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Historical Perspectives

Biodegradable poly(ε-caprolactone)–poly(ethylene glycol) copolymers as drug delivery system

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ABSTRACT

Poly(ε-caprolactone)–poly(ethylene glycol) (PCL–PEG) copolymers are important synthetic biomedical materials with amphiphilicity, controlled biodegradability, and great biocompatibility. They have great potential application in the fields of nanotechnology, tissue engineering, pharmaceutics, and medicinal chemistry. This review introduced several aspects of PCL–PEG copolymers, including synthetic chemistry, PCL–PEG micro/nanoparticles, PCL–PEG hydrogels, and physicochemical and toxicological properties.

Contents

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1. Introduction

Drug delivery is a rapidly developing and evolving discipline underpinned by the principles and development in related fields from classic chemistry to modern biotechnology. It is a key factor contributing to the commercial and therapeutic potential of many drugs and related products, and also performing as the driving force behind the development of many new devices and formulation-based projects (Charman et al., 1999; Langer and Tirrell, 2004). The development of drug delivery technology aims at enhancing the screening and evaluation of new compounds and 'rescuing' failed compounds, optimizing them by improving their effectiveness or tolerability, and simplifying their administration (Rosen and Abribat, 2005; Choi et al., 2006b). In past decades, the application of the synthetic copolymers in drug delivery system (DDS) as drug carriers led to technological advances which bypassed the pharmacokinetic limitations of conventional, rapid release dosage forms. The improved DDS based on synthetic polymers generally appears in three types, micro/nanoparticles, implants (containing hydrogels) and fibres (Lin et al., 1999; Kopecek et al., 2001; Takeuchi et al., 2004; Vargas et al., 2004; Ideta et al., 2005; Croy and Kwon, 2006; Jang et al., 2006b; Lin et al., 2006; Cai et al., 2007; Nakayama et al., 2007). Drug is dissolved, entrapped, adsorbed, attached, or encapsulated in the polymeric material to make controlled release and localized drug delivery possible. Among the synthetic polymers adopted in DDS, biodegradable aliphatic polyesters such as poly(ε-caprolactone) (PCL), poly(lactic acid) (PLA), poly(glycolic acid) (PGA), and their copolymers received considerable attention (Robert and Vancanti, 1993; Chiellini and Solaro, 1996; Jeong et al., 1997; Masahiko, 2002).

PCL is a semi-crystalline, hydrophobic polymer with a relatively polar ester group and five non-polar methylene groups in its repeating unit, which has gained much attention as ideal material for drug delivery and other applications through the decades (Mondrinos et al., 2006; Seregin and Coffer, 2006). And it is mostly synthesized by ring-opening polymerization method from ε-caprolactone (ε-CL) monomers. The high olefin content imparts polyolefin-like properties to PCL (Kim et al., 2004). However, owing to the high degree of crystallinity and hydrophobicity, PCL degrades rather slowly and less biocompatible with soft tissue, which restricts its further clinical application. Therefore, the modification of PCL is proposed. Poly(ethylene glycol) (PEG), for its hydrophilicity, non-toxicity and absence of antigenicity and immunogenicity, can be selected to be attached to PCL, forming PCL–PEG copolymers. Thus, their hydrophilicity, biodegradability and mechanical properties can be improved and may find much wider applications (Koenig and Huang, 1995; Chen et al., 2000; Moon et al., 2002; Huang et al., 2004).

Due to presence of hydrolytically labile ester groups in the macromolecular main chain, PCL is biodegradable under physiological conditions. And it was reported that PCL–PEG copolymers with long PCL chains presented the same crystalline structure as PCL homopolymer, whereas PEG-bearing short PCL blocks retained the crystalline structure of PEG. However, both of them exhibited higher degradability compared with the PCL homopolymer (Wang and Qiu, 1993). In addition to the improved biodegradability, the copolymers showed higher hydrophilicity and better performance in the cell culture studies than the PCL homopolymer. Amphiphilic PCL–PEG polymer can find wide applications as micro/nanoparticles or thermosensitive hydrogels. In this review, we deal with the recent developments in the application of PCL–PEG block polymer in DDS. In Section 2, we focus on the synthesis of different types of PCL–PEG copolymers. In Section 3, we survey the preparation techniques and applications of micro/nanoparticles from PCL–PEG copolymers as drug carriers in DDS. And we refer to novel thermosensitive hydrogels from PCL–PEG copolymer in Section 4, particularly discussing the studies on its gel–sol or sol–gel transition and the recent applications. Finally, conclusion and perspectives were given.

2. PCL/PEG copolymers

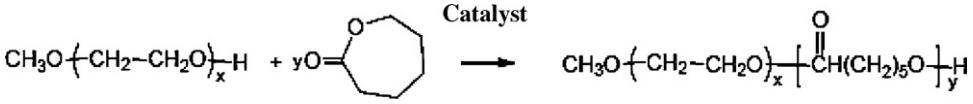
2.1. Synthesis of PCL/PEG copolymers

Synthetic block polymers consisting of hydrophobic PCL (A-block) and hydrophilic PEG (B-block) gained increasing attention, as they allow a modification of physical and chemical properties leading to wide applications in biomedical fields. According to our knowledge, the first who prepared a series of PCL/PEG block copolymers was Perret and Skoulios (1972). They synthesized the copolymers by anionic polymerization using naphthalene–sodium complex as catalyst. Then, a catalyst-free polymerization was introduced to synthesize the PCL/PEG copolymer by Cerrai et al. (1989).

These polymers, according to their structure, can be classified into AB diblock, ABA or BAB triblock, multiblock, star-shape, and graft copolymers. In our review, we limit our discussions of PCL/PEG copolymers to AB diblock, ABA or BAB triblock, and star-shaped block structures.

2.1.1. Synthesis of PCL/PEG diblock copolymers

The most widely used method to synthesize PCL–PEG diblock copolymers was ring-opening polymerization from monomethoxy-PEG (MPEG) and ε-CL with a catalyst, as shown in Scheme 1 (Jedlinski et al., 1993; Youxin and Kissel, 1993; Cerrai et al., 1994; Gan et al., 1996; Rashkov et al., 1996b). Mostly, certain amount of MPEG was first distilled with dried toluene to remove residual water in the solvent. Subsequently, predetermined amounts of ε-CL and stannous octoate were added and the mixture was refluxed for several hours at appropriate temperature with mechanical stirring. Copolymers with different PCL block lengths can be obtained by varying the concentration of ε-CL monomer (Yuan et al., 2000; Choi et al., 2006a). During the polymerization, the moisture in the solvent and the catalyst concentration are important for determining the yield and molecular weight of block copolymers. Therefore, all these PEG and ε-CL monomers should be dried to constant weight before use and the polymerization was supposed to carry out under nitrogen atmosphere or vacuum (Heuschen et al., 1981). When it comes to the adoption of the catalyst, until now, many catalysts, such as stannous octoate, stannous chloride, GeO_2, SnO_2, Sb_2O_3, and SnO, have been used in the polymerization (Deng et al., 1990; Jedlinski et al., 1993; Sawhney et al., 1993; Li et al., 1996; Rashkov et al., 1996a; Nagasaki et al., 1998; Dobrzynski et al., 1999; Kobayashi et al., 2001; Connor et al., 2002; Myers et al., 2002; Nyce et al., 2003; Piao et al., 2003; Deng et al., 2005). Stannous octoate has become the most widely used catalyst because it is commercially available, and easy to handle. Moreover,



Scheme 1. The synthesis of MPEG–PCL diblock copolymer.

it is soluble in common organic solvents and cyclic ester monomers, and is a permitted food additive in numerous countries (Choi et al., 2006a).

2.1.2. The synthesis of PCL–PEG-PCL and PEG-PCL–PEG triblock copolymers

There are two forms of PCL/PEG triblock copolymers: ABA type PCL–PEG–PCL (PCEC) and BAB type PEG–PCL–PEG (PECE) copolymers, respectively. PCEC could be obtained by ring-opening polymerization of ε-CL monomers using dihydroxy PEG as an initiator, with detailed procedures similar to the synthesis of diblock copolymers (Scheme 2) (Cohn et al., 2002; Kim et al., 2004; Lu et al., 2006; Gou et al., 2007).

Compared with PCEC, the synthesis of PECE was carried out by two steps in the presence of coupling agent. It is reported that PECE copolymer was prepared by coupling reaction from PCL diol and PEG using L-lysine methyl ester diisocyanate (LDI) as the chain extender (Zhang and Zhuo, 2005). Under dry argon atmosphere, LDI was first reacted with PCL diol in anhydrous dimethyl-acetamide (DMAC) with stannous octoate as catalyst (Storey et al., 1993; Skarja and Woodhouse, 2000). Then, PEG was added subsequently to continue the reaction for 24 h. Controlling the reaction temperature is a key factor to synthesize the copolymers. Higher reaction temperature led to a faster coupling reaction rate. However, backbiting reaction occurred if the temperature is too high.

Also, PECE copolymers could be prepared from MPEG–PCL diblock copolymer coupled with a coupling agent, such as isophorone diisocyanate (IPDI) and hexamethylene diisocyanate (HMDI) (Scheme 3) (Gong et al., 2007). MPEG–PCL diblock copolymers were first prepared as described above. And the participation of coupling agent could couple the diblock polymer with free hydroxyl group at the end of PCL block, thus forming the PEG–PCL–PEG triblock copolymer.

2.1.3. Synthesis of PCL/PEG star-shaped copolymers

The PCL/PEG star-shaped polymers were successfully synthesized in several ways as reported. Two different synthetic procedures, convergent and divergent methods, used to produce star-shaped block copolymers were studied by Kim et al. (2004). The 3-arm star-shaped block copolymer was synthesized via the convergent method, i.e., pre-synthesized MPEG–PCL diblock copolymer was coupled with a core having three functional groups. First, the MPEG–PCL diblock copolymer was synthesized by the ring-opening polymerization of ε-CL using MPEG as an initiator in the presence of a catalytic amount of stannous octoate. Then, the coupling of MPEG–PCL diblock copolymer and 1,3,5-benzenetricarboxylic acid occurred to form the 3-arm star-shaped PEG–PCL copolymer. Different from the 3-arm star-shaped block copolymer, 4-arm star-shaped block copolymer was prepared using a divergent method. ε-CL was first polymerized using a tetrafunctional initiator, pentaerythritol, in the presence of stannous octoate to produce 4-arm star-shaped PCL having a hydroxyl group at each end of the arm. At the same time, carboxylated PEG was prepared from the reaction of MPEG with succinic anhydride. 4-arm star-shaped PEG–PCL block copolymer was then obtained from the coupling of pre-synthesized 4-arm star-shaped PCL with the carboxylated PEG.

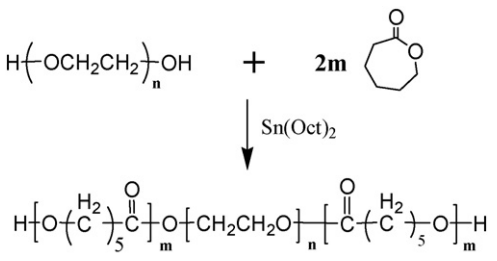
In another study, 3-arm and 4-arm PCL/PEG copolymers were obtained with trihydroxy and tetra-hydroxy PEG (3-arm and 4-arm PEG) respectively in the presence of stannous octoate as catalyst. The various ratios of ε-CL/PEG were used to obtain copolymers with different PCL block lengths (Lu et al., 2006).

