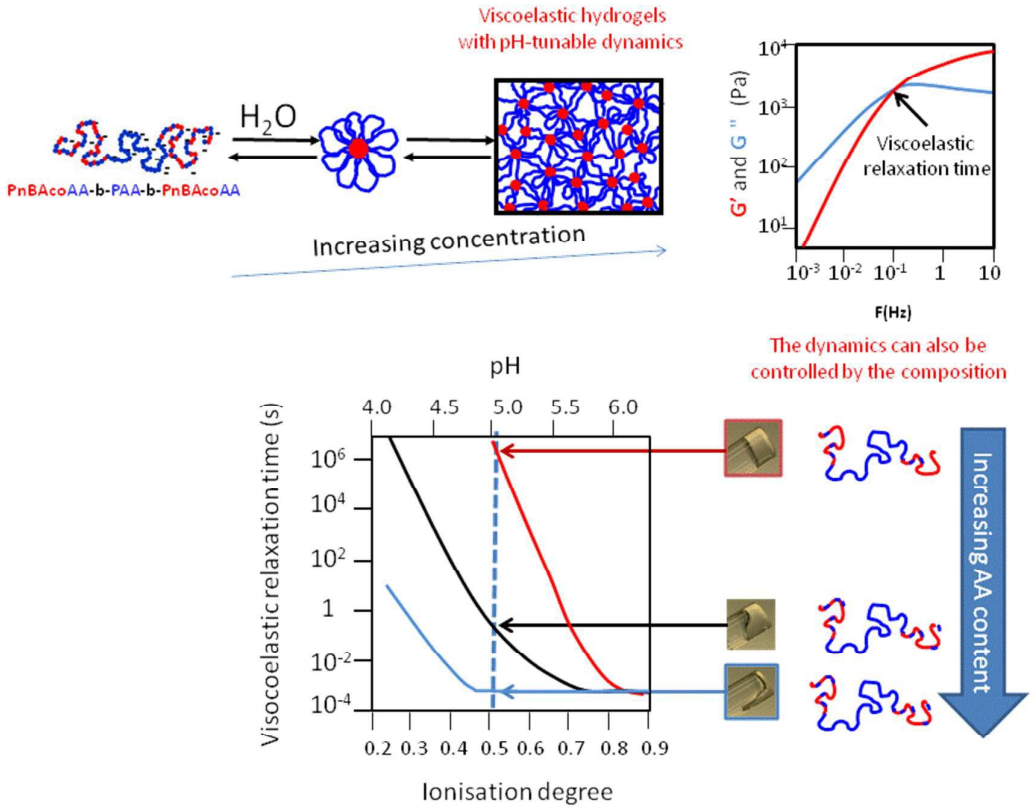


Figure 3. Influence of composition of the end-blocks of P(AA-*co*-nBA)-*b*-PAA-*b*-P(AA-*co*-nBA) aqueous solutions as a function of degree of ionization of the AA moieties controlled by pH. Inspired from references 46 and 49.

In order to improve the mechanical properties of these hydrogels, the double network strategy which appears to be of great benefit for chemical gels, as explained in the introduction, has been attempted by Klymenko et al.[51] They have mixed the amphiphilic copolyelectrolyte previously described, with a neutral amphiphilic triblock based on PEG leading to a double network as evidenced by scattering techniques. Though hydrogels thus formulated still displayed the functionality of each of their individual components, their non linear mechanical properties were not tremendously improved which could be explained by a lack of heterogeneity within the hydrogels and by the absence of enough attractive interactions between both components.

Homologues of these triblock copolyelectrolytes based on styrene (S) and AA have been investigated by Borisova *et al.* as well. The main differences arise from the use of a hydrophobic monomer whose homopolymers display much higher glass transition temperature with respect to PnBA and to the gradient microstructure of the

tempered end-block. It has been shown that the hydrogels based on such self-assembled triblocks displayed dynamics of exchange which depended on the pH (and then on α), as long as the samples were heated above the glass transition temperature of the end-blocks.[52,53]

The nature of the hydrophobic monomers as well as their distribution within the tempered hydrophobic block are obviously of key importance and can even lead to unexpected properties. This fact has been well reported by Popescu et al. who rather than considering a "classical" amphiphilic triblock copolyelectrolyte, have designed an amphiphilic multiblock copolymer (from 3 to 9 blocks) consisting of hydrophobic and hydrophilic blocks respectively based on PnBA and PDMAEMA.[54,55] Once dispersed in water, these copolymers self-assembled within flower-like micelles bridged through PDMAEMA, which resulted in remarkably low critical gel concentration, at least if the end-blocks were PnBA. Furthermore, as a function of pH, the viscosity of aqueous dispersions of the heptablocks exhibited two maxima, whereas the homologue triblocks only displayed a single one. Beheshti et al. have extended a triblock with a PEG central block and two PNIPAM end-blocks with two poly(styrenesulfonate) outer short blocks. The resulting pentablocks may form interconnected physical network at high temperature when carefully adjusting the polymerization degrees of the PEG block,[56] the PSS block preventing any macroscopic phase separation. Hu et al. have combined blocks either consisting of random copolymer of PNIPAM and N,N dimethylacrylamide or pol(4 vinylpyridine) P4VP. The former displays a LCST and the later a UCST, and in turn the resulting multiblocks presented a gel to sol to gel transition upon heating.[57]

Finally, remarkable attempts have been undertaken to simulate the association process towards a 3D network of polyelectrolytes, end-capped by hydrophobic stickers (telechelic polyelectrolytes), by Monte Carlo methods using percolation theory. [58,59] As predicted, the sol-gel transition, induced by increasing concentration, was shifted to lower concentrations upon increasing the hydrophobicity of the stickers for a fully charged polyelectrolyte central block. This is in good agreement with the experimental findings of Charbonneau et al. although the degree of ionization of the central PAA block varied with pH. [47] Moreover, modeling of fully charged and non charged system showed that the charged telechelic polymers are able to form hydrogels at lower concentrations than the non-ionic counterparts.[57]

