Applications of supercritical CO$_2$ in the fabrication of polymer systems for drug delivery and tissue engineering

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Abstract

Supercritical CO$_2$ has the potential to be an excellent environment within which controlled release polymers and dry composites may be formed. The low temperature and dry conditions within the fluid offer obvious advantages in the processing of water, solvent or heat labile molecules. The low viscosity and high diffusivity of scCO$_2$ offer the possibility of novel processing routes for polymer drug composites, but there are still technical challenges to overcome. Moreover, the low solubility of most drug molecules in scCO$_2$ presents both challenges and advantages. This review explores the current methods that use high pressure and scCO$_2$ for the production of drug delivery systems and the more specialized application of the fluid in the formation of highly porous tissue engineering scaffolds.

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1. Introduction

A very wide range of clinically approved pharmaceutical products take advantage of biodegradable polymers to control the rate of drug release within the body [1,2]. The preparation of most systems relies on either melt processing or organic solvent based methods to incorporate into the polymer phase. Liquefaction is required to mix the drug and polymer phases and to form the final delivery system morphology. The use of organic solvents, the formation of interfaces between organic and aqueous phases and the requirement for high temperatures are all factors that are not desirable to retain activity of the drug. Protein therapeutics are one example of a drug class where new factors that are not desirable to retain activity of the drug.

2. Solvent properties of scCO2

CO2 is inexpensive, non-toxic, non-flammable and readily available in high purity from a variety of sources. scCO2 (\(T_s = 31.1 ^\circ C, P_s = 73.8 \) bar) has the unique combination of gas-like diffusivity and liquid-like density, which can be tuned easily by changes in pressure. In recent years, it has been widely used as a solvent, anti-solvent and plasticizer for synthesis, modification and purification of both synthetic and natural polymers [4–9]. The solvent properties of scCO2, i.e. the solubility of polymers in scCO2 and the solubility of CO2 in the polymers, are two key fundamental subjects in this field. Most pharmaceutical compounds and polymers have demonstrated low solubility in scCO2. However, it is the high solubility [10] of CO2 in many polymers that can be particularly advantageous and can lead to dramatic decreases in the glass transition temperature (\(T_g\)) or melting temperature \(T_m\), thus reducing polymer melt viscosity [6,11–15].

2.1. Solubility of polymers in scCO2

While CO2 is a good solvent for most non-polar and some polar low molecular substances, it is a poor solvent for most high molecular weight polymers under mild conditions (\(T<100 ^\circ C, P<350 \) bar), although there are some exceptions such as amorphous fluoropolymers and silicones [4,5]. The significant solubility of fluorinated polymers is due to the interaction of CO2 with the C–F bond, while the flexible — Si–O–Si— moieties in their backbones are responsible for the solubility of siloxane polymers [16]. The polarity of polymers can also be a reason for their limited solubility because CO2 has a quadrupole moment and no permanent dipole [17]. In addition, CO2 has the potential to act as both a weak Lewis acid and Lewis base, and theoretical and experimental evidence indicates that CO2 can participate in hydrogen-bonding interactions. This suggests that CO2 might solubilise dipolar and non-dipolar molecular systems through site-specific solute–solvent interactions. Beckman et al. [9,18] have shown that by careful molecular design and choice of co-monomers to balance the solvent–solute and solute–solvent interactions it is possible to prepare hydrocarbon based copolymers that are CO2-soluble.

