Thermosensitive block copolymer hydrogels based on poly(E-caprolactone) and polyethylene glycol for biomedical applications: State of the art and future perspectives

**Thermosensitive Block Copolymer Hydrogels based on Polycaprolactone and Polyethylene Glycol for Biomedical Applications: State of the Art and Future Perspectives**

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**Abstract**

This review focuses on the challenges associated with the design and development of injectable hydrogels of synthetic origin based on FDA approved blocks, such as polyethylene glycol (PEG) and polycaprolactone (PCL). An overview of recent studies on inverse thermosensitive PEG/PCL hydrogels is provided. These systems have been proposed to overcome the limitations of previously introduced degradable thermosensitive hydrogels (e.g. PEG/poly(lactide-co-glycolide) (PLGA) hydrogels). PEG/PCL hydrogels are advantageous due to their higher gel strength, slower degradation rate and availability in powder form. Particularly, triblock PEG/PCL copolymers have been widely investigated, with PCL-PEG-PCL (PCEC) hydrogels showing superior gel strength and slower degradation kinetics than PEG-PCL-PEG (PECE) hydrogels. Compared to triblock PEG/PCL copolymers, concentrated solutions of multiblock PEG/PCL copolymers were stable due to their slower crystallisation rate. However, the resulting hydrogel gel strength was low. Inverse thermosensitive triblock PEG/PCL hydrogels have been mainly applied in tissue engineering, to decrease tissue adherence or, in combination with bioactive molecules, to promote tissue regeneration. They have also found application as *in situ* drug delivery carriers. On the other hand, the wide potentialities of multiblock PEG/PCL hydrogels, associated with the stability of their water-based solutions under storage, their higher degradation time compared to triblock copolymer hydrogels and the possibility to insert bioactive building blocks along the copolymer chains, have not been fully exploited yet. A critical discussion is provided to highlight advantages and limitations of currently developed themosensitive PEG/PCL hydrogels, suggesting future strategies for the realisation of PEG/PCL-based copolymers with improved performance in the different application fields.

**Keywords**: injectable; inverse thermosensitive; hydrogels; poly(ε-caprolactone); poly(ethylene glycol)

## Introduction

Injectable materials have been widely investigated for tissue engineering applications as they can be applied through minimally invasive surgery and undergo gelling within the target tissue, organ or body cavity. Moreover, as compared to pre-formed implantable devices, injectable hydrogels are able to perfectly fill body defects or cavities. Among injectable systems, stimuli-sensitive polymeric hydrogels, including temperature-sensitive ones, have been widely investigated with potential application as drug, protein, cell delivery carriers and scaffolds for tissue engineering.[1,2,3,4] As compared to injectable materials with chemical gelation mechanism, sol-to-gel transition of temperature sensitive hydrogels occurs in mild conditions: it does not make use of potentially toxic chemical reagents (such as crosslinkers, organic solvents, catalysts and residual unreacted monomers) and is not accompanied by heat release.[1] Temperature-sensitive polymers are generally amphiphilic. In aqueous solution, at proper temperature and higher concentration than "critical gelation concentration" (CGC), they assume a micellar conformation. Subsequent temperature variation of the polymer solution may induce gel formation through micelle aggregation.[1,2] In detail, concentrated aqueous solutions of temperature-sensitive polymers may display an upper critical gelation temperature (UCGT) or a lower critical gelation temperature (LCGT), above which the system undergoes a sharp viscosity decrease (becoming a solution) or increase (becoming a gel), respectively.[4] Once formed, hydrogels with LCGT behaviour are stable in a temperature interval above LCGT ("gel window"); further temperature increase may induce gel-to-turbid sol transition or syneresis. Copolymers based on hydrophobic and hydrophilic segments of the same type may display UCGT or LCGT behaviour depending on the ratio between hydrophobic and hydrophilic segments, molecular weight of the segments and the polymer.[2] Hydrogels with LCGT behaviour, also called inverse thermo-responsive hydrogels based on biocompatible building blocks, show advantages over UCGT hydrogels as injectable systems for biomedical applications, as they are able to encapsulate drugs or cells in mild conditions, by dispersion in aqueous solution at ambient temperature followed by thermal gelation. Inverse thermoresponsive hydrogels of synthetic origin

offer several advantages respect to the ones of natural origin (e.g. based on modified cellulose[1] or chitosan[1]), such as chemical reproducibility, possibility to tailor their chemical properties according to the final requirements and longer *in vivo* persistence generally due to their resistance to enzymatic degradation. Poly(ethylene glycol) (PEG) is the hydrophilic block of many thermoresponsive hydrogels of synthetic origin due to its biocompatibility, FDA approval and its availability in a variety of telechelic end groups. Among PEG based thermoresponsive hydrogels, poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) copolymers (Pluronic) have been widely investigated, as they are available in a wide variety of molecular weights and compositions.[1,5] As a drawback, Pluronic hydrogels are non-biodegradable, poorly stable *in vivo* and unable to provide sustained drug delivery over more than just a few days.[1,6] In an attempt to develop biodegradable PEG-based injectable hydrogels with longer *in vivo* stability, block copolymers based on FDA approved degradable hydrophobic building blocks, such as poly(ε-caprolactone) (PCL) and poly(lactic acid-co-glycolic acid) (PLGA), have been introduced.[7-8910] Synthesised PLGA/PEG tri- and diblock copolymers with inverse thermosensitive behaviour at physiological temperature [1,2,11] were found to be completely reabsorbed *in vivo* approximately within 1-4 weeks, depending on their composition and topology, and to provide sustained release of both hydrophilic and hydrophobic drugs.[1,2] However, PLGA/PEG thermosensitive hydrogels suffer from some drawbacks: copolymers form a sticky paste at room temperature which makes difficult their handling, weighing and transferring; copolymer dissolution in water takes time (from one night to a few days); hydrogel mechanical properties are weak.[12,13,14] Recently, inverse thermoresponsive PEG/PCL hydrogels have attracted a wide interest as a possible alternative to PLGA/PEG thermoresponsive hydrogels. The semi-crystalline nature of PEG/PCL copolymers[16] makes them available in lyophilised powder form at room temperature, which simplifies their handling.[16] Moreover, PEG/PCL copolymers are easily solubilised in water, generally by heating the polymer dispersion in water at a superior temperature than copolymer melting temperature, followed by quenching in ice bath to prepare a transparent homogeneous solution.[16] In addition,

PCL blocks evidence a slower degradation rate than PLGA segments[1,2] which minimizes inflammatory reaction and increases the biostability of incorporated bioactive molecules (e.g. protein, drugs).[15] Finally, due to their partial crystallisation and superior hydrophobicity, PEG/PCL hydrogels display superior gel strength than thermoresponsive PLGA/PEG hydrogels with the same molecular structure (topology, molecular weight of blocks).[2,11,16] As here reviewed, due to their advantageous properties, a wide literature has employed PEG/PCL copolymers as injectable hydrogels for biomedical applications, such as injectable hydrogels for drug release, injectable bone cements in combination with inorganic materials, injectable scaffolds for soft tissue engineering in combination with natural polymers/peptides and anti-adhesive injectable hydrogels preventing post-operative adhesions. The aim of this work is to review the most recent studies on inverse temperature sensitive PEG/PCL block copolymers, focusing on tri- and multiblock copolymers, describing the synthesis methods for their preparation, their physicochemical and rheological properties, gelling mechanisms and main applications with the aim to discuss their potentialities and current limitations, suggesting possible improvements.

**Synthesis Methods**

According to their chemical structure, synthetic block copolymers consisting of PCL (A-block) and PEG (B-block) can be classified into AB diblock, ABA (PCEC) or BAB (PECE) triblock, multiblock, star-shaped and graft copolymers. In this paragraph, the common applied synthesis methods for ABA or BAB triblock and multiblock PEG/PCL copolymers are described. Triblock copolymers can be synthesised by different methods. One method makes use of diblock copolymers. The most widely used method to synthesise PCL-PEG diblock copolymers is ring-opening polymerisation from monomethoxy-PEG (MPEG) and ε-caprolactone (ε-CL), using a catalyst.[17] Stannous octoate $SnOct_2$ is often used as catalyst, mainly because of its high reactivity. American Food and Drug administration accepted it as food additive. Anyway, its cytotoxicity recently caused deep concern about biosafety of the materials synthesized by this catalyst. Seeking

for tin-free catalysts of highly biosafety (eg. Guanidine derivatives) is hence a new challenge in this field.[18] PCL-PEG diblock copolymers can also be obtained by reacting MPEG and PCL at high temperature (i.e. 185 °C), without any catalyst and any solvent.[19] PECE or PCEC triblock copolymers can be obtained by different procedures. PCEC copolymers are usually synthesised by ring-opening polymerisation from dihydroxy PEG and ε-CL using a catalyst, similarly to diblock synthesis (Figure 1a).[20,21,22] On the other hand, PECE copolymers can be obtained by coupling MPEG-PCL diblock copolymers with a coupling agent, as shown in Figure 1b.[23,24]. Both aliphatic and aromatic diisocyanates can be used as coupling reagents. Since the major problem in using an aromatic diisocyanate is the toxicity of the degradation products, aliphatic diisocyanates are generally preferred.[25] The most commonly used aliphatic diisocyanates are lysine diisocyanate (LDI), 1,4-butanediisocyanate (BDI) and hexamethylene diisocyanate (HDI).[18] PEG/PCL multiblock copolymers can be prepared by different methods. One method is based on the condensation reaction of equimolar amounts of PEG diacids and PCL diols for a few hours, at room temperature.[26,27] Another method is one-step condensation polymerisation of PEG and PCL in the presence of a coupling agent (diisocyanate) and a catalyst, as shown in Figure 1c.[28]

