Polymer-Peptide Conjugate Hydrogels; Towards Controlled Drug Delivery

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ABSTRACT

Peptide-based materials exhibit remarkable supramolecular self-assembling behaviour, owing to their overwhelming propensity to form hierarchical structures from α-helices and β-sheets. Coupling a peptide sequence to a synthetic polymer chain allows greater control over the final physical properties of the supramolecular material. So-called ‘polymer-peptide conjugates’ can be used to create biocompatible hydrogels which are held together by reversible physical interactions. Potentially, the hydrogels can be loaded with aqueous-based drug molecules, which can be injected into targeted sites in the body if they can exhibit a gel-sol-gel transition under application and removal of a shear force. In this review, we introduce this topic to readers new to the field of polymer-peptide conjugates, discussing common synthetic strategies and their self-assembling behaviour. The lack of examples of actual drug delivery applications from polymer-peptide conjugates is highlighted in an attempt to incite progress in this area.

Keywords: polymer-peptide conjugates, hydrogels, drug delivery

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1. INTRODUCTION

There is growing interest in combining natural polymers (especially proteins and peptides) with synthetic polymers [1-3]. The use of peptides allows for the incorporation of properties such as biocompatibility and self-assembly, whilst the synthetic polymer component in these materials allows control of physical and chemical properties, such as viscosity and smart behaviour. It has also been shown that the use of synthetic polymers can prolong the lifetimes of materials in the body. Supramolecular organisation of asymmetric polymer-peptide molecules offers a gateway to tailor-made materials suitable for a wide range of applications. So-called polymer-peptide conjugates (PPCs) can be designed to form hydrogels under certain conditions. Industrial viability of such materials increases as the number of amino acids in the peptide sequence decreases (fewer synthetic steps required). Here we will be discussing such PPCs, where the amino acid sequences are 25 or less in length, and their suitability for use in drug delivery. More specifically, this report highlights the lack of examples of PPCs as drug delivery devices, even though their properties appear ideal for such an application. This discussion is intended for those researchers who may be new to the field; as such it is not meant as a comprehensive review of the literature, but rather as an introduction to the area. Throughout this review the single letter abbreviation will be used for amino acids, for example phenylalanine is denoted simply as F. Table 1 lists the abbreviations used in this review.

1.1 Hydrogels

Hydrogels are three-dimensional structures comprised of a water phase immobilised by a scaffold [4]. Due to their similarity to hydrated body tissues, hydrogels are widely used as biomedical and pharmaceutical materials. The three-dimensional structures of hydrogels are networks of polymer chains which are held together by chemical or physical bonds (Figure 1). Chemically-bonded hydrogels are held in place by irreversible covalent bonds linking the polymer chains together. Physically-bonded hydrogels, on the other hand, are held together by reversible interactions such as molecular entanglements, ionic forces, $\pi-\pi$ stacking, hydrogen bonding, and van der Waals forces. Self-assembly into such supramolecular constructs occurs under favourable conditions. Self-assembly formation through noncovalent interactions leads to the spontaneous organisation of molecules into well-defined arrangements [5]. Self-assembling hydrogels have advantages over chemically-bonded hydrogels for application in drug delivery. Most notably,
Table 1. List of abbreviations used throughout this review.