2.2. Solubility of CO2 in polymers

CO2 solubility and diffusivity in polymers are influenced by both the molecular structure (the interaction between CO2 and molecular chains) and the morphology (crystalline or amorphous, related with free volume) of polymers. The solubility of CO2 in many polymers, e.g. poly(methyl methacrylate) PMMA and poly(styrene) PS, has been studied by evaluating CO2 sorption and polymer swelling [19–21]. There was a general perception that sorption and swelling is a purely physical phenomenon until Fourier transform-infrared (FTIR) and ATR-IR spectroscopy were used to study the specific intermolecular interactions between CO2 and polymers [22–24]. While the chain flexibility of polymers can aid dissolution of CO2, carbonyl or ether groups that are accessible in the backbone or on side chains can specifically interact with CO2. Polymers with ether groups in poly(ethylene glycol) (PEG) displayed stronger interaction than polyesters due to weak Lewis acid–base interaction [24]. Although poly(lactic acid) (PLA) and poly(lactic acid-co-glycolic acid) (PLGA) have the same chemical structure in the main chains, the steric hindrance close to the carbonyl group and accessible free volume caused by methyl pendant groups can lead to different behaviour of solubility and interaction. In the case of PLGAs, some methyl groups are substituted with hydrogen which causes much less hindrance for the interaction with CO2. However, the accessible free volume in the polymers was found to have a greater effect on solubility than the interaction between CO2 and the polymers [25]. Therefore, the solubility of CO2 in PLGA copolymers decreases with increasing the glycolic acid content. Moreover, Oliveira et al. [26] studied the solubility of CO2 in PLA with two different L:D contents, and found that CO2 is more soluble in PLA 80:20 (amorphous) than in PLA 98:2 (20% crystallinity).
PGA, PEG and PCL are highly crystalline polymers, and CO₂ does not easily diffuse into these at the temperatures below their melting points, leading to low solubility. In contrast, amorphous poly(DL-lactic acid) (PDLLA) and PLGAs have larger free volume, which allows CO₂ more easily to diffuse into them.

3. Drug delivery applications

3.1. Rapid expansion of scCO₂ (RESS)

RESS has been used to produce a wide range of different drug delivery systems including fibres, microparticles and films. In the RESS process the solute is solubilised in the supercritical fluid [27] and then expanded through a capillary nozzle, typically <150 μm in diameter, into a precipitation chamber. Rapid decompression of the solution leads to supersaturation, nucleation and particle formation. This process can result in the formation of very small uniform particles due to the high supersaturation ratios which can be achieved. Alternatively, larger particles can be produced by controlling factors such as temperature, pressure and nozzle geometry.

Tom et al. were the first to use the RESS process to produce drug encapsulated microparticles [28]. PtLLA and lovastatin were dissolved in scCO₂ and co-precipitated by RESS to form drug entrapped polymeric microparticles and microspheres (10–100 μm). Varying the concentration of lovastatin resulted in a change in product morphology from drug–polymer networks to microspheres. The presence of crystalline needles of lovastatin embedded in the PtLLA microspheres suggested that the drug and polymer precipitated independently.

More recently Kim et al. encapsulated the non-steroidal anti-inflammatory drug naproxen into low molecular weight (2000 Da) PtLLA microparticles using a RESS process [29]. Polymer and drug were co-dissolved in scCO₂ and expanded through a capillary at a pressure of 170–200 bar and temperature of 90–115 °C into a precipitation chamber. This process is therefore different to earlier studies where drug and polymer were dissolved separately in scCO₂ and then co-precipitated [28,30]. Polymeric microparticles (10–90 μm) with embedded naproxen particles (2–5 μm) were successfully prepared. Naproxen was found to dominate the core while the L-PLA coated the surface due to slower precipitation.

The highlighted studies demonstrate the feasibility of the RESS process for preparing polymeric drug delivery systems. However, the main limiting factor is the low solubility of most pharmaceutical compounds and regulatory approved polymers in scCO₂. To overcome this obstacle, co-solvents have been used to modify the polarity of the extracting phase and thus enhance solute solubility in the supercritical phase. Tom and Debenedetti demonstrated that when 1% (w/w) acetone was used as a co-solvent the solubility of PtLLA increased by approximately 500% [31]. Chlorodifluoromethane has also been utilised as a co-solvent for the preparation of PtLLA microparticles and microspheres using RESS [30]. The fluorescent solute pyrene was uniformly incorporated into the microparticles by co-precipitation with the L-PLA. The nozzle geometry (orifice or capillary) was demonstrated to be an important determinant of product morphology. When an orifice was used, dendrites were the most common morphology, whereas microspheres were prepared when capillary nozzles with low length-to-diameter ratios were used.