A two-steps process has been developed based on the initial synthesis of an isocianate (-NCO) end-capped prepolymer, by reaction of PCL diol with a coupling agent (diisocyanate) and a catalyst, under nitrogen atmosphere.[29] Then, PEG is added and the reaction is continued for 24 hours. An alternative two-step process is based on the initial synthesis of PCEC molecules which are then coupled through their terminal hydroxyl groups using terephtaloyl chloride.[16]

Yield of all coupling reactions described above is generally around 80%, indicating an high conversion ratio of the monomers.[18,30] Table 1 collects several types of PEG/PCL copolymers with inverse thermosensitive sol-gel transition, their chemical characteristics and method for synthesis.

## Physicochemical Characterisation

### Thermal Properties

Thermal properties of thermosensitive PEG/PCL block copolymers have been studied by differential scanning calorimetry (DSC). The thermal properties of as-prepared copolymers are useful to understand the correct procedure for their solubilisation: if the material is semi-crystalline at room temperature, solution is prepared by initial dispersion of material powders in water, followed by heating at higher temperature than copolymer melting temperature for enough time to melt the crystalline domains and final quenching to around 4°C.[16] This procedure allows complete copolymer solubilisation. Moreover, the presence of melting and/or glass transition temperature above room temperature in DSC scans of as-prepared copolymer indicates the lyophilizability of the copolymer aqueous solution to powder form, which is advantageous for its handling. In the case of triblock and multiblock PEG/PCL copolymers with low molecular weight PEG (< 1000 g/mol), two close melting endotherms and one exotherm have been recorded during the DSC heating and cooling scans, respectively. [31-32,33] The two melting endotherms have been attributed to the melting of PCL crystalline regions and the melting of recrystallised PCL domains, respectively, while the single exotherm has been attributed to PCL crystallisation. When PEG molecular weight was sufficiently high (≥ 1000 g/mol) in PEG/PCL copolymers, additional thermal events associated with melting and crystallisation of PEG domains could be observed in the DSC heating and cooling scans, respectively.[20,34] On the other hand, for thermosensitive PEG/PCL multiblock copolymers with low PCL:PEG molar ratio (< 3:7 mol:mol), synthesised from PEG with 1000 g/mol or 1500 g/mol molecular weight (showing UCGT behaviour), single melting endotherm and exotherm were observed in DSC scans, associated with melting and crystallisation of PEG segments, respectively.[28] Bae et al. compared the thermal properties of a triblock and a multiblock PEG/PCL copolymer, the latter obtained by coupling the previously synthesised triblock copolymer.[16] In both cases, DSC heating scan revealed a double melting peak while DSC cooling scan showed the

presence of a single exotherm (Figure 2a). Further characterisation by X-ray diffraction analysis demonstrated that, in both cases, PCL segments partially crystallised, whereas PEG segments were amorphous. However, melting enthalpy and crystallisation temperature were significantly reduced in the case of the multiblock copolymer, suggesting its lower crystallisation rate and crystallinity degree. This finding suggests that multiblock copolymers can be more easily solubilised than triblock copolymers due to their lower crystalline fraction.

Thermal properties of copolymer aqueous solutions are interesting as they provide information on any interference of copolymer crystallisation with copolymer solution gelation. However, only few studies are available on thermal properties of copolymer aqueous solutions and gels. Bae et al.[16] investigated the thermal behaviour of concentrated solutions of three- and multiblock PEG/PCL copolymers, incubated at room temperature for 1 h. PCEC opaque gel formed during 1 h incubation of the concentrated solutions at room temperature: DSC analysis of the gel evidenced the presence of an endothermic peak in the heating scan (Figure 2b). On the contrary, the transparent solution of PEG/PCL multiblock copolymer did not show any thermal transition, due to the slower crystallisation rate of the multiblock copolymer compared to PCEC (Figure 2b). Therefore, differently from PEG/PCL multiblock hydrogels, gelling mechanism of concentrated solutions of triblock PEG/PCL copolymers may be influenced by crystallisation, due to their relatively fast crystallisation rate compared to multiblock copolymers. Similarly, Payyappilly et al.[35] showed that PECE hydrogels (30 % concentration) partially crystallised, showing a melting peak at around 39°C (Figure 2c). As a consequence, aqueous solutions of PECE and PCEC copolymers showed time- and temperature-dependant gelling mechanism, whereas multiblock PEG/PCL copolymer hydrogels only evidenced temperature sensitive gelation.[16]

**Sol–Gel Transition.**

Tube Inverting Test

The tube inverting test is the most commonly used method to characterise the sol-gel-sol transitions of LCGT systems. It is based on the preparation of copolymer solutions with varying concentrations within vials. Once prepared, each solution is subjected to a controlled heating to a certain temperature and kept at this temperature for a certain time, after which the vial is inverted. The sol or gel state is assessed by observing if the sample respectively flows or does not flow because of its weight, within a defined period of time (generally 1 min). Result interpretation is critical as a viscous sol and a gel with a low yield point can be hardly distinguished from a gel or a sol, respectively.[23,36,37] Results are influenced by sample volume and dimensions of the vial,[23,36,37] therefore a comparative evaluation of previous reports on tube inverting test is complicated by heterogeneity of experimental conditions. In a set of experiments, comparable results can be obtained by testing samples with the same weight and using vials with the same geometry and size. The inversion time is another fundamental parameter to be carefully selected: a viscous sol can move only a few millimeters within a short inversion time and can be wrongly defined as a gel. To avoid similar mistakes, a sufficiently extended inversion time have to be selected.[36] By analysing samples prepared in the same conditions, the tube inverting test provides a tool to obtain a temperature-concentration phase diagram (Figure 3a). If the polymer concentration is higher than CGC, the sample undergoes a sol-to-gel transition with increasing temperature over LCGT. A further temperature increase over UCGT determines a gel-to-sol transition. Several research groups investigated the effects of different factors, such as copolymer chemical composition and solubilisation parameters, on the sol-gel-sol transitions of PEG/PCL block copolymers.[20,21,23,24 32,38-39,40] Table 2 summarises the effects of these factors on the sol-gel-sol transitions of PECE and PCEC copolymers. At the same copolymer molecular weight and PEG/PCL ratio, PCEC copolymers are characterised by a larger gel window and a lower CGC than PECE copolymers. In

PCEC copolymers, PCL-block aggregation occurs at lower temperatures than in PECE triblock copolymers and, as a consequence, hydrophobic interactions responsible for sol-gel transition occur at lower temperatures.[20,37] In both PECE and PCEC copolymers, an increase in PCL molecular weight (being PEG molecular weight kept constant) makes the gel region wider and CGC lower, because the micellar structure becomes more stable and breaks at higher temperatures with increasing PCL block length.[20] In contrast, longer PEG blocks hamper micelle formation, thus resulting in a higher sol-gel transition temperature. The effects of total molecular weight increase (being PEG/PCL ratio kept constant) are the results of the concomitant increase in both PCL and PEG molecular weight.[20] In both PCEC- and PECE-based aqueous solutions an increase in concentration results in LCGT decrease, because micelle packing and aggregation occur at lower temperatures. Both salting-out and salting-in salts affect the gelation process in a concentration-dependant manner (influence increases with increasing salt concentration).

Salting-out salts (i.e. NaCl) cause gelation window shift to lower temperatures, without affecting CGC. Salting-in salt (i.e. NaSCN) effect is opposite. Salting-out salts reduce hydrogen bonds between water and copolymer chains, enhancing the hydrophobic interactions between micelles and leading to a sol-to-gel transition at a lower temperature.[24,40] On the contrary, salting-in salts are capable of solubilising the hydrophobic PCL block in water, thus inducing gelation at higher temperatures. PEG addition (concentration ranging between 0.2 wt% and 5 wt%) to an aqueous PECE copolymer solution induces a shift of the gelation window to lower temperatures, because PEG chains act as bridges between PECE-based micelles, thus leading to enhanced intermicellar interactions and gelation at lower temperatures.[24,40] Gel-to-sol transition measured during cooling evidences an hysteresis respect to sol-to-gel transition measured during heating and the gelation window shifts to lower temperatures in both PECE- and PCEC-based solutions.[23,24,32] Hysteresis has been attributed to two main reasons: (i) PCL crystallisation in water solution is very slow and may occur only after long time (this also explains the effect of aging time on gelation behaviour[16,23,24]) and (ii) hydrophobic interactions of the melt and swollen PCL block clusters

become weaker on cooling.[23,24, 32] Finally, also solubilisation temperature affects the sol-gel-sol transitions, since different solubilisation protocols result in different heating processes during tube inverting tests and a different degree of crystallinity of the solubilised polymer.[23]

On the contrary, PEG/PCL multiblock copolymers have not been sufficiently investigated up to date. In general, sol-gel-sol transitions of PEG/PCL multiblock copolymers depend on several concurrent parameters, such as molecular weight of the whole polymer, PEG/PCL ratio, composition, diisocyanate/diol ratio.[28] For instance, CGC and UCGT of PEG/PCL multiblock copolymer hydrogels have been found to decrease and increase with increasing molecular weight, respectively.[27] In addition, Bae et al. have reported that phase diagram of PEG/PCL multiblock copolymer aqueous solutions was independent on solution annealing time at 10°C, due to their low crystallisation rate.[16] Further information has been not provided suggesting the need for a future more in-deep study on thermosensitive PEG/PCL multiblock copolymer hydrogels.