<table>
<thead>
<tr>
<th>Abbreviations</th>
<th>Amino Acids</th>
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<tbody>
<tr>
<td>AGET</td>
<td>Activators Generated by Electron Transfer</td>
</tr>
<tr>
<td>ARGET</td>
<td>Activator Regenerated by Electron Transfer</td>
</tr>
<tr>
<td>ATRP</td>
<td>Atom Transfer Radical Polymerisation</td>
</tr>
<tr>
<td>CRP</td>
<td>Controlled Radical Polymerisation</td>
</tr>
<tr>
<td>CTA</td>
<td>Chain Transfer Agent</td>
</tr>
<tr>
<td>CuBr</td>
<td>copper (I) bromide</td>
</tr>
<tr>
<td>CuBr₂</td>
<td>copper (II) bromide</td>
</tr>
<tr>
<td>DCC</td>
<td>N,N-dicyclohexylcarbodiimide</td>
</tr>
<tr>
<td>DCM</td>
<td>dichloromethane</td>
</tr>
<tr>
<td>dmG</td>
<td>N,N-dimethylglycine</td>
</tr>
<tr>
<td>Fmoc</td>
<td>Fluorenylmethoxycarbonyl</td>
</tr>
<tr>
<td>GPC</td>
<td>Gel Permeation Chromatography</td>
</tr>
<tr>
<td>HEMA</td>
<td>2-(hydroxyethyl) methacrylate</td>
</tr>
<tr>
<td>ICAR</td>
<td>Initiators for Continuous Activator Regeneration</td>
</tr>
<tr>
<td>LCST</td>
<td>Lower Critical Solution Temperature</td>
</tr>
<tr>
<td>mPEO</td>
<td>monomethoxy poly(ethylene oxide)</td>
</tr>
<tr>
<td>nBA</td>
<td>N-butyl acrylate</td>
</tr>
<tr>
<td>NIPAAm</td>
<td>N-isopropylacrylamide (also poly[N-isopropylacrylamide])</td>
</tr>
<tr>
<td>oligoEO</td>
<td>oligo(ethylene oxide)</td>
</tr>
<tr>
<td>PEO</td>
<td>poly(ethylene oxide) / poly(ethylene glycol)</td>
</tr>
<tr>
<td>PHPMA</td>
<td>poly[N-(2-hydroxypropyl)methacrylamide]</td>
</tr>
<tr>
<td>PPC</td>
<td>Polymer-Peptide Conjugate</td>
</tr>
<tr>
<td>RAFT</td>
<td>Reversible Addition-Fragmentation Chain Transfer</td>
</tr>
<tr>
<td>SPPS</td>
<td>Solid Phase Peptide Synthesis</td>
</tr>
<tr>
<td>TFA</td>
<td>trifluoroacetic acid</td>
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Figure 1. Molecular structures of (a) chemically- and (b) physically-bound hydrogels.
physical bonding is reversible, meaning that these hydrogels can be rendered injectable (via a gel-sol-gel transition during injection).

1.2 Polymer-Peptide Conjugates

PPCs are hybrid materials which covalently combine peptide sequences with synthetic polymer chains (Figure 2). There has been a great deal of interest in the last twenty years or so on the combination of biological and synthetic polymers with a wide range of applications.

Many biological entities, e.g. oligonucleotides [6], saccharides [7] and lipids [8], have been investigated for conjugation with synthetic polymers, but it is amino acid-containing species as conjugates which have received the majority of attention [2]. While biologically-inspired polymers such as synthetic proteins and polypeptides have been conjugated to polymers, this review concerns the combination of the simplest biological units, those of short amino acid sequences (peptides) with polymers. The reader is directed elsewhere for work describing protein and polypeptide-based polymer conjugates [9-19]. Furthermore, the attachment of a hydrophobic alkyl chain to a hydrophilic peptidic segment to produce ‘peptide amphiphiles’ (PAs) are also not discussed [20]. However, it should be noted that this is not a reflection on the ability, or lack thereof, of these materials to act as drug delivery scaffolds/vehicles, but more to highlight the desperate need for progress in the area of polymer-peptide conjugates.

There are examples of biological polymer-synthetic polymer conjugates for drug delivery, where the biological entity itself may be the active species, i.e. a drug or pro-drug. The delivery of the peptide via this method is the essence of the Ringsdorf model of drug delivery [21], which can be traced back to a landmark paper in 1975 [22]. Alternatively, the peptide may be used for therapeutic targeting, for example via biorecognition of the peptide motif [23, 24]. Both of these strategies are beyond the scope of this review.

Herein we discuss PPCs which contain peptide sequences of no more than 25 amino acids. Generally, the greater the length of the amino acid chain, the more expensive the cost of synthesis. This is due to the increased number of synthetic and (potentially) purification steps, and is even more of a concern when considering commercial processes. Therefore control of self-assembly with the lowest number of amino acids is favourable [25]. There are several reports of hydrogel-forming PPCs with amino acid sequences greater than 25; selected examples include human fibrin coiled-coil sequences conjugated to PEO.

Figure 2. A schematic of a polymer-peptide conjugate.
[26], collagen-mimetic linked peptides to four-armed stars of PEO [27], and pentaheptad peptides joined to poly [(N-(2-hydroxypropyl)methacrylamide) (PHPMA)] [28, 29].

Jatzekewitz [30] published a report in 1954, on what is thought to be the first successful PPC, which sparked a steady interest in the literature for the following 30 years. However, in recent years the interest in PPCs has exploded as their potential applications have become realistic. This activity was increased further with incredible advances in coupling mechanisms and other synthetic strategies (discussed in Section 2) [31]. New materials are being produced with greater fidelity, greater purity and novel properties, which leads to an even broader range of potential applications.

