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Review

In situ forming implants — an attractive formulation principle for parenteral depot formulations

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ABSTRACT

In the area of parenteral controlled release formulations, in situ forming implants (ISFI) are attractive alternatives to preformed implants and microparticles. ISFI avoid the use of large needles or microsurgery and they can be manufactured in simple steps with a low requirement of equipment and processes. They are injected as low viscous solutions and transform in the body to a gel or solid depot. Different triggers can be used to stimulate this transformation: (1) in situ cross-linking, (2) in situ solidifying organogels, and (3) in situ phase separation. The review discusses the principles and the pros and cons of each strategy. It also gives examples of clinically used products or systems which are currently in clinical trials.

Although the principle of ISFI is so attractive, key issues remain to be solved. They include (i) variability of the implant shape and structure, (ii) avoidance of burst release during implant formation, and (iii) toxicity issues. Unfortunately, until now our knowledge concerning the detailed processes of the implant formation is still very limited. This is due to the fact that the processes of implant formation and degradation, drug release and tissue response are complex, heterogeneous, interconnected and not easy to follow, especially in vivo. Despite this statement, many efforts are made in industry and academia to improve current approaches. New materials and approaches enter the preclinical and clinical phases and one can be sure, that ISFI will gain further clinical importance within the next years.

Contents

1. Introduction

Due to the steadily increasing number of biotechnology-based drugs and compounds which cannot be administered via the oral route, parenteral drug delivery systems received significant research interest in the last two decades. Although intensive efforts have been devoted to alternative application routes (e.g. pulmonal, transdermal, oral, nasal), poor and highly variable absorption remains as the key issue of the alternative administration routes. In addition, further problems may arise (e.g. increased antibody formation, impact of smoking, cough or food…). Significant progress has also been made to tackle the main concerns of parenteral administration:

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the safety and the pain. Parenteral delivery systems can be designed to provide flexible delivery characteristics. Many drugs combine a high activity with a short half-life. Parenteral depot formulations are therefore a formulation option to avoid a constant infusion or a very high frequency of injections. Depot formulations with release kinetics from days, over weeks, months to even years have been developed [1]. Thereby parenteral depot systems enhance patient compliance by diminishing the frequency of applications. Furthermore, these depot formulations can minimize undesirable side effects caused by fluctuating drug blood levels which are typical of immediate release products [1]. In the case of localized parenteral delivery systems that allow for the drug to deposit directly at the site of action, the drug dosage and therefore the system toxicity can be reduced. Typical applications of parenteral depot systems include the treatment of hormone sensitive breast or prostate cancers (with GnrH agonists or antagonists), local chemotherapy, the local treatment of infections or the local delivery to the eye.

Various types of parenteral dosage forms are available, such as solutions, emulsions [2], liposomes [3], micelles [4], implants [5], microparticles [6], nanoparticles and nanocapsules [7]. However, only implants and microparticles gained importance as controlled release systems [8]. Preformed implants are made by melt extrusion administered subcutaneously by a special application device or through a larger needle. In the case of non-biodegradable systems (e.g. Vantas®, Viadur®), the implants have to be removed after the release periods. In the case of biodegradable materials, the polymers degrade during and after the release processes to monomers which are metabolized and excreted. Typical preformed subcutaneous implants are 10 mm long and of cylindrical shape with a diameter of 1 mm (e.g. Zoladex® and generic formulations for the treatment of hormone sensitive breast and prostate cancer). They are injected through a 16 gauge needle (outer diameter 1.65 mm). Smaller implants are used for the treatment of eye diseases [9]. For subcutaneous implants, also larger sizes have been commercialized. The non-biodegradable one year implant Vantas® has a length of 35 mm and a thickness of 3 mm. Preformed implants permit a rapid administration, however, the large diameters of the injection needles cause fear and discomfort for the patient. Microparticulate systems can be given to patients with smaller needles, which is more comfortable to the patient. Most widely, emulsion–solvent evaporation, spray drying and phase separation technologies are used for their manufacturing. Supercritical techniques will certainly become more important within the next years [10]. Microparticles are often filled in two chamber syringes which separate the dispersion medium from the particles to prevent premature degradation. The disadvantages of microparticulate systems include complex and more expensive production processes and – compared to preformed implants – a more time consuming and difficult administration procedure with the danger of incomplete dispersion of the microparticles, syringe clogging and the administration of an incomplete dose. Due to the drawbacks of preformed implants and microparticles, large efforts have been made to develop alternative depot systems with the following characteristics: (i) rapid, painless and easy administration through small needle sizes, and (ii) easy manufacturing at low costs. As a result, an increasing number of injectable and biodegradable in situ forming systems have been developed as alternatives [11–13]. Prior to injection the in situ forming systems represent a low viscous and injectable system. Once administered these low viscous polymeric formulation solidify into a semi-solid or solid depot. Thus it turns into a 'solid' dosage form as it is illustrated in Fig. 1 for a thermally-induced gelling system.

Biodegradable implants formed from injectable fluids have one general advantage compared to pre-shaped parenteral depot systems. From the patient's point of view, the application of in situ forming implants (ISFI) is less invasive and so less painful. An improved patient compliance and comfort can be achieved by the avoidance of invasive techniques in the implantation and removal of the implants. These characteristics encouraged many researchers to investigate their use

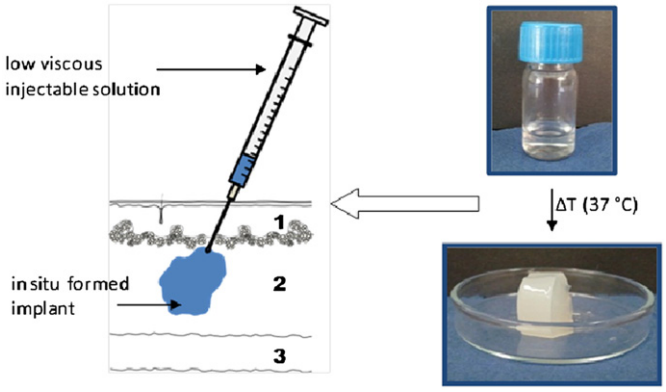

Fig. 1. Example of an in situ forming thermally-induced gelling system (1 epidermis and dermis, 2 subcutis, 3 muscle).

for various purposes. ISFI have been investigated for controlled drug delivery in systemic treatments as well as localized therapies. In addition ISFI have found applications in tissue engineering, three dimensional cell culturing, cell transplantation, or for orthopedic and dental administrations [12–15].

In situ forming systems can be classified according to their mechanisms of implant formation into (Fig. 2):

- in situ cross-linked polymer systems
- in situ solidifying organogels and
- in situ phase separation systems.

2. In situ cross-linked systems

In situ forming cross-linked polymer networks can be achieved by photo-initiated polymerization [16,17], chemical cross-linking with cross-linking agents [18] or physical cross-linking [19] of specific monomers. There are several issues that must be considered. In particular the demands for in vivo reaction conditions are quite restricted, such as the need of non-toxic monomers, cross-linking agents and solvents, oxygen rich environments, narrow range of physiologically acceptable temperatures and suitable rates of rapid polymerization [12].