Rheological Characterisation.

Rheological tests are carried out to study the sol–gel–sol phase transition of LCGT systems by analysing the behaviour of the storage modulus (G') and loss modulus (G'') as a function of temperature (temperature ramp test), generally by means of a parallel plate rheometer. Plates with different diameter and various gaps between the plates have been used; data have been generally collected under a controlled stress, at a predefined frequency (typically 4 dyn/cm$^2$ and 1 rad/s, respectively) and using different heating rates.[20,38] Sol–gel–sol transition of aqueous copolymer solutions can be identified by a marked change in G' and G'' moduli. In sol state, the value of G' is relatively low (G'<G''): when sol–gel transition occurs, the value of G' increases and the temperature at which G' becomes higher than G'' is defined as the temperature of sol–gel transition. For a certain temperature interval above sol-gel transition temperature, G' and G'' values are approximately stable. However, at higher temperature, the value of G' dramatically decreases and the temperature at which G' becomes lower than G'' is defined as the gel–sol transition

temperature.[20,38,41] Figure 3b reports the behaviour of G' as a function of temperature (temperature ramp test) for PEG-PCL-PEG (550-2190-550 Da) copolymer solutions at different concentrations.[38] The rheological behaviour of the system depends on various factors: 1) the concentration of the aqueous copolymer solution;[41] 2) the physicochemical properties of the copolymer (molecular weight of the copolymer and of the single blocks; ratio between hydrophilic and hydrophobic blocks[20]) and 3) type of copolymer (di-; three-, multiblock copolymer).[42] For instance, the rheological properties of two different triblock copolymers, PCEC (980-1000-980 Da) and PECE (550-2190-550 Da), having similar overall molecular weight and total PEG and PCL block length, were investigated.[43] In detail, at the same aqueous solution concentration of the copolymers (20 % (w/v)), the PCEC-type formed micelles with loops or intermicellar bridges (as discussed in "Gelation mechanisms" paragraph) which were responsible for higher G' than PECE – type (10000-20000 Pa vs 100 Pa). Bae et al.[16] compared the rheological behaviour of a PCEC and a PEG/PCL multiblock copolymer containing PCEC units. In the gel state, G' of PCEC and the multiblock copolymer was around 10000 Pa and 100 Pa, respectively. The higher PCEC copolymer gel strength was attributed to copolymer partial crystallisation during gelling, as described in "Thermal properties" paragraph. In the gel state, G' and G'' showed similar values, suggesting the semisolid nature of the gel.[16] Soft tissue elasticity ranges from myocardial tissue (~ 45 Pa)[44], spinal cord tissue (50 Pa for bovine and 200 Pa for human tissue)[45], brain (200-1000 Pa)[46], fat (2500-3500 Pa), striated muscle (~10000 Pa)[46], cartilage (~20000-30000 Pa) [46] to precalcified bone (~ 40000 Pa).[46] PEG/PCL hydrogels are potentially suitable for the delivery of cells and/or therapeutic molecules for the treatment of soft tissues with similar mechanical properties, such as myocardial tissue, spinal cord and brain for PECE and PEG/PCL multiblock hydrogels and striated muscle and cartilage in the case of PCEC hydrogels.

Rheological characterization is also helpful to measure the molecular weight of hydrogel chains between the crosslinks ($M_c$), according to the following equation[35]:

$$M_c = \rho \cdot R \cdot T / G'$$

Where G' is the elastic modulus of the linear region of the modulus vs. stress sweep plot, ρ is polymer concentration in the hydrogel, R is molar gas constant and T is the test temperature. $M_c$ decreases with increasing polymer concentration in hydrogel, affecting hydrogel permeability to molecules. The value of $M_c$ can be used to calculate the hydrogel mesh size according to Canal and Peppas equation.[47, 48]

Gelation Mechanisms

Although gelation mechanism of PEG/PCL copolymers differs depending on their chemical structure, amphiphilicity is the key property for temperature dependent gelation. Due to their chemical composition, after solubilisation in water-based solvents, PEG/PCL copolymers take micelle-like conformation provided with hydrophobic core and hydrophilic shell, to minimise the free energy of the system.[49] Micelle formation is possible at higher concentration and temperature than CGC and critical micellisation temperature (CMT), respectively. Following micellisation, temperature induced gelation occurs according to different mechanisms depending on polymeric chemical structure.2 PECE copolymers in water-based solution at lower temperatures than LCGT form regular micelles with an internal core of PCL and an external shell of PEG.2 At constant temperature, micelles remain in a dynamic equilibrium with unimers.[50] By increasing temperature, micelle hydrophobic core is partially dehydrated and micelles become more rigid. A further increase in temperature leads to gelation at LCGT with destruction of the spherical shape of micelles.[50] Gelation of PECE copolymers is mainly driven by hydrophobic interactions between PCL blocks, that aggregate each other to minimise their surface contact with water (Figure 4a). Aggregation forces depend on entropy, which increases with increasing temperature over LCGT.[51] Relatively long PEG blocks interfere with hydrophobic interactions, thus decreasing sol-gel temperature due to a steric effect.[52]

PCEC copolymers show a similar sol-gel transition with increasing temperature, although the gelation mechanism is slightly different. After solubilisation in an aqueous medium, at temperature

below the LCGT, they form micelles with possible intermicellar PEG bridges.2 By increasing temperature, a network of micelles forms leading to gelation (Figure 4b). Even for PCEC copolymers, hydrophobic interactions between PCL are responsible for gel formation. Not all PEG chains form intermicellar bridges in PCEC hydrogels: some of them simply bend outside a micelle while PCL blocks arrange into the core of the same micelle. By increasing temperature above UCGT, both PECE and PCEC gels undergo a gel-to-turbid sol transition, with destruction of micellar structure.2

Due to their complex chemical structure, PEG/PCL multiblock copolymers, after solubilisation in an aqueous medium, take a different conformation than PECE and PCEC copolymers. Figure 4c shows the associated micelle model proposed by Loh et al. with polymeric chain entanglements into a network structure.[53] By increasing temperature over LCGT, gelation occurs, driven by hydrophobic interactions between PCL blocks.[54] The temperature at which PEG/PCL copolymers undergo sol-gel transition mainly depends on polymer concentration and hydrophobic block length. At increasing polymer concentration and PCL block length, hydrophobic interactions increase leading to a decrease of gelation temperature.[55]

While for PEG/PCL triblock copolymers, crystallisation may interfere with hydrogel formation,[16] the slower crystallisation rate[16] of PEG/PCL multiblock copolymers accounts for a crystallisation-independent gelation mechanism.

## In Vitro and in Vivo Degradation Properties

### *In Vitro Degradation of PCL-PEG Copolymers*

Chemical degradation of hydrogels can occur via hydrolytic and enzymatic mechanisms, simultaneously present *in vivo*. Hydrolysis is a passive reaction while enzymatic degradation is driven by specific enzymes able to accelerate the degradation process, allowing a closer reproduction of *in vivo* conditions.[17,56] *In vitro* hydrolytic degradation tests have been generally

carried out by sample incubation in phosphate buffered saline (PBS), with medium refreshment at predefined time intervals and sample analysis in terms of molecular weight, weight loss and thermal properties. Huang et al.[57] reported that, during *in vitro* hydrolytic degradation test, the molecular weight of PCL-PEG-PCL copolymers showed a monomodal distribution which average value continuously decreased as a function of time. This behaviour suggested the occurrence of homogeneous hydrolysis. Soluble oligomers formed during degradation were responsible for the measured time dependent sample weight loss. *In vitro* hydrolytic degradation is affected by many experimental factors. In detail, degradation time decreases with decreasing the initial polymer molecular weight, with increasing test temperature and in acidic media.[20,38,56] In *in vitro* enzymatic degradation tests, lipase has been generally used due to its ability to selectively degrade the ester bonds of PCL.[58, 59] Li et al.[59] studied the degradation of PCL and PEG/PCL diblock and triblock copolymers and demonstrated that PEG incorporation did not alter enzymatic degradation properties. *In vitro* degradation tests are not intended to substitute *in vivo* tests. Indeed, due to the high complexity of body fluids that is hard to be reproduced *in vitro*, it is common to obtain different results when polymer degradation studies are carried out both *in vitro* and *in vivo*. Nevertheless, *in vitro* analysis may (i) provide preliminary information about biomaterial degradation rate that could be useful to plan *in vivo* studies and (ii) isolate the contribution of one body fluid component on polymer degradation kinetics, as in *in vitro* enzymatic tests with a selected enzyme, such as lipase.

