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Review article

In situ forming parenteral drug delivery systems: an overview

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Abstract

Biodegradable injectable in situ forming drug delivery systems represent an attractive alternative to microspheres and implants as parenteral depot systems. Their importance will grow as numerous proteins will lose their patent protection in the near future. These devices may offer attractive opportunities for protein delivery and could possibly extend the patent life of protein drugs. The controlled release of bioactive macromolecules via (semi-) solid in situ forming systems has a number of advantages, such as ease of administration, less complicated fabrication, and less stressful manufacturing conditions for sensitive drug molecules. For these reasons, a number of polymeric drug delivery systems with the ability to form a drug reservoir at the injection site are under investigation. Here, we review various strategies used for the preparation of in situ forming parenteral drug depots and their potential benefits/draw-backs, especially with regard to the delivery of protein drug candidates.

Keywords: In situ forming implants; Protein delivery systems; Biodegradable polymers; Thermoplastic pastes; In situ cross-linking; Solvent precipitation; Thermally induced gelation; Ion-mediated gelation

1. Introduction

Liquid formulations generating a (semi-) solid depot after subcutaneous injection, also designated as implants, are an attractive delivery system for parenteral application for a number of reasons. First, the application is less invasive and painful compared to implants, which require local anesthesia and a small surgical intervention. Secondly, localized or systemic drug delivery can be achieved for prolonged periods of time, typically ranging from one to several months.

The field of in situ forming implants has grown exponentially in recent years, in parallel to the development of new protein therapeutics. Caused by the explosion of genomic information and the final mapping of the human genome [1] a large number of peptides and proteins may become candidates for therapeutic application. The understanding of protein functions in the ethiopathology of currently incurable diseases could lead to new therapeutic approaches. Hence, the development of new injectable drug delivery systems which protect proteins against denaturation in body fluids and allow sustained release profiles are in great demand.

There are also important economic reasons to consider because development of an injectable controlled-release formulation can extend the patent life of a drug. Since a number of recombinant proteins (biogenerics) fall into this category, parenteral delivery systems are an attractive proposition. Oral administration of generic proteins is still a formidable challenge and it remains to be seen if such a route of administration is feasible [2].

Generally, parenteral depot systems could minimize side effects by achieving constant, 'infusion-like' plasma-level time profiles, especially important for proteins with narrow therapeutic indices. A dose reduction resulting from the avoidance of peaks and valleys, as well as the enhancement of patient compliance by reducing the frequency of application, are further potential benefits. From a manufacturing point of view, in situ forming depot systems offer the advantage that they are relatively simple to manufacture from polymers adapted for this approach. Compared to microspheres, which have to be washed and isolated after preparation, operating expenses for the production of in situ forming applications are marginal, thus lowering investment and manufacturing costs.

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Our objective is to discuss the different strategies used to prepare in situ forming devices (ISFD). The main focus will be placed on approaches for the delivery of hydrophilic macromolecular drugs, especially proteins. The basis for comparison are biodegradable microspheres, which have been shown to provide prolonged drug release for a variety of commercial products, including luteinizing hormone–releasing hormone (LHRH) agonists, somatostatin analogues, and human growth hormone [3–5]. ISFD are developed as an alternative to implants and microspheres.

The origin of ISFD can be traced back to work of the Southern Research Institute in the early 1980s, where injectable depot formulations for antibiotics were used for the local treatment of periodontal diseases [6,7]. These studies were continued by ATRIX Laboratories, Fort Collins, CO, USA. Their product Eligard™, containing leuprorelin in an ISFD, has received approval by the FDA [8]. Another player who entered the field recently is ALZA Corporation, Mountain View, CA, USA. Their technology is very similar to ATRIX, but utilizes special solvents, such as benzyl benzoate and benzyl alcohol [9]. The third player AP PHARMA, Redwood City, CA, USA, has invented biodegradable poly(ortho ester)s with low melting temperatures to be used as injectable thermoplastic pastes [10]. Finally, MacroMed, Salt Lake City, UT, USA, developed ABA and BAB triblock copolymers containing PLGA A-blocks and PEG B-blocks as thermally induced gelling systems for parenteral application [11]. The list of companies is not exhaustive and many more competitors are expected to emerge in the foreseeable future. The effective technologies will be discussed below.

In this review, injectable in situ forming implants are classified into four categories, according to their mechanism of depot formation: (1) thermoplastic pastes, (2) in situ cross-linked polymer systems, (3) in situ polymer precipitation, and (4) thermally induced gelling systems.

The coverage of the literature is not intended to be comprehensive; rather, select examples will be discussed to highlight the performance of this class of parenteral delivery systems. A comparative analysis will emphasize the challenges that are associated with the delivery of proteins.

2. Thermoplastic pastes

Semi-solid polymers can be injected as a melt and form a depot upon cooling to body temperature. The requirements for such ISFD include low melting or glass transition temperatures in the range of 25–65°C and an intrinsic viscosity in the range of 0.05–0.8 dl/g [12–14]. Below the viscosity threshold of 0.05 dl/g no delayed diffusion could be observed, whereas above 0.8 dl/g the ISFD was no longer injectable using a needle. At injection temperatures above 37°C but below 65°C these polymers behave like viscous fluids which solidify to highly viscous depots. Drugs are incorporated into the molten polymer by mixing without the application of solvents. Thermoplastic pastes (TP) allow local drug delivery at sites of surgical interventions for the delivery of antibiotic or cytotoxic agents. Alternatively, they can be used to generate a subcutaneous drug reservoir from which diffusion occurs into the systemic circulation.