2.1. Photo-initiated polymerized systems

Photo-initiated polymerization fulfills many of the requirements for in vivo polymerization. The initial materials are liquid solutions, which can be easily placed. Afterward the rapid polymerization at

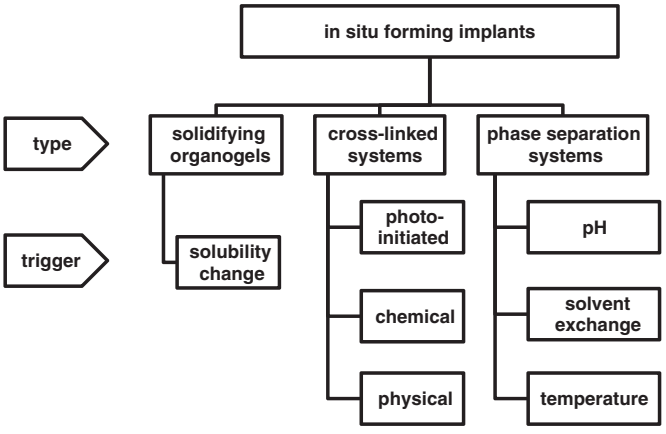

Fig. 2. Overview of in situ forming implant technologies. Modified from Refs. [12,13].

physiological temperatures forms the polymer matrix of the required dimension. Photopolymerizable systems are used for various biomedical applications. Dentists polymerize dimethacrylate monomers in combination with silica particles for caries tooth restorations as alternatives to mercury amalgam fillings [20]. Other photo-initiated polymerized systems have been investigated for orthopedic applications [21,22], tissue adhesion prevention [23,24], cell transplantation [25], tissue engineering [26] and drug delivery [25,27]. In general these systems need monomers with at least two free radical polymerizable regions, photosensitive initiators (e.g. eosin dyes) and ultraviolet (UV) or visible light. In the latter case the light sources are placed at the injection site by fiber optic cables.

To develop degradable polymers, meth(acrylate) groups were coupled to various water soluble polymers. For examples ABA triblock copolymers of poly(N-(2-hydroxypropyl) methacrylamide lactate) A blocks and poly(ethylene glycol) (PEG) B blocks have been used to encapsulate mesenchymal stem cells [26]. Sawhney et al. developed water-soluble macromers based on AB block copolymers of PEG and poly(α-hydroxy acids) with terminal acrylate groups. On treated wound site they were photo-polymerized from buffered saline solution. The resulting adherent hydrogel film formed an even coating on the tissue as a non-adhesive barrier to prevent postoperative adhesions [23]. Photo-crosslinkable water soluble chitosan was obtained by introduction of lactose and photo-reactive azide groups in chitosan. When exposed to UV light the modified chitosan formed an insoluble, transparent and adhesive hydrogel within 60 s [28]. The resulted hydrogel degraded within 1 month in vivo. Toxicity tests for mutagenicity and cytotoxicity have shown the safety of the modified chitosan and its hydrogel. Paclitaxel was retained in this chitosan hydrogel and remained biologically active for 21 days [29]. However an initial burst release was observed in the first 24 h, followed by a slow release in the following 21 days due to the biodegradation of the gel.

In principle photo-initiated polymerization causes the creation of reactive species. To minimize the possible adverse side effects to the surrounding tissue and to protect the bioactive ingredients, low photo-initiator concentrations and low-intensity UV light should be used. However, on the other hand, long exposure times should be avoided. Therefore, the focus is on specific cross linking structures with good biocompatibility. The obvious downfall of the clinical application of in situ photo-polymerized systems is the low penetration depth of UV and visible light in living tissue. The depth of injection strongly impacts the cross-linking efficacy and therefore the reproducibility of gel formation after transdermal illumination [30]. As a result, the use of in situ gels formed by UV irradiation is mainly limited to dental applications. Furthermore these systems can be used for minimal invasive surgery attendant therapies, e.g. peritumoral injection after tumor restriction and during bone or cartilage augmentation surgery by the use of catheters or laproscopic devices allowing direct exposure of the formulations to UV light.

2.2. Physical cross-linked systems

The physical cross-linking of polymers leads to the formation of a covalent network structure. They are formed by inter- and intramolecular bonding via hydrogen bonds or various charge interactions, such as ionic interaction or polyelectrolyte complex (PEC) interactions [13,18].

Alginates are anionic linear polysaccharides derived from brown sea weed. The most important feature of the alginate is the gelation in the presence of divalent cations, such as calcium [25]. This property has led to a wide use in cell transplantation vehicles to grow tissues or as wound dressings. However, high molecular weight alginates were reported to be non-biodegradable. Moreover high calcium concentrations (>5 mmol/l) have inhibited the growth of cells in culture [25]. Under physiological conditions only the calcium concentration in the eye is sufficient to induce gelation. So far the use of in situ cross-linked alginate gels was limited to ophthalmic drug delivery [12,13].

Chitosan, a linear polycationic polymer, forms hydrogel networks through ionic bridges between the polymeric chains with negatively charged components. This cross-linker may either be metallic anions, as molybdenum (VI) or anionic molecules, in particular tripolyphosphate, citrate or oxalate [18,31,32]. The nature of these interactions is the same as in PEC. Generally in ionic cross-linking the reacting entities are ions or ionic molecules with a well-defined molecular weight, whereas PECs are formed by polymers with a broad molecular weight distribution. Hydrogels formed by ionic cross-linked chitosan possess a pH-sensitive swelling behavior and exhibit drug release by diffusion through their porous structure. They are generally thought to be well-tolerated and have been used for controlled release of various drugs [18,33]. However their main disadvantages are the impact of the drug and environment on the implant formation process, the risk of premature dissolution of the gel due to the highly ion and pH-sensitive swelling and possibly low mechanical stability.

2.3. Chemically cross-linked systems

While physical cross-linking yields to reversible networks, chemical cross-linking is characterized by the formation of polymer networks via covalent links. However chemical modification of the polymers or the addition of cross-linking entities is needed. Cross-linking agents like benzoyl peroxide, dialdehydes (e.g. glyoxal and glutaraldehyde), oxalic acid or genipin were used to covalently link acrylic ester terminated pre-polymers of poly(lactide) (PLA), poly(ε-caprolactone) (PCL), chitosan and gelatin [12,18,32,34]. The toxicity of the cross-linking agents is the obstacle in the use of these ISFI. Their dispersion into the body may be associated with severe side effects, even at low concentrations [18,35]. Among them benzoyl peroxide, in its function as a free radical precursor, is described to induce tumor promotion [34]. The dialdehydes and oxalic acid are considered to be toxic [34]. The naturally occurring genipin has been investigated as a substitute [34]. It is an aglycone derivative from the iridoid glycoside geniposide. It can be found in fruits and is commonly used in herbal medicine and as a food dye. In vitro genipin has been tested to be non-cytotoxic. After injection in rats it has been shown to be biocompatible [18]. However many of the described reagents yield to rather slow network forming reactions and possess a marked exothermic character, which limits their practical use.