*In Vivo Degradation of PCL-PEG Copolymers*

*In vivo* degradation tests have been performed to get an insight into the material behaviour after implantation: several *in vivo* factors, such as pH, enzymes, mechanical stress and temperature, influence degradation kinetics.[17] Tests have been usually carried out through subcutaneous injection of the concentrated copolymer solution into rats. Histological analysis have been generally conducted at different time intervals to monitor degradation.[38,60] For example, Ma et al.[21] studied *in*

*vivo* gel formation and degradation of a concentrated solution of a PCEC copolymer ($M_n$: 4200 Da; 20%), injected into the dorsal area of Kunming experimental mice through a 25-G needle. A gel immediately formed at the injection site. Gel size decreased during the period of observation and disappeared 45 days after injection. On the other hand, degradation behaviour of a PECE hydrogel ($M_n$: 3408 Da; 30%), injected subcutaneously in rats, was also evaluated.[61] At predetermined time steps, the rats were sacrificed, the injection site was carefully cut and opened and photos of the *in situ* formed gel were taken. PECE hydrogel was found to completely degrade within 2 weeks. To authors' knowledge, *in vivo* degradation experiments on PEG/PCL multiblock copolymer hydrogels have not been reported. However, due to the higher molecular weight of PEG/PCL multiblock copolymers respect to triblock copolymers (Table 1), it can be expected that the formers show a longer *in vivo* persistence time.

**Main Applications**

Concentrated solutions of amphiphilic PEG/PCL copolymers have been mainly used as injectable systems for drug delivery and tissue engineering. Having a micelle-like structure, gels may incorporate both hydrophobic drugs, which can be solubilised into the micelle core, and hydrophilic drugs, which can be loaded into the micelle shell.[20,21,32,62,63] Concerning drug delivery kinetics, release of low molecular weight hydrophilic drugs such as timolol maleate (TM)[40] and vitamin B12 ($VB_{12}$)[24] generally showed significant initial burst release (>20%) and complete release within one week due to drug solubilisation in the hydrophilic micelle shell and the larger hydrogel mesh size as compared to the hydrodynamic drug diameter (Figure 5a). Release rate of low molecular weight hydrophilic drugs was affected by two major factors, i.e. hydrophobic/hydrophilic balance and total molecular weight of the polymer: release kinetics was found to decrease with increasing total molecular weight and the hydrophobic/ hydrophilic ratio[40]. The release of hydrophilic drugs can be controlled by additives. Mishra et al.[40] used poly(vinyl alcohol) (PVA) and PCL additives to modify TM release rate from PECE hydrogels. The use of polymer additives was effective only on a

copolymer with moderate hydrophobic aggregation tendency ($PEG_{750}$-$PCL_{3750}$-$PEG_{750}$). Hydrophilic high molecular weight PVA reduced hydrogel pore size, whereas hydrophobic low molecular weight PCL promoted micelle aggregation. As TM is hydrophilic, its release rate was affected by PVA addition, leading to reduced burst release (from 44.9±0.4% to 22.3±1.9%) and cumulative release within 316 h (from 86.4±0.8% to 73.7±1.8%).

On the other hand, release of protein drugs was slower as compared to low molecular weight hydrophilic drugs, due to their higher drug hydrodynamic diameter and the possible intermolecular interactions (mainly through Van der Walls interactions) occurring between the high molecular weight drug and the hydrogel.[32] Moreover, hydrogels were found to protect bovine serum albumin (BSA), used as a model protein drug, from degradation in an acidic environment and to preserve its bioactivity within the release time.[24] Payyappilly et al.[35] evaluated the release of insulin from a PECE hydrogel. Insulin release kinetics decreased with increasing PECE hydrogel concentration from 15 % to 30% due to a reduction of the hydrogel network mesh size (Figure 5b). Moreover, the hydrogel preserved insulin bioactivity, as suggested by far-UV circular dichroism analysis which showed unchanged secondary structure of released insulin. Finally, PECE hydrogel was semicrystalline with a melting peak at about 39-40°C, therefore an increase in temperature from 37°C to 40°C caused an increase in the insulin diffusion coefficient as schematically shown in Figure 6. Therefore, PECE hydrogel could be promising for a pulsatile drug release following a pulsatile change in temperature.

Finally, the release of hydrophobic drugs from PCL/PEG hydrogels has also been evaluated.[20] Hydrophobic drugs were mostly solubilised in the micelle hydrophobic core of the hydrogel, which slowed down their cumulative release rate as compared to hydrophilic drugs.[20]

Injectable hydrogels for drug release are particularly advantageous for cancer treatment, as they allow localised drug delivery through a minimally invasive treatment. Anticancer drugs such as doxorubicin, paclitaxel and honokiol, are hydrophobic agents. Wang et al.[64] have proposed two different approaches for the incorporation of hydrophobic drugs within PEG/PCL hydrogels (Figure

7). According to the traditional method (formulation-1), hydrophobic drug is dispersed into a previously prepared copolymer solution, which is then converted into a gel by increasing the temperature to 37°C. An alternative method (formulation-2) has been proposed based on the preliminary preparation of drug-loaded copolymer micelles. Subsequently, particles are solubilised and gel is formed by increasing temperature to 37°C. Formulation-2 method is promising for clinical application. However, easily solubilised copolymers at mild conditions (e.g. at room temperature) should be employed to avoid degradation of temperature sensitive drugs. For this reason, Wang et al.[64] have synthesised modified PCL-PEG-PCL copolymers with progressively reduced hydrophobicity, crystallinity degree and melting temperature, by the incorporation of increased amounts of 1,4,8-trioxa[4.6]spiro-9-undecanone moieties into PCL blocks (PETC copolymers).

Recently, PCEC hydrogels have also been proposed for a combined local radiotherapy and chemotherapy, using a radioactive nuclide (Re-188-Tin colloid) and a chemotherapeutic drug (liposomal doxorubicin).[65] Both agents showed a sustained release for at least 10 d. The system was tested *in vivo* by intratumoral injection in mice with hepatocellular carcinoma, showing advantages in focusing radiotherapy and chemotherapy locally, with 80% tumour growth inhibition within 32 days.

In tissue engineering applications, injectable PEG/PCL hydrogels have been used as antiadhesive materials, mainly to prevent post-surgical abdominal adhesion.[34,66,67,68] Undesirable adhesions after surgery may induce severe complications, such as pain, functional obstruction and difficult re-operative surgery. Degradable injectable hydrogels are advantageous as antiadhesive materials as they are easy to be applied. Kim et al. prepared PECE/hyaluronic acid blends (0.1-2 wt.% hyaluronic acid) to be used as injectable antiadhesive hydrogels.[34] PECE/hyaluronic acid hydrogels showed accelerated gelation as compared to pure PECE hydrogels due to an entropy loss effect, attributed to attractive interaction between hyaluronic acid and PEG chains and their confinement into the PECE micelle shell (entropy loss). Moreover, PECE/hyaluronic acid hydrogels showed

lower mechanical properties than PECE hydrogels attributed to a weaker micellar aggregation tendency.

On the other hand, due to their intrinsically porous structure and biomimetic hydrated nature, injectable PEG/PCL hydrogels have been used as injectable scaffolds in combination with bioactive materials. For instance, thermosensitive hydrogels have received much attention in bone tissue engineering. As an example, PECE hydrogels were added with nano-hydroxyapatite powder (10-30 wt.%) to prepare injectable materials for bone tissue engineering.[69] The same authors added collagen into nano-hydroxyapatite/PECE hydrogel to improve material potentialities for bone regeneration.[70] Composites with 60 wt.% PECE, 10 wt.% nano-hydroxyapatite and 30 wt.% collagen preserved their thermosensitivity. Osteogenic capacity was assessed by injecting the composite hydrogel into cranial defects of New Zealand white rabbits up to 20 weeks.

Ni et al. [71,72] have prepared an injectable acellular bone matrix (ABM)/PECE composite hydrogel to enhance bone regeneration in cranial defects, due to osteoinductive properties of ABM.

Table 3 and 4 collect main examples of drug delivery and tissue engineering applications of PEG/PCL triblock copolymer injectable hydrogels, respectively.