In many circumstances, even in the presence of co-solvents, the solubility of most pharmaceutical compounds is still far too low to make RESS a viable process. Furthermore, the higher temperatures which are often required for RESS make it unsuitable for labile polymers and drugs. To circumvent this problem, scCO₂ can be used as an alternative to conventional solvents to coat drug particles with polymer [32–34]. This process takes advantage of the differing solubility of drug and polymer in CO₂–solvent mix. The solubility of the coating material is much higher than that of the drug so the polymer precipitates onto preformed drug particles on decompression. An example of this is the rapid expansion from a supercritical solution with a non-solvent (RESS-N) process. This has been used to encapsulate both low molecular weight drugs and proteins into polymeric microparticles [33,34]. RESS-N utilises polar organic solvents, such as low molecular weight alcohols (methanol, ethanol and 1-propanol), as co-solvents. These solvents greatly increase the solubility of many pharmaceutical polymers (including PEG, PMMA, PEG-PPG-PEG, and ethylcellulose) in scCO₂ due to their hydrogen-bonding capacity. Furthermore, as they are non-solvents for the polymer, they do not induce swelling or microparticle aggregation. Proteins (lysozyme and lipase) containing microparticles were prepared by spraying a suspension of protein in scCO₂ and dissolved polymer at a temperature of 35 °C and a pressure of 200 bar through a nozzle to atmospheric pressure [34]. PEG, PMMA, PtLLA, PtXLGA, and PEG–PPG–PEG triblock polymers were used as coating materials. Particles were fabricated in the range from 8 to 62 μm. More recently the RESS-N process has been used to encapsulate the low molecular weight drugs p-acetamidophenol, acetylsalicylic acid, 1,3-dimethylxanthine, flavone and 3-hydroxyflavone into PEG, PMMA, ethylcellulose and PtLLA microparticles with an average size of 14.6 μm [33]. Although RESS-N is a promising technique, drug release from coated microparticles has not been reported to date so it is unclear if coating using this technique can modify dissolution kinetics.

A similar RESS process has been successfully used to coat bovine serum albumin (BSA) microparticles with the lipidic compounds Dynasan® n 114 or Gelucire® 50-02 [32]. BSA and the coating material were placed in the autoclave and scCO₂ (35 or 45 °C and 200 Bar) introduced to dissolve the coating material whilst stirring. Cooling induced a phase change from supercritical to liquid CO₂ and reduction in solvent strength which resulted in the coating material being precipitated onto the preformed drug particles. Whilst coating with Gelucire® 50-02 produced a homogeneous coat and protein was released continuously over 24 h, coating with Dynasan® 114 did not give a continuous coat which led to rapid in vitro release of BSA (70% in 30 min).

Another application of the RESS process in the field of drug delivery is impregnation of polymeric materials with pharmaceutical agents (see Kikic and Vecchione for a review) [35]. Impregnation utilises the high diffusivity and low surface
tension of supercritical fluids. Polymer impregnation can be achieved when the active substance is soluble in the supercritical fluid (SCF) and the polymer is swollen by the SCF. If the drug has high affinity for the polymer matrix (high partition coefficient between the polymer and SCF phase), or specific interaction with the polymer, the drug can be molecularly dispersed within the polymeric matrix. Drugs with low affinity for the polymer can be precipitated and deposited into the matrix on decompression. In this scenario the drug will recrystallise in the matrix and will not be molecularly dispersed.