One of the most interesting properties of these conjugates is their ability to self-assemble to form complex three dimensional networks. These networks can entangle to form an array nanostructures [4], which are physically-bound owing to the propensity of the peptide units to aggregate.

1.3 Polymer-Peptide Conjugate Hydrogels

PPC hydrogels are physically-bonded networks capable of imbibing large quantities of water. As with proteins, the physical bonding can lead to secondary structure formation, e.g. α-helices and β-sheets, which can lead to tertiary structure formation. Advantages of using PPC hydrogels for biomaterials include the combination of the best properties of peptides and those of synthetic polymers [1]. The peptide component provides increased functional control, well-defined homogeneous hierarchical structures, consistent mechanical properties, and supportive folding/unfolding transitions [1, 32]. It is the peptide component that drives the self-assembly in these materials. Synthetic polymers can provide enhanced biocompatibility (e.g. non-reactivity, low clearance times), enzymatic degradation resistance and adaptability [1, 32]. The combination of peptides and synthetic polymers can provide materials with properties superior to those of the individual components [1]. Common methods of self-assembly to give PPC hydrogels include; solvent exchange, direct rehydration, temperature-switch, and salt-triggered processes. Various structures can be obtained depending upon the conditions of assembly, e.g. temperature, solvent, or molar ratio of polymer to peptide.

1.4 Drug Delivery

Certain PPC hydrogels undergo a gel-sol transition under shear force, yet reform a gel on removal of the force, i.e. they are injectable [33]. Such behaviour is described as thixotropic, that is shear-thinning (viscous flow under shear stress) and recovery when relaxed (self-healing) [34], and is a highly desirable property for drug delivery applications. This is a major advantage over chemically cross-linked gels which require precise incision (and subsequent removal) for practical application in biological environments. Clearly, this behaviour is particularly useful for tissue engineering and drug delivery, where the application of the hydrogel through a syringe is highly desirable. In drug delivery, therapeutic molecules can be very easily incorporated by preparation of the precursor solution prior to injection [35]. Furthermore, PPC hydrogels can be designed so that they can be easily broken down into their unimolecular components after use and, as such, can both be passed from the body and have higher efficiency.
of delivery of the drug package (compared to drugs covalently bound to polymers) [36]. A major disadvantage of PPCs is that comprehensive design rules remain somewhat undefined.

With judicious choice of the peptide and polymer components, PPCs can be rendered stimuli-responsive. The hydrogel will thus respond to changes in the surrounding environment, e.g. physical (temperature, ionic strength and solvent), chemical (pH, salt effect) or biochemical (enzyme presence) [37]. On the appropriate trigger, stimuli-responsive materials change their characteristic properties; these transformations are normally reversible once the stimulus is removed. This stimuli-responsive behaviour of PPC hydrogels can be exploited as a mechanism for drug delivery.

The remainder of this review is divided into two parts; (i) a description of the methods commonly used to synthesise PPCs, and (ii) the potential application of PPC hydrogels for drug delivery where the PPC hydrogel is employed as a scaffold/reservoir for the active drug molecules or components.

2. SYNTHETIC STRATEGIES

The ability to choose a specific sequence of amino acids, alongside a range of synthetic polymers, allows for the design of various morphologies and materials, such as fibres [38-41], three-dimensional hydrogels [42-44], nanosponges [45, 46], microgels/nanogels [47, 48], vesicles [49] and micelles [50]. Peptide motifs allow self-assembly to occur in a manner in which the structural arrangements are well-defined, and in which the resulting properties are more controllable.

It should be noted, however, that amino acid and monomer selection design rules are not entirely straightforward, as structural analogues often behave very differently and are hugely affected by purity [51]. There is also a need to consider the effect of the species which are used in the synthesis of PPCs as these have been shown to affect hydrogel formation. For example, the protecting species fluorenylmethoxycarbonyl (Fmoc) is known to impact upon the formation of hydrogels [52].

There are many methods employed for the synthesis of the precursor peptides (i.e. amino acid coupling strategies) and polymers (i.e. polymerisation techniques) for use in PPCs which are beyond the scope of this paper and are detailed in the literature. Conjugation strategies fall into two distinct approaches: convergent and divergent. A convergent approach involves both building blocks synthesised separately and then joined via an additional ligation step. By contrast, a divergent approach entails either the modification of the peptide or polymer block allowing ‘growth’ of the other component from the first functional unit. Section 2.1 details convergent strategies, whilst Section 2.2 details divergent strategies. Figure 3a-d illustrates some of the most common coupling methods used to synthesise PPCs via a convergent approach, whilst Figure 3e demonstrates an example of a divergent approach.