Intratumoral injection of Taxol™ or application of the paste within tumor resection sites are examples for the TP approach. Polyanhydrides, such as poly(bis(*p*-carboxyphenoxy)propane-sebacic acid) [15], polycaprolactone (PCL) [16] and ABA triblock copolymers PLA–PEG–PLA [17] have been investigated in this context. In all cases, paclitaxel release over more than 60 d was achieved; however, the rate of drug release was very low. Attempts were made to accelerate drug release by using blends of the polymer with low molecular weight compounds, such as PEG, gelatin or albumin. This usually led to a higher drug burst, but not to higher release rates [18]. Another problem confronted was the high melting temperature of TP requiring injection temperatures greater than 60°C. This led to very painful injections [19] and necrosis at the injection site resulting in the encapsulation of the depot by scar tissue, which inhibited paclitaxel diffusion.

Poly(ortho esters), POE, have emerged as a polymer class with interesting properties for TP due to their good biocompatibility [20]. The most recent development in this field are block-copolymers containing triethylene glycol and glycolide leading to POEs with a self-catalyzed degradation behavior [10,21,22]. Low molecular weight POEs have relatively low softening temperatures in the range of 35–45°C and seem to be especially well suited for TP since they degrade by surface erosion, a feature not seen with the polymers discussed above. The most successful family of POEs was obtained by the reaction between a diketene acetale and a polyol. The use of triethylene glycol as polyol component produced somewhat hydrophilic polymers, whereas hydrophobic materials could be obtained by using 1,10-decanediol [2,23,24]. Mixing of the polymers with drugs occurs at room temperature using a three-roll mill in the absence of any solvents. Incorporation of approximately 20% (w/w) PEG-monomethyl ether facilitated the injection of the semi-solid POEs without requiring special equipment (hydraulic syringe) through small bore needle sizes, not larger than 22 gauge [2,23–25]. Both, the release and stability of different model proteins in such systems were investigated [25]. It was observed that protein release from semi-solid, self-catalyzed POEs is a complex progress which can be adapted by the proper pH and buffering capacity of the protein solution. Fig. 1 shows the effect of different buffers on the release of α-lactalbumin from a POE matrix. The first application of semi-solid POEs in human volunteers showed that these were biocompatible when placed in the periodontal cavity, although the retention profiles were not fully satisfying as drug was released within approximately 5 days [2]. This latest generation of POEs is clearly superior to the polyesters described above for the following reasons: (a) low melting temperatures in the range

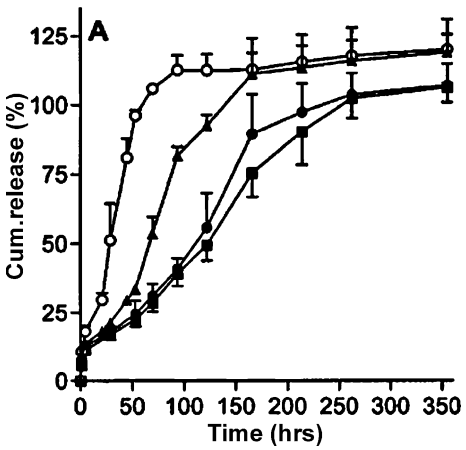

Fig. 1. Cumulative release of a POE matrix containing α-lactalbumin colyophilized at 5 mg/ml protein concentration with selected buffers: (▲) 5 mM citrate buffer (pH 6); (○) histidine buffer (pH 6); (●) histidine buffer (pH 7); or (■) HEPES buffer (pH 7) (from Van De Weert et al. [25]).

of 25–45°C can be designed by polymer modification to overcome the drawback of high injection temperatures; (b) degradation by surface erosion avoids an acidic microenvironment which is deleterious for proteins and DNA; and (c) avoidance of organic solvents in the formulation.

3. In situ cross-linked polymer systems

The formation of a cross-linked polymer network is advantageous, because of the possibility to control the diffusion of hydrophilic macromolecules. Such a system could ideally release peptides and proteins over a prolonged period of time. In situ cross-linking implants have been a challenging objective, as polymers containing double-bonds and free radical-initiation are necessary. These two factors are detrimental to living tissue and further to the encapsulated drug. Thus, protection of the bioactive agents during the cross-linking reaction is necessary. This could be achieved by encapsulation into fast degrading gelatin microparticles [26,27]. As a potentially useful application one could mention biodegradable hydrogels produced from the macromer, PEG-oligo-glycolyl-acrylate, using a photo-initiator, such as eosin and visible light [28–30]. The controlled release of proteins was observed over a period of several days. These hydrogels are restricted to surgical sites accessible to a light source as they form with difficulty after injection into the body.