More importantly, Monte Carlo simulation can provide additional information on the conformational transitions of the telechelic chains occurring during the gelation process. Four types of chain conformations can be distinguished: free, dangling, loop and bridge which affect the network structure and thus the rheological properties of the gel (i.e. the plateau modulus is proportional to the number of bridges). Thus the fraction of the different conformations could be monitored as a function of concentration keeping constant the hydrophobic interaction energy of the stickers and the charge density of the polyelectrolyte chain. It was indicated that the fraction of bridges increases rapidly with concentration, reaching to about 40 % of the polymer chains in the gelation threshold and more that 60 % well above it (free-standing gel).[59]

2) Peptide-based hydrogels

Another fascinating and rapidly developed field in the context of reversible (physically crosslinked) hydrogels, constitutes the self-assembling peptide-based nanofibrous 3D networks, named also scaffolds, as they are designed mainly for the purpose of tissue engineering and drug delivery biomedical applications.[60,61] Polypeptides are very attractive macromolecules thanks to their protein-mimetic properties that can be integrated to the peptide-based hydrogels endowing them with some advantages, regarding the conventional synthetic polymer-based counterparts namely biocompatibility, biodegradability and biofunctionality with respect to cell attachment, proliferation, differentiation and infiltration. More importantly, and concerning self-assembling capability and stimuli responsiveness, novel bottom-up strategies have been emerged due to the secondary conformations (i.e. α-helix, β-sheet) that amino acid sequences can adopt, depending on the environmental conditions. Due to the amino acid (basic repeating unit of peptide systems) diversity and nature, a large variety of non-covalent interactions are involved in self-assembly process at molecular level that is hydrophobicity, H-bonding, π-π stacking, electrostatic attractions/repulsions, metal-ligand interactions etc, acting either separately or complementary or even antagonistically that remarkably enrich the molecular design capability towards "tailor-made" hydrogels for specific biomedical applications.

The peptide-based nanofibrous hydrogels can be classified into several categories depending on the size and molecular architecture/topology of the involving

amino acid sequences that constitutes the building blocks of the gelator. That is: low molecular weight gelators (LMWG),[62] peptide amphiphiles (PA),[63] self-assembling peptides (SAP) also named "molecular lego",[64,65] copolypeptides (CPP)[66] and peptide hybrid copolymer (PHC) also referred as "macromolecular chimeras",[67] each of them exhibiting advantages and drawbacks. All of them undergo hierarchical self-assembly in aqueous media leading primarily to the formation of nanofibrous 3D structures, following more or less similar supermolecular principles. For instance, a common feature of fibrillogenesis in most of the cases is the formation of discrete β-sheet secondary conformations of the amino acid sequences, that develop in higher order fibrillar nanostructures of high aspect ratio mainly driven by hydrophobic interactions and stabilized by hydrogen bonding. In principle, the β-sheet formation is favored if amino acid sequences display alternating polar and nonpolar residues.

Among the large diversity of peptide-based building blocks that lead to 3D fibrous networks, herein we discuss those related with amphiphilic molecules of relatively long chains that are conventional polymer-polypeptide block copolymer hybrids and copolypeptides. The main synthetic strategy to prepare the polypeptide segments, in the context of chain growth polymerization, is the ring opening of amino acid N-carboxyanhydrites initiated by an amine group which permits long amino acid sequences.[68-70] Concerning PHC, two main synthetic roots have been developed so far, either by using amine end-functionalized macromolecules (usually polyethylene oxide) as macroinitiator or by grafting peptides onto a polymer backbone chain.

2a) Polymer hybrid copolymers

One of the strategies that have been developed so far and leads to responsive hydrogels concerns the design of graft copolymers constituted of poly[N-(2-hydroxypropyl)methacrylamide], HPMA, hydrophilic main chain, grafted with peptide domains (protein-like motifs) that undergoes association leading to 3D networks.[71] In principle the responsiveness of these kinds of hydrogels is related to the defined structure of the peptide physical cross-links. By designing peptide grafts as to form coiled coil associations, self-assembled hydrogels were formed in PBS (phosphate buffer) at neutral pH from two graft copolymers bearing distinct pentaheptad peptides that create dimerization motifs constituting the physical crosslinks (Fig. 4).[72,73] These hydrogels are sensitive to guanidine hydrochloride

(GdnHCl) which disassemble them by coiled-coil denaturation. The hydrogel was recovered by removing GdnHCl by dialysis.

Another pathway towards self-assembled hydrogels, following the same concept, was to use β-sheet peptides as grafting segments of the HPMA. Initially a HPMA-g-CGGbeta11 PHC was designed and evaluated as gelator.[74] Rheological investigations showed that an elastic gel was formed in acidic pH and at concentrations above 3 wt% polymer. The graft copolymer self-assembled into a 3D network, mediated by the formation of β-sheet that created antiparallel arrangements among the pendant peptides of different macromolecules (Fig. 4). TEM revealed fibril formation with minimal lateral aggregation and SEM of freeze-dried gel showed a particular morphology of the network characterized by long-range order and uniform aligned lamellae.