**Discussion**

Several PCEC and PECE triblock copolymers were synthesised with inverse thermosensitive properties of their concentrated solutions at physiological temperature. PECE and PCEC copolymers showed $M_n$ of around 2000-8500 Da and PEG:PCL ratio of 1:1-1:4 wt.:wt. (Tables 1, 3, 4). PCEC copolymers resulted advantageous over PECE copolymers as they could be synthesised in one step without the use of a coupling reagent, they showed a wider "gel window" (i.e. temperature interval of gel stability), a longer *in vivo* resorption time (up to 6 weeks[61]) and a superior gel strength.[43] Despite the great potential of reverse thermoresponsive triblock PEG/PCL systems as injectable hydrogels for tissue engineering and drug delivery, one major disadvantage is represented by the short stability of the sol phase at ambient temperature, due to progressive crystallisation of

dissolved copolymer, leading to the formation of an opaque gel ("low-temperature gel").[16] Multiblock PEG/PCL copolymers were introduced both to increase the *in vivo* persistence time of the injectable hydrogels due to their higher molecular weight (Table 1) and to slow down crystallisation rate of copolymer blocks, as it could interfere with the gelling mechanism.[16] In general, in PEG/PCL copolymers, both PCL or PEG segments can crystallise depending on PEG:PCL ratio and single block molecular weight.[28] However, crystallisation rate and crystallinity degree of multiblock copolymers are significantly lower than those of triblock copolymers, due to their superior molecular weight. In addition, the use of rigid or cumbersome coupling molecules between the blocks, such as terephthaloyl chloride, may further hinder crystallisation, increasing the stability of the sol phase.[16] On the other hand, although water solutions of triblock PEG/PCL copolymers were found to be stable at room temperature for a limited time-period at 37°C (< 1 h for $PCL_{1000}$-$PEG_{1000}$-$PCL_{1000}$[16]), they converted into gels with higher storage modulus than that of PEG/PCL multiblock copolymer (~10000 Pa vs. ~100 Pa [16]). For this reason, triblock PCEC copolymers could be advantageous for applications where gels with higher modulus are required, such as the therapeutic treatment of striated muscles and cartilage. On the other hand, PECE and PEG/PCL multiblock copolymers showed suitable gel strength to be used for the treatment of myocardial tissue, spinal cord and brain.

As compared to amorphous PEG/PLGA copolymers, which form a sticky paste at room temperature, the semi-crystalline nature of PEG/PCL block copolymers allows material preparation in powder form, which is not only advantageous for material handling, weighing and transferring, but also for the preparation of freeze-dried pharmaceutical formulations to be re-dissolved to prepare hydrogels.

One limitation of thermogelling PEG/PCL hydrogels as carriers for drug delivery arises from burst release, especially in the case of hydrophilic low molecular weight drugs. The use of hydrophobic or hydrophilic polymeric additives interacting with PEG/PCL copolymer can affect sol-to-gel state transitions and allows a modulation of drug delivery kinetics.[40] On the other hand, a simplified

method to incorporate hydrophobic drugs within injectable hydrogels could be based on drug loading into copolymer micellar particles before their solubilisation.[64] Although the method improves drug loading within the hydrogel, it is not suitable for highly crystalline copolymers requiring a heating-quenching method for solubilisation. For this reason, PEG/PCL triblock copolymers should be modified by copolymerisation to decrease their hydrophobicity and crystallinity degree to apply this drug loading method.[64]

Moreover, due to their neutral properties, PEG/PCL copolymers were found to be sub-optimal for the delivery of ionic proteins, peptides or DNA. For such applications, hydrogels which are both pH and temperature sensitive could be advantageous: at low (cationic hydrogels) or high (anionic hydrogels) pH, they are in a sol-state and possess ionized groups that may interact with encapsulated charged molecules; on the other hand, at physiological conditions (37°C; pH: 7.4), they become gels.[73]

Another general disadvantage of amphiphilic copolymers containing polyester units is their sensitivity to hydrolysis: for this reason, storage conditions should avoid copolymer degradation and, once prepared, hydrogels should be administered within a short time. Additives such as $Mg(OH)_2$ could be used to reduce *in vivo* degradation rate by neutralization of acidic products of polyester block degradation.[74]

To the authors' knowledge, reports on possible applications of thermosensitive PEG/PCL multiblock hydrogels are lacking in the scientific literature. Due to their higher molecular weight and expected longer degradation time, they could be suitable for tissue engineering applications. Although multiblock PEG/PCL copolymers can be synthesised using a diisocyanate as a coupling reagent between the blocks,[28] synthesis of polyurethanes incorporating combinations of PEG and PCL diols, or di- / triblock PEG/PCL copolymers in associations with different chain extender blocks could allow the tailoring of the physicochemical properties of the injectable material depending on the final application.

## Conclusion

Injectable PEG/PCL hydrogels have been extensively studied. PECE hydrogels showed lower gel strength and shorter *in vivo* stability than PCEC hydrogels. Due to their different degradation rate, PECE and PCEC hydrogels were applied as *in situ* drug delivery carriers for rapid or sustained drug release, respectively. Moreover, both PECE and PCEC copolymers were used as injectable anti-adhesive tissue interfaces or blended with bioactive components to obtain injectable scaffolds. Due to the different gel strength, each of these systems is suitable for the treatment of tissues with distinct mechanical properties. A few PEG/PCL multiblock copolymer hydrogels were also prepared and characterised. Although their potential biomedical uses have not been investigated up to now, they could be promising as injectable scaffolds for soft tissue regeneration, due to their relatively slow degradation rate and low gel strength. Their properties could be enhanced through functionalisation or blending with bioactive molecules guiding cell behaviour.

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**Figures**

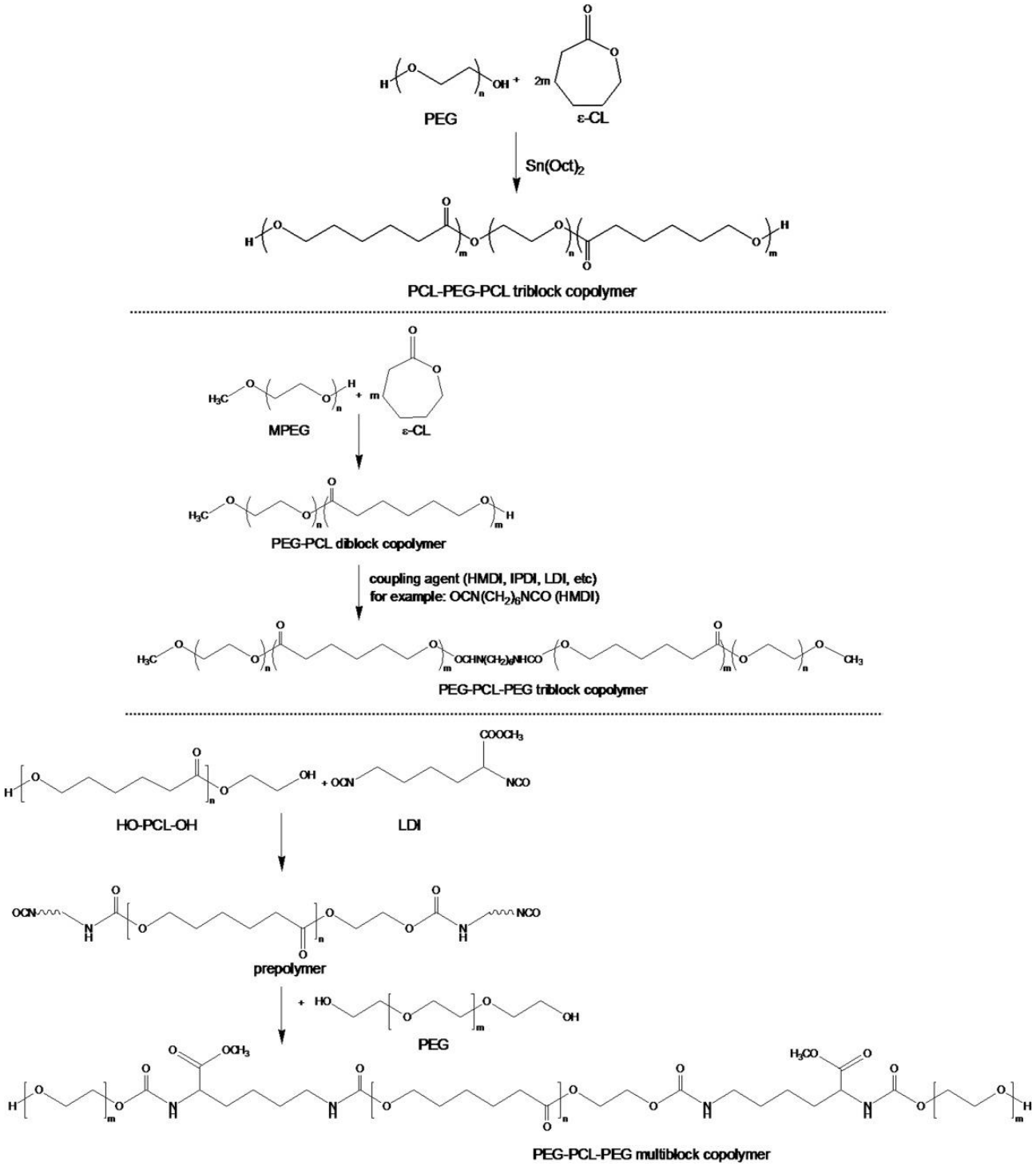


*Figure 1*. Schemes of chemical synthesis for: a) PCEC: ring opening polymerisation of ε-CL using PEG and a catalyst. (Reprinted with permission.[22] Copyright 2007, Wiley Periodicals); b) PECE: reaction between PEG-PCL diblock copolymer, previously obtained by ring opening polymerisation of ε-CL using MPEG and a catalyst, and a diisocyanate coupling agent. (Reprinted with permission.[17] Copyright 2009, Elsevier); c) multiblock PEG/PCL copolymers: reaction of PCL diol with a diisocynate, leading to NCO terminated prepolymer which is then made to react with PEG diol. (Reprinted with permission.[29] Copyright 2005, Elsevier).