The cross-linking of thiol functional groups in different polymers, a process which does not require radical-initiated polymerization, was also studied. Thiols are known to be unstable as they can be easily oxidized to disulfides by oxygen in the air [31]. Some research groups recently immobilized thiol groups on water-soluble polymers, such as chitosan and deacylated gellan gum (DGG) for in situ hydrogel formation [32,33]. Unfortunately the gelation requires several hours to occur, probably too long for in situ use. Because of the important role of disulfide bonds in protein and peptide chemistry, Qiu et al. suggested that disulfide bond formation with the polymer should be avoided not to interfere with the complex protein structure by unwanted inter- and intrachain disulfides bonds [31]. As a result, they developed a new poly(ethylene glycol)-based copolymer containing multiple thiol (–SH) groups [31,34]. These are cross-linked by vinylsulfones forming thioether bonds, as shown in Fig. 2. The resulting hydrogel, DepoGel™, contains more than 90% water and is therefore capable of entrapping proteins. After subcutaneous injection of the polymer hydrogel formulation containing erythropoietin (EPO) into female New Zealand white rabbits, the EPO levels in plasma were sustained for at least 2 weeks. The conservation of the EPO bioactivity after release is shown in Fig. 3 demonstrating the biological effects of the released EPO on increased hematocrit levels in rabbits. This result indicates that the cross-linking chemistry did not interfere with the EPO thiol groups. First safety studies showed that the gel caused minimal inflammatory cell response when injected subcutaneously into rats, rabbits and dogs. One potential drawback of this system is the need of surgical excision after the drug release, since the polymers are not biodegradable. It has not yet been shown if this approach can be adapted for biodegradable polymeric systems.

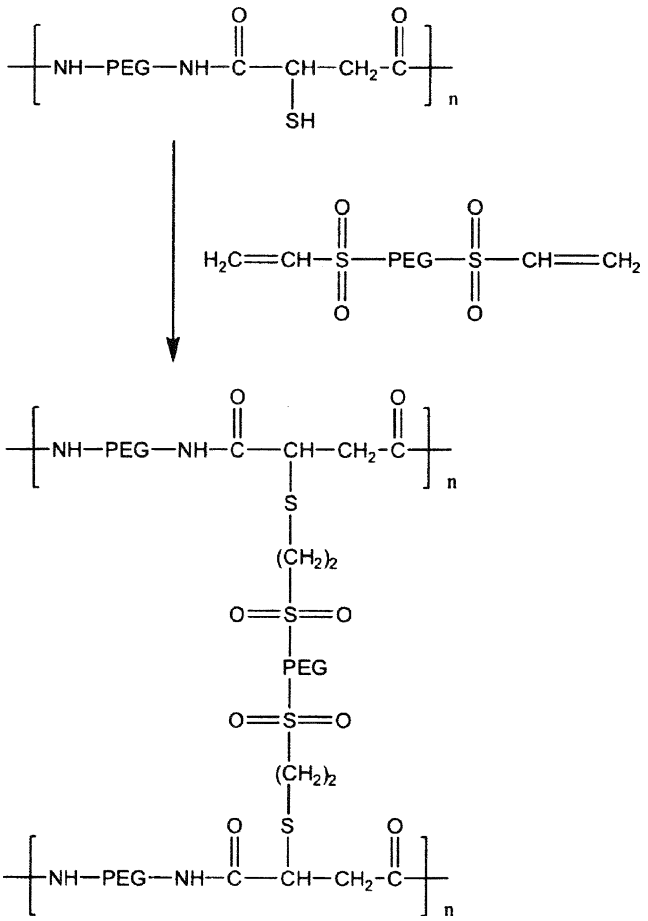

Fig. 2. Preparation of polymer hydrogel through chemical cross-linking PEG-based thiol-containing copolymer. Thioether bonds are formed to cross-link polymer chains (from Qiu et al. [31]).

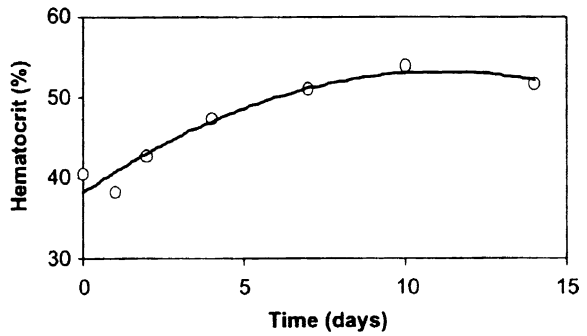

Fig. 3. The biological effects of the released EPO on hematocrit level in rabbits (from Qiu et al. [31]).

Ion-mediated gelation has been reported for a number of polymers, e.g. alginates/calcium ions or chitosan/phosphate ions [35–37]. In both cases cross-linked polymer networks are generated but usually not in an 'in situ' situation. The concentrations of the counter ion available under physiological conditions are usually insufficient for cross-linking of the above-mentioned polymers. Only the calcium concentration in the eye led to in situ formation of alginate formulations [37].

In our group, we recently developed drug-loaded nanoparticles which are capable of forming a hydrogel in the presence of ions. This process occurred under physiological conditions, thus no additional ions were necessary. We assume that this ion-mediated gelation is due to the positive surface charge of nanoparticles, since they were formulated using a novel amine-modified branched polyester. These polymers consist of a poly(vinyl alcohol) backbone modified with amines (DEAPA: diethylaminopropylamine, DMAPA: dimethylaminopropylamine, DEAEA: diethylaminoethylamine) using N,N′-carbonyldiimidazole (CDI) linker chemistry [38]. The hydrophilic backbones were grafted with poly(D,L-lactide-co-glycolide), PLGA, side chains of either 10 or 20 repeating units. Modification of the degree of amine-substitution, the PLGA side chain lengths and/or the graft density resulted in polymer degradation times, which could be varied in a vast range lasting from several hours to days to months. Fig. 4 summarizes the synthetic scheme of biodegradable