Neither radiation nor radical-initiated polymerization is required for the cross-linking of thiol functional groups. Thiols are known to easily oxidize to disulfides in the presence of oxygen. Thiol groups were coupled to polyacrylates, chitosan and deacetylated gellan gum [36–38]. These thiolated polymers, also known as “thiomers”, form inter- and intramolecular disulfide bonds within the polymer. The results are strong cohesive dosage forms (e.g. microparticles or gels) with high stability, prolonged disintegration times and controlled release of embedded peptides [38,39]. The formation of disulfide bonds with mucus glycoproteins yielded to strong mucoadhesive properties. With this, the permeation of peptides through the mucus could be improved. By the inhibitory properties of some thiomers toward peptidases the pharmacological efficiency of peptides was enhanced. Nasally applied thiomer-gels lead to a bioavailability of 2.75% of human growth hormone, whereas the corresponding unmodified gels had only a very marginal or no effect at all [38]. But in situ gelation of thiomers often requires several hours and the formation of unwanted disulfide bond of the polymers with proteins cannot be completely excluded. Based on the thiomer concept hydrogels with thioether-bonds in PEG-copolymers where used to entrap erythropoietin (EPO) [40]. These polymers consist of multiple thiol groups cross-linked by vinylsulfones. In vivo studies showed that the resulting hydrogel (DepoGel™) did not alter the EPO bioactivity. EPO

plasma levels were sustained for 2 weeks. But the major drawback is the non-biodegradability of the polymer.

Covalent cross-linking without employing any extraneous cross-linking agents can further be obtained by condensation of natural proteins and polysaccharides. Balakhrishnan et al. developed an injectable biocompatible and biodegradable system of oxidized alginate and gelatin that undergo self-cross-linking within few seconds in the presence of small concentrations of borax [25,41]. Cross-linking is predominantly due to Schiff's base formation between the amino-groups of the lysine side groups of gelatin and the available aldehydes of the oxidized alginate. The rapid gelation in the presence of borax could be attributed to the slightly alkaline pH of the medium. This pH facilitated the Schiff's reaction as well as the complexation ability of borax with alginate hydroxyl groups. The systems have been used multifariously as a drug delivery vehicle, tissue engineering scaffold and as wound dressing [25,41].

3. In situ solidifying organogels

Organogels are semi-solid systems consisting of a three-dimensional network of self-assembled gelator fibers and a continuous liquid organic phase. The gels are prepared by dissolving the gelator, in concentrations up to 15 wt.%, in the heated solvent. Upon cooling the solubility of the gelator decreases. The gelator self-assembles into aggregates, which form the network by intermolecular physical interactions [42]. As an example, L-alanine fatty acid derivatives form highly viscous gels in safflower oil by Van der Waals interaction and hydrogen bonding [43,44]. To decrease their viscosity and to facilitate injection 10 wt.% of N-methyl-2-pyrrolidone (NMP) was added to the system. This N-stearoyl-L-alanine methylester (SAM) based gels were used for the long term delivery of leuprolide [44]. The system slowly degraded and released the luteinizing hormone-releasing hormone (LHRH) agonist within a period of 14–25 days. In rats chemical castration was induced up to 50 days [44]. The same system was further used to sustain the release of rivastigmine, a cholinesterase inhibitor used against Alzheimer's disease. After subcutaneous injection the drug was released up to 11 days in the therapeutic range. The initial burst within the first 24 h was below 15% and was five-fold lower compared to the control oil formulation [43]. In both studies SAM based organogels possessed a good biocompatibility profile over the evaluation period.

Other extensively investigated organogels are formed from glycerol fatty acid esters, such as glycerol monooleate (GMO), glycerol monopalmitostearate (Precirol®) and glycerol monolinoleate [12]. These amphiphilic, water insoluble lipids swell in water and form various types of lyotropic liquid crystals. From a water content of 35 wt.% onward they build cubic phases with a gel-like character. These systems are highly viscous and the drug release depends on the water solubility of the incorporated drug. Insulin was released from GMO within 4 days, with an initial release of about 70% during the first 24 h. Somatostatine was released in only 6 h. To ease injectability and to extend the release duration from the gels, vegetable oils were mixed to the glycerol fatty acid esters. Gao et al. incorporated glycolyzed apricot kernel oil in Precirol® to control the release of the lipophilic contraceptive steroids levonorgestrel and ethinyl estradiol. In vivo studies in rats showed that the subcutaneous injected organogels completely blocked the estrous cycle up to 40 days [45]. The duration of the pharmacological effect, between 5 and 6 weeks, was the same as the time needed for biodegradation of GMO by lipases. This fact suggests an erosion controlled release from the implant. An inflammatory reaction was histologically observed for 1 week and disappeared afterward. Despite their low costs and biodegradation by lipases, the lack of toxicity data of the ingredients, as well as the purity and stability of oil and waxes currently limits the use of organogels in drug delivery. Another disadvantage is the high initial viscosity of the cubic phases and the need to apply heat for mixing and possible differences between the melting points of waxes and oils which promote phase separation. This led to the development of low viscosity injectable cubic phase-forming formulations [46]. Mixtures of monoglyceride, water and water-miscible organic cosolvents formed the cubic phase after contact with aqueous fluids. The in situ cubic phase-forming systems could be successfully sterilized by autoclaving and aseptic filtration without losing their phase behavior and sustained release properties.

4. In situ phase separation systems

Another strategy to form injectable drug depots is the phenomenon of phase separation from a solution. Hereby polymers undergo abrupt changes in their solubility in response to changes in their environmental temperature, pH or by solvent removal.

4.1. pH-induced gelling systems

Sol-to-gel transitions induced by changes in environmental pH are related to polymers containing ionizable functional groups. Chitosan is a biocompatible and biodegradable pH-dependent cationic polymer [47]. The amino-polysaccharide is soluble in acidic solutions and phase separates at pH > 6 to form hydrogels, through deprotonation of primary amino groups. However without further chemical cross-linking the gels possess low mechanical stability and rapid release of low molecular weight drugs [18,33]. Poly(methacrylic acid) (PMA) and PEG form water-insoluble interpolymeric complexes (IPCs) at pH < 5.8 [48]. The complexes result from hydrogen bonding between carboxylic acid groups from PMA and ether groups from PEG. These bonds are interrupted and the IPC re-dissolves in water when at least 20–50% of a non-aqueous co-solvent is added. This IPC solution transforms into a gel at physiological pH when water replaces the co-solvent. By time encapsulated drug is released as a result of dissociation of the IPC into the individual water soluble components, which were cleared via the renal pathway. Concentrations above 30 wt.% and below 60 wt.% IPC were required to form the gel and to facilitate injection. IPC dissolved in 1:1:2 NMP/ethanol/water was able to entrap and to control the release of macromolecules like fluorescein-labeled insulin and albumin for up to 6 days in vitro.