More studies of synthesizing star-shaped PCL/PEG copolymers were carried out. PCL was attached to polyamidoamine (PAMAM) dendrimer to form the inner core by ring-opening polymerization. And the PEG copolymer was then attached to the PCL terminus by an ester-forming reaction (Wang et al., 2005a,b, 2008). Furthermore, 5,10,15,20-tetrakis(4-aminophenyl)-21*H*,23*H*-chlorin (TAPC) has been chosen as a initiator to form chlorin-core star block copolymer. And the activated MPEG–PCL–COCl was conjugated to the amino group of TAPC (Peng et al., 2008).

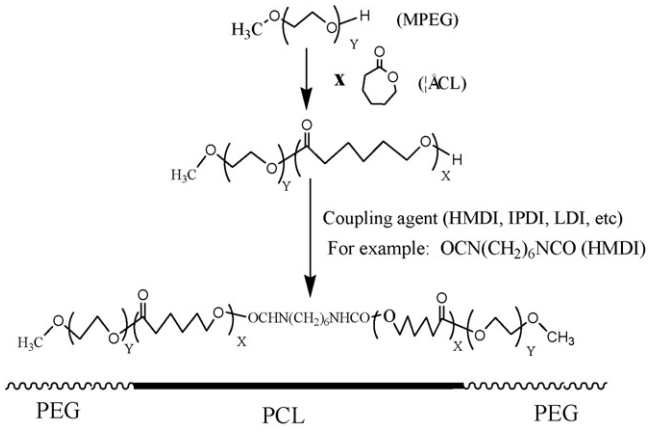
2.2. The biocompatibility of PCL/PEG copolymers

The fundamental understanding of cellular and tissue responses, which accounts for the biocompatibility of materials intended to be used in close contact to biological systems, is important for the design of new polymeric drug delivery systems. Therefore, the biocompatibility of PCL/PEG copolymers was investigated extensively.

It has been revealed that PCL/PEG copolymers showed better performance in the cell culture studies than the PCL homopolymer (Huang et al., 2004). Furthermore, the influence of PEG segments composition on biocompatibility of PCL/PEG copolymer was carried out. The biological responses to the spin cast surface of different PCL/PEG diblock copolymers were investigated *in vitro*. The results showed that surface hydrophilicity improved with the increase in



Scheme 2. Synthesis scheme of the PCL–PEG–PCL copolymer. Reproduced from Ref. Huang et al. (2008), with permission.



Scheme 3. Synthesis scheme of PEG–PCL–PEG copolymers.

PEG segments in diblock copolymers and that bacteria adhesion was inhibited by increase in PEG contents. In the mean time, the number of adhered endothelial cells, platelets and monocytes on diblock copolymer surfaces was enhanced in an increased composition of PEG segments in PCL–PEG, where, nevertheless, the platelet and monocyte activation was reduced. With increase in PEG/PCL ratio, PCL–PEG had better cellular response as well as lower degree of platelet and monocyte activation. The study demonstrated that surface nanotopography could influence not only cell adhesion and growth but also platelet and monocyte activation (Hsu et al., 2004). The PEG segments were also concerned with *in vivo* pharmacokinetics and biodistribution in mice. The PEGylated PCL copolymers, while forming micelles, decreased drug uptake by the liver and kidney, and also prolonged drug retention in the blood (Lin et al., 2005b). It is believed that the surface grafted, flexible and hydrophilic chains of PEG form dense conformational clouds to prevent protein (opsonin) adsorption to these nanoparticles thus avoid being captured by the mononuclear phagocyte system (MPS) (Monfardini and Veronese, 1998).

Not only the PEG composition determines its biocompatibility, PEG configurations also have great effect on its biocompatibility, which was looked into by a series of investigation of the cell attachment, cell uptake and cell viability of PCL–PEG nanoparticles with PEG in different configurations (Hu et al., 2007). And a slight difference in biocompatibility of MPEG–PCL diblock copolymer and PCEC triblock copolymer came into light.

PCEC triblock copolymers have been investigated in a series of studies by the group of Cerrai et al. under *in vitro* conditions. PCEC copolymers were evaluated for their cytotoxicity in 3T3 mouse fibroblasts. Also, hemocompatibility was studied by measuring the activation of plasma prekallikrein to kallikrein and tested by the contact activation of thrombocytes, measuring the release of platelet factor 4 and β-thromboglobulin. The results suggested that the triblock copolymers were generally cyto- and hemocompatible, being of great potential as biomaterials (Cerrai et al., 1989). *In vitro* cytotoxicity of PCEC and PECE copolymer were investigated by the group of Gong et al. (in press-b,d), and the results indicated that the two kinds of PCL/PEG copolymer were both biocompatible with very low cytotoxicity (Fig. 1).

The biocompatibility of materials was studied in various fields according to different applications. The cytotoxicity of nanoparticles or micelles based on PCL–PEG copolymers were examined by different types of cells such as MCF-7 cells (Ameller et al., 2004), normal human fibroblast cells (Lin et al., 2005a) and HepG2 cells (Hu et al., 2007), respectively. All the results confirmed their low cytotoxicity. In addition to that, biocompatibility of PCL–PEG copolymer, while being applied as biofunctionalized nanofibers, semi-interpenetrated hydrogels, or gene carrier with alpha-cyclodextrin (alpha-CD) molecules, was studied respectively (Shuai et al., 2005; Zhu and Chan-Park, 2005; Grafahrend et al., 2008).

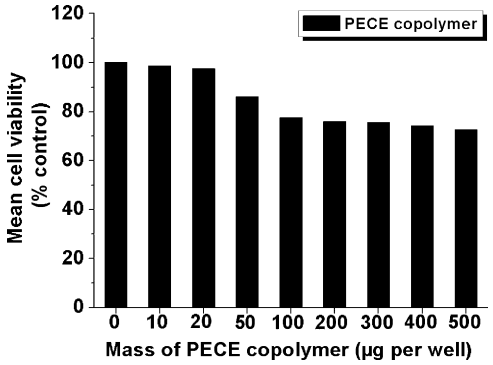


Fig. 1. HEK 293 cell viability assay of PEG–PCL–PEG copolymer. Reproduced from Gong et al. (in press-d), with permission.

Besides, *in vivo* toxicity evaluation of PCL/PEG copolymers were also widely studied. The close contact with biological systems in the application of PCL/PEG copolymers made the compatibility of the materials essential. Recently, an acute oral toxicity evaluation of biodegradable and pH-sensitive hydrogel based on PCL, PEG and methylacrylic acid (MA) (P (CL-MA-EG) hydrogel) was carried out (Chen et al., 2008b). In acute oral toxicity test, mice were orally administered with a total 15 g/kg body weight (b.w.) of P (CL-MA-EG) hydrogels, and were observed continuously for 14 days. No mortality or significant signs of acute toxicity was observed during the whole observation period, and no macroscopic alteration was found in the organs. Histopathological analysis of various organs also did not show any significant pathological changes. It suggested that the studied P (CL-MA-EG) hydrogel in this article were nontoxic after acute oral administration and it might be a promising candidate as a novel oral drug carrier (Chen et al., 2008b). Furthermore, by the group of Gong et al. (in press-d), acute toxicity test and histopathological study were performed in BALB/c mice by intrapleural, intraperitoneal or subcutaneous administration of PECE hydrogel (30 wt%), respectively. The dose of intrapleural, intraperitoneal or subcutaneous administration was up to 10 g/kg body weight (b.w.), 25 g/kg b.w. and 25 g/kg b.w., respectively, and the mice were observed continuously for 14 days. No mortality or significant signs of acute toxicity was observed during the whole observation period and there is no significant lesion to be shown in histopathologic study of major organs. And histopathological study of tissue at injection site showed no significant inflammatory reaction and histopathological changes after 7 days (Fig. 2).

2.3. The degradation of PCL/PEG copolymers

There are two ways by which polymer bonds can be cleaved: passively by hydrolysis or actively by enzymatic reaction (Gijpferich, 1996), which indicates two modes for the degradation

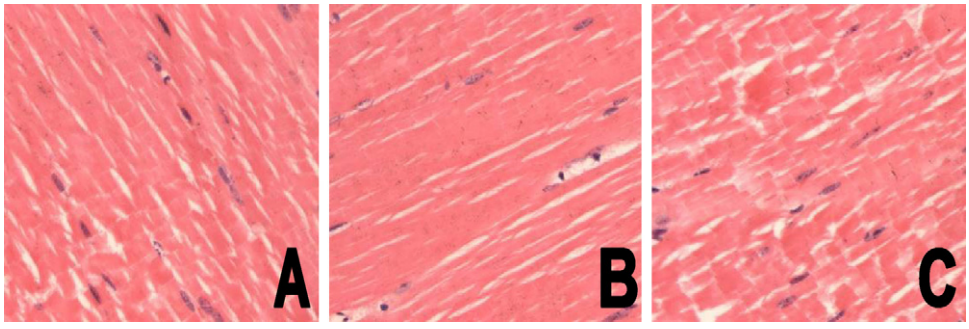


Fig. 2. Photograph of tissue samples from injection site after PECE hydrogel subcutaneous injection (×400). (A) Control group; (B) 7 days after injection; (C) 14 days after injection. Reproduced from Gong et al. (in press-d), with permission.

Scheme 4. The proposed hydrolytic degradation process of polyesters. Reproduced from Qian et al. (2004), with permission.

of PCL/PEG copolymer. It is mainly the type of bond within the polymer backbone that determines the type and rate of polymer erosion. Therefore, the ester bonds in PCL/PEG copolymer made hydrolysis an important mode of degradation (Scheme 4). The hydrolytic degradation behaviors of both PCL homopolymer and PCL–PEG copolymer were studied in detail (Li et al., 1998). However, a general problem related to biodegradable polymers is the fact that the *in vitro* degradation data frequently do not sympathize with the data of *in vivo* degradation experiments due to the appearance of enzymes (Wu et al., 1994). It was reported that the *in vivo* degradation of PCL is significantly accelerated compared to the *in vitro* experiments (Sun et al., 2006). This is assigned to the optimum concentration of PCL-degrading enzymes, namely lipases, in the body. Further factors influencing the *in vivo* degradation kinetics of polymers are mechanical stress, temperature, pH, the presence of ions and of species modifying the enzyme activity (He et al., 2005). The enzymatic degradation of PCL polymers has been investigated, especially in the presence of lipase-type enzymes (Mochizuki et al., 1995; Gan et al., 1997, 1999). Wu et al. (2000) claimed that the presence of the microbial *Pseudomonas* lipase enhanced the degradation rate of PCL nanoparticles by a factor of 1000 as compared with their purely hydrolytic degradation. Highly crystalline PCL was reported to totally degrade in 4 days in the presence of *Pseudomonas* lipase.

And an investigation concerned with enzymatic biodegradation of PCL–PEG diblock and triblock copolymers with the aim of identifying the effect of PEG incorporation on the biodegradation characteristics of PCL was carried out (Li et al., 2002). The result demonstrated that, in the presence of *Pseudomonas* lipase, the enzymatic degradation of PCL was not altered by the incorporation of PEG for both the diblock and triblock copolymers compared to a PCL homopolymer.

A further investigation of the biodegration behavior of PCL–PEG block polymer was identified by degradation tests *in vitro* and *in vivo*. The tests *in vitro* and *in vivo* were carried out by immersing samples in pH 5.0, 7.2 and 9.0 buffer solutions with or without lipase at 25.0, 37.0 and 50.0 °C, and implanting samples in the back or small intestine of rats. It was found that the degradation rate of the PCL–PEG increased with increase in PEG content, temperature, acidity or alkalinity, and it was accelerated by the presence of enzyme. The fastest degradation rate was observed in the physiological condition of the sample being implanted in the body of animals (Bei et al., 1998).