Gueny and Akgem impregnated PDLLGA with 5-fluourouracil (5FU) and \( \beta \)-estradiol for the purpose of controlled release [36]. Macroporous foams or microporous particles could be prepared depending on if the system was depressurised rapidly or slowly. Drug loadings of 26.8 mg/g and 0.49 mg/g could be achieved at 55 °C 207 bar for \( \beta \)-estradiol and 5FU respectively. The majority of the drug was released rapidly from the matrix suggesting the bulk of the drug was adsorbed to the surface rather than encapsulated.

Kazarian and Martirosyan used ATR-IR spectroscopy to monitor ibuprofen impregnation of poly(vinyl pyrrolidone) (PVP) films [37]. It was demonstrated that ibuprofen was molecularly dispersed in the polymer matrix, with the drug molecules interacting with carbonyl group of PVP. In a similar study the RESS process was used to impregnate indomethacin into chitosan thermost sets for controlled release applications [38]. Chitosan and indomethacin powders were physically mixed and then exposed to \( \text{scCO}_2 \) in an autoclave. SCF impregnation resulted in indomethacin being amorphously dispersed throughout the chitosan matrix. FTIR data suggested that the aliphatic carbonyl group of indomethacin interacted with NH2 groups of chitosan. Following supercritical fluid processing, the dissolution rate of indomethacin was markedly increased. Van Hees et al. used the RESS process to prepare drug cyclodextran inclusion complexes to improve the solubility of the poorly soluble drugs [39,40]. Piroxicam-\( \beta \)-cyclodextran inclusion complex and miconazole cyclodextran complexes were prepared by physically mixing the compounds prior to exposure to \( \text{scCO}_2 \). The \( \text{scCO}_2 \) carries the drug into cyclodextran cavity, substituting water molecules with the less polar guest.

### 3.2 Anti-solvent applications

\( \text{scCO}_2 \) is a relatively poor solvent for most polymers and pharmaceutical compounds. Although co-solvents can be used to increase solubility in \( \text{CO}_2 \), or the process adapted to coat preformed drug particles (as discussed above), in the vast majority of circumstances solubility is still too low to make RESS a practical process. To overcome these problems the anti-solvent or non-solvent properties of carbon dioxide can be exploited. Many different anti-solvent techniques have been used for preparation of polymeric drug delivery systems. These include: GAS, SAS, PCA, ASES and SEDS. These techniques use a dense gas as an anti-solvent to precipitate a solute which is dissolved in an organic solvent. Precipitation occurs as the gas is absorbed by the organic solvent thus expanding the liquid phase and reducing the solvent power until nucleation and particle formation occur. The supercritical anti-solvent techniques offer greater flexibility than the RESS process and consequently have been studied more thoroughly for preparation of drug delivery systems.

#### 3.2.1 Biodegradable polymer microparticles

The majority of anti-solvent studies have focussed on encapsulation of pharmaceuticals into biodegradable poly(esters) such as PLA and PLGA. Bleich and co-workers were amongst the first to demonstrate the potential of anti-solvent techniques for microencapsulation [41]. Hyoscine butylbromide was encapsulated into PlLA microparticles by co-precipitation of drug and polymer from a solution of methanol and methylene chloride using the ASES process. A maximum drug loading of 19.8% (w/w) was achieved. Similarly Sze et al. used ASES to microencapsulate para-hydroxybenzoic acid (\( \beta \)-HBA) into PlLA [42]. Drug encapsulation efficiency was relatively poor, but could be modestly improved from 7.0% to 9.2% by spraying the drug solution and polymer solution through separate channels using co-axial nozzle assembly, rather than co-dissolving the drug and polymer and spraying the mixture through a single nozzle. Particles consisted of fibrous \( \beta \)-HBA embedded within polymeric microparticles. Temperature was found to be an important parameter in determining particle morphology. Whereas discrete particles were formed at 25 °C, at temperatures above the critical point the number of deformed or agglomerated particles increased due to the plasticization effect of \( \text{CO}_2 \).