4.2. Thermoplastic pastes

Thermoplastic pastes are based on polymers having a melting point or glass transition temperature in the range of 25 °C to 65 °C. Before injection these polymers are heated above their glass transition or melting point and then injected as a liquid. In the body they form a semi-solid depot upon cooling down to body temperature. At an injection temperature above 37 °C and below 65 °C these polymers behave like viscous fluids and flow easily when pushed. Drugs are incorporated in the molten polymer by simple mixing without the use of organic solvents. Originally biodegradable thermoplastic pastes have been prepared from polymers such as polyanhydrides, PCL, PLA, and their blends with PEG as well as PLA–PEG–PLA triblock copolymers. But some of these polymers possess high transition points resulting in high injection temperatures partly above 60 °C. The injections were very painful and lead to necrosis at the injection site [49]. Further the encapsulation of the depot by scar tissue was observed, which inhibited the drug diffusion. Another disadvantage is the general slow drug release from these implants. As an approach to solve this problems Heller et al. developed a biodegradable semi-solid poly(ortho ester) (POE) [50,51]. Low molecular weight POEs, with softening temperatures in the range of 35 °C to 45 °C, have shown to be highly biocompatible in animals as well as in humans [52]. Thus they are semi-solid at room temperature; therapeutic agents can be incorporated by simple mixing without heating or the use of solvents. To be injectable through a needle their intrinsic viscosity should not exceed 0.8 dl/g. POE possessing intrinsic viscosities below 0.5 dl/g failed to delay drug release [53]. The

incorporation of lactic acid units in the backbone of the hydrophobic block polymers led to a self-catalyzed degradation behavior. POEs underwent predominantly surface erosion. Depending on the polymer structure, drug release could be modulated from days to weeks [51]. Although POE showed good biocompatibility in pre-clinical studies full evaluation of their biodegradation and toxicity still has to be performed. Another point is to test the system stability and the stability of the incorporated drugs. So far they are not approved for parenteral application.

4.3. Thermally-induced gelling systems

Thermally-induced gelling systems undergo sol-to-gel transition in water when the temperature increases. Thermosensitive polymers are especially attractive as they do not require organic solvents, copolymerization agents or externally applied triggers for their gelation at physiological conditions. In spite of this, temperature induced phase transitions are controlled by the temperature dependence of certain molecular interactions, such as hydrogen bonds or hydrophobic effects. At the lower critical solution temperature (LCST) hydrogen bonding between water and a polymer becomes unfavorable compared to interactions between the polymers themselves. The solvated macromolecules lose their water of hydration and polymer–polymer interactions increase. The polymers associate to a network structure, reflected by a sharp rise in system viscosity. Ideally the aqueous polymer solutions flow freely at room temperature and become a gel at body temperature. The shape of the gel depot and the drug release profile, e.g. initial burst of the incorporated drug are directly related to the kinetics of the sol-to-gel transitions and should therefore be fast [11,15]. Both synthetic and natural occurring materials may possess a temperature controlled gelation behavior.

The most studied synthetic polymers are copolymers of poly(ethylene oxide) (PEO) and poly(propylene oxide) (PPO) (PEO–PPO–PEO; known as Poloxamers) and copolymers of poly(N-isopropyl acrylamide) (PNIPAAM) [11,54–58]. However the use of these systems is limited because they are not biodegradable. Aqueous solutions of PNIPAAM undergo reversible sol-to-gel transition at 32 °C. The LCST can be shifted to 37 °C by the addition of salts and surfactants [11]. The opaque PNIPAAM gels do not dissolve or swell in water. With increased temperature, the gels expel water and shrink in mass. PNIPAAM is not suitable for implantation purposes in human due to its cytotoxicity, non-biodegradability and platelet activation upon contact with blood. Poloxamers are widely used as solubilizer and thickening agents. So far Poloxamer 188 is the only representative of these co-polymers approved for parenteral administration. Concentrations of Poloxamer 188 above 20 wt.% are needed to achieve gel formation. The high polymer content often changes the osmolarity and consequently negatively influences the tolerability of the formulations. Although Poloxamers are usually regarded as non-toxic, studies reported a dose dependent systemic side effect. The chronic administration elevated the plasma levels of cholesterol and triglycerides [12]. Among the commercially available PEO–PPO–PEO copolymers, Poloxamer 407 (Pluronic® F127) has been reported to be less toxic. Aqueous solutions with a concentration between 16 and 30 wt.% form semisolid transparent gels and have been examined for various biomedical applications [59]. But the delivery periods rarely exceeded a few days and the gels suffered from weak mechanical strength [11]. The physical cross-linking between the micelles was so weak that the gels were rapidly eroded by dissolution from the surface. A 25 wt.% Poloxamer 407 gel was completely dissolved in PBS medium within 4 h [60]. An increase in concentration to 35 wt.% yielded to 50% reduction in gel size within the same time period. After implantation in rats Poloxamer 407 gels could not be observed after 2 days [61]. In addition, the incorporated drug, salt concentration in the media and additives, such as PEG or poly(vinyl pyrrolidone) (PVP) strongly influenced the sol-to-gel transition behavior [59]. These characteristics result in a low reproducibility and limit the use of Poloxamer gels to short-term therapies.

Block copolymers of PEG and poly(lactide-co-glycolide) (PLGA) were proposed as alternative and biodegradable materials. At temperatures below 15 °C both PEG–PLGA–PEG (BAB) and PLGA–PEG–PLGA (ABA) copolymers assemble to micellar structures in water, whereas the hydrophobic PLGA forms the inner core. Gelation at body temperature occurs via a micellar aggregation mechanism. Polymer concentrations ranging from 10 to 30 wt.% were needed to achieve a reverse thermal gelation. The micellar properties and the LCST depend on several factors such as the length and composition of the blocks [59]. The hydrophobicity of the incorporated drugs strongly affects their release profiles. Jeong et al. studied the release kinetics of ketoprofen and spironolacton from ABA hydrogels [59]. The hydrophilic ketoprofen was released continuously over 2 weeks and the release rate depended on the initial polymer concentration. More hydrophobic spironolacton was released over 2 months. BTG International Ltd. (formerly Macromed Inc.) distributes low molecular weight ABA and BAB triblock copolymers under the trademark ReGel®. The block copolymers have been intensively investigated for solubilization and stabilization of water-insoluble (e.g. paclitaxel and cyclosporine A) drugs and proteins, including zink-insulin and porcine growth hormone [62]. The solubility of paclitaxel was increased by 400 folds in aqueous formulations containing 23 wt.% PLGA–PEG–PLGA. The resulting gels released paclitaxel in a controlled fashion over 50 days in vitro [62]. In vivo ReGel® formulation was completely resorbed from the injection site within 4–6 weeks. The efficiency of paclitaxel loaded ReGel® (OncoGel®, 6 mg/ml paclitaxel) against human breast cancer was superior compared to a maximum tolerated dose of a commercial product (Taxol®). The direct intratumoral injection of OncoGel® and the slow drug clearance from the injection site resulted in minimal paclitaxel distribution to other organs and less drug-related adverse effects than in the Taxol® group. OncoGel® has reached clinical phase II studies for the treatment of esophageal cancer, brain cancer and breast cancer [15]. However, in the esophageal cancer trial, the results were negative: the paclitaxel gel did not show any impact on the primary endpoint of the overall tumor response in a phase IIb study exploring its use as a neoadjuvant therapy to standard chemotherapy and radiation therapy before surgery in patients with esophageal cancer. Study follow-up for the secondary outcome measure of patient survival has been discontinued as there can be no anticipated impact. Based on these results, BTG International Ltd. will not seek a partner for OncoGel®, though “additional uses for the ReGel™ drug delivery technology used in OncoGel® will be explored” [63].