Some studies on degradation of PCL/PEG copolymers were focused on the degradation of PCL and the way in which the products metabolizing *in vivo* (Gan et al., 1997, 1999; Yu et al., 2008). The mechanism of chemical degradation of PCL has already been reported, and the products of thermal degradation which led to an unzipping depolymerization of the copolymer were H_2O, CO_2, and 5-hexenoic acids (Persenaire et al., 2001). And there was some work done to reveal the metabolism of the degraded PCL. Woodward et al. (1985) studied *in vivo* adsorption of low molecular weight PCL at cellular level. They found that the PCL pieces was ingested and digested ultimately by phagocyte and giant cell. They concluded that the degradation of PCL in the second stage mainly involved intracellular phagocytosis. Another long-term *in vivo* excretion study of PCL discovered that PCL implant gradually lost strength and broke into pieces. In the next step, low molecular weight PCL pieces were metabolized by unknown process and ultimately excreted from the body through urine and feces. The material did not accumulate in any body organs (Sun et al., 2006).

3. PCL/PEG micro/nanoparticles

The PCL/PEG block copolymers have been intensively studied on their application as controlled drug delivery devices (Edlund and Albertsson, 2002; Sinha et al., 2004). Drug encapsulated into biodegradable polymer matrix exhibits improved drug delivery behaviors and has more advantages compared to conventional dosage forms. Their therapeutic values have been enormously increased in the form of microparticles and nanoparticles by improving the therapeutic effect, prolonging the biological activity, controlling the drug release rate, and decreasing the administration frequency (Jeong et al., 2003; Ding et al., 2005). Meanwhile, PCL/PEG micro/nanoparticles also have potential application in making injectable aqueous formulation for hydrophobic drug (Kim et al., 1998; Shin et al., 1998; Verger et al., 1998; Ryu et al., 2000; Jeong et al., 2004; Singh and Muthu, 2007). Therefore, the studies, concerned with PCL/PEG micro/nanoparticles from the preparation method to the application in DDS, caught considerable attention.

3.1. Preparation of drug-loaded micro/nanoparticles based on PCL/PEG copolymers

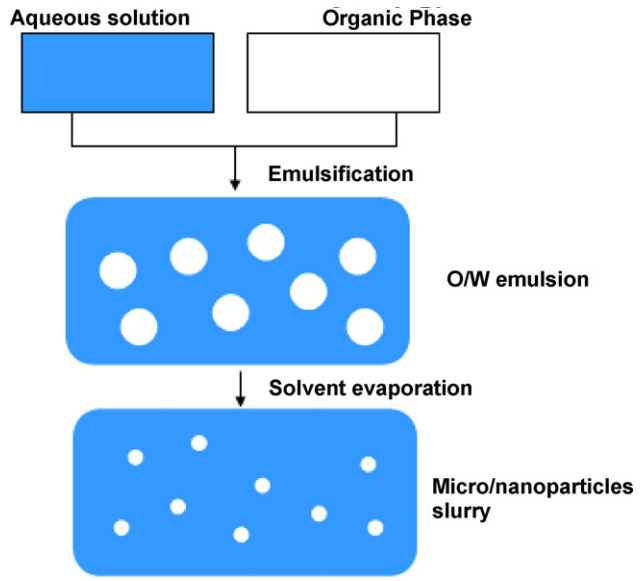
In biomedical field, the particles smaller than 1000 nm are normally called nanoparticles, whereas the commonly mentioned nanoparticles in the field of materials science are those particles at the size between 1 and 200 nm. The so-called microparticles usually refer to those particles have sizes of several to hundreds of micrometers. When biodegradable polymeric micro/nanoparticles are used in advanced drug delivery system, the properties of carriers (such as particle size, shapes, molecular structure and molecular weight) would greatly determine their application by affecting the body distribution, circulation time, release behavior, therapeutic value, etc. (Narasimhan and Langer, 1996; Hirosue et al., 2001; Kost and Langer, 2001). Therefore, it is very necessary to develop advanced methods to prepare biomedical micro/nanoparticles with ideal properties.

3.1.1. Emulsion solvent extraction method

Emulsion solvent extraction method has been widely used to prepare drug-loaded polymeric micro/nanoparticles for its adaptability to different polymer matrixes and flexibility to be applied for the encapsulation of both hydrophobic and hydrophilic substances (Sergio et al., 2005; Gou et al., 2007, 2008b,c,d). The techniques are based on an emulsification to form small droplets and a following removal of the organic phase of emulsion by evaporation or diffusion. Several ways of preparing micro/nanoparticles, including solvent evaporation, solvent diffusion and nanoprecipitation could be concluded as solvent extraction method.

When polymeric micro/nanoparticles functioned as carriers for lipophilic drug, the simple oil in water (O/W) emulsion solvent evaporation method was mostly applied to prepare particles (Scheme 5). First, drug and polymer are co-dissolved in volatiles organic solvent such as dichloromethane and acetic ether (Et Ac). Next is to make the organic phase well dispersed in an aqueous phase containing surfactants or/and stabilizers by stirring. Then, the organic drops were solidified and micro/nanoparticles formed due to the extraction of the solvent by evaporation. The drug-loading particles were purified afterwards by dialysis or washing to remove the residual surfactants and organic solvents, and were lyophilized.

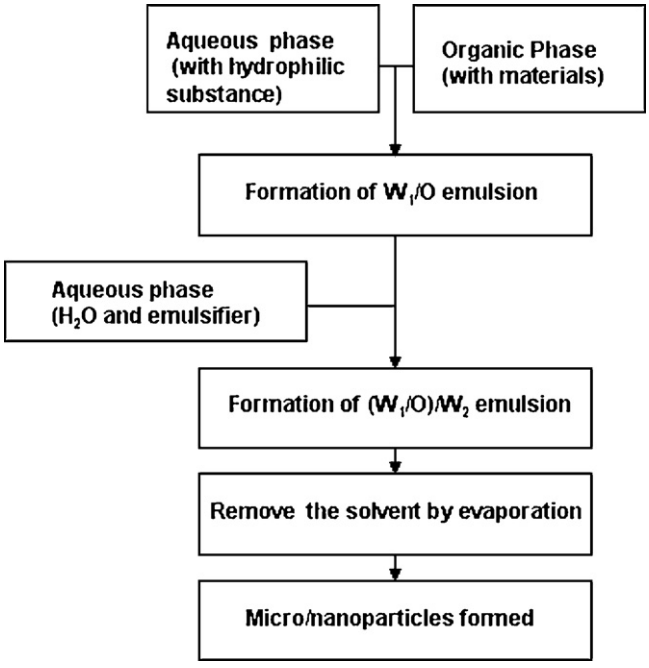
Beyond single-emulsion solvent extraction method mentioned above, multi-emulsion solvent extraction method was also widely studied to prepare micro/nanoparticles from polymers loading various kinds of drugs especially hydrophilic drugs such as proteins, peptides and genes (Zarnbaux et al., 1998; Zhou et al., 2003; Kim and Bae, 2004; Jia et al., 2008). The most extensively used multi-



Scheme 5. The preparation of micro/nanoparticles by solvent evaporation method.

emulsion solvent extraction method was water in oil in water ($W_1/O/W_2$) emulsion solvent extraction method (Scheme 6). Previously, different kinds of PCEC microspheres containing human serum albumin (HSA) were successfully prepared by a double emulsion ($W_1/O/W_2$) evaporation method (Zhou et al., 2003) and the experimental parameters of the methods were investigated (Zarnbaux et al., 1998). Similarly, microspheres based on PCL–PEG copolymer containing bovine serum albumin were prepared by adopting double emulsion method (Kim and Bae, 2004).

Otherwise, the emulsion solvent diffusion method is also a useful way to prepare polymer micro/nanoparticles relying on the concept of "solvent extraction". This technology could be concluded in a two-step process, which included initial production of an O/W emulsion and a following dilution to cause a deposition of the polymer and drug around the droplets, thus leading to the formation



Scheme 6. The preparation of micro/nanoparticles by modified two-step solvent evaporation method. (W/O/W emulsion).

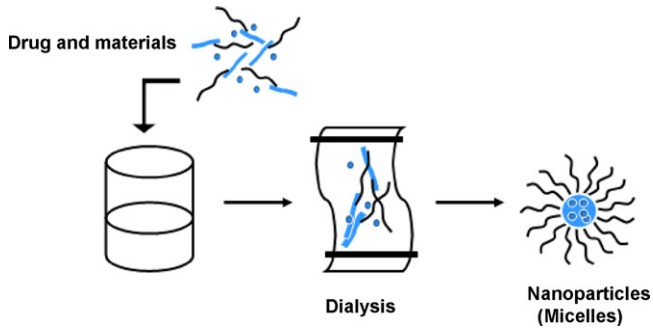
of micro/nanoparticles. In this technology, partially water-soluble solvent such as Et Ac or propylene carbonate (PC) should be applied rather than dichloromethane. Gou et al. have prepared the magnetic PCEC composite microspheres by solvent diffusion method. PCEC and the obtained ferrofluid were dissolved in dimethylsulfoxide (DMSO) to be well dispersed. In the next step, the suspension was dropped into PVA aqueous solution under ultrasonic. DMSO was miscible with water while PCEC was dissolved in DMSO but insoluble in water. When PCEC/DMSO solution was dropt into aqueous PVA solution under ultrasound dispersion, the PCEC/DMSO micro-drops formed and the PCEC microspheres were obtained due to the extraction of DMSO for it could be miscible with aqueous phase (Gou et al., 2008e).

When it comes to the preparation of nanoparticles from PCL/PEG copolymers, despite the emulsion techniques mentioned above, the so-called nanoprecipitation method (Verger et al., 1998) could be applied as well. This technique was concluded as one of the solvent extraction methods. It was reported that nanoprecipitation has been applied to prepare PEG–PCL–PEG particles containing 4′-demethylepipodophyllotoxin (DMEP) (Zhang and Zhuo, 2005). Briefly, triblock copolymers and DMEP were dissolved in acetone. And the organic phase was added dropwise into distilled water under moderate stirring. Then the acetone was removed under reduced pressure. Finally, the suspension obtained was passed through 0.45 μm filter to remove aggregates and then freeze-dried. All the prepared nanoparticles were smaller than 100 nm with narrow distribution. Similarly, based on PCL–PEG diblock copolymers, all-trans-retinoic acid (atRA)-loaded nanoparticles (Jeong et al., 2004), maleimide-functional particles (Gindy et al., 2008a) and busulfan encapsulated micelles (Jeong et al., 2004; Layre et al., 2006) were successfully prepared by nanoprecipitation. Moreover, the research of PCL–PEG nanoparticles for use as multimodal carriers for drugs and imaging agents was carried out. PCL–PEG nanoparticles simultaneously co-encapsulating hydrophobic organic actives and inorganic imaging nanostructures were prepared using the flash nanoprecipitation process in a multi-inlet vortex mixer. Obtained composite nanoparticles are produced with tunable sizes between 75 nm and 275 nm, narrow particle size distributions, high encapsulation efficiencies, specified component compositions, and long-term stability (Gindy et al., 2008b).

When emulsion solvent extraction method was employed to prepare drug-loaded polymeric micro/nanoparticles, the emulsification and the solvent extract process would greatly affect the properties of obtained particles. We could control the emulsification process by adjusting the polymer concentration, drug/polymer mass ratio in feed, organic/water phase volume ratio and stirring intensity, in other words, controlling the solvent extraction speed. Although emulsion solvent extraction method was highlighted as a versatile method to prepare drug-loaded polymer micro/nanoparticles, some problems with this method such as residual surfactants, the addition of stabilizers or organics solvent should also be considered while processing (Zarnbaux et al., 1998).