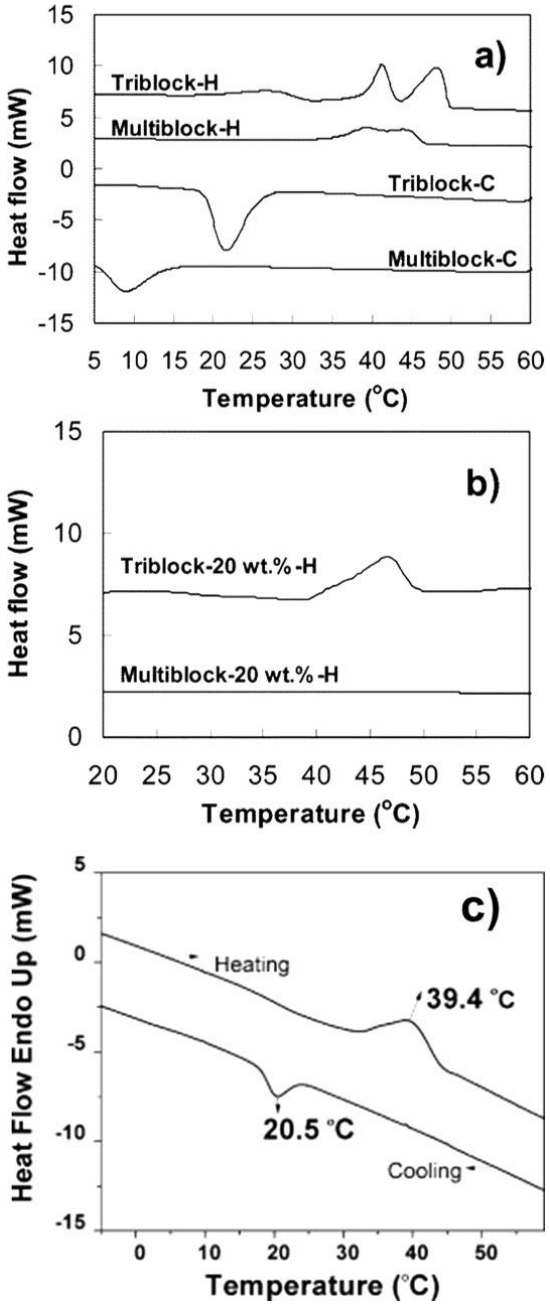


*Figure 2.* DSC heating and cooling traces for: (a) PCEC and PEG/PCL multiblock copolymers and (b) their aqueous solutions with 20 % (wt./v) concentration, after storage at room temperature for 1 h (Reprinted with permission.[16] Copyright © 2006, American Chemical Society); (c) PECE hydrogels (Reprinted with permission.[35] Copyright © 2013 Wiley Periodicals, Inc.). H indicates the first heating scan, C indicates subsequent cooling scan.

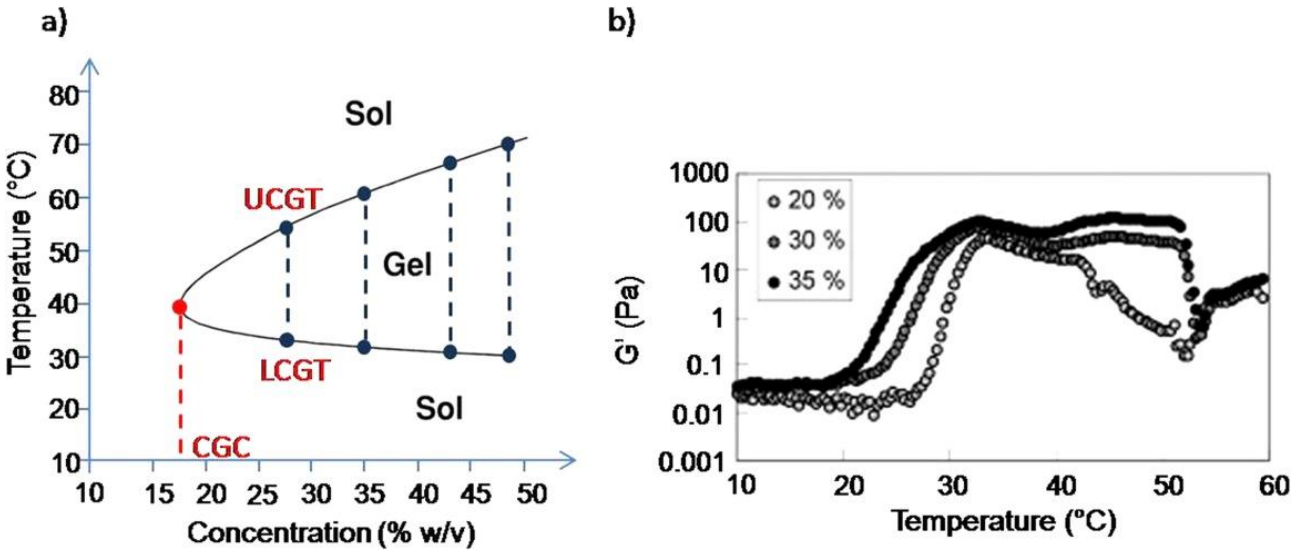


*Figure 3*. Characterisation of sol-gel-sol transition of LCGT hydrogels: a) phase diagram obtained by tube inverting test; b) rheological characterisation of PECE hydrogels: behaviour of G' as a function of temperature (temperature ramp test) and solution concentration. (Reprinted with permission.[38] Copyright 2005, American Chemical Society).

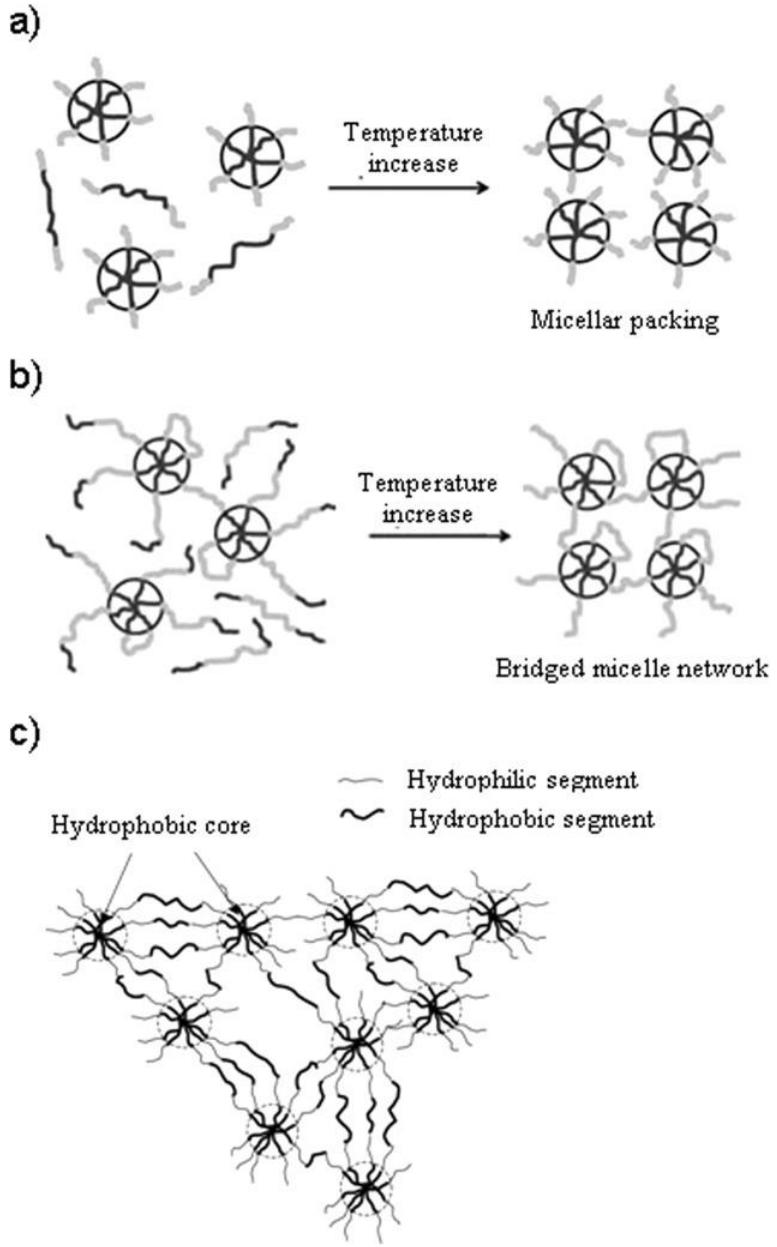


*Figure 4*. Gelation mechanism of a) PECE copolymers.(Reprinted with permission.[2] Copyright 2010, WILEY-VCH Verlag GmbH & Co. KGaA); b) PCEC copolymers (Reprinted with permission.[2] Copyright 2010, WILEY-VCH Verlag GmbH & Co. KGaA); c) PEG/PCL multiblock copolymers.(Adapted with permission.[53] Copyright 2007, American Chemical Society).