Bodmeier and co-workers assessed the suitability of PCA for the preparation of drug entrapped polymeric microparticles [43]. Initially the swelling behaviour of a range of polymers in carbon dioxide was investigated. It was found that amorphous polymers with low glass transition temperatures agglomerated even at low temperatures. The more crystalline PlLA was therefore selected to study further. Microparticles were favoured at moderate temperatures, low polymer concentrations (1%), high pressure and high \( \text{CO}_2 \) flow rates, whereas fibres formed at high polymer concentrations and low flow rates. Chlorpheniramine and indomethacin were encapsulated into the particles but encapsulation efficiency was low. More recently Falk et al. have used the PCA technique to encapsulate gentamycin, naloxone and naltrexone into PlLA microparticles [44]. To improve solubility in methylene chloride, gentamycin and naltrexone were solubilised in methylene chloride by hydrophobic ion pairing (counter ions replaced with an anionic detergent sodium bis-2-ethylhexyl sulfosuccinate). Drug encapsulated PlLA microparticles (0.2–1 \( \mu \)m) were prepared by expanding the drug/polymer solution through a vibrating nozzle (120 kHz). Ion paired drugs were encapsulated with high efficiency whereas the encapsulation efficiency of the hydrophobic drug rifampicin, which was not ion paired, was much lower due to its higher solubility in \( \text{CO}_2 \). In a further publication Falk and Randolph demonstrated that increasing post-precipitation \( \text{CO}_2 \) flow rate and volume decreased residual organic solvent levels [45]. Interestingly, increasing \( \text{CO}_2 \) co-flow rate during precipitation increased residual solvent content as it led to a less crystalline product and thus reduced diffusivity of methylene chloride through the polymer matrix.
The SEDS process has also been exploited for the preparation of drug loaded polymeric microparticles [46,47]. Ghaderi et al. used the SEDS process to encapsulate hydrocortisone into discrete sub 10 μm particles PoLGA, PLLA and PoLGA [47]. Chen et al encapsulated 5FU into L-PLA microparticles [46]. Sub-micron particles (980 nm) were prepared with low residual methylene chloride content (46 ppm). A drug load of 3.05% and encapsulation efficiency of 17.8% were achieved. Drug was released rapidly (89.5% in first 36 h followed by 5.5% in next 36 h) by a diffusion mechanism.

In addition to small drug molecules, anti-solvent techniques have also been exploited to encapsulate peptide, protein and even plasmid DNA into polymeric microparticles. Engwicht et al. used ASES to encapsulate BSA or estriol in to PoLGA 50:50 or a triblock co-polymer of b-poly-L-lactide-co-DL-lactide-co-glycolide (62.5:12.5:25) microparticles [48]. Polymers were dissolved in a mixture of 2,2,2-trifluoroethanol and methylene chloride and the drug dissolved in methanol prior to mixing with the polymer solution. PLGA formed small agglomerated particles due to the plasticization effect of CO₂ and residual solvent, whereas the triblock polymer formed larger individual particles (sub 10 μm). High loading efficiency of BSA and estriol (over 100% due to extraction of LMW polymer species) was observed for both polymers. Approximately 50% burst release occurred in first 24 h due to drug at or near the microparticle surface.