3.1.2. Dialysis method

Dialysis method was intensively used to prepare micro/nanoparticles, especially nano-sized micelles. Common procedure was to dissolve the block copolymer and drug in water miscible organic solvent (such as N,N-dimethylformamide (DMF) or DMSO) followed by dialysis of this solution against water. During dialysis, self-assembly of block copolymers took place and drug was encapsulated in the micelle cores (Scheme 7). At the same time the semi-permeable membrane allowed the removal of unloaded drugs. Numerous results have been reported based on dialysis method (Kim et al., 1998, 2001, 2003b; Shin et al., 1998; Kim and Kim, 2001; Kim and Lee, 2001; Lee et al., 2003; Park et al., 2005b; Lu et al., 2007).



Scheme 7. The preparation of nanoparticles by dialysis.

Clonazepam (CNZ)-loaded core–shell type nanoparticles of PCEC triblock copolymer were prepared by dialysis method. The solution of PCEC and CNZ was dialyzed with molecular weight cut-off (MWCO) 12,000 g/mol dialysis tube against distilled water (Ryu et al., 2000). And a similar way was used to prepare PCL–PEG micelles loading indomethacin (Kim et al., 1998; Shin et al., 1998) and, hydroxycamptothecin (Shi et al., 2005) by slight changes in solvent and MWCO of dialysis tube. The particle sizes of obtained micelles ranged from 30 nm to 100 nm. The nanoparticles obtained from dialysis were mostly with average sizes around 100 nm, the folate-conjugated MPEG/PCL micelles containing paclitaxel were prepared in same way. The size of the folate-conjugated MPEG/PCL micelles formed was about 50–130 nm, depending on the molecular weight of block copolymers, and was maintained at less than 150 nm even after loading with paclitaxel (PTX) (Park et al., 2005a). However this technique could be time-consuming for that dialysis commonly continued for 24 or 48 h, and the distilled water was supposed to exchange for intervals of hours.

It is necessary to adopt the proper way to prepare particles for the method of preparation might affect the physicochemical properties of obtained particles. Two types of MPEG–PCL particles gained by dialysis method and by nanoprecipitation were compared. The study carried out by Letchford et al. (2009) has focused on how the physicochemical properties of MPEG–PCL particles affected the drug releasing behavior. The particles obtained through dialysis were called micelles while particles from nanoprecipitation were defined as nanospheres, both containing PTX as model drug. The micelles solubilized a maximum of 1.6% (w/w) of PTX and released 92% of their drug payload over 7 days, as compared to the nanospheres, which solubilized a maximum of 3% (w/w) of PTX and released 60% over the same period of time. *In vitro* human plasma distribution of nanoparticulate paclitaxel was also investigated, which indicated the formulation of nanoparticles might affect the drug releasing behavior and distribution.

3.1.3. Spray-drying method

Another kind of method to prepare micro/nanoparticles from polymers is spray-drying, which was widely used in collecting the dried particles from colloids system. The control of the spray-drying conditions is very essential, which include the size of nozzle, inlet temperature, liquid flow, and compressed spray air flow (Goud et al., 2005). When the liquid was fed to the nozzle with peristaltic pump, atomization occurred by the force of the compressed air, disrupting the liquid into small droplets. The droplets, together with hot air, were blown into a chamber where the solvent in the droplets was evaporated and discharged out through an exhaust tube. The dry product was then collected in a collection bottle (electronic precipitator) (Wang et al., 2005a,b). The preparation process of microspheres by spray-drying method is shown in Scheme 8. Wagenaar and Muller (1994) claimed that spray-drying allows the formation of particles in the micrometer size range, is rapid, easy to scale-up, and less dependent on the solubility characteristics of the drug and polymer than other techniques. And the microspheres obtained in this way presented stable drug recovery and morphological characteristics after 5 months of room temperature storage (Owusu-Ababio and Rogers, 1996; Raffin et al., 2002).

3.1.4. Others

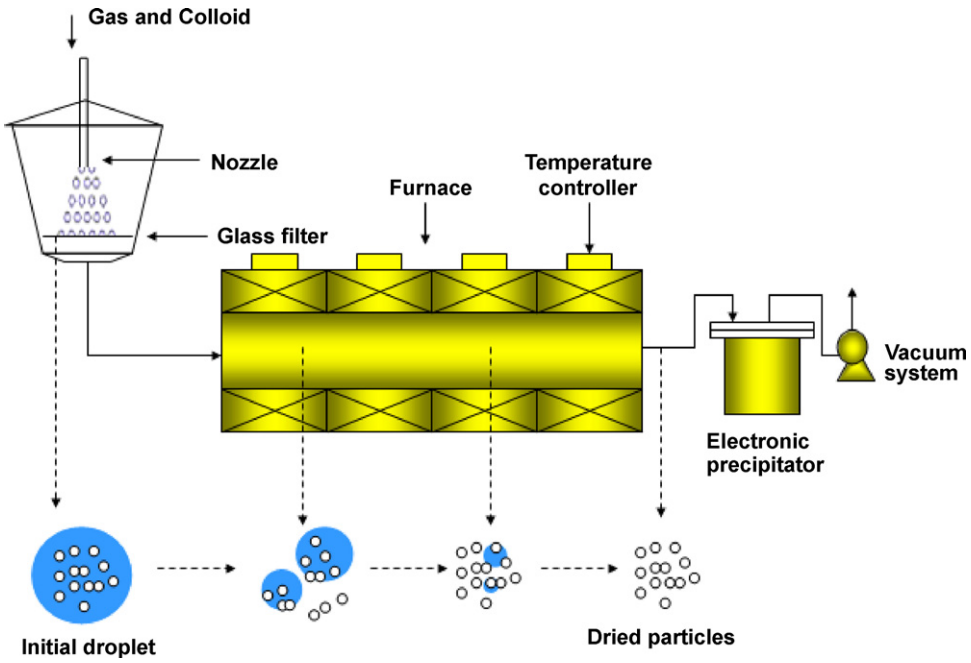
There are other ways to prepare micro/nanoparticles. As reported by Hoang et al. (2009), micelles composed of MPEG–PCL were prepared by the thin-film hydration method. Typically, 50 mg of MPEG–PCL was dissolved in DMF by stirring at room temperature for 24 h. The copolymer solution was dried under nitrogen at room temperature and left under vacuum overnight. 1 mL of phosphate buffered saline (PBS) (0.01 M, pH 7.4) at 60 °C was then used to hydrate the copolymer film. The solution was vortexed, stirred for 72 h at room temperature and sonicated for 1 h prior to use. Micelles formed were of the mean diameters of 57.7 ± 5.4 nm and retained their size and monomodal size distribution over the 14-day incubation period in the presence and absence of physiologically relevant concentrations of BSA. Otherwise, studies on micelles formed by thermal-induced self-assembling without any organic solvents were carried out (Wei et al., 2009). PCEC copolymers were dissolved in distilled water at 50 °C. Five minutes later, PCEC micelles formed due to thermal-induced self-assembling. Drugs loaded PCEC micelles were prepared by direct dissolution method assisted by ultrasonication. The obtained micelles were spherical in shape and of about 60 nm in diameter. This method could be considered highly welcome in preparation of micelles due to absence of toxicity organic solvents.

3.2. Application of micro/nanoparticles

3.2.1. Delivery of small molecular drugs

Generally, amphiphilic block copolymers composed of hydrophilic and hydrophobic segments can form a micelle-like structure with a hydrophobic inner core and a hydrophilic outer shell in selective solvent (Price et al., 1989; Hamad and Qutubuddin, 1990; Malmsten and Lindman, 1992; Quintana et al., 1992; Gao and Eisenberg, 1993; Marchal-Heussler et al., 1993; Munk et al., 1993; Piskin et al., 1995; Lemoine et al., 1996; Tanodekaew et al., 1997). In PCL/PEG polymeric micelles, hydrophobic core formed by PCL is surrounded by water-soluble polar groups of PEG that extended into an aqueous medium. Therefore, the drugs with a hydrophobic character can be easily incorporated into the core of nanoparticles by covalent or non-covalent bonding through hydrophobic interactions in aqueous media (Kim et al., 1998; Shin et al., 1998; Verger et al., 1998; Ryu et al., 2000; Jeong et al., 2004; Singh and Muthu, 2007).

Micro/nanoparticles from PCL/PEG copolymers had wide application in improving the solubility of hydrophobic drugs. Various water-insoluble drugs were "rescued" by encapsulation in micelles or nanospheres. For instance, micelles composed of MPEG-PCL of about 20 nm in size were obtained. Drug encapsulation of poorly water-soluble drugs belonging to biopharmaceutics classification system (BCS) class 11 (ketoprofen and furosemide) was evaluated. Experimental solubility for these two drugs showed a significant enhancement such that a maximum value of 23.4 mg/ml was obtained for ketoprofen in a 10% (w/v) micellar solution as compared to 0.14 mg in water. In the case of furosemide, the solubility increased from 0.04 mg/ml in water to about 3.2 mg/ml in a 10% (w/v) micellar solution (Dwan'isa et al., 2008). Also some studies on enhancing the solubility of geldanamycin with MPEG–PCL micelles were reported (Forrest et al., 2006), which inhibits heat shock protein 90 (Hsp90) and has shown significant antitumor activity *in vivo*. The fatty acid prodrugs of geldanamycin were encapsulated in PEG–PCL micelle, the aqueous solubility of which was increased



Scheme 8. The preparation of micro/nanoparticles by spray-drying method.

to 2 mg/ml and exhibited high activity against MCF-7 breast cancer cells. Other micelles containing small drugs such as indomethacin (IMC) and rapamycin were prepared and both showed ideal profiles of drug releasing and *in vivo* study. The investigation of the IMC pharmacokinetics in the IMC-loaded nanoparticles was carried out using the rats as animal model. Pharmacokinetics parameters such as the mean residence time (*MRT*, h), the steady-state volume of distribution (V_{DDS}, 1), the terminal half-time and the plasma clearance of IMC were determined, leading to a conclusion that MEPG–PCL nanoparticles have a significant potential for sustained release and the enhancement of circulation time of loaded drug by prolonging terminal half-life, increasing *MRT* and V_{DDS} of IMC (Kim et al., 2001). In another study, Laird Forrest et al. prepared an injectable formulation of rapamycin using amphiphilic block copolymer micelles of PEG–PCL (Yatscoff et al., 1995). The obtained micelles were stable in the presence of physiological serum albumin and demonstrated sustained release over several days, and the addition of α-tocopherol dramatically increased the release time of the vehicle in serum albumin (Forrest et al., 2005).

There were other papers which have reported on the studies concerned with PCL/PEG copolymers as carriers for hydrophobic drugs. Wei et al. (2009) found a significant difference between rapid release of free honokiol and much slower and sustained release of honokiol (HK) loaded PCL–PEG–PCL micelles in the *in vitro* release tests (shown in Fig. 3). Besides, micelles of MPEG–PCL as vehicles for the solubilization and controlled delivery of cyclosporine A were prepared by Aliabadi et al. (2005). With the same material, solubilization of fenofibrate was achieved (Jette et al., 2004). Spontaneously self-assembled micelles encapsulating ketoprofen and furosemide were obtained using PEG-PCL modified with trimethylene carbonate (Latere et al., 2008), also, PEG-P(CL-co-TMC) micelles containing risperidone were studied for oral delivery (Ould-Ouali et al., 2005).