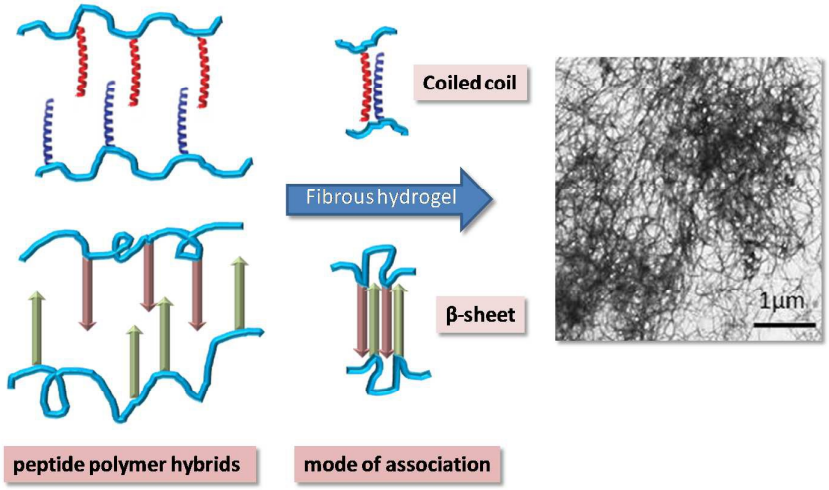


Figure 4. Schematic representation of the self-assembly of graft copolymers hybrids (PHC type) through the formation of either coiled coil or β-sheet motifs that leads to fibrous hydrogels (cryoTEM image). Inspired from references 70 and 71.

In order to shift the gelation pH to neutral, hybrid hydrogels, arising from co-assembly of a mixture of HPMA graft copolymers bearing complementary β-sheet peptides, were designed more recently.[75] Circular dichroism (CD) investigation suggested that the hydrogel formation was triggered through association of the complementary β-sheet motifs, grafted in the different polymers, through electrostatic attractions of oppositely charged amino acid residues. Upon mixing of aqueous solutions of the complementary graft copolymers, a fibrous network was formed trough the hierarchical co-assembly of the β-sheet tapes. Time sweep oscillatory test

of 3 wt% polymer concentration showed that the elastic modulus G´rose to 20 Pa in a few minutes and reached a plateau value of about 300 Pa after 4 h. Preliminary in vitro studies demonstrated that long-term culture maintained cell viability and promoted cell proliferation, suggesting potential application as scaffold.

Injectable biodegredable hydrogels from linear PHC block copolymers constituted of biocompatible polymers, e.g. Poly(ethylene glycol) PEG, conjugated with polypeptides have been developed the recent years. The thermogelling properties, e.g. sol-gel transition temperature, gel modulus, critical gel concentration and the nanoassembly patterns can be tuned by controlling the stereochemistry of polypeptide (nature composition and length of the amino acid segments), the end-capping by hydrophobic moieties along with the nature and length of the conventional polymer. [76,77] In order to introduce pH sensitivity, ionic moieties were incorporated along the polymer backbone.[78] Providing that one of the goals to design these kinds of injectable hydrogels is tissue engineering, the requirement of significant population of fibrous nanostructures similar to natural extracellular matrix could be controlled by the secondary structure of the peptide constituents. For instance, 3D fibrous networks formed by self-assembly of PA-PLX-PA [PA is polyalanine and PLX is poly(propylene glycol- poly(ethylene glycol)- poly(propylene glycol)] was evaluated for chondrocytes 3D culture. By comparing samples differing in the stereochemistry of PA, (L-PA, L/DL-PA, DL-PA) it was shown that the L/DL-PA counterpart was proven to yield an excellent 3D system for the proliferation and differentiation of the chondrocytes. This system formed a nanofibrous matrix based on *β*-sheet PA structures with suitable fiber density for the chondrocytes 3D culture.[79]

In onother gelator design, Jeong *et al* reported the thermogeling behavior of a PLX end-capped by poly-(alanine-*co*-leucine) (PAL) hydrophobic polypeptides. The PAL-PLX-PAL association in water led to PAL forming hydrophobic cores in where the α-helical secondary structure of the polypeptides was adopted, decreasing the molecular motion of the PLX and in turn the sol-to-gel transition in the effective range of 20-50 ^{o}C. The polymer was degraded by proteolytic enzymes such as matrix metalloproteinase and elastase, whereas it was quite stable against cathepsin B, cathepsin C, and chymotrypsin or in phosphate-buffered saline (control). The in situ formed gel in the subcutaneous layer of rats showed a duration of about 47 days, and H&E (hematoxylin/eosin) staining study suggests the histocompatibility of the gel in vivo with a marginal inflammation response of capsule formation. A model drug of

bovine serum albumin was released over 1 month by the preset-gel injection method. The thermogelling PAL-PLX-PAL could be a promising biocompatible material for minimally invasive injectable drug delivery.[80]

Two series of Poly(ethylene glycol)-b-poly(O-benzyl-L-tyrosine) (PEG-PBTyr) block copolymers with various lengths of PEG (M_w 2000 and 5000 g/mol) and polypeptide block (from 6 to 23 Tyr residues) were prepared and evaluated as thermo-hydrogelator.[81] The length ratio of the blocks was proven critical, regarding the ability of these diblocks to form hydrogels. As shown, only PEG2000-$PBTyr_6$ was able to form thermoresponsive transparent hydrogels at a range of concentrations and temperatures. The sol-to-gel transition temperature depends on polymer concentration and rises upon concentration decrease. Cryogenic TEM revealed the formation of a continuous 3D network of fibers even at concentrations as low as 0.25 wt%. The fiber formation relies on the β-sheet conformations adopted by the PBTyr blocks, as confirmed by CD. The hydrogelation was attributed to a combination of factors including amphiphilic balance of blocks and β-sheet conformations, the packing of which was strengthened by increasing temperature.