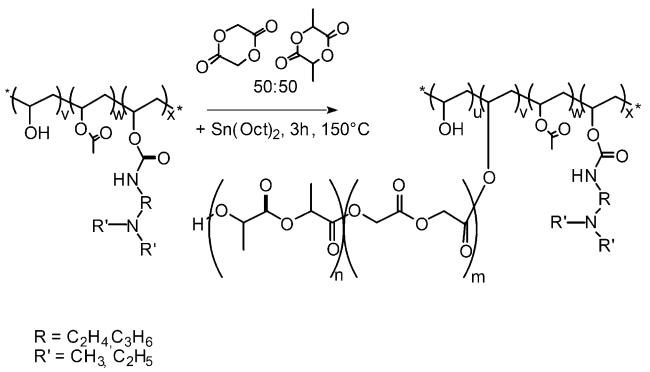

Fig. 4. Synthetic scheme of amine-modified poly(vinyl alcohols)-*g*-poly(D,L-lactide-co-glycolide)s.

amine-modified poly(vinyl alcohols)-*g*-poly(D,L-lactide-co-glycolide). Drug loaded or plain nanoparticles were formed by a solvent displacement method [39]. Nanoparticle formulation using this method was achieved without high shear homogenization processes, which destroy sensitive molecules such as DNA or proteins. Drug loading of the nanoparticles with hydrophilic macromolecules was reached either by incorporation during the preparation process (plasmid-DNA) or by adsorption on the surface of the particles (insulin). The hydrogel formation in situ provides the opportunity to load the depot with some additional drug which could lead to a controlled initial dose followed by the sustained release out of the particles. The resulting drug release from these devices then depends on the degradation profile of the polymer and the electrostatic interactions of the drug with the polymer. These factors can be adjusted by varying the degree of amine-modification and the PLGA side chain length of the polymer used.

The encapsulation of proteins in such nanoparticles, the factors influencing the formation of the depot, and the release properties are currently under investigation.

4. In situ polymer precipitation

The concept of ISFD based on polymer precipitation was first developed by Dunn and co-workers in 1990 [7]. A water-insoluble and biodegradable polymer is dissolved in a biocompatible organic solvent to which a drug is added forming a solution or suspension after mixing. When this formulation is injected into the body the water miscible organic solvent dissipates and water penetrates into the organic phase. This leads to phase separation and precipitation of the polymer forming a depot at the site of injection. This method has been developed by ARTIX Laboratories and is designated as the Atrigel™ technology [40–42]. The most advanced product using Atrigel™ as a drug carrier, Eligard™, contains the LHRH agonist leuprolide acetate (7.5, 22.5 or 30 mg) and PLGA 75/25 dissolved in *N*-methyl-2-pyrrolidone (NMP) in a 45:55 (m/m) polymer:NMP ratio [8,41–45]. This system led to suppression of testosterone levels in dogs for approximately 91 days. It was not affected by variations in polymer concentration (40–50%) or drug loading (4–6%) but rather by the molecular weight of the PLGA. Clinical studies demonstrated that a depot containing 22.5 mg leuprolide produced and maintained an effective suppression of serum testosterone below the medical castrate level of 50 ng/dl or less. Fig. 5 shows the serum testosterone concentrations during treatment with subcutaneous injections of Eligard™ 22.5 mg given at 3-month intervals.

The phase inversion dynamics of PLGA solutions is a complicated phenomenon directly affected by solvent properties. Several other organic solvents for the polymers have been studied, such as propylene glycol, acetone, dimethyl sulfoxide (DMSO), tetrahydrofuran, 2-pyrrolidone and

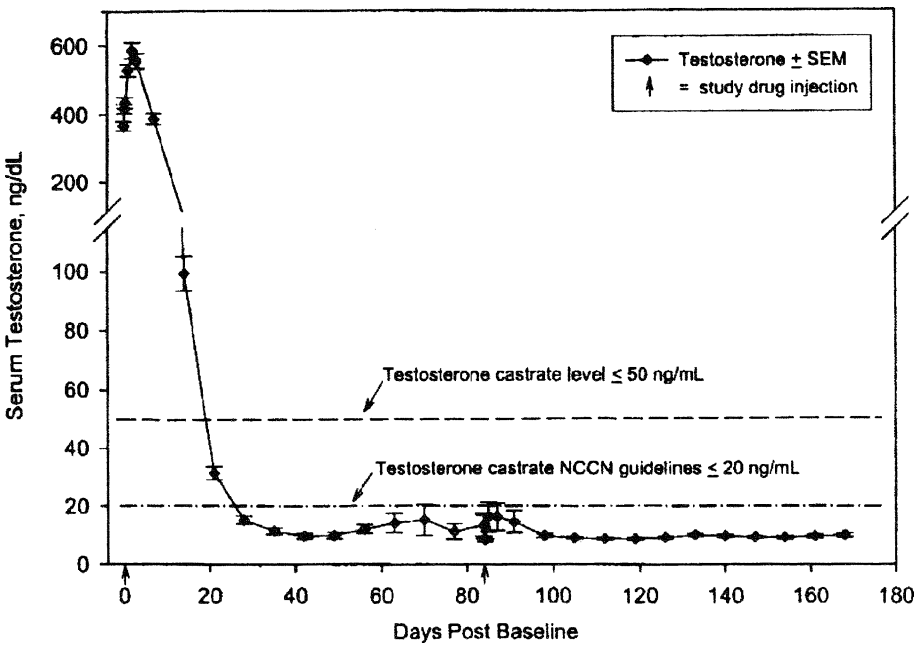

Fig. 5. Serum testosterone concentrations during treatment with subcutaneous injections of Eligard™ 22.5 mg given at 3-month intervals from baseline day 0 through month 6 (from Chu et al.[44]).

glycofurol, in an attempt to minimize the initial drug burst. The effect of solvent choice, however, was not clearly defined. The main factors seem to be the polymer type and the molecular weight. It should be noted that most organic solvents are poorly tolerated and cause pain at the injection site.