Invaluable insight on the delivery of hydrophobic drugs for cancer chemotherapy has been gained through the research on polymeric micro/nanoparticles. Widely used anticancer drugs were either solubilized or made controlled released via incorporated into micro/nanoparticles. Hydrophobic drugs such as hydroxycamptothecin (Zhang et al., 2006), honokiol (Gou et al., 2008c) and paclitaxel (Park et al., 2005b) were made into nanoparticles of high encapsulation efficiency and stayed stable in aqueous solution.

In addition to that, MPEG–PCL nanoparticles containing hydrophilic drug doxorubicin were also prepared to optimize drug releasing profiles (Hsieh et al., 2008; Yadav et al., 2008). There were some efforts made on reducing the toxicity and enhancing the circulation time of anti-cancer agents (Shi et al., 2005; Li et al., 2008). Cis-Dichlorodiamminoplatinum (II)(cisplatin) has demonstrated extraordinary activities against a variety of solid tumors. However, the clinical efficacy is contrasted by its toxicity profile. Cisplatin was incorporated into the core–shell structure MPEG–PCL nanoparticles. Controlled release of cisplatin was observed in a sustained manner. Compared with free cisplatin, cisplatin-loaded nanoparticles exhibited superior antitumor effect by delaying tumor growth when delivered intratumorally in the *in vivo* evaluation (Li et al., 2008). Also, micelles loading hydroxycamptothecin (HCPT) from

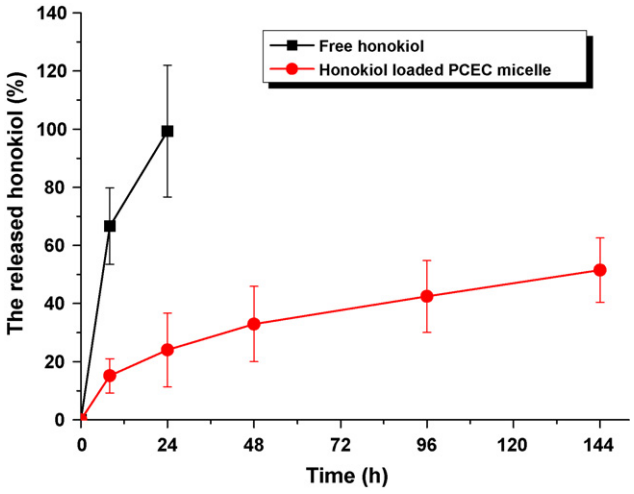


Fig. 3. Drug release profiles of free HK and HK-loaded micelles in PBS solution at pH 7.4. Reproduced from Wei et al. (2009), with permission.

MPEG–PCL copolymers were prepared and the change of pharmacokinetic characteristics of HCPT could lead to more powerful antitumor activity and less toxicity of the drug (Shi et al., 2005).

In the mean time, the cancer-targeting issues were studied intensively for reducing the side effects in cancer chemotherapy. Noticeably, some research concerned with folate as targeting groups was reported (Park et al., 2005a; Chen et al., 2008a; Yang et al., 2008b). Park et al. (2005a) have prepared folate-conjugated MPEG–PCL micelles containing paclitaxel. The paclitaxel-loaded folate-conjugated MPEG–PCL micelles (PFOL50) have exhibited much higher cytotoxicity for cancer cells, such as MCF-7 and HeLa cells, than MPEG–PCL micelles without the folate group. Similarly, folate-conjugated MPEG–PCL nanospheres were also prepared containing the same drug (Park et al., 2005b). Chen et al. have succeeded in synthesizing the hyperbranched core–shell polymers with aliphatic polyester Boltorn H40 as the core, PCL as the inner part, PEG as the outer shell and folate incorporated to the surface to achieve tumor cell targeting property. 5-Fluorouracil and paclitaxel were encapsulated in the nanoparticles by dialysis method. The drug release property and the targeting of the drug-loaded nanoparticles to different cells were evaluated *in vitro*. The results showed that the drug-loaded nanoparticles exhibited enhanced cell inhibition because folate targeting increased the cytotoxicity of drug-loaded nanoparticles against folate receptor expressing tumor cells (Chen et al., 2008a). In addition to this, Superparamagnetic iron oxide (SPIO) and doxorubicin (DOX) were coencapsulated within the folate-conjugated MPEG–PCL micelles. The result of cell culture experiment indicated that folate attachment to micelles resulted in the recognition of the micelles by tumor cells overexpressing folate receptors, leading to facilitation in cellular uptake of micelles, and the transport efficiency of the SPIO-loaded and folate-functionalized micelles into the tumor cells can be further enhanced by applying an external magnetic field to the cells (Yang et al., 2008a).

Other specific application of PCL/PEG micro/nanoparticles such as multimodal carriers for drugs and imaging agents was reported. MPEG–PCL nanoparticles co-encapsulating hydrophobic organic actives (beta-carotene) and inorganic imaging nanostructures (Au) were obtained with narrow particle size distributions between 75 nm and 275 nm. The technique was anticipated to be applied to a variety of hydrophobic active compounds, fluorescent dyes, and inorganic nanostructures, yielding nanoparticles for combined therapy and multimodal imaging applications (Gindy et al., 2008b). Another novel drug delivery system for hydrophobic photosensitizers in photodynamic therapy (PDT) was accomplished using MPEG–PCL micelles containing protoporphyrin IX (PpIX). The spherical micelles have a high PpIX-loading efficiency of 82.4% and a narrow size distribution with a mean diameter of 52.2 ± 6.4 nm. The cellular uptake of PpIX in RIF-1 cells using PpIX micelles was approximately two-fold higher than that for free PpIX. Results showed that PpIX micelles have markedly increased photocytotoxicity over that with free PpIX (Li et al., 2007).

3.2.2. Delivery of biomacromolecules

3.2.2.1. Protein delivery. Protein could be bound to charged polymeric nanoparticles for electrostatic interactions, and this provided an important method to load protein onto the surface of nanoparticles (Kazzaz et al., 2000; Cui and Mumper, 2002; Zhou et al., 2003; Ataman-Onal et al., 2006). MPEG–PCL (PECL) microspheres containing human serum albumin (HSA) were prepared by solvent extraction method based on the formation of double w/o/w emulsion. And all the PECL microspheres achieved higher HSA loading efficiency compared with PCL microspheres. In order to obtain high HSA loading in maleimide-functional MPEG-PCL nanospheres, a study has been done to reveal how the reaction parameters (reaction order and docking constant) describing the

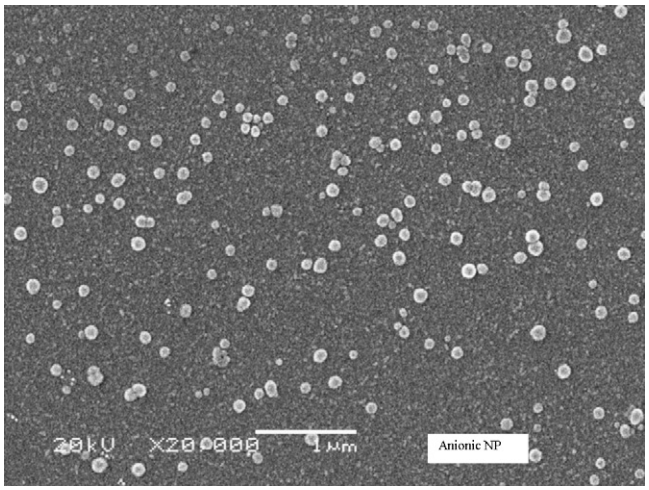


Fig. 4. The SEM photograph of the prepared blank anionic PCEC nanoparticles. Reproduced from Gou et al. (2007), with permission.

extent of ligand conjugation were determined (Zhou et al., 2003). The result suggested that the particle formation process, using block copolymer-directed kinetically controlled assembly and surface functionalization represented a versatile new platform for the preparation of bioconjugated nanospheres with accurate control of ligand density and minimal processing steps.

When it comes to vaccine delivery via PEG/PCL micro/nanoparticles, series of works about PCEC nanoparticles in vaccine delivery system in immunotherapy of cancer were carried out by Gou et al. (2007, 2008a,b). Anionic PCEC nanoparticles were prepared by modified emulsion solvent evaporation method, and the morphology of prepared nanoparticles could be observed from Fig. 4. And human basic fibroblast growth factor (bFGF), as model protein, was absorbed onto the surface of nanoparticles thanks to the electrostatic interaction. *In vitro* release study indicated a controlled release of bFGF from PCEC–bFGF complex, which was presented in Fig. 5. Then the complex was injected s.c. into BALb/c mice at 20 μg/dose on weeks 0, 1, 2 and 3. The result indicated that high level of bFGF special antibody was obtained at week 5, which implied that anionic PCEC nanoparticles modified by SDS were an efficient adjuvant for bFGF to create strong humoral immunity. Furthermore, to improve the capacity of anionic PCEC nanoparticles to enhance humoral immunity, mannan, as a receptor of dendritic cells (DCs), was attached to the surface of PCEC nanoparticles during the preparation (Gou et al., 2008b). Then, the mannan coated anionic PCEC nanoparticles were employed to enhance humoral immunity against bFGF, and antibody titer was proved four times than that created by PCEC-bFGF complex which was probably because that mannan-PCEC nanoparticles could target the antigen

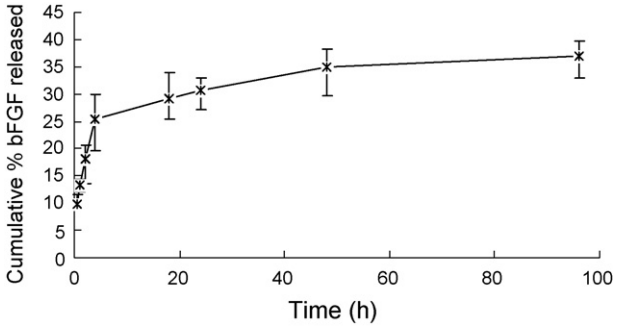


Fig. 5. *In vitro* release behavior of bFGF from bFGF/PCEC nanoparticle complexes. Reproduced from Gou et al. (2007), with permission.

to DCs, because there are mannan-receptors in DCs. Moreover, the antibody against bFGF was obtained and it was kept at a high level for a long time more than 11 weeks which indicated that bFGF encapsulated PCEC nanoparticles might have potential application in single-dose vaccine delivery system (Jia et al., 2008).

In another study, MPEG–PCL polymersomes were conjugated with mouse-anti-rat monoclonal antibody OX26 (OX26-PO) to achieve great blood–brain barrier (BBB) permeability. The result of brain delivery in rats proved that the increase of surface OX26 density of OX26-PO decreased blood AUC. The optimized OX26 number conjugated per polymersome was 34, which can acquire the greatest blood–brain barrier (BBB) permeability surface area product and percentage of injected dose per gram brain (%ID/g brain). Furthermore, NC-1900, as a model peptide, was encapsulated into OX26(34)-PO and improved the scopolamine-induced learning and memory impairments in a water maze task via i.v. administration (Pang et al., 2008).