Elvassore et al. prepared insulin and insulin/PEG loaded PLLA (102,000 MW) nanoparticles by GAS anti-solvent [49]. PLLA was dissolved in methylene chloride and then mixed with a solution of PEG and insulin co-dissolved in DMSO (50:50 solvent ratio). Nanoparticles (400–600 nm) were prepared in high yield and high drug encapsulation efficiency (up to 93.4%) with very low residual methylene chloride (8 ppm) and DMSO content (300 ppm). Insulin extracted from the particles maintained >80% of its hypoglycaemic activity in vivo. When PEGs >1900 MW were incorporated insulin was released in a burst over 80 h, whereas when low MW PEGs were used no burst occurred and insulin was released slowly over 1500 h. As the L-PLA used in these studies had a very high MW (102,000) and degraded extremely slowly with <3% of encapsulated insulin being released over the 1500 hour study period. In a more recent report it was demonstrated that insulin dissolution profile could be improved using high concentrations of low MW PEG (350, 750 or 1900 Da) [50]. Using a semi-continuous gas anti-solvent precipitation technique, under optimised conditions, insulin loaded PLA/PEG nanoparticles were prepared in high yield (>70%) and with high encapsulation efficiency (over 90%). Encapsulation efficiency was reduced as PEG 1900 content increased or as PEG MW increased at 67% PEG loading. Drug release rate increased as PEG content increased with 100% of insulin released over 3–4 months when particles were produced with a PEG 1900 content of 67 or 75%, but only 10% at a 23% PEG load (Fig. 1).

Sze et al. encapsulated lysozyme into PL-LA microparticles using an ASES technique [42]. Lysozyme (dissolved in DMSO) and PL-LA (dissolved in methylene chloride) were sprayed though separate channels using a co-axial nozzle. A loading of 3.7% (w/w) was achieved under optimised conditions, but the encapsulation efficiency was only 14.6%.

More recently chitosan–plasmid DNA complexes have been prepared using a supercritical anti-solvent technique [51]. The complexes were prepared by spraying a chitosan–DNA complex solution through a V shaped nozzle, with the anti-solvent flowing through one side and the drug polymer solution through the other. A mixture of ethanol and scCO₂ were used as the anti-solvent. The ethanol functioned as a co-solvent to allow the water to be miscible with carbon dioxide. Inclusion of chitosan reduced DNA degradation during processing, increased yield and enhanced luciferase expression in the murine lung when compared to powder sprayed without chitosan or plasmid DNA solutions.

The majority of drug microencapsulation studies have focussed on the semi-crystalline polymer Pt.LA, whereas as far fewer studies have successfully produced drug entrapped microparticles from amorphous polymers such as PDLLGA. Pt.LA has very limited applications in the field of drug delivery due to extremely long in vivo degradation times (months to years). It is only suitable for delivery of extremely potent drugs over prolonged periods of time, applications that require infrequent administration, for example vaccines, or drug eluting medical devices. Most controlled release drug delivery applications require dosing frequencies between once weekly and once every 6 months. Dose frequency is determined by the stability of the drug in the polymeric system and the potency of the therapeutic. For drug delivery systems faster degrading
amorphous polymers such as PDL-LGA and PDL-LA are therefore required. However, carbon dioxide has a higher solubility in amorphous polymers resulting in slower mass transfer and rate of solidification. Consequently, microparticles tend to aggregate and flocculate due to the plasticizing effect of residual carbon dioxide [43,52–54]. The plasticizing effect can be further enhanced by the presence of residual organic solvents in the final product.

To overcome the plasticization effect caused by residual CO₂ and solvent, Ghaderi et al. used combinations of scCO₂ and supercritical nitrogen [47]. The nitrogen decreased the interaction between carbon dioxide and the polymer inside nozzle thereby increasing the dispersive effect of the nozzle. Discrete particles <10 μm were produced from PDL-LGA, over 10 times smaller those prepared in the absence of nitrogen [54]. Decreased polymer solubility in the CO₂–N₂ mixture resulted in faster particle solidification rates, less aggregation and hence smaller particles. Hydrocortisone was entrapped into PDL-LGA particles with an encapsulation efficiency of 22%. There was no change in particle size between drug loaded and non-loaded particles.