2.1 Convergent Strategies (Direct Conjugation)

There are a range of methods available for introducing peptide sequences to synthetic polymers (Figure 3). Coupling can take place either in solution or solid phase. When dealing with peptides, most experimentalists tend to favour the solid phase approach for the peptide synthesis, and then will undergo conjugation in either
the solid or solution phase. The following section provides examples of common coupling methods used to link polymers to peptides; (i) succinimide, (ii) Schiff base, (iii) click chemistry, and (iv) thiol-maleimide. Complementary group conjugation is one of the most prevalent routes for coupling reactions. This approach involves exploitation of the reactivity of the amine (N-terminus) or carboxylic acid group(s) (C-terminus) of the peptide, and the end group of the synthetic polymer which can be functionalised during, or post, polymer synthesis. Further, any side groups with appropriate functionality can be targeted as potential conjugation sites.

2.1.1 Succinimide

A good example of complementary group conjugation is demonstrated using N-hydroxsuccinimide functional polymers (Figure 3a). Succinimidyl esters react readily with amines and are especially useful as they can couple to either the terminal amine group of a peptide, or be directed to an amine side group (in lysine-containing amino acid sequences) by using a protecting group strategy. Polymers can be functionalised with succinimidyl esters, such as succinimidyl carbonates with relative ease, and many are commercially available, with monomethoxypoly(ethylene oxide) derivatives being the most

Figure 3. Some of the most common convergent strategies used to synthesise polymer-peptide conjugates; (a) Succinimide coupling, (b) Schiff base coupling, (c) Azide-alkyne click chemistry, and (d) Thiol-maleimide coupling. (e) An example of a divergent approach synthesising a peptide sequence from a polymer support.
commonly used polymers for PPCs [53]. Further, it is also possible to modify the peptide to include a succinimidyl moiety and then directly couple this to an appropriately functionalised polymer [54]. Alternatively, benzotriazoles can be used, as they behave in a similar manner. Whilst the succinimidyl esters have a wide use, conjugating PEO-succinimidyl succinate introduces a second ester group in the polymer backbone [9, 55]. After conjugation to a peptide this group remains in the final product and is highly susceptible to hydrolysis, which would result in detachment of the PEO and the loss of its beneficial properties. This drawback applies to all conjugates containing readily hydrolysable groups, and must be considered during synthesis and conjugation, depending on their intended application.

2.1.2 Schiff base
Modifying the polymer end group to an aldehyde affords another potential conjugation route [31, 34] via reductive amination (Figure 3b). The coupling of the polymer-aldehyde derivative and peptide results in the formation of a Schiff base, which, when treated with a reducing agent (such as sodium cyanoborohydride), produces an amine (or an amide depending on the R group). Roberts reported work describing the partial selectivity of mPEO-propionaldehyde by modifying the pH [9, 56]. This resulted in the aldehyde group preferentially binding to the N-terminal amine, due to the more acidic nature of the α-amine in comparison with other nucleophilic species.

2.1.3 Click chemistry
Click chemistry is used to describe a set of orthogonal reactions amongst which is the widely reported copper-catalysed Huisgen reaction between azides and alkynes (Figure 3c) [57-65]. Click chemistry is noted for its robustness, insensitivity to a wide variety of functional groups, reliability, and ease of purification. This area of bioconjugation has received a large amount of attention. For example, van Dijk and co-workers [66] reported the conjugation between alkyne functionalised-PO and azide-functionalised alanyl-phenylalanyl-lysyl-(2-azidoethyl)-amide, in which the conjugation took place in the presence of sodium ascorbate and copper (II) sulfate. The resulting conjugate was star shaped and formed supramolecular hydrogels. Tzokova et al. reported the synthesis of short chain of PEO-peptide conjugates using click chemistry, but unlike the previous example, the peptide was modified to include the terminal alkyne group [67].

The major disadvantage of this approach is the catalyst system, which most commonly contains copper. This presents a problem for a system required to interact in a biological environment and as such, materials need to be extensively purified to ensure no toxic contaminants remain. Furthermore, amino acids have an affinity to complex with metals [68], which could affect the conjugation rate and make purification difficult.

2.1.4 Thiol-maleimide
Another common coupling strategy, which could be considered a “click” reaction, exploits the reactivity of the side group in cysteine; the thiol. The allyl or vinyl groups in maleimides can react with the thiol group in an addition reaction to form a stable carbon-sulfur-carbon linkage (Figure 3d). Cysteine, like lysine, can be engineered into the peptide sequence during synthesis. However, there is the potential problem
of forming disulfide bridges, which in general, are susceptible to degradation in a biological environment [69]. Further, the maleimide moiety can be hydrolysed and undergo ring opening to form maleic acid.