3.2.2.2. Nucleic acid. Non-viral gene therapy is widely believed to be able to overcome the problems inherent to current viral-based therapies, including immune and toxic reactions as well as the potential for viral recombination (Liu and Huang, 2002). Many approaches are being explored to improve the efficiency of non-viral gene delivery systems, seeking for different carrier formulations for plasmid DNA delivery. The PCL/PEG copolymers were intensively studied for its potential to be non-vial gene carriers (Jang et al., 2006a; Vroman et al., 2007; Huang et al., 2008). Research aimed at optimizing the carrier properties of PCL/PEG micro/nanoparticles has been reported. Noticeably, the investigation to evaluate the participation of PEG segments in gene delivery system composed of PCL was carried out. PCL/DNA polyplexes were compared with PCL/DNA combined with a PCL–PEG copolymer to form novel PEG–PCL/DNA formation with a hydrophilic corona on the surface. The cytotoxicity, transfection efficiency and cellular uptake of polyplexes and their association with PEG were evaluated on HeLa cells. The degree of interaction of copolymer with plasmid DNA was very high. Association of polyplexes with PCL–PEG copolymer led to a small increase in particle size and a sharp decrease of charge surface. Cytotoxicity, transfection efficiency and cellular uptake were significantly reduced relative to unshielded copolymer/DNA complexes. In conclusion, the PEGylated formulations might be an attractive approach for the *in vivo* application (Vroman et al., 2007). Furthermore, to reveal the effect of charge on the vehicle properties for systemic gene delivery, two types of MPEG–PCL nanoparticles containing pGL3-Control (plasmid DNA) were prepared using nonionic amphiphilic block copolymers and ionic amphiphilic block copolymers containing a terminal cationic group. The hydroxy groups of MPEG–PCL block copolymer were then modified into an amine group to synthesize an amine-terminated MPEG-PCL diblock copolymer (AMPEG/PCL). DNA was incorporated into the polymeric nanoparticles by physical entrapment and electrostatic interaction. Based on *in vitro* cytotoxicity tests, the DNA-loaded MPEG/PCL and AMPEG/PCL nanoparticles did not induce any remarkable cytotoxicity against normal human fibroblasts. AMPEG/PCL nanoparticles with cationic charge groups exhibited somewhat higher DNA transfection efficiencies in comparison with the nonionic MPEG/PCL nanoparticles of the same molecular structure except for the terminal amine groups (pb0.05). These results indicated that AMPEG/PCL nanoparticles with low density of primary amine groups could effectively release the DNA within cells and thus be useful as a carrier for DNA delivery (Jang et al., 2006a). Despite the effect of PEG participation and the charge on the properties of PCL/PEG micro/nanoparticles as gene carriers, the consideration of pDNA/nanoparticles mass ratio on carrier behavior was reported by performing experiments on cationic PCEC nanoparticles (Huang et al., 2008). The effect of polymer concentration on the particle size of prepared cationic PCEC nanoparticles was presented in Fig. 6. Particle size increased almost linearly with the increase of polymer concentration. Effect of pDNA/nanoparticles mass ratio on the size of complex was investigated and the result showed that pDNA/nanoparticles mass ratio could be adjusted to obtain pDNA/nanoparticles complex in optimized size for a better transfer. The prepared cationic PCEC nanoparticles are able to absorb plasmid DNA to form pDNA-nanoparticles complex and the pDNA could released from the complex in an extended profile *in vitro* (Fig. 7) (Huang et al., 2008).

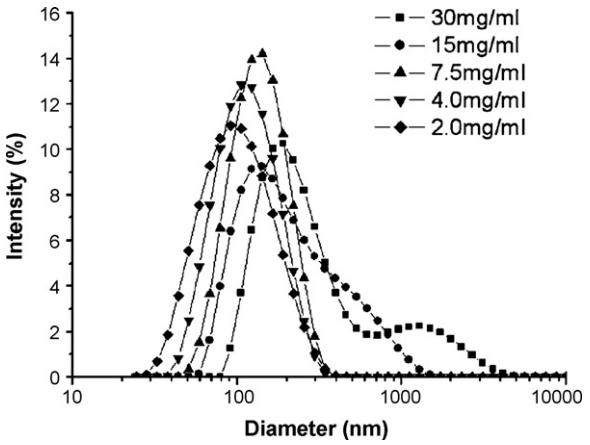


Fig. 6. The particle size distribution spectra of cationic PCEC nanoparticles formed at various polymer concentrations. Reproduced from Huang et al. (2008), with permission.

PCL/PEG micro/nanoparticles also represented as one of the promising polymeric vehicles for small interfering RNA (siRNA) delivery. Sun et al. have designed triblock copolymer consisting of PCL/PEG and cationic poly(2-aminoethyl ethylene phosphate) (PPEEA) block (mPEG45-b-PCL100-b-PPEEA12). Copolymer self-assembled into micelle nanoparticles (MNPs) with siRNA binding to PPEEA block. The loading of siRNA to MNPs is convenient and does not significantly alter the particle size distribution. The siRNA loaded MNPs mediated effective siRNA delivery and resulted in remarkably efficient gene silencing in HEK293 cells in the presence of serum (Sun et al., 2008). In addition to that, specific siRNAs that target estrogen receptor alpha (ERalpha) were encapsulated in PEG–PCL–malic acid (PEG–PCL/MA) nanocapsules (NCs). The intravenous injection of these NCs into estradiol-stimulated MCF-7 cell xenografts led to a significant decrease in tumor growth and a decrease in ERalpha expression in tumor cells (Bouclier et al., 2008).

4. PCL/PEG hydrogel

4.1. Introduction of PCL–PEG hydrogel

Hydrogels are a special class of materials that absorb a considerable amount of water while maintaining their integrity in water. In

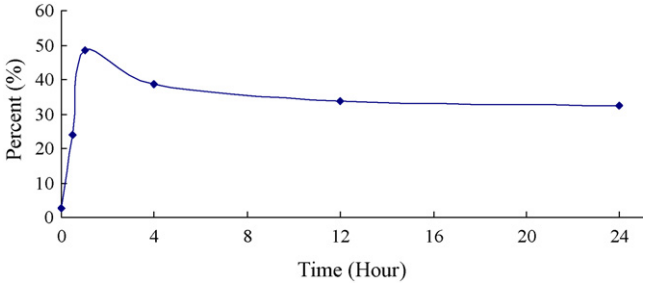


Fig. 7. Release behavior of pGFP from pGFP/PCEC nanoparticle complexes *in vitro*. Reproduced from Huang et al. (2008), with permission.

past decades, the stimuli-sensitive copolymer hydrogel has gained increasing attention owing to their biodegradability, biocompatibility, and smart responsibility to the environmental stimulus, including chemical substances and changes in temperature, pH, light, pressure, electric field, etc. Among them, especially, the thermosensitive physical crosslinked hydrogels and pH-sensitive hydrogels, consisted of hydrophobic PCL (A) blocks and hydrophilic PEG (B) blocks, have been extensively studied because of their potential biomedical applications in advanced drug delivery system, including controlled drug delivery, cell encapsulation and tissue repair (Jeong et al., 1997; Markland et al., 1999; Torres-Lugo and Peppas, 1999; Lee et al., 2001; Jeong et al., 2002; Kissel et al., 2002; Ichikawa and Peppas, 2003; Kim et al., 2003a; Li et al., 2003a,b; Song et al., 2004; Chao et al., 2006, 2008; Liu et al., 2007a,b; Chen et al., 2008b; Wang et al., 2009).

PCL and PEG have been widely used in biomedical field, because they are materials that are biocompatible and have been used in several FDA approved products. Since Perret and Skoulios (1972) firstly prepared a series of block copolymers containing PEG and PCL, these copolymers consisting of PCL blocks and PEG blocks have been widely studied (Martini et al., 1994; Bogdanov et al., 1998; Ryu et al., 2000; An et al., 2001; Huang et al., 2004; Bae et al., 2005; Li et al., 2005, 2006). Due to combination of great advantages of PEG and PCL, the PCL–PEG copolymer might have great application in biomedical fields.

4.2. Thermosensitive hydrogels

As advanced smart drug delivery systems, thermosensitive hydrogels act as a very important role. At or below ambient temperature, the thermosensitive hydrogels is a free flowing sol. Then, by *in vivo* injection, the hydrogel forms a non-flowing gel at body temperature. Upon incorporation of pharmaceutical agents into the hydrogel, the systems could potentially act as sustained drug release depot *in vivo* (Kamath and Park, 1993; Bromberg and Ron, 1998; Qiu and Park, 2001; Jeong et al., 2002; Gong et al., 2007, 2009a,b, in press-a,b,c,d). Administration of drugs *in situ* assisted by injectable thermosensitive hydrogel is an interesting route for drug local delivery.

In previous contributions, the gel–sol or sol–gel phase transition behavior studies were mostly investigated using the rheometer and test tube-inverting method. According to the studies of Liu et al., the obtained aqueous PEG–PCL block copolymer system was enclosed in a vial with one screw cap and kept at 4 °C for 12 h prior to measurement. The temperature was increased by 2 °C each step, and samples were immersed for 15 min at a measuring temperature. The sol–gel transition temperature was determined by tilting the vials 90 °C for 1 min. If there was no flow, it was regarded as a gel state (Hwang et al., 2005).

In respect of thermosensitive hydrogels based on PCL–PEG–PCL copolymer, Martini et al. (1994) investigated the micellization and gelation properties of these triblock copolymers in aqueous solution, and they found the sol–gel transition on cooling such copolymer aqueous solution at given concentration. However, An et al. (2001) reported that the PCL–PEG–PCL system did not show lower gel–sol transition at any composition and the sol–gel transition on cooling. Just recently, Bae et al. (2005) found that PCL–PEG–PCL triblock copolymer aqueous solution (>15 wt%) underwent the sol–gel–sol transition as the temperature increased from 10 °C to 60 °C. Liu et al. (2007a) found that the gel–sol phase transition behavior of PCL–PEG–PCL copolymers was dependent on hydrophilic/hydrophobic balance in macromolecular structure, as well as on heating history of copolymer aqueous solution. In their further contribution, by adjusting the PCL/PEG balance and molecular weight of PCL–PEG–PCL copolymer, Gong et al. reported the sol–gel–sol transition of PCL–PEG–PCL hydrogels as the temperature increased (shown in Fig. 8).

PEG–PCL–PEG hydrogel is another PCL/PEG hydrogel widely investigated recently. Hwang et al. (2005) found that the aqueous solution of PEG–PCL–PEG triblock copolymers (>15 wt%) undergoing "clear sol–gel–turbid sol" transition as the temperature increases from 20 °C to 60 °C. Gong et al. (2007) reported the gel–sol transition of the PEG–PCL–PEG triblock copolymers aqueous solution. They found that the gel–sol phase transition was depended on macromolecular weight, hydrophilic/hydrophobic balance in macromolecular structure, as well as some other factors, including heating history, volume, and aging time of copolymers aqueous solutions and dissolution temperature of the copolymers. Moreover, in their further study, they observed sol–gel–sol transition of PECE copolymers and more factors affecting the transition diagram were investigated in detail (Gong et al., 2009a). The sol–gel–sol transition behavior of the copolymers depended on a number of factors, such as macromolecular weight, hydrophilic/hydrophobic balance (PEG/PCL ratio) in the molecular structure, the treatment manner of hydrous copolymers, topology of the triblock copolymers, and the solution composition of the hydrogel.

Phase transition behaviors of PCL–PEG–PCL or PEG–PCL–PEG hydrogels are very much dependent on chemical composition (PCL/PEG ratio in molecular structure and total molecular weight of the copolymers) and thermal history of the copolymers solution. PCL–PEG–PCL and PEG–PCL–PEG hydrogels with different PCL/PEG ratio, total molecular weight, or thermal history will show quite different phase transition behaviors, resulting in the difference of phase transition behavior described between previous works.