Also diblock copolymers of PEG–PLGA form micelles in water that undergo sol-to-gel transitions at 30 °C [64,65]. To enable water solubility, the polymers are composed of long PEG blocks combined with shorter PLGA blocks. In vitro the PEG–PLGA copolymers dissolved within 1 week. Whereas more durable gel depots were formed in vivo. By modifying the copolymer composition the persistence of the gels could be varied from 1 week up to 2 months [64]. However PLA and PLGA degrade to their monomeric acids, that yield in an acidic environment [66,67]. To circumvent this limitation Hyun et al. developed methoxy poly(ethylene glycol) (MPEG) PCL diblock copolymers that underwent sol-to-gel phase transitions within few seconds under physiological conditions [61]. Their degradation did not result in an acidic environment. In vivo the MPEG-PCL gels maintained their structural integrity longer than 30 days. The release of fluorescein isothiocyanate-labeled bovine serum albumin (FITC-BSA) was sustained within these 30 days. The following studies showed a good in vivo efficacy of paclitaxel and doxorubicin loaded MPEG-PCL gels after intratumoral injection [68,69]. Both systems were more efficacious in inhibiting the growth of the subcutaneous tumors than the injection of the pure drug solutions and biodistribution results implied fewer off-target side effects.

As mentioned above thermo-sensitive gels can be prepared in almost the same manner with naturally occurring polymers. Cellulose itself is not soluble in water. By introducing hydrophilic groups, the cellulose derivatives become water soluble. When cellulose derivatives have an optimum balance of hydrophilic and hydrophobic moieties, they gel at elevated temperatures in aqueous solutions. Typical examples are methylcellulose (MC), hydroxypropylcellulose (HPC) and hydroxypropylmethylcellulose (HPMC). The LCST depends on the substitution at the hydroxyl group, e.g. MC shows phase transition between 40 °C and 50 °C, HPMC between 75 °C and 90 °C and HPC at about 55 °C [11,59,70]. The phase transition temperatures can be modulated by varying the molar substitution of cellulose or addition of salts. For example the addition of sodium chloride lowered the LCST of MC to 32 °C–34 °C. However the thermal gelling properties of cellulose derivatives are not sufficiently fast for injectable drug delivery systems. Gumpta et al. blended MC with hyaluronan (HA) to achieve a fast-gelling injectable material for the localized delivery to the injured spinal cord [71]. The blend of HA with MC formed a shear thinning gel prior to injection. The gel strength increased after injection due to phase transition of MC at body temperature. In vivo rat studies showed a biocompatibility in the intrathecal space for 1 month. However, cellulose derivatives are not biodegradable and therefore, their use is limited to lower molecular weights which can be excreted.

Chenite et al. developed thermally sensitive neutral solutions based on combinations of chitosan with polyol salts (e.g. ß-glycerolphosphate, ß-GP) [72]. Without additives chitosan is soluble in acidic solution and phase separates at pH > 6. The addition of ß-GP to acidic chitosan solutions allows rising the pH to neutral without phase separation. These systems are thermosensitive and form hydrogels at temperatures around 37 °C and above. Thermosensitive formulations are obtained at low polymer concentrations. Mainly the degree of deacetylation (DD) of chitosan and its molecular weight distribution as well as chitosan impurities impact the stability of the sol as well as the gelation time and temperature. The chitosan/ß-GP systems could deliver macromolecular drugs from several hours to days [73,74]. However the high porosity of the gel yielded to a complete release of hydrophilic low molecular weight compounds within hours. To achieve a sustained release, independent from the drug molecular weight, hydrophilic compounds were first incorporated into liposomes. In the following the liposomes were mixed with the chitosan/ß-GP solution [75]. The release rate of the hydrophilic molecules could be controlled by the size and compositions of the liposomes. Besides pharmaceutical applications, the chitosan/ß-GP hydrogels were evaluated for tissue engineering purposes. The gel was capable of maintaining the bioactivity and releasing bone morphogenic protein (BMP) [72]. Chitosan/ß-GP systems were also evaluated for cartilage repair. Entrapped chondrocytes in solidified chitosan/ß-GP solutions initiated and proliferated the deposition of a functional matrix in an animal model [59]. BioSyntech Inc. developed a hybrid implant, named BST-Car-Gel® to improve cartilage healing. The mixture of chitosan/ß-GP and autologous blood solidifies within minutes via normal blood coagulation and the chitosan mediated mechanism [76]. The clinical trials in Canada, Korea and Spain of this product started in 2006 (ClinicalTrials.gov Identifier NCT00314236). The data analysis is currently ongoing, however, the recruitment for a follow up study (NCT01246895) suggests a positive outcome. Another BioSyntech Inc. product currently tested in human studies is BST-DermOn®. By using the hemostatic, bacteriostatic and mucoadhesive properties of chitosan, the chitosan/GP hydrogels were tested as an adjective therapy in topical wound therapy. Creating a moist healing environment, the hydrogels promote the healing of long lasting chronic wounds [77]. However in 2010 BioSyntech Inc. and Bio Syntech Canada Inc. got bankrupt. The Superior Court of the province of Quebec granted an order appointing PricewaterhouseCoopers Inc. as the interim receiver, who further sold it to Piramal Healthcare, an Indian company, so that the further development of the products is unclear.

4.4. Systems based on phase separation by solvent exchange

Only in situ polymer precipitation systems based on solvent removal have become commercially available so far (Table 1).