2.2 Divergent Strategies

It is possible to use a divergent strategy to synthesise PPCs where a sequence is built up from a polymer substrate (Figure 3e) or a polymer grown from a peptidic macroinitiator. Consequently, the following section is divided into two: peptide growth from polymers (2.2.1), and polymer growth from peptides (2.2.2). However, it should be noted that the latter approach is only possible (to date) using radical polymerisation systems due to their tolerance of biological groups, with the two most common CRP techniques being reversible addition-fragmentation chain transfer polymerisation (RAFT) [70], and atom transfer radical polymerisation (ATRP) [71, 72].

2.2.1 Peptide growth from polymers

There are a group of commercial resins that have PEO preloaded via an acid-labile linker (usually a benzyl ester). The length of the peptide chain however, heavily determines the solubility of the conjugate and makes isolation difficult [2]. For example, Hentschel reported conjugation involving bound PEO and free amino acids. Here the PEO was part of a Tentagel-PAP resin, and was functionalised with a terminal amine group [73]. Protected amino acids were directly conjugated to this group through standard Fmoc and DCC chemistry [74]. The newly formed PPC was then cleaved from the resin using TFA (Figure 3e). Using a resin makes isolation and purification easier, however scaling up can be more difficult due to associated costs.

2.2.2 Polymer growth from peptides

Monomers can be polymerised from a peptidic macroinitiator system, also referred to as a “grafting from” approach. This allows for easier purification as only unreacted reagents will need to be removed, as opposed to the “grafting to” method, in which the unreacted reagents may include the presynthesised polymer, peptide and coupling reagents. Controlled radical polymerisation (CRP) provides a useful approach toward the formation of well-defined polymers from peptidic initiators. CRP is commonly used in the synthesis of PPCs, either in the solution or solid phase, with the former favoured due to lack of control in the latter [75]. One of the greatest advantages of the CRP techniques arises from the ability to introduce functional groups on the chain end of the polymers via selection of appropriate functional reagents (e.g. initiators or chain transfer agents, CTA) or through post-polymerisation modification. Whilst such techniques are commonly exploited to produce functional polymers for conjugation (via the “grafting to” method), this part of the review focuses on CRP using peptidic initiators/CTAs only. Readers are directed to the work of Le Droumaguet and Nicolas for further reading on controlled radical techniques [31].

2.2.2.1 Atom transfer radical polymerisation

ATRP has been used in the synthesis of a whole host of designer materials, including polymer brushes [76, 77], pH-responsive vesicles [78], hybrid nanoparticles[79], macromonomers[80, 81], non-fouling gold surfaces [82] and many macromolecules used in biomedical applications [83]. Since the discovery of ATRP there has been an evolution of the
technique to overcome some of its initial drawbacks. These newer protocols include AGET [84], ARGET [85], and ICAR [86].

There are numerous examples of peptide-based ATRP initiators in the literature. One particular example by Rettig et al. [75] focused on the synthesis of a peptide macroinitiator which was used to prepare peptide-poly(n-butyl acrylate). Oligopeptide synthesis was performed using standard SPPS protocols [74] with the ATRP initiating group introduced at the terminal amine of the oligopeptide. This was followed by cleavage from the solid support. The oligopeptide macroinitiator initiated the polymerisation of nBA in degassed DMSO at 60°C, with a CuBr/CuBr₂/PMDETA catalyst system. CuBr₂ helped to mediate control of the reaction and PMDETA ensured that the amine groups (which can act as multidentate ligands) of the oligopeptide did not associate with the copper complex. This unwanted effect was observed when the authors increased the peptide concentration; a decrease in the overall rate of polymerisation was observed. GPC analysis of the final product revealed a molar mass dispersity of 1.19, highlighting a good level of control. Further, this highlights the tolerance of RAFT with respect to complex, multifunctional peptide structures.

2.2.2.3 Other polymerisation techniques

Preparation of thermoresponsive peptide-PNIPAAm hydrogels has recently been reported by Maslovskis et al. [89]. The authors used AIBN as an initiator for the free radical polymerisation of NIPAAm. Thiol-terminated oligopeptide was added to the reaction mixture as a chain transfer agent. Unreacted peptide was excluded from the final conjugate by dialysing against water for several days. It was found that varying the quantity of peptide offered control over the composition of the conjugate. However, as a controlled polymerisation technique was not used, the final conjugate was not as well-defined as those examples described in Sections 2.2.2.1 and 2.2.2.2.