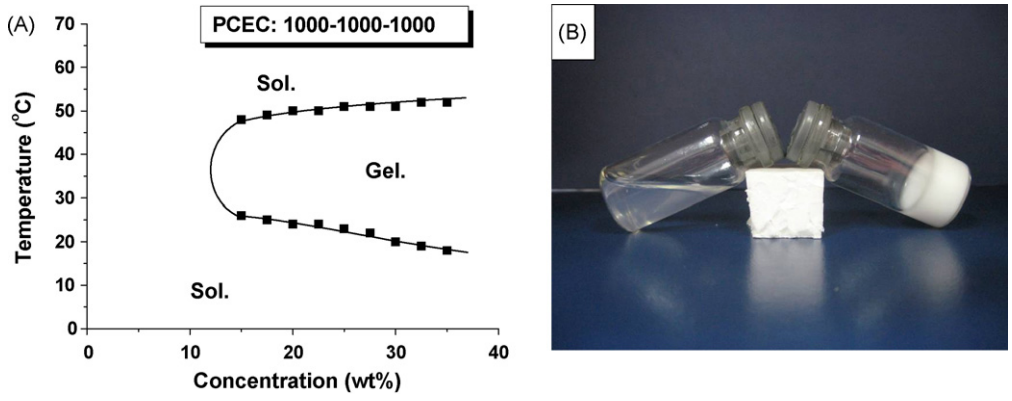


Fig. 8. Sol–gel–sol phase transition of PCEC hydrogel. (A) Sol–gel–sol phase transition diagram. (B) Photograph of PCEC hydrogel (20 wt%) at 10 °C (left) and at 37 °C (right). Reproduced from Gong et al. (in press-b), with permission.

Copolymers with much higher PCL/PEG ratio (e.g., >2.5) are quite hydrophobic and are partially soluble in water, whereas copolymers with much lower PCL/PEG ratio are too hydrophilic and freely soluble in water. Therefore, in order to develop hydrogels with lower sol–gel transition temperature at around physiological temperature, chemical composition of copolymers and thermal history of copolymer aqueous solution should be chosen carefully.

Mechanistic studies on the phase transitions and characterization of the sol and gel state were reported using various instrumental techniques, such as ultrasound velocity, dynamic and static light scattering, small angle neutron scattering, rheology, and microcalorimetry (Jeong et al., 1999). The general thermogelation mechanisms include: solvent-induced gelation, partial crystallization, coil-to-helix transition, hydrophobic association, and micelle packing, which serve as reversible physical crosslinking points to form hydrogel (Lee et al., 2001; Jeong et al., 2002). Recently, Bae et al. (2005) claimed that the mechanism of sol–gel transition or gel–sol transition for PCL–PEG–PCL hydrogel were micellar affregation or increase in the molecular motion of PCL accompanying the core–shell structure breakage. And later, Hwang et al. (2005) proposed the similar mechanism of sol–gel–sol transition for PEG–PCL–PEG hydrogel. Liu et al. (2007b) reported that the gel–sol transition of PCL–PEG–PCL hydrogel was micelle packing driven by hydrophobic interactions and crystallization of PCL blocks. More recently, Gong et al. (in press-c) proposed the similar mechanism, and the schematic diagram was shown in Fig. 9.

4.3. pH-sensitive hydrogels

Currently, therapeutic drugs are administrated mainly by intramuscular or intravenous injections due to their delicate physicochemical characteristics in aqueous solutions and susceptibility to be degraded in acidic gastric fluid and biological fluids where there are many kinds of enzymes. In order to increase bioavailability of drugs by oral route, pH-sensitive hydrogels are used as the drug delivery system (Peppas and Klier, 1991; Markland et al., 1999; Torres-Lugo and Peppas, 1999; Ichikawa and Peppas, 2003; Kim et al., 2003a; Chao et al., 2006, 2008; Chen et al., 2008b; Wang et al., 2009). The pH-sensitive hydrogels contain pendant acidic or alkaline groups which can accept or release protons in response to changes of environmental pH (known as polyanionic and polycationic hydrogel, respectively). Acidic groups become ionized at high pH and swell more if crosslinked, whereas alkaline groups becomes ionized at low pH. Hydrogels made from these copolymers by crosslinking display significant differences in swelling properties depending on the pH of the environment. Furthermore, the significant differences of pH between the stomach

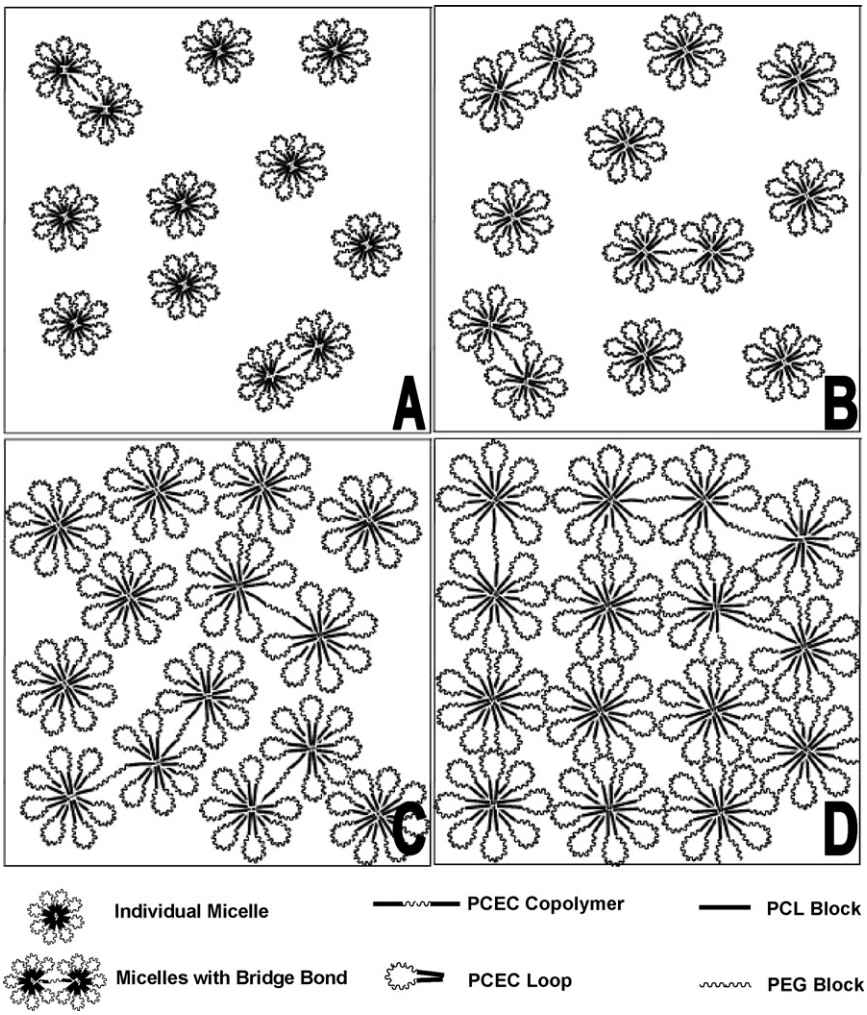


Fig. 9. A schematic diagram of the micellar aggregation mechanism for PCEC copolymer aqueous solution as temperature increase. (A) At low temperature, micelles with small size flow freely in the aqueous solution. (B) The micelle size slightly increases as temperature increases. (C) When the hydrogel concentration was above CGC, with further increase in temperature to around sol–gel transition temperature, the micelle size increase rapidly resulting in sol–gel transition. (D) The aggregation and packing between micelles increases to form more dense gel by raising temperature. Reproduced from Gong et al. (in press-c), with permission.

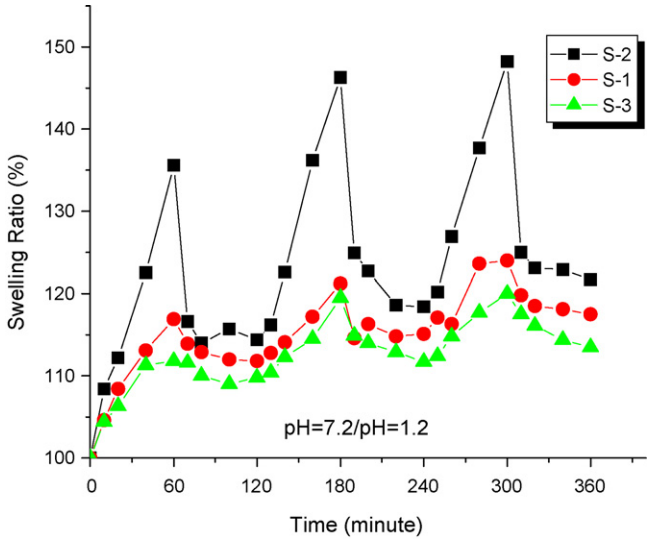


Fig. 10. Effect of MA and PCL content on the dynamic swelling/deswelling behavior of hydrogels in aqueous medium with pH = 7.2/1.2 at an interval of 60 min (37 °C). Reproduced from Chao et al. (2008), with permission.

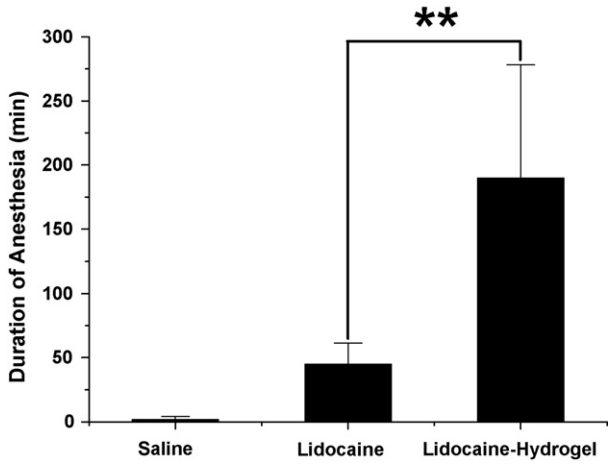


Fig. 11. Latency of tail flick test after subcutaneous administration of normal saline, lidocaine, and lidocaine loaded PCEC hydrogel. Error bars represent the standard deviation ($n = 6$). Lidocaine loaded PCEC hydrogel produced significantly local enduring antinociceptive effects compared with lidocaine aqueous solution ($^{**}p < 0.01$). Reproduced from Gong et al. (in press-c), with permission.

and the intestine are large enough to elicite pH-sensitive response of the hydrogels.

Polyanionic hydrogels swell minimal in the stomach condition, and therefore the drug release is also minimal in the stomach. When the drug delivery system is in intestinal region, the swelling of the hydrogel is increase due to increase in pH, and thus the drugs could permeate out. The properties of polyanionic hydrogels could protect drugs from acidic degradation, and delivery them in intestinal region. Peppas and Klier (1991) developed a novel hydrogel with PEG and poly(methacrylic acid) (PMA), which has excellent pH-sensitive properties. Poly(methacrylic acid–ethylene glycol) (P(MAA–g-EG)) copolymer exhibits unique properties due to its ability to form reversible hydrogen-bonded complexes. This complexation results in changes of swelling properties upon change in pH value of surrounding environment. At acidic environment, the acidic protons of the carboxyl groups in PMA segments interact with the ether oxygen in PEG chains through hydrogen bonding to form highly compact network, which lead to the shrinkage of the hydrogels. When pH increased to alkaline value, the carboxyl groups become ionized and the complexation disassociates, which results in swelling of the network. This behavior presents potential application of such polymer in pH-sensitive drug delivery systems. But unfortunately, P(MAA–g-EG) hydrogel is not biodegradable. As a result, its clinical use is greatly limited. Chao et al. (2008) introduce PCL into the backbone of P(MA–g-EG) hydrogel, and developed a novel biodegradable pH-sensitive P(CL-MA-EG) hydrogel. The swelling behavior, pH sensitivity, and degradation behavior of the hydrogels were studied in detail (shown in Fig. 10). Unlike polyanionic hydrogels mentioned above, polycationic hydrogels, which swell more under acidic conditions, could be ideal local drug delivery systems in the stomach. Patel and Amiji (1996) reported the preparation and application of chitosan-PEG hydrogel for delivery of antibiotics in the stomach.