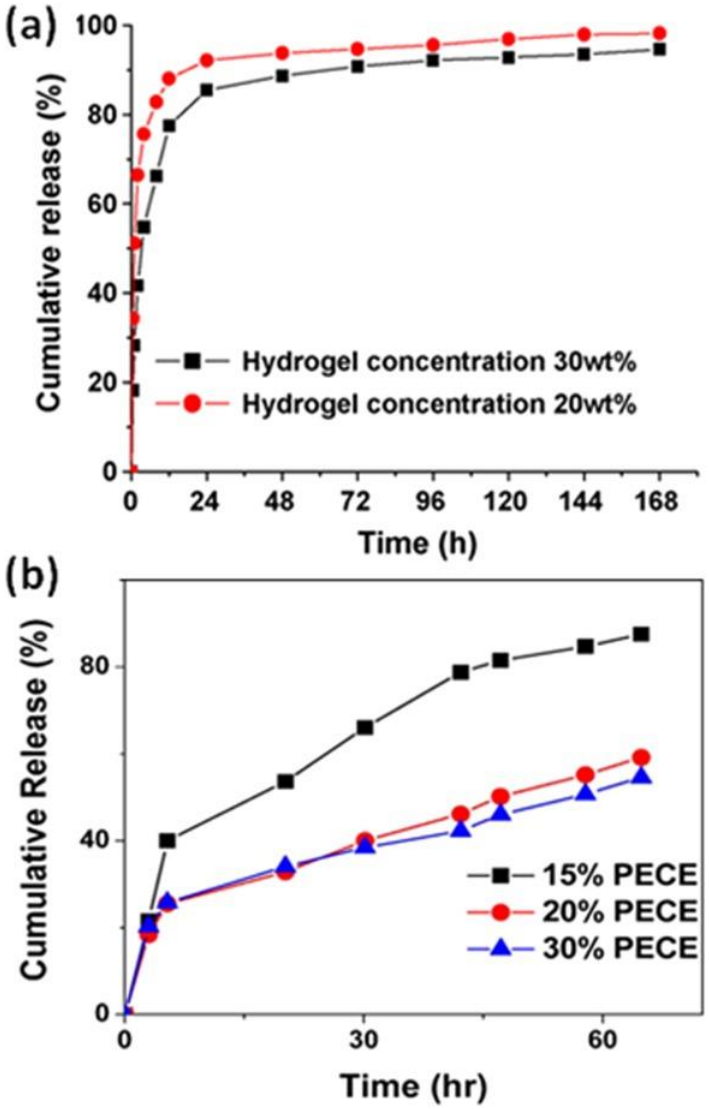


*Figure 5*. Controlled release of vitamin B12 (reprinted with permission.[32] Copyright 2009, Elsevier), (a) and insulin from PECE hydrogels with different concentrations (Reprinted with permission.[35] Copyright © 2013 Wiley Periodicals, Inc.) (b). Colour figure can be viewed in the online issue.

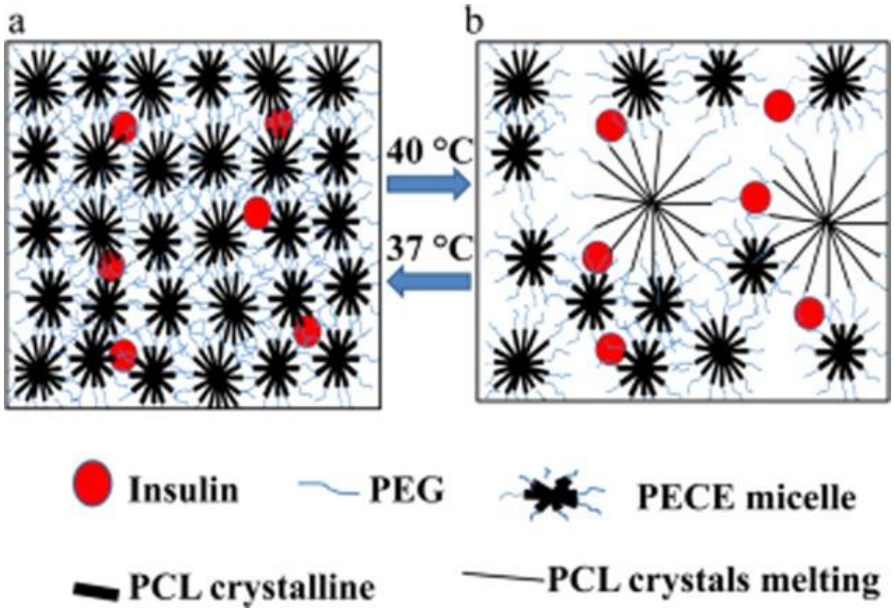


*Figure 6.* Schematic representation of release of incorporated insulin from a PECE hydrogels at (a) 34°C and (b) 40°C. At 40°C, crystalline domains melt, increasing hydrogel mesh size. Colour figure can be viewed in the online issue. (Reprinted with permission.[35] Copyright 2007, American Chemical Society).

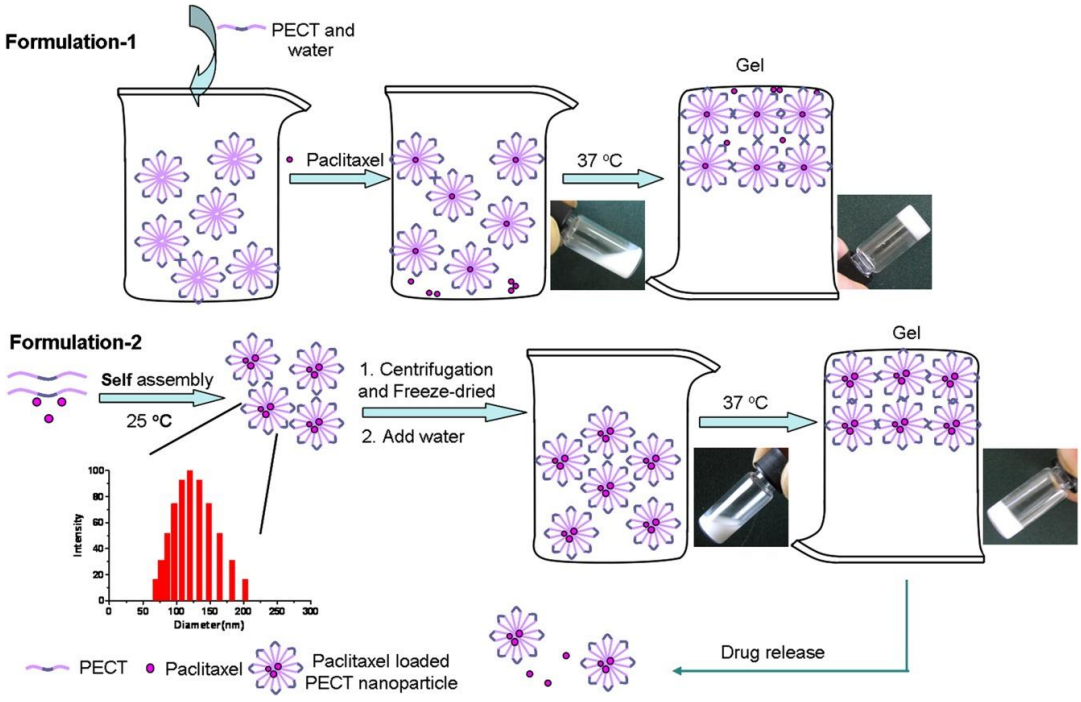


*Figure 7.* Schematic representation of possible incorporation of paclitaxel within PETC thermosensitive hydrogels. (Reprinted with permission. [64] Copyright 2012, Elsevier). Colour figure can be viewed in the online issue.