Also the drug burst is directly related to the dynamics of the phase inversion. Brodbeck et al. demonstrated that protein release kinetic from ISFD was influenced by solution thermodynamics, e.g. solvent strength and water miscibility [46,47]. They studied NMP, triacetin and ethyl benzoate ternary phase systems with PLGA and water. NMP exhibited a rapid phase inversion associated with a high drug burst, due to the formation of a porous rubbery gel structure. In contrast, other solvents, such as triacetin and ethyl benzoate, both weak solvents for PLGA, yielded low phase inversion rates, resulting in a slow gelation which reduced the drug burst of proteins significantly. Therefore, solvent type and polymer concentration are the most critical factors determining the release profile obtained under in vitro and possibly also in vivo conditions. Usually high concentrations of the polymer in the order of 40–50% (w/w) are necessary to suppress the initial drug burst. Fig. 6 shows the effect of different polymer concentrations of high and low molecular weight PLGA systems on the initial drug burst. Injection volumes usually are in the order of 1–5 ml.

The solvent NMP has raised concerns with respect to biocompatibility. Therefore, the myotoxicity of the preferred Atrigel™ solvents, namely NMP, DMSO and 2-pyrrolidine, were compared in the isolated rodent skeletal muscle using creatine kinase efflux as a toxicity criterion. After 120 min of exposure, both NMP and DMSO exhibited a high myotoxic potential similar to the positive control, phenytoin. These results were confirmed in vivo after intramuscular injections in rats, pointing to a potentially harmful acute myotoxicity of these formulations [48]. These findings are in contrast to data obtained in rhesus monkeys after an injection of 4 ml Atrigel. No acute toxicity was observed in these experiments and the tissue reaction seemed to be similar to those reported for biodegradable implants prepared as preformed solids, such as microspheres or rods [49].

Alternative approaches to reduce the unwanted local irritation potential of in situ precipitating systems were developed by Kranz et al., who prepared O/O emulsion systems using an internal polymer phase (drug, biodegradable polymer and organic solvent) and peanut oil as external phase claiming the in situ formation of microspheres at the injection site [48]. Similarly, PLGA was dissolved in triacetin/PEG, and emulsified into miglyol, yielding protein-encapsulated embryonic microspheres with in situ forming properties [50,51]. The data observed were in line with a study showing that oil/drug formulations cause less local muscle damage than aqueous/drug formulations [48].

ALZA Corporation has recently entered the field of ISFD, capitalizing on the investigations with more lipophilic solvents such as benzyl benzoate, thought to be less irritating [52]. A comparative study using different hydrophilic and hydrophobic solvent systems for ISFD was recently reported by Cleland [53]. In this study, homogenous solutions of poly(D,L-lactide), PLA, with the protein were obtained when benzyl alcohol/benzyl benzoate mixtures were used. It should be noted that the drug burst was significantly higher in rats with the so-called Polylactid Depot (PLAD) system compared to microspheres with recombinant human vascular endothelial growth factor

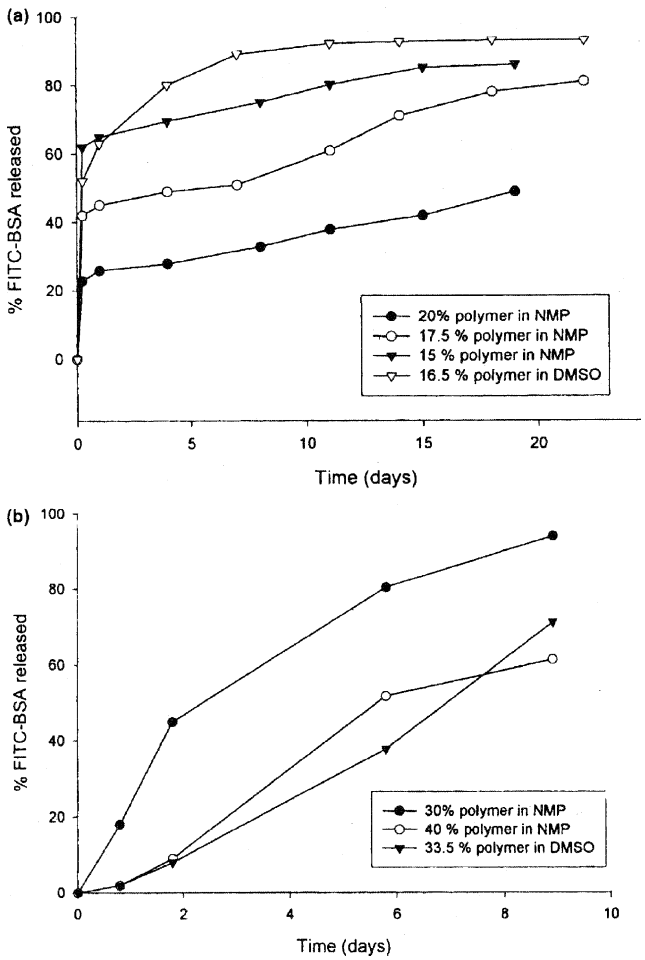

Fig. 6. (a) Release of FITC-BSA from high molecular weight PLGA systems in PBS. (b) Release of FITC-BSA from low molecular weight PLGA systems in PBS (Reproduced from Kranz et al. [48]).