Similar thermogelation behavior is exhibited by another hybrid triblock hydrogelator ODLAG-PEG-ODLAG, (ODLAG is oligo DL-allylglycine), which self-assemble in aqueous media upon heating forming β-sheet-based fibrous hydrogel. The opaque gel although it was formed above a T_{gel} observed at T>80 ^oC, remained stable at room temperature without the appearance of a gel-to-sol transition. However sonication or the presence of enzymes induced a gel-to-sol transition. Again the hydrophilic balance and the length of polypeptide (only 6 residues per block lead to gelation) were critical as observed in the previous system.[82]

Another important factor affecting the gel properties in PHC thermogeling systems is the length of side alkyl groups in PEG-poly(L-alkyl-glutamate) copolymers. Four diblock copolymers contained different hydrophobic side ester groups (methyl, ethyl, n-propyl, n-butyl) in the polypeptide block were thus evaluated. All copolymers underwent sol-gel transitions upon rising temperature. However the lower critical gelation temperature (CGT) was varied, depending on the alkyl group, following the order: ethyl<methyl< n-propyl<n-butyl. At a fixed polymer concentration of 6 wt% PBS solution, the elastic modulus raised abruptly, firstly in the "ethyl" sample, at about 24 ^oC, followed by the "methyl" sample at about 43 ^oC, whereas no gelation was observed with the other two samples up to 50 ^oC. This behavior was attributed to

the significant effect of the alkyl group length on the secondary conformation of the poly(L-alkyl-glutamate) blocks, that is the increase of side group length promoted conformational transition from *β*-sheet to α-helix that influenced the CGT.[83]

Thermosensitive hydrogels based on PEG-poly(γ-propargyl-L-glutamate) (PEG-PPLG) capable to be functionalized by azide-modified bioactive molecules, such as biotin and galactose, were designed and their biofunctionality was evaluated.[84] Concentrated solutions in PBS underwent sol-to-gel transitions upon heating. The transition temperature increased with decreasing the polypeptide length. The biofunctionalized copolymers retained sol−gel phase transition properties near body temperature. In contrast to biotin, the hydrophilic galactose promoted cell adhesion on the hydrogel surface.

Thermoresponsive polypeptides comprising OEgylated L-glutamic acid sequences [poly(L-EG_xGlu)],[85,86] where x represents the number of ethylene glycol repeating units, have been incorporated as building block in PHC to design tunable hydrogelators. In a series of PEG_{44}-Poly(L-EG_2Glu)$_x$ diblock copolymers, when the degree of polymerization (x) of Poly(L-EG_xGlu) was lower than 30 the copolymer self-assembled into nanoribbons forming a hydrogel whereas for x>40, it formed micelles with the polypeptide adopting mainly a helical conformation.[29,87] TEM, CD and FTIR suggested that the formation of sufficient *β*-sheet content accounted for the nanoribbon assemblies forming a continuous network. The thermosensitivity was manifested as mechanical strengthening on heating.

Further tuning of the LCST and the hydrogel properties of these PHC systems were attempted by replacing poly(L-EG_2Glu) with a random copolypeptide copolymerizing L-EG_2Glu with L-Alanine.[88] The PEG-Poly(L-EG_2Glu-*co*-L-Ala) diblock copolymers formed elastic hydrogels (C_P=7 wt %) at room temperature showing that the CGT is lower than 20 ^oC. Comparing the rheological properties of various gelators it was indicated that the increase of L-Alanine content resulted to an increase of elastic modulus. More importantly the hydrogel underwent gel-to-sol transition upon heating which contrasted to the behavior of conventional thermoresponsive (LCST-based) hydrogels. This was attributed to the weakening of hydrogen bonding that stabilizes the *β*-sheet structures responsible for the nanoribbon mediated network.

Star-shaped polypeptides comprising poly(L-EG_2Glu) arms emanated from a dendrimer core were designed as gelators. These stars can spontaneously self-

assemble into hydrogels in water. The hydrogel properties were strongly dependent on the arm length and arm number. For similar arm length, the sample with 32 arms had lower CGC than that with 8 arms. In contrast to the hydrogel system driven by β-sheet conformations, in the star polypeptide systems the ordered packing of rigid α-helices accounted for the formation of fibrils, which further entangled and branched to form 3D networks. The as formed hydrogels showed shear thinning and rapid recovery properties and could be used as injectable hydrogels for controlled peptide drug release systems.[89]

Another strategy involving star-shaped PHC is the functionalization of the arm ends of an 8armed PEG by collagen-like peptides which act as the physical crosslinks for the polymer system through triple helix formation. Three stars differing in the number of repeating triads [Pro-Hyp-Gly (POG) inspired from natural collagen] were evaluated. All the systems formed hydrogels at room temperature due to the formation of triple helices that interconnect the stars into a 3D network. Although the storage modulus is similar for the different samples, the thermal stability of the hydrogels affected by the length of the peptide conjugates. The triple helices of the peptides exhibit melting temperature (T_m) which increased with the peptide length. Thus the network disrupted upon heating, following the same trend as the T_m of the collagen peptides. [90]

Two component systems comprising two different macromolecules enabling to form a 3D network by specific complexation, constitutes another route to prepare peptide-based hydrogels. 4armed star PEG conjugated at the arm ends by short peptide motifs, capable to interact with heparin to form non-covalent hydrogels, have been designed and studied regarding tenability of gel properties and biofunctionality.[91] Mixtures of starPEG-peptide conjugates with 14kDa heparin at a 1:1 ratio in physiological PBS were prepared and evaluated. Hydrogels were formed when the peptide motifs, [$(BA)_n$ type: where B is a basic residue, either arginine or lysine] can form α-helical structures upon interacting, through electrostatic interactions, with heparin. Simple variables govern the system properties that can be easily tuned by changing the number of $(BA)_n$ repeats, by adjusting the concentration of each component, or by introducing simple mutations. More importantly, stable hydrogels can be formed in the presence of large quantities of cells in cell culture medium at 37 °C, and cells embedded within the hydrogel survive and are

metabolically active. More recently it was shown that this kind of hydrogels can be used as injectable drug delivery systems, capable to capture and release peptide tags.[92]