*Table 1.* Main chemical features and methods of synthesis of PEG/PCL triblock and multiblock copolymers exhibiting an inverse thermosensitive sol-gel transition.

| **Copolymer molecular weight ($M_n$) (g/mol)** | **PCL block molecular weight (g/mol)** | **PEG block molecular weight (g/mol)** | **PEG:PCL ratio (wt:wt)** | **Polydispersity index** | **Synthesis method** | **Ref.** |
|---|---|---|---|---|---|---|
| | **PEG/PCL triblock copolymers** | | | | | |
| | **PCL-PEG-PCL** | | | | | |
| 2500[b] | 700 | 1000 | 1: 1.5[b] | 1.36[b] | Ring-opening copolymerisation of ε-CL initiated by PEG using $Sn(Oct)_2$ as the catalyst. | 20 |
| 3120[b] | 1000 | 1000 | 1:2.12[b] | 1.51 [b] | | |
| 3520[b] | 1000 | 1500 | 1: 1.35[b] | 1.25 [b] | | |
| 4500[b] | 1500 | 1500 | 1: 2[b] | 1.56 [b] | | |
| 2760[b] | 1000 | 600 | 1: 3.6[b] | 1.67 [b] | | |
| 4000[b] | 1100 | 1500 | 1: 1.67 [b] | 1.2 [b] | As above. | 21 |
| 4200[b] | 1250 | 1500 | 1: 1.8 [b] | 1.3 [b] | | |
| 4560[b] | 1500 | 1500 | 1: 2.04[b] | 1.3 [b] | | |
| 2600[b] | 750 | 1000 | 1: 1.6[b] | 1.1 [b] | | |
| | **PEG-PCL-PEG** | | | | | |
| 2212[b] | 750 | 750 | 1: 2.128[b] | - | Ring opening polymerisation of ε-CL initiated by MPEG and coupling reagent IPDI. | 23 |
| 2680 [b] | 1000 | 750 | 1: 1.461[b] | - | | |
| 2965[b] | 1500 | 750 | 1: 0.971[b] | - | | |
| 8198[b] | 4000 | 2000 | 1: 1.034 [b] | - | | |
| 2130[b] | 1100 | 550 | 1: 0.990 [b] | - | | |
| 3236[b] | 2000 | 550 | 1: 2.067 [b] | 1.26 [b] | As above using HDMI as coupling reagent. | 24 |
| 4634[b] | 3000 | 750 | 1: 2.052[b] | 1.18 [b] | | |
| 3914[b] | 2500 | 750 | 1: 1.642 [b] | 1.24 [b] | | |
| 3230[b] | 2000 | 550 | 1: 2.076 [b] | 1.26 [b] | As above. | 20 |
| | **PEG/PCL multiblock copolymers** | | | | | |
| 12700 [a] | ~1000 | 1000 | ~1:2 | 2.3[b] | PCEC units (prepared by ring opening polymerisation using $Sn(Oct)_2$.) coupled by terephtaloyl chloride. | 16 |

[a] Calculated from gel permeation chromatography (GPC)
[b] Calculated from Nuclear Magnetic Resonance Analysis ($^1$H NMR)

*Table 2.* Dependence of sol-gel-sol behaviour of triblock PEG/PCL-based copolymers on experimental parameters.

| **VARIABLE PARAMETERS** | **CONSTANT PARAMETERS** | **SAMPLE** | | **REF.** |
|---|---|---|---|---|
| | | **PECE** | **PCEC** | |
| Molecular topology | - copolymer molecular weight<br>- PEG/PCL ratio | CGC of PCEC < CGC of PECE<br>LCGT of PCEC < LCGT of PECE<br>UCGT of PCEC > UCGT of PECE | | 20, 32 |
| PCL molecular weight ↑ | - PEG block molecular weight | CGC ↓<br>LCGT ↓<br>UCGT ↑ | | 20, 21, 23, 32 |
| PEG molecular weight ↑ | - PCL block molecular weight | CGC ↑<br>LCGT ↑<br>UCGT ↑ | LCGT ↑<br>UCGT ↑ | 20, 39 |
| Copolymer molecular weight ↑ | - PEG/PCL ratio | CGC ↓<br>LCGT ↑<br>UCGT ↑ | LCGT ↑<br>UCGT ↑ | 20, 23, 32 |
| Solution concentration | - copolymer<br>- solubilisation parameters | LCGT ↓<br>UCGT ↑ | | 21 |
| Solvent:<br>-water<br>-normal saline solution<br>-glucose solution | - copolymer<br>- solubilisation parameters | Similar CGC in the analysed solvents<br>LCGT and UCGT in saline and glucose solutions < in water | | 24, 38, 32 |
| Additives:<br>-salting-in salts (e.g. NaSCN)<br>-salting-out salts (e.g. NaCl)<br>-PEG with $M_n$: 2,000 Da<br>-PCL with $M_w$: 550 Da<br>-PVA with $M_w$: 30,000-70,000 Da | - copolymer<br>- solubilisation parameters | LCGT ↑, UCGT ↑ with salting-in salts<br>LCGT ↑, UCGT ↓ with salting-out salts<br>No variation in CGC for both salts<br>In PECE copolymers with PEG addition (0.5-2 wt.%):<br>LCGT ↓; UCGT ↓; no variation in CGC<br>In PECE copolymers with PCL or PVA addition (5 wt.%): CGC ↓; LCGT ↓; UCGT ↑ | | 24, 40 |
| pH | - copolymer<br>- solubilisation parameters | No variation in CGC, LCGT and UCGT in acidic respect to neutral media. | - | 24 |
| Annealing time of the solution | - copolymer<br>- solubilisation parameters | Annealing at 4 °C (0-24 h): CGC, LCGT and UCGT unchanged | Annealing at 10°C (2-60 min):<br>LCGT ↓ as a function of time;<br>UCGT and CGC unchanged | 16, 23, 24 |
| Heating history | - copolymer<br>- solubilisation parameters | LCGT measured by heating solution > LCGT measured by cooling gel (hysteresis)<br>UCGT measured by heating solution > UCGT measured by cooling gel (hysteresis) | | 23, 24, 38 |
| Solubilization temperature | - copolymer<br>- solubilisation parameters | Temperature decrease from 35°C to room temperature:<br>CGC, LCGT, UCGT ↓ | - | 23 |

*Table 3.* Main applications of triblock PEG/PCL copolymers as injectable drug delivery carriers.

| **$M_n$ (Da)** | **Concentration (% w/v)** | **Drug** | **Objective of the study** | **Ref.** |
|---|---|---|---|---|
| **PECE copolymers** | | | | |
| 5250<br>3500 | 25 | Timolol maleate | Analysis of polymeric additives on sol–gel transition and drug release. | 40 |
| 3300<br>3100<br>4500 | 20, 30 | -$VB_{12}$<br>-Honokiol<br>-Bovine serum albumine | Analysis of *in vitro* release of hydrophilic, hydrophobic and protein drugs. | 32 |
| 3408 | 5, 10, 15 | No drug | Evaluation of PECE hydrogel for sustained ophthalmic drug delivery. | 62 |
| 3694 | 15, 20, 30 | Insulin | Evaluation of PECE hydrogel for pulsatile release of insulin | 35 |
| **PCEC copolymers** | | | | |
| 3120 | 20, 30 | -$VB_{12}$<br>-Honokiol<br>-Bovine serum albumine | Analysis of *in vitro* release rate of hydrophilic, hydrophobic and protein drugs. | 20 |
| 3120 | 20 | Lidocaine | Demonstration of controlled drug release in vivo | 20 |
| 3700<br>4000<br>4500<br>2500 | 15, 20, 25 | Bovine serum albumine | Analysis of in vitro release of protein drugs and their stability. | 21 |
| | 20 | Horseradish peroxidase | | |
| 2590;<br>3412 | 15, 20, 25, 30 | -Naltrexone Hydrochloride<br>-$VB_{12}$ | Analysis of in vitro release as a function of solution concentration and loaded drug | 63 |
| 2502 | 30 | Doxorubicin | Preparation of a PCEC hydrogel for radiotherapy combined with chemotherapy | 65 |

*Table 4.* Main applications of triblock PEG/PCL copolymers in tissue engineering.

| **$M_n$ (Da)** | **Concentration (% w/v)** | **Objective of the study** | **Ref.** |
|---|---|---|---|
| **PECE copolymers** | | | |
| 3300 | 20 | Injectable PECE/hydroxyapatite (10, 20, 30 wt.%.) hydrogels for bone regeneration. | [69] |
| 3300 | 30 | Injectable PECE/collagen/hydroxyapatite (60/10/30 wt./wt./wt.) hydrogels for bone regeneration. | [70] |
| 3150 | >15 | Injectable acellular bone matrix (ABM)/PECE (10/90; 20/80; 30/70 wt./wt.) composites by incorporating the ABM granules into the PECE hydrogel for bone regeneration. | [71] |
| 3150 | - | Injectable PECE/acellular bone matrix composite hydrogels for bone regeneration in cranial defects | [72] |
| 3630 | 35 | Preparation of a PECE hydrogel to prevent post-surgical abdominal adhesion. | [66] |
| **PCEC copolymers** | | | |
| - | 20 | Preparation of a PCEC hydrogel to prevent postoperative adhesion. | [67] |
| 2340-3620 | 20 | Preparation of PCEC/sodium hyaluronate blend hydrogels for injectable anti-adhesive applications | [34] |
| 3100 | 20 | Preparation of thermosensitive hydrogel to prevent postoperative peritoneal adhesion | [68] |