Examples include Atridox® which is used for the periodontal delivery of doxycycline [78] and Eligard®, a subcutaneous depot of leuprolide for the treatment of prostate cancer [79]. This concept, conceived by Dunn et al., employs biodegradable carriers dissolved in or diluted with water miscible, physiological compatible organic solvents [80]. Prior to injection the drug is added and forms an injectable solution or dispersion. After subcutaneous injection of the formulation into the body the organic solvent dissipates into the surrounding tissue as aqueous body fluids penetrate into the implant. This leads to phase separation and precipitation of the polymer, forming a depot at the injection site. The active pharmaceutical ingredient (API) gets entrapped within the matrix as it solidifies and is released by diffusion processes or as the implant biodegrades. The systems based on phase separation by solvent removal have been used in a large number of drug delivery applications ranging from small molecules (e.g. lidocaine [81], aspirin [82]), over peptides [83] to proteins [84,85]. Standard "hydrophobic" biodegradable polymers, such as polyhydroxyacids, polyanhydrides, polyorthoesters and others can be used as carriers. However PLA, PLGA and PCL are preferably used because of their approval by the American Food and Drug Administration (FDA) and their long history of clinical use.

The Atrigel® technology, introduced by Dunn et al., dissolved the polymers in organic solvents with high water miscibility. Solvents include NMP, dimethylsulfoxide (DMSO), propylene glycol, acetone, tetrahydrofuran (THF), 2-pyrrolidone [80], ethyl acetate [84], glycofurol [86] or low molecular weight PEG [87]. NMP is most frequently used because of its solvating ability. Polymer concentrations in the prepared formulations range from 10 wt.% to 80 wt.%. Both, the concentration of the polymer in solution and its molecular weight affect the viscosity of the implant forming solutions [88]. The phase inversion dynamics are a complex phenomenon and are directly affected by the solvent properties. After contact with the aqueous medium the high water miscibility of the solvents results in a fast phase inversion of the polymer solutions. Generally the solidification of these systems takes place in

Table 1
In situ forming implants that are commercially available or in clinical development.

Mechanism of formation	Product	Drug	Company	Administration	Indication	Regulatory status
In situ phase separation						
Solvent exchange	Eligard® (Atrigel® technology)	Leuprolide acetate	Sanofi-Aventis	sc	Advanced prostate cancer	Approved by FDA
	Atridox® (Atrigel® technology)	Doxycycline hyclate	TOLMAR	into the peridontal pocket	Adult parodontitis	Approved by FDA
	POSIDUR™ (SABER™ technology)	Bupivacaine	DURECT	at the surgical site prior wound closure	Postoperative pain	Clinical phase III
	Relday™ (SABER™ technology)	Risperidone	DURECT	im	Schizophrenia and bipolar disorder	Pre-clinical phase
Thermally-induced	Oncogel® (ReGel® technology)	Paclitaxel	BTG	intratumoral injection	Anticancer therapy	Clinical phase II

the order of seconds to minutes. Fast phase inversion of polymer solutions and high solvent affinity to the precipitant result in implants with large finger pores, directly related to high initial drug release [88]. The influence of several formulation parameters on the drug burst, like polymer type, type of solvent or co-solvent and additives, have been studied in detail in vitro [89,90]. The main factors of influence are the type of the polymer and its molecular weight. High polymer concentrations (40 wt.%–50 wt.%) decreased the initial release. However these solutions were highly viscous, so that the injectability was impaired. The affinity of the API for the solvent–water versus the solvent–polymer phase has also a strong impact on the burst release. Graham et al. discovered that protein release can be modified by varying the aqueous miscibility of injectable depots [91,92]. Reducing the solvent/non-solvent affinity of PLGA solutions slowed the rate of phase inversion and yield to a more uniform release. Typical solvents include benzyl benzoate, ethyl benzoate, triacetin [85], triethyl citrate [93] or benzyl alcohol [94]. Polymer solidification in these slow phase inverting systems takes from hours to days. The morphology of the depot is more or less uniformly dense with a smaller pore size. However the solution viscosities of these systems are in a range that makes injection difficult without a previous warm-up to 37 °C.

Implants can also be formed from biodegradable non-polymeric carriers, e.g. sucrose acetate isobutyrate (SAIB). SAIB is a water insoluble high viscous (100,000 mPa·s) compound, approved as a food additive to stabilize emulsions. Diluted with small amounts (15%–35%) of pharmaceutically acceptable organic solvents, like ethanol, triacetin or NMP, it can be injected as low-viscosity formulations (50 mPa·s to 200 mPa·s). After injection the solvent diffuses away, leaving a viscous adhesive matrix of SAIB with incorporated active ingredients and additives. Various drugs have been tested for release by this delivery system including human growth hormone and risperidon [95,96]. The SAIB system is patented by Southern Biosystems and licensed to DURECT under the trade name SABER™. The SABER™ system has the advantage of low cost for raw material and ease of manufacturing. SABER™-Bupivacaine (POSIDUR™) has been developed for the local treatment of post-surgical pain and is in clinical phase III. Injected during surgery it continuously releases bupivacaine and in this way provides up to 3 days of uninterrupted local anesthesia.

The central issues of the solvent removal in situ precipitation system are the potential of unwanted local irritations due to the use of high amounts of organic solvent, and the lag between the injection of the solutions and the formation of the solid implant. Biocompatibility studies of NMP/PLGA and DMSO/PLGA solutions in rhesus monkey showed no acute toxicity and tissue reaction similar to that for other biodegradable polymers [97]. The biocompatibility of PLA, dissolved in benzyl benzoate and benzyl alcohol, in rabbits showed normal inflammatory and foreign body reactions similar to blank and pure API solutions. Neither necrosis nor tissue damage was observed [94]. Contradictory myotoxicity studies showed a high acute myotoxic potential, comparable to the positive control for NMP, DMSO, benzyl alcohol, triethyl citrate and their polymeric solutions. 2-Pyrrolidone, triacetin and propylene carbonate caused less muscle damage, whereas the myotoxicity of ethyl acetate was comparable to the negative control [98,99]. Another major concern is the stability of polymers, mainly PLGA, and the drug in the in situ forming systems. Dong et al. observed a faster degradation of PLGA with increased storage temperature and water content of the solvents [89]. For these reasons, polymeric solutions should be stored at 4 °C and the drugs ought be added as dry powders to the solution prior to injection. Additionally sensitive drugs like proteins may denaturate in the organic solvent. To overcome these limitations the group of Bodmeier followed a different approach: they dispersed the drug containing polymer solutions in a second external phase composed of oil for injection [98]. The polymer solution droplets solidified after contact with aqueous fluids to form microparticles in situ. The viscosity of in situ forming microparticle (ISM) systems is determined by the viscosity of the second phase. In comparison to ISFI, ISM can be prepared with higher polymer concentrations and the use of less organic solvent maintaining the injectability of the system. Further the ISM showed reduced initial release [100] and myotoxicity [98,99]. The main obstacle is the relatively low ISM emulsion stability [101]. Voigt reported that the coalescence of polymer solution droplets have yielded to in situ formation of implant-like matrices and complicated injection.

5. Work in progress

Despite the attractive features of ISFI and the existence of clinically used systems, serious constraints do still exist. The main obstacles in the development of ISFI are:

1. the variability in the shape of the formed implants
2. the suppression of the burst release
3. the toxicity of the matrix forming materials and solvents used.