Inspired from the charge-driven coacervate hydrogels, formed by mixing of ABA with CBC conventional triblock copolymers, bearing oppositely charged anionic A and cationic B blocks in stoichiometric ratio,[10] a novel system comprising PGA-PEG-PGA and PLL-PEG-PLL ionic PHC have been reported recently.[93] Due to the strong and highly efficient electrostatic interactions, robust and high modulus non-fibrous hydrogels were obtained within a few seconds at concentrations as low as 3–5 wt % polymer after mixing the cationic and anionic PHC solutions. Physical association of the oppositely charged polypeptide end-blocks led to the formation of coacervate domains bridged by the PEG middle blocks, provided thus a 3D network formation which resulted in gelation. The hydrogel properties could be tuned by varying pH, polymer composition and concentration. An interesting unexpected effect opposed to the conventional systems, was the increase of storage modulus upon increasing ionic strength. This was attributed to the hydrophobic interactions exerted by the polypeptides after partial electrostatic screening that preserved the complexes. These peptide-based hydrogels can also encapsulate intact cells without significantly compromising cell viability, suggesting that they encompass excellent cytocompatibility. In vivo evaluation performed in rats with subcutaneous injection, indicated that the hydrogels were formed and degraded, while (H&E) staining suggested good biocompatibility in vivo (Fig 5).[93]

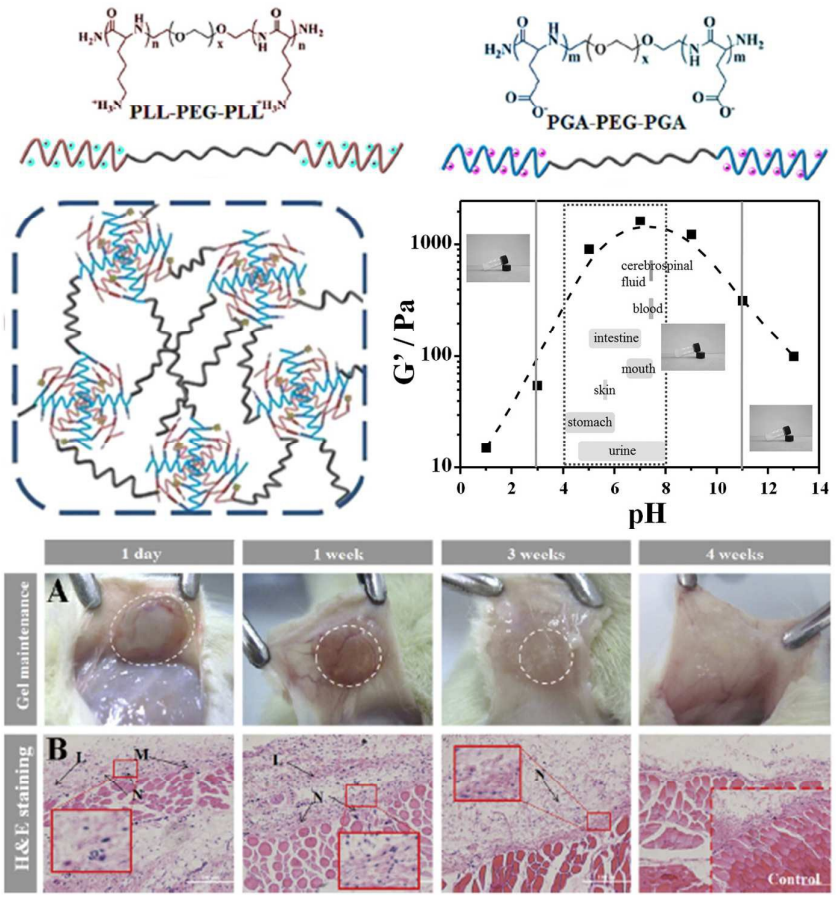


Figure 5. pH-responsive coacervate hydrogels from PGA-PEG-PGA/PLL-PEG-PLL mixtures. In vivo 10 wt% GA_{83}–EG_{91}–GA_{83}/LL_{78}–EG_{91}–LL_{78} hydrogel formation and degradation in the subcutaneous tissue at different intervals. (B) Images of H&E stained surrounding tissues at indicated days for the examination of the inflammation reaction (L: lymphocytes; M: macrophages; N: neutrophils). Scale bar, 200 lm. From ref. 93 with permission of Elsevier.

Physically crosslinked networks, formed by PLGA-PNIPAM-PLGA triblock PHC with a diamino-terminated PEO in an organic solvent through acid-base proton transfer and successive ionic binding, is another two component system that leads to interesting thermoresponsive hydrogels upon absorbing water. The PLGA polypeptides adopted α-helix conformation which was maintained after complexation, while the PNIPAM central block exhibited the known thermosensitivity. Thus the networks show reversible thermal responsiveness due to the desorption of water when heating the sample above the LCST of PNIPAM and fast swelling-deswelling processes can be easily achieved in aqueous media, which might be promising for drug delivery applications.[94]

PNIPAM has been used as thermosensitive block in an 8-arm PEG with 1 arm conjugated with PNIPAM and the other 7 arms conjugated with proline-rich peptide domains.[95] This associative star hybrid was used as a thermo-induced reinforcing secondary network formed *in situ* within a self-assembled shear-thinning hydrogel with peptide-based, physical crosslinks, in order to retard material biodegradation and to prolong cell retention time. Human adipose-derived stem cells were transplanted into the subcutaneous space of a murine model using hand-injection through a 28-gauge syringe needle. Cells delivered within the double-network hydrogel were significantly protected from mechanical damage and enhanced in vivo cell retention rates compared to delivery within saline and single network hydrogels. This strategy constitute a nice paradigm of the so-called adaptable hydrogels [96] that have recently emerged as a promising platform for 3D cell encapsulation and culture, enhancing thereby potential regenerative medicine therapies.