3. POLYMER-PEPTIDE CONJUGATE HYDROGELS SUITABLE FOR DRUG DELIVERY

PPCs have a wide range of applications,
such as bio-sensors, artificial enzymes and photonic and nano-electronic devices [3]. PPC hydrogels have been investigated for a range of applications, including scaffolds for tissue engineering/regeneration [90]. Hydrogels have long been recognised as vehicles for drug delivery [91, 92], but self-assembling hydrogels may lead the way towards better hydrogel drug delivery vehicles [93], owing to the reversibility of their structure formation. Here we focus on the use of peptide motifs as structural design tools for the construction of hydrogels via nanostructure formation (e.g. tubes, fibrils and tapes). Most of the literature on PPCs discusses materials which have potential for application in drug delivery, but does not demonstrate their use as such. Thus, the following examples of PPC hydrogels have the potential for use as protective matrices/reservoirs in drug delivery applications. The following sections are divided into PPC hydrogels based on poly(ethylene oxide) (PEO) and PPC hydrogels based on other polymers.

3.1 Poly(ethylene oxide)-Based Conjugates

A wide range of polymers have been investigated for conjugation to peptides for pharmaceuticals, but those utilising poly(ethylene oxide) (PEO) have received the most attention [2, 36]. This is arguably due to the biocompatible properties of PEO [9, 53]. PEO is non-toxic, non-immunogenic, water soluble, inexpensive, has well known physicochemical properties, and is FDA approved [94]. Combining a biological entity with PEO is often referred to as PEGylation [95]. Although PEO has been successfully used by many groups, the lack of attachment sites is a potential drawback associated with its use for PPC hydrogel formation. Examples of PEO-containing PPCs are presented herein.

Tzokova et al. [67] demonstrated the synthesis of PEO-F$_4$ and the subsequent self-assembly into hydrogels via nanotube formation. The F$_4$ hydrophobic motif was shown to control the formation of self-assembly via antiparallel $\beta$-sheets and $\pi-\pi$ stacking of the phenyl groups. In an extension of this work the same group attempted to produce a set of design rules for PEO-peptide conjugates based on phenylalanine and valine [73]. Different length low molecular weight PEO samples (350, 1200, and 1800 Da) were conjugated to F$_4$ and V$_4$. This work indicated that both the PEO length and the nature of the peptide significantly affect the self-assembly of such PPCs. PEO-F$_4$ conjugates produced nanotubes, fibres, and wormlike micelles as the length of the PEO block was increased, respectively. For the homologous PEO-V$_4$ series, $\beta$-sheet formation dominated, and the self-assembled structures were closer to those formed by peptides alone regardless of the PEO chain length. Larger PEO (3000 and 5000 Da)-F$_4$ PPCs were synthesised by Castelletto and Hamley [96]. Low concentration aqueous solutions of these PPCs were shown to self-assemble, due to hydrophobic interactions of aromatic phenylalanine residues. At higher solution concentrations $\beta$-sheet formation was observed, where self-assembled straight fibril structures were seen. However, the analogous F$_2$-containing PPC (PEO 3000 Da), also synthesised by this group, did not exhibit self-assembly behaviour. This indicates that not only is a sufficient hydrophilic/hydrophobic balance needed, but the number of amino acid repeats must also be above a critical value for self-assembly to occur [96].

In other work, Hamley et al. [97] synthesised peptide-PEO-peptide PPCs with a central PEO component (1500 Da)
flanked by Y2 or F2 dipeptide sequences. The hydrophobic dipeptides were selected for their simplicity, and allow direct comparison with the F4 sequence. It was found that self-assembly was dependent on the nature of the dipeptides and the terminal-end group used (Fmoc or amine). Water insoluble PPCs were produced when both ends were capped with the hydrophobic Fmoc group. By contrast, PPCs end-capped with NH2 groups at both ends were soluble in water. However, neither of these materials gave rise to self-assembling PPCs. The telechelic PPCs (NH2-Y2-PEO-Y2-Fmoc and NH2-F2-PEO-F2-Fmoc) self-assembled via β-sheet formation, to produce fibril-based hydrogels. A balance of hydrophobic and hydrophilic end-capping was required for this application. NH2-Y2-PEO35-Y2-Fmoc was found to be thermoresponsive with a switchable transition near body temperature. This is clearly a valuable feature for a drug delivery device.