To sum it up it can be said that an ideal in situ forming implant should:

- Possess a low viscosity of the implant solutions to ensure a good injectability.
- Allow a simple drug load.
- Contain only biodegradable and biocompatible excipients.
- Possess good system stability.
- Yield a low variability of drug release with a low initial burst.

So far none of the presented systems fulfill all of the mentioned requirements (Table 2).

Table 2
Overview on in situ forming implants.

Delivery system		General issues
In situ cross-linked systems	Photo-initiated polymerization	• Low penetration depth of irradiated light • Formation of reactive oxygen species • Toxicity of unreacted monomers • Lack of toxicity data • Modification of drug
	Physical cross-linked	• Weak mechanical stability of the gels • Slow kinetics of cross linking
	Chemically cross-linked	• Toxicity of the cross-linking agents • Lack of toxicity data • Modification of drug
In situ solidifying organogels		• Stability and purity of the used lipids
In situ phase separation systems	pH-induced gelling	• Weak mechanical stability of the gels
	Thermoplastic pastes	• High temperature at time of injection • Small temperature window • Lack of toxicity data
	Thermally-induced gelling	• High burst release • Slow kinetics of gelling • Lack of toxicity data
	Polymer precipitation by solvent exchange	• Toxicity of organic solvents • Lack of toxicity data • High burst release

As mentioned previously one of the major challenge is the reliable formation of the implant with respect to size, shape and structure. It is a result of several parameters which come from different sources as illustrated in Fig. 3.

For example, PLGA–NMP based in situ polymer precipitation systems can form rod like structures if they are injected rapidly. Spherical implants are formed after slow injection. The solvent exchange kinetics and associated drug release strongly depend on the resulting implant to surface area [102,103]. Besides the strong impact on the diffusion controlled release, the implant degradation will be affected due to autocatalytic effects in larger depots [104]. The main hindrance for the use of ISFI for drug delivery purposes especially for low molecular weight drugs is the low reproducibility of the implant shape under in vivo conditions with different persons administering the implants. One possible approach to solve this problem is the use of multiparticulate systems like in situ forming microparticle developed by Kranz and Bodmeier [100]. On the other hand, the ability of ISFI to fill irregular defects makes them very attractive for tissue engineering purposes, such as cartilage and bone augmentation. Furthermore in situ forming implants can improve the site directed delivery of other drug dosage forms, as it has been shown for microparticles [105], liposomes [75] or nanocapsules [106] suspended in chitosan-based thermally-induced gelling systems.

The other big challenge in translating ISFI systems from bench to bed side is to control the initial burst release, especially for in situ polymer precipitating systems. Several formulation parameters have been examined in order to minimize the burst effect within these systems, e.g. increasing the molecular weight and the concentration of polymers or to vary the type of solvents or co-solvents [88,90]. However the increase in the concentration and molecular weight of PLGA impedes the syringeability of the formulations [107]. New strategies to minimize the initial release rates are to alter the precipitation rate of PLGA by combining relatively hydrophobic cosolvents (e.g. triacetin) or aliphatic esters (e.g. ethyl heptanoate) with more hydrophilic solvents like NMP or DMSO [108–110]. Another interesting starting point is the development of hybrid implants that combine different matrixes, such as SAIB and PLA or PLGA [111] or incorporate solid lipids like glycerol monostearate into the PLGA-based implants [107]. Poly(ethylene carbonate) (PEC), a novel surface-eroding biomaterial has been recently tested as an alternative matrix for ISFI [112]. Since drug is released upon erosion from the implant surface the authors postulate a more regular release compared to non-linear release profiles with a high initial release of the PLGA-based implants. But to really control the drug release characteristics it is important to quantify not only the release rates, but more importantly the dynamics of the phase separation process both in vitro and in vivo under physiological conditions. Unfortunately, until now the knowledge concerning the detailed processes of the implant formation is still very limited. This is due to the fact that the processes of implant formation and degradation, drug release and tissue response are complex, heterogeneous, interconnected and not easy to follow, especially in vivo. The in vitro–in vivo correlation is often not known and a difficult topic for biodegradable polymers in general. Most frequently applied analytical methods for the characterization of implants, such as the different kinds of chromatography, infrared spectroscopy, differential scanning calorimetry and optical microscopy with or without histological staining are either limited to in vitro experiments or require the surgical extraction of implants by killing the animal. Additionally the required special sample preparation may lead to artifacts [91,104,113]. Another common imaging technique to study the implant's morphology is scanning electron microscopy [114]. One shortcoming of this technique is its destructive character, since the sample preparation involves freezing and sectioning of the implants. In addition, all these procedures exclude continuous studies with the same sample on a single animal. These conventional in vivo experiments require a large number of animals, which does not comply with the international efforts to reduce the number of animal experiments. Most solvent diffusion study protocols are also limited to in vitro evaluation as the amount of solvent released from the implants is detected in the incubation media by either determination of their refraction index [111], electric conductivity [115] or by HPLC–UV [110]. Also the dark-ground video imaging technique developed by Graham et al. to visualize and quantify the phase inversion dynamics of several PLGA solutions [91,92] is limited to in vitro experiments and the evaluation of thin films over a short time scale (minutes to a few hours). During the last years, successful trials have been made to follow non-invasively the implant formation in vivo, at least in preclinical models, by the magnetic resonance based methods electron paramagnetic resonance (EPR) spectroscopy and magnetic resonance imaging (MRI) [116–120] and ultrasound imaging [121,122]. In 2010 Solorio et al. introduced diagnostic ultrasound to visualize and quantify non-invasively the process of implant formation by polymer precipitation in vitro and in vivo [121]. The solidification of the implant was visible by a change of the ultrasound contrast [121]. It has been shown, that the application side impacts the shape of the implant [123]. However ultrasound imaging is limited by the resolution of the images and therefore suited for the macroscale analysis of the implants and cannot measure the solvent exchange process. Four years ago, in 2008 EPR spectroscopy was applied to quantify both the kinetics of the solvent/non-solvent exchange and polymer precipitation and to correlate the in vitro and in vivo data. Using spin probes as reporter molecules, it was possible to measure the solvent exchange, as the hyperfine splitting of the nitroxides depended on the polarity of the solvents. In addition information about the direct microviscosity of the reporter molecules (= molecular mobility) could be obtained. Therefore, precipitation of the model drug within the implant could further be followed. Using pH sensitive nitroxides it was also possible to follow the microacidity inside degrading implants, pH values as low as pH 2 have been observed in vivo [123]. MRI was able to track the implant formation and erosion as well as responses of the body (e.g. edema, encapsulation). The characterization of key processes of the implant formation is an important point to understand and optimize the in situ polymer precipitation systems. Also the direct implant environment plays a crucial role during implant formation process [92]. Kranz et al. compared the in vitro bupivacaine release from in situ forming implants and in situ forming microparticles obtained by different in vitro methods [124]. The initial drug release decreased along with increased contact of implant surface to the medium in the following order: direct injection method > dialysis bag > vial method (water contact restricted to one site). So far no regulatory guidelines have been established for experimental conditions to assess the performance of parenteral controlled drug delivery systems and ISFI in special. Various experimental setups have been reported in the literature,