Block copolypeptides

A novel area, in the context of designing block copolypeptides capable to form self-assembling hydrogels, has been emerged the recent years.[97,98] Initially amphiphilic block copolymers comprising ionic hydrophilic long polypeptides covalent bonding with hydrophobic shorter blocks were synthesized by ring opening polymerization and sequential addition of α-amino-NCA monomers [99] and evaluated as gelators in aqueous media.[40,100-102] In particular, series of copolypeptides comprising positively charged poly(L-lysine, K) and hydrophobic poly(L-leucine, L), differing in macromolecular architecture (diblock, K_mL_n, triblock, $K_mL_nK_o$, pentablock $K_mL_nK_oL_nK_o$) and composition, were studied in terms of self-assembly mechanism and rheological properties. In the samples that formed hydrogels the macromolecules self-assemble into disorganized fibrillar supramolecular nanostructures (Fig 6). The poly(L-leucine) blocks, adopting α-helical/rod-like conformations, are packed perpendicular to the fibril long axes and the charged poly(L-lysine) blocks are arranged at the outer surface of the fibrils as a dense brush. The width of the fibrils is determined by the balance among the electrostatic repulsions, hydrophobic edge energy and degree of fibril twist. CryoTEM confirmed the fibrillar network as well as the coexistence of entangled/branched fibrils yielding percolated network with elastic properties. The degree of polymerization (DP) of both blocks is critical for the netwok (gel) formation. The length of L block must be

suitable to form stable α-helices (i.e. DP of L must be at least 20) and the length of K block must be long enough to avoid flat membrane formation (i.e. DP of K must be 100-150 minimum). At constant overall polypeptide DP, the gelation concentration decreases and the gel strength increases with increasing DP of the hydrophobic helix-forming block.[43] Furthermore, the macromolecular architecture, influences the network structure and in turn the rheological properties. The $K_mL_nK_oL_nK_m$ pentablock

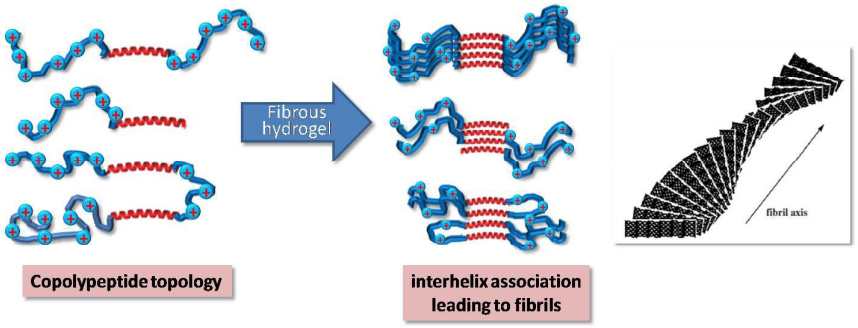


Figure 6. Schematic representation of the hierarchical self-assembly of copolypeptides constituted of the positively charged poly(L-lysine, K) and the hydrophobic poly(L-leucine, L), of deferent macromolecular topologies (diblock, K_mL_n, triblock, $K_mL_nK_o$, pentablock $K_mL_nK_oL_nK_o$) that form α-helical-based fibrillar supramolecular networks. Inspired from references 100-102.

architecture gave substantial enhancement of rheological properties, regarding the corresponding diblock and triblock counterparts due to bridging for long K_o central block.[44] Overall, the gel properties can be tuned by macromolecular design thanks to the flexibility of the synthetic procedure. These CPP-based tunable hydrogels exhibit a number of desirable properties, i.e. low gelation concentration, microporosity, injectability, degradability by enzymes and stability in the presence of ions, [103] making them good candidates for bioapplications. Recent *in vitro* and *in vivo* experiments showed that diblock K_mL_n CPP hydrogels (HG) can serve as depots for sustained local delivery of hydrophilic [104,105] and hydrophobic [106] effector molecules for investigative and potential therapeutic applications in healthy as well as in injured central nervous systems (Figure 7, inspired from ref 105).

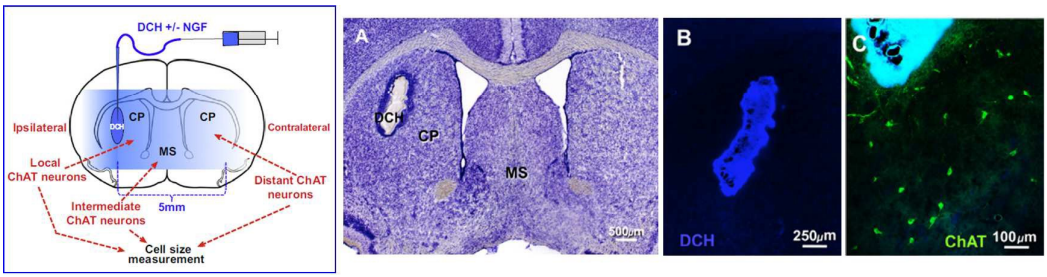


Figure 7. Schematic of experimental design to evaluate release of bioactive protein from HG depots in vivo. NGF was used as a bioactive protein known to induce hypertrophy of basal forebrain cholinergic neurons in the caudate putamen (CP) and medial septum (MS). Depots of HG with NGF were injected into the CP on one side. Appearance of HG depot and cholinergic neurons in mouse forebrain. (A) Cresyl violet stained mouse forebrain showing a typical HG depot in CP on one side at 1 week after HG injection. (B) Detail of HG depot labeled with conjugated blue fluorescent dye (AMCA-X) in tissue section neighboring to that shown in A. From ref 105 with permission of Elsevier.