(rh-VEGF) [54]. In the case of recombinant human growth hormone (rh-GH), however, more constant release profiles were obtained lasting about 7 days [55,56]. The modification of the sucrose acetate isobutyrate (SABER) technology of Southern Biosystems with ethanol and benzyl alcohol also allowed the incorporation of PLA as a release modifier. This modification of SABER led to a reduction of the drug burst. With increasing polymer concentrations of up to 10%, the drug burst of rh-GH in rats could be significantly suppressed exhibiting again a 7 day release profile [57].

These investigations demonstrate a strong commercial interest in the ISFD technology. The approach taken by ALZA in collaboration with Genentech relies on the optimization of solvent properties used for preparation of the in situ forming gels. It should be noted that only 'hydrophobic' polyesters, such as low molecular weight PLA are likely candidates for in situ polymer precipitation. Further, protein stability represents a limitation of these formulations. Thus, once mixed Eligard™ should be discarded if not administered within 30 min, as a result of protein denaturation in the organic solvent [58]. For these reasons, proteins were added as dry powders together with different stabilizers. The drug was not the only component susceptible to degradation and aging during storage. Wang et al. observed polymer degradation, when the polymer solution in NMP was incubated at 25 and 37°C for approximately 26 weeks [59]. The same system dissolved in benzyl benzoate (BB) additionally exhibited increasing viscosities of PLGA due to thermoreversible gelation. In a practical sense, structure formation may be minimized or eliminated by storing PLGA solutions at 10°C, which would prevent degradation.

Again, more data are required to allow a fundamental assessment of this approach.

5. Thermally induced gelling systems

Numerous polymers show abrupt changes in solubility as a function of environmental temperature. The prototype of a thermosensitive polymer is poly(*N*-isopropyl acryl amide), poly-NIPAAM, which exhibits a rather sharp lower critical solution temperature, LCST, of approximately 32°C [60]. Unfortunately, poly-NIPAAM is not suitable for biomedical applications due to its well-known cytotoxicity. Moreover, poly-NIPAAM is non-biodegradable.

Triblock poly(ethylene oxide)–poly(propylene oxide)–poly(ethylene oxide) copolymers, PEO–PPO–PEO, known as poloxamers or Pluronics®, have shown gelation at body temperature when highly concentrated polymer solutions >15% (w/w) were injected [61,62]. These concentrations of a surfactant, however, lead to notable cytotoxicity [63] and, furthermore, they increase the plasma cholesterol and triglycerol levels in rats after intraperitoneal injection [64].

MacroMed developed thermosensitive biodegradable polymers based on ABA and BAB triblock co-polymers, in which A denotes the hydrophobic polyester block and B denotes the hydrophilic poly(ethylene glycol) block. Low molecular weight polymers of this polymer class are water-soluble and yield a temperature-dependent reversible gel–sol transition. The aqueous polymer solution (sol) of PEG–PLA–PEG (Mr 5000-2040-5000) is loaded with drug at 45°C and then injected into animals to form a gel at body temperature continuously releasing hydrophilic model substances, such as fluorescein isothiocyanate dextran (FITC-dextran), over 10–20 days [65,66].

The synthesis of PEG–PLA/PLGA–PEG is quite complex and careful control of the molecular weight is essential since this parameter affects thermosensitivity in a critical way. The phase diagram of PEG–PLA/PLGA–PEG is affected by numerous parameters and it should be noted that the LCST shifts are a function of block-lengths and block composition [67]. Monomethoxy-PEG is polymerized with L-lactide or glycolide in toluene solution. The resulting AB diblock copolymers are then coupled using hexamethylene diisocyanate. The polymers are purified by fractional precipitation as shown schematically in Fig. 7.

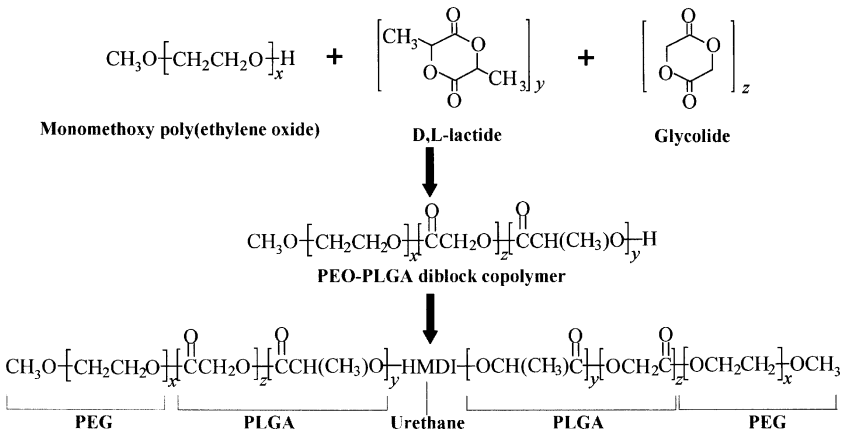

Fig. 7. Synthetic scheme of PEG–PLGA–PEG triblock copolymers with thermally induced gelation properties (from Lee [70]).

Both ABA and BAB triblock copolymers have then been claimed by MacroMed as thermosensitive liquid drug carrier systems with gelation properties [11,68].