4.4. Application of PCL–PEG hydrogel

Hydrogel as an amphiphilic material is a potential candidate of implanted delivery system for long-term delivery of hydrophilic small molecular, protein, or nucleic acid drugs which rapidly degraded in the presence of proteolytic enzymes (Sawhney et al., 1993).

Ha et al. (1997) studied the bioerodible hydrogel for albumin delivery which was successfully prepared from semi-interpenetrating polymer networks (SIPNs) composed of PCL–PEG macromer. Cho et al. also reported the hydrogel based on SIPNs composed of PCL and PEG macromer containing tetracycline. It was found that water absorption increased with PEG content due to great hydrophilicity of PEG. And the drug release can be controlled by PEG content in PCL–PEG SIPNs, concentration of macromers in solution during SIPNs preparation, and molecular weight of PEG (Cho et al., 1996). Recently, Gong et al. (in press-c) report the *in vitro* release behaviors of hydrophilic small molecular and protein drugs from PCL–PEG–PCL hydrogel, and *in vivo* release behavior of Lidocaine were also observed by anaesthesia assay using tail flick latency (TFL) test (shown in Figs. 11 and 12). In other investigations, pH-responsive hydrogels are studied to increase bioavailability of protein and peptide drug. pH/temperature-sensitive injectable hydrogel of pentablock copolymer PAE–PCL–PEG–PCL–PAE was manufactured by using poly(beta-amino ester) (PAE) as a pH-sensitive moiety to conjugate to the temperature-sensitive PCEC copolymer. And the complexes were made ionic by PAE, thus being capable of loading anionic biomolecule such as insulin. The pharmacokinetic release of insulin from the complex gel *in vivo* on male Sprague–Dawley (SD) rats was studied (Huynh et al., 2008). Recently, another pH-sensitive hydrogel P(CL-MAA-EG) from PCL, methacrylic acid and PEG was successfully synthesized as well (Chao et al., 2008).

Due to great hydrophilicity, thermosensitive hydrogels have been widely used as drug delivery system for biomacromolecules such as proteins and pDNA. However, it is difficult for such hydrogel to deliver poorly soluble drugs. Therefore, a novel composite system, nanoparticles in hydrogel, was proposed owing to their unique potentials via combining the characteristics of a hydrogel system (e.g., amphiphilicity and *in situ* sustained release ability) with a nanoparticle (e.g., capability of encapsulating hydrophobic agent and very small size) (Hamidi et al., 2008). A composite drug delivery system: PCEC nanoparticles in thermosensitive hydrogel, was demonstrated for topical administration of poorly soluble drug. Briefly, poorly soluble drug was incorporated into PCEC nanoparticles by solvent evaporation method. Then F127 was dissolved in the drug-loaded PCEC nanoparticles slurry at the concentration of 20% at 4 °C. When the F127 hydrous matrix containing drug-loaded PCEC nanoparticles was injected into body, with the increase of temperature, F127 gel formed. As a result, an

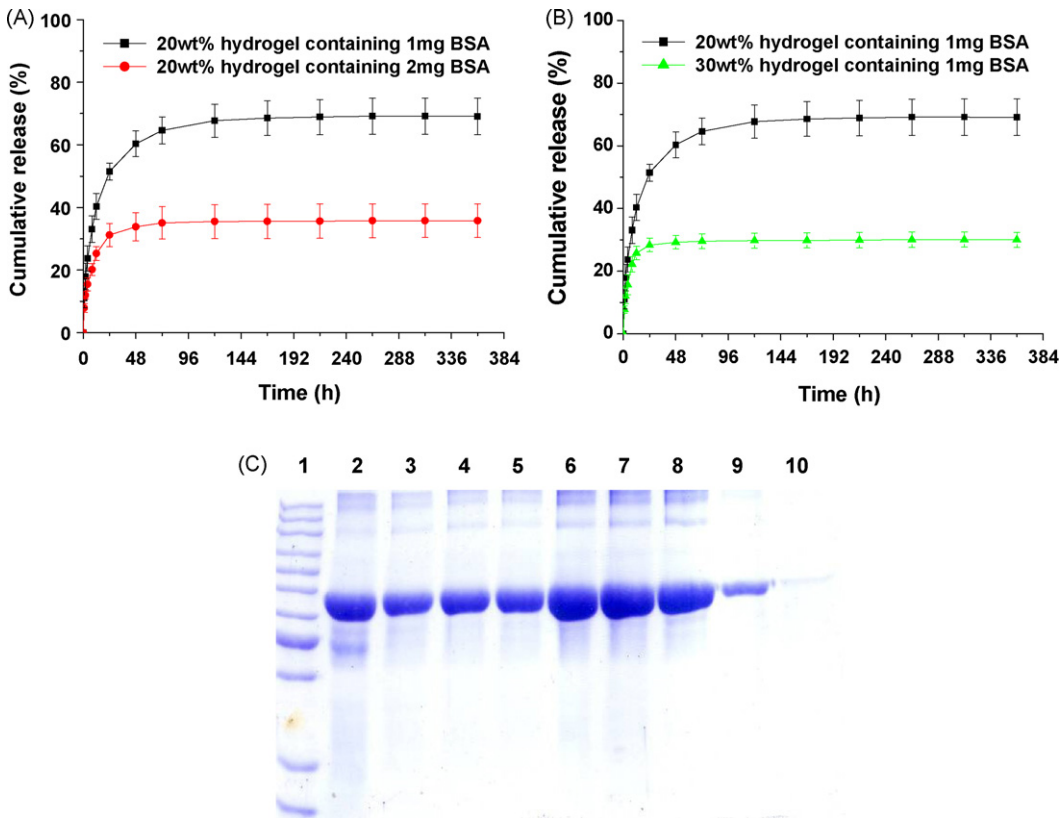


Fig. 12. *In vitro* release behavior of BSA from PCEC hydrogel (S2). Error bars represent the standard deviation ($n = 3$). (A) Effect of initial BSA loading amount; (B) Effect of hydrogel concentration; (C) SDS-PAGE results of BSA *in vitro* release profile; Lane 1: marker; Lane 2: Standard BSA; Lane 3: 1 h; Lane 4: 4 h; Lane 5: 12 h; Lane 6: 24 h; Lane 7: 48 h; Lane 8: 72 h; Lane 9: 168 h; Lane 10: 360 h. Reproduced from Gong et al. (in press-c), with permission.

injectable drug delivery system, nanoparticles in thermosensitive hydrogel, for topical administration of poorly soluble drugs was successfully demonstrated and it was showed in Fig. 13 (Gou et al., 2008c). Further, Gou et al prepared another composite hydrogel drug delivery system: honokiol loaded PCEC nanoparticles in PECE hydrogel[184]. In their work, the authors obtained nanoparticles and hydrogels from PCL–PEG copolymer by varying the chemical structure, macromolecular weight, PCL/PEG molar ratio, and topography structure. And the hydrophobic chemotherapeutic honokiol was processed into nanoparticles at first, and then was dispersed in PECE hydrogels. The honokiol was released from the composite hydrogel in an extended profile. Such nanoparticle-in-hydrogel composite drug delivery system might have great potential application in pharmaceutical field.

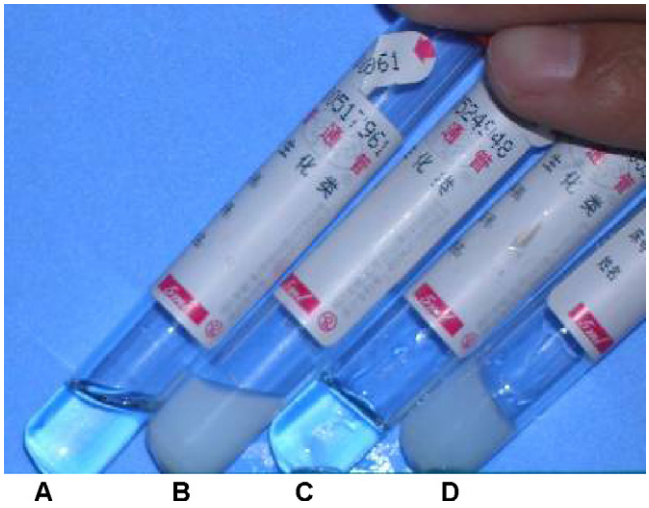


Fig. 13. Direct observation of obtained drug delivery system: nanoparticles in thermosensitive hydrogel. After the tube was taken out of the water bath, the photo was taken immediately to avoid the phase transition take place at room temperature. (A) F127 sol, at the concentration of 20%, at 4 °C; (B) F127 sol at the concentration of 20% containing honokiol loaded nanoparticles slurry at 4 °C; (C) F127 gel, at the concentration of 20%, at 37 °C; (D) F127 gel, at the concentration of 20%, containing hokiol loaded nanoparticles slurry at 37 °C. Reproduced from Gou et al. (2008c), with permission.

5. Conclusion and perspectives

The tremendous progress has been attained in the evaluating and optimizing the application of PCL–PEG copolymers in DDS. The reported studies on the PCL–PEG copolymers involved syntheses of the copolymers, preparation and application as micro/nanoparticles and thermosensitive hydrogels carrying various substances. Owing to the appearance of PEG segment in PCL–PEG copolymer, the degradation and biocompatibility of the PCL polyester has been improved.

The synthetic procedures vary in reactants and catalysts to obtain different types of di- or triblock copolymers of PCL–PEG. The obtained copolymers are able to be characterized and the study on their degradation and biocompatibility was performed. From the obtained copolymers, the fabrication of micro/nanoparticles can be adopted and modified from numerable techniques according to the attributes of materials and loaded substances. The investigation of thermosensitive hydrogel from PCL–PEG copolymer particularly focused on the gel–sol or sol–gel transition behavior and their potential as a candidate of implantable delivery system.

Despite considerable research efforts and impressive progress made in recent years, the question of evaluation of toxicity

of PCEC nanoparticles remains to debate, for a further and complete evaluation needed to be worked on the biocompatibility issue of the nanoparticles. Meanwhile, developing advanced method to prepared drug-loaded PCL/PEG micro/nanoparticles is a field to be worked on. Application of ligand modified PCL/PEG micro/nanoparticles in cancer target therapy was highlighted and would attract much attention in future studies. Numerous challenges remain in the application of PCL–PEG micro/nanoparticles in vaccine and the mechanism of improved immunity enhanced by polymeric nanoparticles is unclear. Making drugs based on PCL/PEG vectors to market might be challenging but exciting and promising as well. So far, PCL–PEG micro/nanoparticles containing various types of drugs have been prepared, which made the application of ligand linked PCL–PEG particles as target drug delivery system possible. In addition, developing a transdermal and oral needle-free protein and DNA vaccine delivery based on PCL–PEG micro/nanoparticles and hydrogels is another issue. The ability to address these challenges will be facilitated by advances in biology and materials science, and advances in materials characterization will aid in understanding how materials interface with cells and tissues. Although many advances have been made, much work still remains before micro/nanoparticles or hydrogel of PCL–PEG optimized as useful devices for efficient drug delivery.

Acknowledgments

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