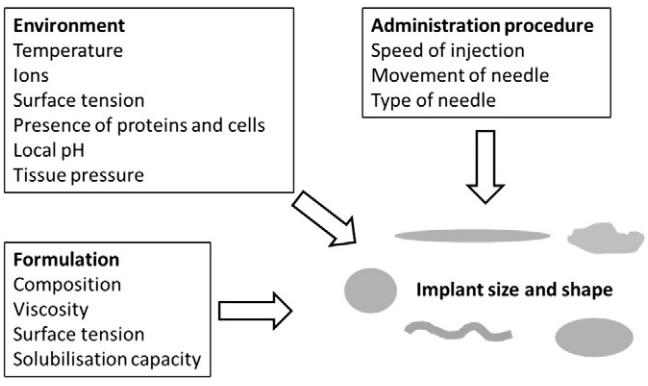

Fig. 3. Sources of the variability of the implant shape and size.

e.g. the use of agitated or non-agitated vials, dialysis membranes or agarose gels [92,124]. Further the composition of the release medium and the device to bulk fluid ratio directly affect the performance of the systems [88,125–127]. Therefore special cautions to the experimental conditions have to be paid when comparing results. An optimal in vitro method that assures sink conditions, reproducibility of sampling, and prediction of in vivo release has not been developed yet. The last point to consider is the safety of the ISFI with respect to the toxicity of their components and the stability of the formulations. The toxicity of the molecular linkers and the radical species formed are the main hindrance for a broader use of implants formed by chemical or photo-initiated cross-linked polymers. Whereas the toxicity of the organic solvents used for in situ polymer precipitation systems is another issue. Several solvents like low molecular PEGs, ethyl acetate or ethyl benzoate have been introduced as more compatible solvents compared to NMP [98]. But recently Schoenhammer et al. reported that low molecular PEGs accelerate the degradation of PLGA in solution as a result of trans-esterification [128]. So the stability of PLGA in PLGA/PEG 400 solutions may be beside edema formation [117] another obstacle in its use as an alternative solvent. The long-term stability of PLGA or PLGA-based polymer solutions is a general issue that has not been resolved yet. Two different approaches have been followed to overcome these toxicity and stability limitations. One attempt is to search for new solvents, e.g. PEG-dialkylether [128], which do cause no or only minor polymer alterations [128]. The alternative approach is to develop new degradable biomaterials that need less or no organic solvents. Gurny's group synthesized alkyl substituted polylactides. Instead of lactic acid, the more hydrophobic 2-hydroxyoctanoic acid is used as a monomer. The resulting polymers are regarded as “hexyl-substituted polylactides” (“hexPLA”) and possess unique physicochemical properties [129]. They are semi-solid and injectable without or with only small (<5%) amounts of NMP. The hexPLA systems can be sterilized by dry heat and degrade in a continuous manner without the formation of acidic microenvironments. It is a lipophilic, viscous solution, which is injectable. HexPLA is biodegradable and able to control the release of drugs from days to several weeks [130,131]. It is certainly a new and very promising approach and a real innovation in the field of drug delivery.

Aware of the problems caused by the organic solvent toxicity many researchers are oriented toward environmentally responsive hydrogels. Of special interest were hydrogels that respond to temperature changes in their environment as the sole stimulus for their gelation. Ideally the aqueous solutions are liquid at ambient temperature and gel under physiological conditions [75,132]. Many new formulations based on this principle have been proposed for drug delivery [74,133–135], tissue engineering [14,136] and cell encapsulation [72,76,137] purposes. Among the natural polymers that exhibit gelation upon temperature change, particularly chitosan-based systems have been studied in detail in the past decade [73,138–142]. But for a more practical application chitosan-based thermally-induced gelling systems should be optimized to faster gelation times. The gelation rate can be modified by varying the composition of chitosan/ß-GP systems. Faster gelation can be achieved by using chitosan with a high DD [73], increasing the chitosan or ß-GP content [143]. However increasing the content of chitosan and above all the content of ß-GP will increase the osmolarity of the systems [143,144]. Especially ß-GP concentrations above 10% (w/v) produce a hypertonic environment [143] that may lead to local irritations after injection. With time the osmolarity of the gel will be reduced by water exchange and diffusion of ß-GP out of the gel [144]. The pH-value of the chitosan/ß-GP systems is another factor that influences the gelling behavior [145]. The addition of Na_2HPO_4 increases the pH value to 7.3–7.4 and accelerated the gelation time. Also co-crosslinking with the chemical cross-linker genipin yielded to a reduction of the gelation time [146]. The other side of the picture is that acceleration of the sol-to-gel transition negatively affects the storage stability of the systems [73]. Schuetz et al. suggested freezing as a conceivable storage method [147]. The authors further proposed replacing ß-GP by trehalose or mannitol and subsequent freeze-drying. The reconstituted solutions from the lyophilizate still possessed their thermosetting properties and were injectable. The strength of the thermally-induced chitosan-based gelling systems lie beside their potential in drug delivery to act as a vehicle for other drug delivery systems or cells, especially for tumor treatment or tissue engineering purposes. Recent applications include the local delivery of liposomes [148], microparticles [105] and the use of in vivo scaffold for rat bone marrow mesenchymal stem cells [149] or as a vehicle to immobilize chondrocytes for cartilage repair [150]. But the major obstacle of all chitosan-based drug delivery systems is that chitosan is not approved by the FDA for drug delivery purposes or a generally regarded as safe (GRAS) material [151]. However, monographs do now exist for chitosan HCl. Chitosan is not a uniform chemical substance. Instead the natural polysaccharide describes a steadily increasing group of chemical entities with differing Mw, DD and chemical modifications that all possess different distribution, degradation and toxicological profiles [151]. Several companies supply chitosan in various grades of purity, Mw and DD. The quality and characteristics of chitosan strongly depends on the manufacturing process. Therefore thorough analytical description of chitosan is necessary to achieve a product of constant quality. The sole specification of DD is not sufficient as this parameter strongly depends on the analytical method employed [152]. Augsten and Mäder reported a high batch-to-batch variability of chitosan of the same DD from the same supplier [153]. Availability of high and constant quality of chitosan types is an urgent need for further development. Apart from the natural biomaterial chitosan many synthetic amphiphilic block copolymers have been extensively studied to form thermo-sensitive hydrogels, including ABA triblock copolymers of PEG and PLGA, PCL or modified PCL with PEG as the B block [154]. But their biological compatibility needs further evaluation.

The decrease of variability and the improvement to provide a burst free, controlled drug release with predictable biological fate of a nontoxic carrier will be the main challenge for the future development of ISFI. Many efforts are made in industry and academia to improve the current approaches. New materials and approaches enter the preclinical and clinical phases and one can be sure, that ISFI will gain further clinical importance within the next years.

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