The carrier formulation of MacroMed, ReGel®, 23% (w/w) ABA-triblock copolymer (PLGA–PEG–PLGA) in phosphate-buffered saline (pH 7.4), has entered the market. MacroMed distributes OncoGel®, which contains paclitaxel at a concentration of 6 mg/g ReGel® for intratumoral injection, followed by a continuous drug release over a period of 6 weeks. The clear advantage is the ability to solubilize the water-insoluble drug substances, such as paclitaxel, which allows a prolonged release for more than 50 days. ReGel® also exhibited sustained release kinetics for protein drugs. Release data were published by Zentner et al. [69]. The products discussed included paclitaxel and proteins, such as insulin, rh-GH, granulocyte colony-simulating factor (G-CSF) and recombinant hepatitis B surface antigen (rHBsAG). The in vitro release data for G-CSF, rh-GH and insulin show some drug burst in the order of 20%, which is also reflected in the plasma-level data. Again more experimental data would be helpful to appreciate the merits of this type of protein delivery system.

Sol–gel transitions occur around 30°C at polymer concentrations of 15–23% (w/w) in aqueous solution. Below the LCST, the system behaves as a Newtonian fluid and changes to a visco-elastic state with a 4-fold increase in absolute value of viscosity after gelation. The mechanism by which thermoreversible gelation occurs is thought to be different in BAB and ABA triblock copolymers. In both cases micellar structures are obtained in the sol state, as shown in the Fig. 8. The gelation is driven by entropy, as elevated temperatures decrease the hydration

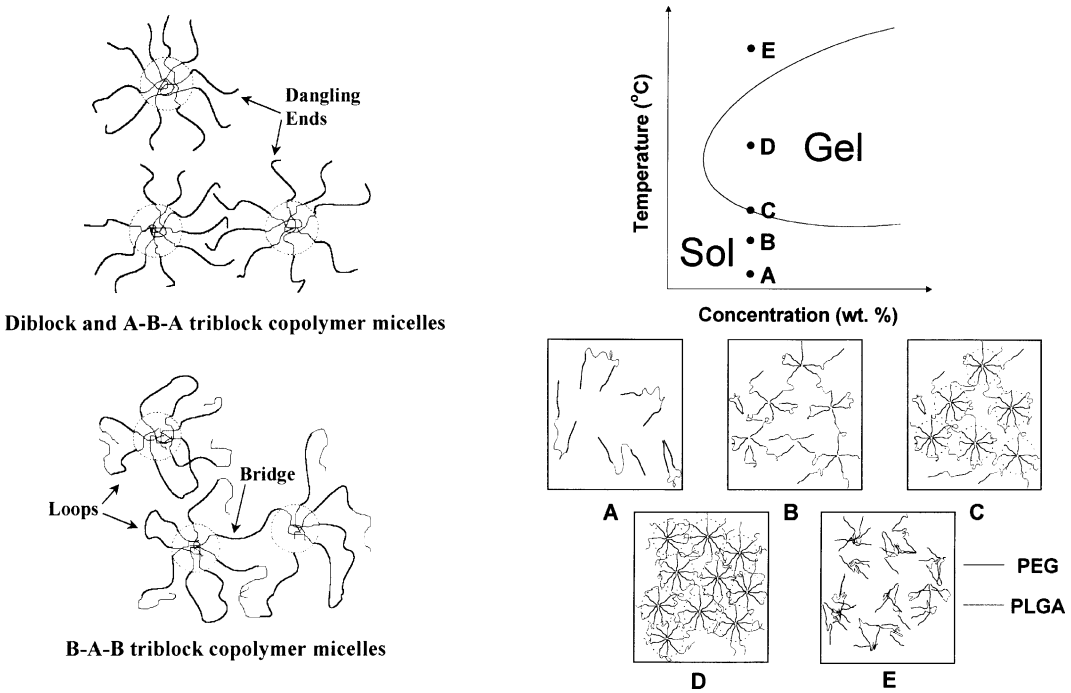

Fig. 8. Possible micellar chain topologies of PEG–PLGA ABA- and BAB triblock copolymers in water (from Lee [70]).

of the PEG chains. This results in reduced water–polymer hydrogen bonding and thus, a more hydrophobic character [67]. Hydrophobic A-blocks consisting of PLA or PLGA will associate leading to a core-corona structure with dangling PEG chains in the case of BAB polymers and PEG loops in the case of ABA polymers. Gelation of the triblock copolymers is the result of dense micelle packing and phase mixing of the corona (PEG) with the core leading to chain entanglement in the ABA type or micellar bridging in the BAB type. The latter mechanism could lead to irreversible aggregation.

The physicochemical properties of these thermosensitive gel systems are anything but straightforward. The ratio of hydrophilic (PEG) and hydrophobic (PLGA/PLA) segments, block-length, hydrophobicity (PCL > PLA > PLGA), polydispersity and stereo-regularity (amorphous/-semi-crystalline) of the hydrophobic A-block are critical factors. Slight changes can have drastic effects on micellar properties [11,70].

Another aspect deserves some consideration, namely the loading of the gels with hydrophilic drugs. At the sol–gel transition state, the system's volume will contract leading to the expulsion of the aqueous phase in which proteins are dissolved. This effect causes some initial drug burst and only those proteins associated with or dissolved in the lipophilic core do not experience this push-out effect [69].

It is interesting to note that ABA polymers were investigated more intensively than the BAB type, probably because they are more easily produced in a one-step synthesis.

6. Summary

The physicochemical properties of the drug candidates will drastically influence the choice of suitable injectable in situ forming delivery systems. Lipophilic and water-insoluble candidates, such as paclitaxel and cyclosporine A, could virtually be incorporated into any of the systems discussed in more detail below. Therefore, the considerations will be limited to hydrophilic macromolecular drugs typically represented by proteins.

The second aspect concerns the indication and intended duration of treatment. Obviously, prolonged treatment of cancer patients sets a different scenario for risk/benefit analysis than, for example, the treatment of dwarfism in children.