Recently, nonionic CPP-based hydrogels, exhibiting reversible thermo responsiveness, have been designed following similar criteria, applied for the ionic copolypeptides previously described.[107] Diblock CPP composed of hydrophilic poly(γ-[2-(2-methoxyethoxy)ethyl]-*rac*-glutamate) (E^{P2}) and thermo-induced hydrophobic poly(γ-[2-(2-methoxyethoxy)ethyl]-L-glutamate-*stat*-L-leucine) segments self-assemble upon heating forming α-helix-based fibrous hydrogels. A key innovation in successful development of these thermoresponsive BCPP was the use of statistical copolymerization of thermo-sensitive residues E^{P2} with helicogenic hydrophobic residues (leucine), to prepare thermoresponsive segments that adopted stable α-helical conformations. Control of the composition of these segments allows tuning of the gelation temperature as has been shown in conventional block/random copolymers.[108,109]

Amphiphilic block CPPs comprising K (Lys) and L (Leu) residues, prepared by solid-phase peptide synthesis and thus having low DP (16-mers), showed significantly different self-assembly nanostructures with respect to those reported from the higher DP counterparts described above. The $L_4K_8L_4$ CPP, was hierarchically self-assembled into nanofibers based on stable β-sheet secondary conformations that were formed upon partial neutralization of the Lys residues at around pH 9. By tuning the peptide sequence and environmental conditions, such as solution pH, a rich polymorphism of 3D-nanostructures was obtained through self-assembly.[110] Therefore, it seems that beyond others the number of repeating units of

CPP, especially when passing to the oligopeptide regime, is critical since it affects remarkably the mechanism of hierarchical self-assembly.

In a later report of the same group, the same $L_4K_8L_4$ was integrated to a three armed star CPP and its self-assembling ability in aqueous media was investigated at different pH conditions. As revealed by a combination of analytical tools, the branched peptide showed a pH-dependent conformation forming shape-specific, β-sheet-based nanofibers with morphologically kinked structures under specific pH conditions.[111] Thus, exploring a novel peptide building unit of different topology (star vs linear) and understanding the relationship between molecular structures and the consequent morphology of the self-assembled nano-objects, constitutes a nice paradigm for bottom-up strategies towards fabrication of peptide-based fibrous 3D scaffolds.

Very recently a triblock CPP, composed of a pH-responsive poly(L-glutamic acid) (E) central block, end-capped by two hydrophobic poly(L-alanine) blocks (A) was synthesized by ring opening polymerization of protected amino acid N-carboxy anhydrides and sequential monomer addition using a NH_2-$(CH_2)_6$-NH_2 bifunctional initiator.[112] The peptide combination and its molecular peculiarity (N-termini at both ends and a hydrophobic spacer in the middle of the PGA central block) render this $[A_5E_6$-$(CH_2)_6$-$E_5A_5]$ CPP different from those reported above.

This CPP self-assembles in aqueous media, forming various nanostructures through hydrophobic and H-bonding interactions depending on the environmental conditions, i.e. pH, ionic strength and temperature. At elevated concentrations and at physiological pH and salinity conditions, the triblock aqueous solutions underwent a sol-gel transition only after heating and slow cooling thermal treatment, forming opaque stiff gels. Systematic investigation as a function of concentration revealed a phase diagram constituted of three regimes in the temperature versus concentration space, i.e transparent sol, opaque sol, and opaque gel. Yet a critical concentration of 4.5 wt% was determined. Below this value, clear solutions were obtained for all temperatures. This novel phase behavior was ascribed to a mechanism occurred in heat-induced denaturation of protein primary aggregates. Structural investigation by electron microscopy showed that the copolypeptide chains underwent hierarchical growth of fibrils into giant bundles of superfibers, that were disrupted and rearranged into a supermolecular network of twisted rigid superfibers upon thermal treatment. Interestingly, upon switching pH from 7.4 to 4 the β-sheet-based twisted nanofibers

were transformed to untwisted α-helix-based giant nanobelts (Fig. 8). It seems that subtle differentiation of the molecular structure regarding the similar $L_4K_8L_4$ topology of the previous system results to completely different nanostructuration.[112] At physiological conditions of interest, self-supporting opaque hydrogels were obtained characterized by high elasticity and fast recovery from shear thinning. In addition, upon tuning the CPP concentration, the gelation can be adjusted to physiological temperature, making it a promising candidate as biocompatible injectable hydrogel for biomedical applications, i.e. scaffolds for tissue engineering or controlled drug delivery.

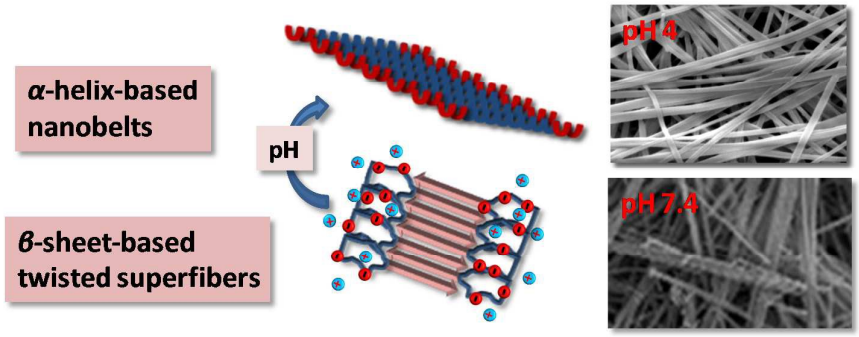


Figure 8. Schematic representation of the hierarchicaly self-assembled $Pala_5$-PGA_{11}-$Pala_5$ triblock copolypeptide at different pH. In the left, SEM images of the discovered structures after freeze drying of the peptide aqueous hydrogel formulations. Inspired from reference 112.

Concluding Remarks

This article highlights the recent advances on the subject of responsive, to pH and/or temperature, self-assembling hydrogels focusing on two types of gelators namely conventional block copolymers, bearing ionogenic repeating units, and polypeptide-based associative segmented (block) macromolecules.