Börner and co-workers [38, 98] synthesised PEO-based (~3150 Da) PPCs containing the peptide sequence (TV)2. This peptide sequence was chosen as it is known to form β-sheet structures. Here a peptide-PEO-peptide structure was selected, but in this example a “template” bridged the peptide and polymer components (Figure 4). The template was designed to preorganise the peptide sequences into the appropriate geometry to enhance the aggregation tendencies of the PPC. Each peptide was end-capped with dmG, to yield a cationic PPC with an overall structure of [dmG-(TV)2-NH]2-template-PEO72 (Figure 4). The PPCs self-assembled to form nanofibres, which aggregated into well-defined bundles. This work suggested that these PPCs can be tailor-made to target different structures. In this example, it was noted that the fibres formed were stiff, and so may also have applications in biomaterial fibre-reinforcement. Hentschel et al. [99] expanded the investigation of TV peptide sequences. Here PPCs containing PEO (~3150 Da) and the (TV)4 sequence were synthesised. These PPCs self-assembled via β-sheet formation, but tape structures, rather than fibrils were formed. Secondary growth was occasionally noted on top of the primary tapes, and this is currently under further investigation. Interestingly the introduction of ester segments disrupted the amide backbone, which was re-established via selective rearrangement (O to N acyl switch, as illustrated in Figure 5) post-synthesis. This work demonstrated that the aggregation tendency (during the synthesis of the PPC) could be temporarily suppressed via the integration of multiple “switch-peptide” backbone defects, which further expands the peptide sequences available for synthesis.

A conjugate containing low molecular weight oligoEO (three repeat units) and a short peptide sequence (I3) was synthesised by Ganesh et al. [55, 100]. It was found that self-assembly occurred via β-sheet formation to give fibrils which entangled to produce gel-like aggregates. The PEO-peptide and PEO-peptide-PEO structures that were synthesised produced differences in secondary structure, and the conformation of these polymer-peptides was dependent on the concentration and the position of oxyethylene groups.

Substantially longer peptide sequences (>15 amino acids) have also been investigated. Rösler et al. [101], for example, synthesised PEO-peptide and PEO-peptide-PEO PPCs (Figure 6). PEO (2600 Da) was used in the PEO-peptide, with the addition of PEO (750 Da) for the PEO-peptide-PEO conjugates. The peptide
sequences were composed of alternating hydrophilic and hydrophobic α-amino acids (G[KL], K[Q], XLQLXLQGG, where X was D or Q). It was reported that the amphiphilic β-strand sequences mediated the self-assembly of these PPCs into lamellar superstructures. Hamley et al. [88] extended this work using a slightly different alternating sequence of hydrophilic and hydrophobic α-amino acids, which were based on the GELELEQQKLKLKLKG sequence, to synthesise PEO-peptide PPCs.

The polymers self-assembled to form rod-like structures in twisted, helical tapes, which further stacked into fibrillar rods. These examples illustrate that the combination of a longer peptide sequence containing biological motifs with a synthetic polymer provides self-assembling materials, with the potential to interface with biological systems.

Even longer peptide sequences (>20 amino acids) have been incorporated into PPCs. Two PPCs; one comprising of PEO

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**Figure 4.** A peptide-PEO-peptide structure, where a “template” bridges the peptide and polymer components introduced to control the orientation of self-assembly [38, 98], where V and T represent valine and threonine, respectively.

**Figure 5.** Reaction mechanism of O to N acyl switch used in the work of Hentschel et al. [99], where G, W, V and T represent glycine, tryptophan, valine and threonine, respectively.
(750 Da) and peptide sequences containing a folding motif (GEAK[LAEIEAK]$_2$LAEIY), and the second having a longer PEO component (2,000 Da) were investigated in the work by Vandermeulen et al. [102]. These PPCs favoured the formation of coiled-coils, and self-assembled into well-defined supramolecular aggregates. An equilibrium was established between the unimeric, dimeric and tetrameric coiled-coil assemblies, which was found to be dependent upon concentration, temperature, solvent, and the molecular weight of the PEO component. The same group carried out further examination of PPCs comprised of PEO and the LAEIEAK-based coiled-coil sequence [54]. This LAEIEAK sequence was used as a starting point for the work and a series of PPCs were obtained by exchanging amino acid residues in specific heptad repeat positions. These conjugates retained their ability to form coiled-coils, highlighting the dominant amino acids required for coiled-coil formation, and aggregates were formed under appropriate conditions. A degree of pH-sensitivity could be induced by alteration of the amino acid sequence. Further investigation of these polymers was proposed as being directed towards biomedical applications, in particular as drug delivery vehicles. Burkoth et al. [103, 104] also investigated PPCs containing peptides over 20 amino acids in length. Here, the conjugation of a synthetic amyloid peptide sequence (YEVHHQLVFFAEDVGSNKGAIIGL) to PEO (3000 Da) was investigated. These PPCs self-assembled via β-sheet formation to form fibrils. In this case, the PEO was thought to shield the C-terminus hydrophobic domain of the peptide sequence, preventing extensive fibril-fibril aggregation. Manipulation of this material during the synthesis (e.g. peptide sequence alteration) should allow substantial tertiary structural engineering.