6.1. Thermoplastic pastes

Most disadvantages of this system have been overcome by the new generation of poly(ortho esters) developed by AP Pharma. These polymers are semi-solid at room temperature, hence heating for drug incorporation and injection is no longer necessary. Injection is possible through needles no larger than 22 gauge, mostly used for intramuscular application. The protein drugs could be mixed into the systems in a dry and, therefore, stabilized state; thus, neither protein degradation can occur in aqueous solutions nor are irritating organic solvents needed. Shrinkage or swelling upon injection is thought to be marginal and, therefore, the initial drug burst is expected to be lower than in the other types of ISFD. An additional advantage consists of the self-catalyzed degradation by surface erosion. Thus, reasonable protein stability and continuous release were observed [25].

These promising properties for the delivery of protein drugs have to be further investigated in vitro and in vivo. Still, animal data concerning biodegradation, biocompatibility and toxicity are yet to be performed before a full evaluation of this technique can be made. Moreover, stability studies are required to decide whether the systems can be formulated as a pre-mixed ready to use paste or whether the proteins have to be reconstituted directly prior to injection.

Thermoplastic pastes can also be obtained by blending PLGA with plasticizers, leading to systems similar to those used in in situ polymer precipitation. Triacetin/PEG was an example for such an ISFD.

6.2. In situ cross-linked polymer systems

The use of free-radical initiated polymerization is controversial for at least two reasons. First, the risk of tumor promotion caused by the initiators, such as benzoyl peroxide, and, secondly, the degradation of biological active agents by free radicals. The latter could be overcome by the encapsulation of the proteins before incorporation into the cross-linking matrix. This would result in additional factors influencing the matrix properties, making the release out of these systems difficult to predict. Additionally, in the case of photopolymerization, the application is limited to subcutaneous administration, due to the absorption of the initiating light by the tissue.

The thiolation of biocompatible polymers is an interesting approach in the field of in situ cross-linkable deliver systems. DepoGel™ showed good biocompatibility and release properties without initial drug bursts. However, there is little data available as of yet and the non-degradability represents a drawback.

We consider the ion-mediated gelation of aqueous nanoparticle suspensions to be a very promising approach. The preparation of the nanoparticles in a one-step process is relatively easy, comprising only short or no contact of the bioactive agent with organic solvents. No additional cross-linking reagents are necessary. Besides, the injection of an aqueous suspension is a considerably less problematic process compared to the injection of viscous polymer solutions in organic solvents. Currently, further investigations are ongoing with regard to the drug encapsulation, the depot formation, the release mechanism, and the shelf life.

Table 1
In situ forming parenteral drug delivery systems which reached the state of approval or are under clinical investigation

	Thermoplastic paste	Thermogelling system	Polymer precipitation
Injection	Semi-solid paste	Aqueous sol	Organic solution
Depot formation	Solidification	Sol–gel transition	Phase separation
Polymer	POE	ABA and BAB	PLGA
Drug loading	Dry powder	Aqueous solution	Organic solution
Protein stability	High	Medium	Medium
Drug burst	Low	Medium	High
Release	Surface erosion	Pore diffusion/bulk erosion	Pore diffusion/bulk erosion
Local tolerance	High	High	Low
Injection pain	Low	Low	High

6.3. In situ polymer precipitation

This technique is by far the most straightforward approach to parenteral depot systems. The Atrigel™ system for leuprolide could rather be the exception than the rule. Its inherent concern is associated with the use of organic solvents. Injections of such solvents are painful and may lead to local myotoxic effects. Strategies to reduce these effects by using benzyl alcohol/benzyl benzoate mixtures may hold some promise, since benzyl alcohol has local anesthetic effects.

Another intrinsic problem of in situ precipitation is the drug burst commonly observed with hydrophilic macromolecular drugs. The addition of stabilizers and excipients may help to some extent. Still, little is known about stability of proteins in these formulations. There are still some open questions with regard to the reduction of drug burst by changes in formulation and excipients, the reduction of local tissue damage, and the solubility of polymers in more acceptable solvent mixtures. Concerning the long degradation times of PLGA, other polymers remain to be investigated for the adaptation of this approach to further indications.

6.4. Thermally induced gelation

The ABA and BAB triblock copolymers developed by MacroMed are very attractive, because of the ease of administration (aqueous sol) using conventional syringes. The release of proteins from the ReGel® has not been studied in great detail, but it seems that some initial drug release is difficult to avoid. The biocompatibility and toxicity do not seem to be problematic.

Further investigations with regard to the stability of proteins in the aqueous polymer solutions, the shelf life of the formulations, and in vivo release data for proteins are ongoing.

7. Conclusions

Continuous advances in biotechnology and drug development will produce more pharmaceutically active agents that will be difficult to administer by conventional means. In situ forming parenteral depot systems have emerged as a very attractive approach for the controlled release of bioactive macromolecules. Several systems have reached the stage of commercialization and many candidates are under clinical investigation (Table 1). The development has been very rapid in the recent years and ISFD are a true success story for the field of drug delivery. From a physicochemical point of view, ISFD are anything but straightforward and the need for more fundamental studies to exploit the full potential of this approach can only be emphasized. It is imaginable that the properties of ISFD are not fully utilized yet. Which one of the strategies discussed above will be suitable for a specific protein under consideration depends on numerous factors. Hence, it has to be seen which formulation can be adapted for a broad range of proteins and thus, will lead the field of ISFD in the future.

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