Concerning the first category, several trends appeared in the last five years. One is dealing with macromolecules that associate intermolecularly through coulombic interactions between oppositely charged segments to create a 3D network. This strategy comprises two different directions namely co-assembly (mixture of different macromolecular chains enclosed oppositely charged segments) and self-assembly (block polyampholyte). In the first case, a stoichiometric ratio of the opposite moieties favors best network formation while in the second case, remarkable charge

asymmetry is needed. Both systems are sensitive to ionic strength and pH, when weak polyelectrolytes are involved, which affects the electrostatic interactions and in turn the gel properties. A drawback of the co-assembly with respect to self-assembly systems is that the polymer concentration needed for gelation is relatively high (ca 10 wt%), likely because of the non-ionized hydrophilic mid block of the involved triblocks.

Another interesting trend is the use of random copolymer building blocks in the place of homopolymers, which endows the gelators with additional combining properties.[107] For instance, by introducing weak ionic moieties in the thermo-sensitive associative blocks (which become stickers above their LCST) of a triblock gelator, the formed hydrogel is endowed with additional pH and ionic strength sensitivity. Moreover, the control of the mol percentage of the random blocks, can tune the LCST and in turn the thermo-inducing sol-gel transition that is critical for biomedical applications.

The control of the hydrophobicity of the stickers is another interesting example for tuning the rheological properties of the resulted hydrogels. This was achieved by replacing the hydrophobic end-block of a polyelectrolyte with statistical copolymer segments, comprising weak anionic repeating units. Thus by varying pH, the hydrophobic attraction of the stickers and thus their exchange dynamics can be affected remarkably. Therefore the gel properties, like the relaxation time (τ) of the network, can vary several orders of magnitude with the degree of ionization of the electrolyte moieties (controlled by pH) affecting strongly the viscoelasticity of the gel. Consequently a dynamic network (low τ) can be transformed to a kinetically frozen one (high τ) and vice versa simply by changing the pH of the medium.

As far as the peptide-based gelators are concerned, after the elucidation and improvement of the ring opening polymerization of the NCA-amino acids, which made easier the synthesis of polypeptide sequences, novel gelators were designed, targeting "smart" hydrogels with real biodegradability. We highlighted herein two types of these gelators namely polymer/peptide hybrids and copolypeptides. One of the main differences with the conventional copolymeric gelators is the network formation mechanism, which in most of the cases, leads to entangled nanofibers, either arisen from β-sheet or α-helix-based hierarchical self-assembly. In this case, the topology of the conventional triblock copolymers hydrophobic-hydrophilic-hydrophobic required to form a 3D network, is not a prerequisite.

Two main strategies were followed towards the design of hydrogelators constituted of conventional polymeric blocks and peptides. The first one led mainly to PEO-based PHCs that exhibited thermo-responsiveness, tuned by various factors namely length and nature of the peptide (either homo or random copolymer), including the choice of the ester pendant groups of the amino acid residues, the polymer/peptide composition and the macromolecular topology (diblock, triblock, star-shaped). The other one was dealing with graft copolymers composed of a polymer backbone grafted by protein-like peptides (capable to form either β-strands or α-helices) as the associative pendant blocks.

Moreover, the development of synthetic pathways toward well-defined block copolypeptides, of tunable molecular futures (e.g. narrow molecular weight distribution, end-group fidelity), allowed the design of hydrogelators endowed with responsiveness, that rely to peptide secondary structural transitions (e.g. random coil-to-α-helix) triggered by pH and/or ionic strength. In general, the choice of the amino acid sequences, determining the secondary structure that will be adopted at the desired environmental conditions and their topology on the macromolecule, are critical factors that affect the hierarchical self-assembly of these gelators toward fibrous 3D networks. In all cases we found very promising gel properties (e.g. injectability)[113, 114] that combined with their biodegredabilty and biofuncionality, renders these hydrogelators as very promising candidates for biomedical applications either as scaffolds for tissue engineering[115,116] or drug and protein delivery formulations.[117,118]

Taking advantage of the immense skills of conventional block copolymer hydrogelators, namely macromolecular design, self-assembling mechanism, gel rheological properties, responsiveness to various triggers and innovative applications, the development of novel self-assembling gelators integrating the new knowledge emerging from the peptide-based systems, opens new horizons towards bio-inspired technologies including tissue engineering, biomedical devices, sensors, biospecific interactive surfaces and so on. The future of the field of entirely biocompatible and biodegredable self-assembling hydrogels is thus promising. However new exciting challenges remain to be addressed, like for instance the deeper understanding of the principles of the hierarchically self assembly peptide-based systems, so as to be able to rationalize an effective macromolecular design for specific applications. Yet, the development of dynamic gelling system, inspired from the supramolecular chemistry,

by using reversible dynamic covalent bonding, will offer additional opportunities towards innovative "smart" biofunctional hydrogelators.

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Recent trends on responsive self-assembling hydrogels: from polyions to peptide-based polymeric gelators

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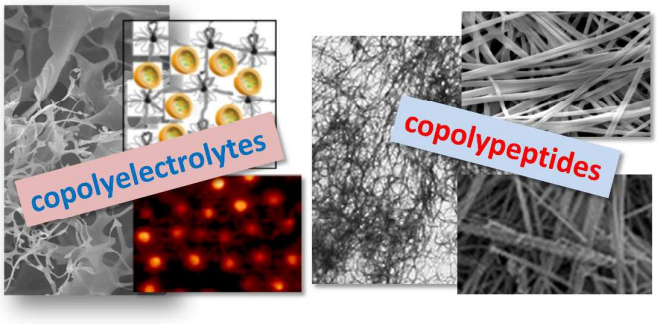

This review article highlights the recent advances on the pH and/or temperature responsive self-assembling hydrogels focusing on two types of gelators namely conventional block copolymers, bearing ionogenic repeating units, and polypeptide-based associative segmented (block) macromolecules.