### 3.2 Conjugates Comprising Alternative Polymers

Although PEO has been widely used in PPCs featured across the literature, other polymers have also have also received attention. Poly(N-isopropylacrylamide) (PNIPAAm) has been extensively used for biomaterials [105]. A PPC comprising of PNIPAAm and the octapeptide FEFEFKFK was reported by Tirelli and co-workers [106]. The peptide was selected as it is

![Figure 6. PEO-peptide and PEO-peptide-PEO polymer-peptide conjugates designed by Rössler et al [101].](image-url)
known to form β-sheet rich fibrillar hydrogels. This PPC had a molecular weight of 18.3 kDa composed of 98.5 mol% polymer and formed gels, where the gelation effect was attributed to the behaviour of the peptide. The hydrogel exhibited double thermo-responsive behaviour, which would be beneficial in drug delivery applications. Tirelli and co-workers [89] expanded the range of PNIPAAm-FEFEFKFK PPCs by using different molecular weight polymer components. In this work hydrogels were obtainable when using small amounts of the PPCs to dope pure PNIPAAm. This work demonstrated that polymer/PPC mixtures formed temperature-sensitive self-supporting hydrogels, when above a critical solution concentration. LCST transition was controlled by the PNIPAAm component and was not found to be significantly influenced by the presence of the peptide. The properties of the gels were tuneable via variation of the peptide or polymer component. For example, the elastic modulus of the conjugate was proportional to the quantity of peptide present in the macromolecules. Careful tuning of the molecular composition of these materials will allow hydrogels to be produced with properties specifically designed for drug delivery.

2-hydroxyethyl methacrylate (HEMA) is another polymer well known for its use in biomaterials [92]. A PPC comprised of HEMA and the peptide GRGDS was synthesised by Mei et al. [107], where the RGD component of this sequence is the well-studied cell-binding motif. The PPC was found to form hydrogels, although the mechanism of formation was not discussed. This work illustrates that the biocompatible polymer, HEMA, can be used in self-assembling PPCs, and potentially for drug delivery applications.

Poly[N-(2-hydroxypropyl)methacrylamide] (PHPMA) is a non-immunogenic, neutral, hydrophilic polymer which has been employed in anticancer drug delivery. Radu et al. [108] conjugated PHPMA to the amino acid sequence QQRFOWQFEQQ. This sequence was selected as it is known to form antiparallel β-sheets which self-organise into fibrils in aqueous solutions. The ability of the peptide to form β-sheet nanostructures was retained upon conjugation to low molecular weight PHPMA, demonstrating that PHPMA can be utilised as a self-assembling biomaterial, extending the range of polymer components available for PPCs.

4. SUMMARY

There is an incredible body of work behind the synthesis of polymer-peptide conjugates, with relatively recent discoveries in controlled radical polymerisation processes and ligation chemistries, alongside the long-standing methods in amino acid coupling. Similarly, there have been great advances in understanding and manipulating the self-assembling behaviour of PPCs. Although this area of research remains somewhat in its infancy, our understanding is frequently being expanded and a generic set of design rules for PPCs will be available in the near future. Consequently, we are now in a position to design macromolecules based on polymers and peptides with controllable properties in terms of routes to gelation and physical characteristics of the final hydrogel. The potential for injection of thixotropic biocompatible materials, coupled with their ability to accommodate copious volumes of aqueous media, renders them ideal scaffolds for targeted drug delivery. However, as discussed throughout this
review, there are still no reports to this end. It is our intention to incite progress in the use of relatively short polymer-peptide conjugates in the field of supramolecular drug delivery. With the toolkit of available materials ever-increasing in size and the ability to control the precise properties of the devices by macromolecular design of the individual components, this area of research is set to explode in terms of productivity in the upcoming years.

REFERENCES