Young et al. successfully produced PLGA particles using a modified PCA technique in which PLGA was dissolved in methylene chloride and then sprayed into a vapour CO₂ phase over a liquid CO₂ phase [55]. Temperature was found to be an extremely important determinant of final product morphology. At higher temperatures PLGA remained highly plasticized (T_g decreased from 45 °C to approximately −40 °C at 276 K in the presence of carbon dioxide) resulting in agglomerated particles. While at temperatures of below −30 °C dissolution rate of methylene chloride with CO₂ decreased also resulting in large agglomerates (>1000 μm). At −20 °C uniform spherical particles were prepared. Polymer concentration was also an extremely important determinant of particle size. As polymer concentration increased, particle size increased (from 0.5–5 μm at 1% to 5–70 μm at 5%) due to increased viscosity and slower precipitation rates. At concentrations >10% large agglomerates were formed.

A drawback of the anti-solvent techniques is that they require the pharmaceutical agent to be soluble in a solvent which is miscible with scCO₂. This limits the use to compounds that are soluble in organic solvents. While this does not create a problem in the case of low molecular weight hydrophobic compounds, more complex hydrophilic agents, such as proteins and peptides, are insoluble in most organic solvents. Consequently, CO₂ miscible solvents such as DMSO must be used to solubilise the biological molecule. When dissolved in such solvents proteins can refold and irreversibly change their secondary structure resulting in loss of functional activity and risk of immunogenicity. Two approaches have been used to overcome this limitation. The first is to use ethanol as a cosolvent to allow the water to be miscible with carbon dioxide [51,56]. Although proteins have been sprayed to produce stable micron-sized powders using such approach [56,57], most commonly used biodegradable polymers are not water soluble so this technique is unlikely to be suitable.

A second approach was taken by Young et al. [55] where lysozyme was encapsulated into PLLA or PDL-LGA microspheres using a PCA technique. Rather than dissolving lysozyme in a solvent, lysozyme particles were suspended in a solution of polymer dissolved in methylene chloride. Suspension of protein in organic solvents generally causes much less degradation than dissolution. Particles were formed by spraying the protein–polymer suspension through a capillary nozzle into a carbon dioxide vapour phase above a liquid CO₂ phase (Fig. 2). Lysozyme was successfully encapsulated at a 10:1 by weight of polymer to protein into PLLA particles, but drug dissolution was not investigated.

### 3.3. Particles from gas saturated solutions (PGSS)

The PGSS process is an alternative supercritical fluid processing technology that can be used to form particles from polymers or other materials that are plasticized by a supercritical fluid. First developed for coatings [58] the technique has been applied also to the preparation of particles of the small molecule drug nifedipine [59,60] which plasticizes well in scCO₂. More recently the technique has been developed to produce drug particles entrapped within polymeric microparticles, fibres or implants or scaffolds. The PGSS process utilises the ability of scCO₂ to plasticize amorphous polymers such as PDL-LGA and PDL-LA. The addition of CO₂ markedly lowers the T_g of the polymer causing it to liquefy at near ambient temperatures. Furthermore, the presence of CO₂ significantly lowers the viscosity of the polymer melt which enables drug particles to be

![Fig. 2. SEM micrograph (bottom) and optical micrograph (top) of PLGA microspheres formed by spraying at 0.5 mL/min a 5.0/0.5% PLGA/lysozyme suspension in CH₂ Cl₂ through a 100 μm capillary nozzle into CO₂ flowing at 25 mL/min at −20 °C. Reproduced with kind permission from [55].](image-url)
homogeneously mixed in by means of a stirring device. In these processes, the drug itself is usually protein based and is not plasticized or modified in any way by the scCO2. If the liquefied mixture is sprayed into a collection chamber fibres or particles can be produced [61–63], whereas if the process is conducted in a mould, the polymer will foam on depressurisation leading to the production of a porous scaffold or monolith [64,65].

The PGSS process has many advantages over other supercritical fluid and emulsion technologies. These include: the absence of solvents at any stage during processing, the fact that neither the polymer nor drug need be soluble in the scCO2 and the mild processing conditions (typically <40 °C and 150 bar). Furthermore, as there are no solvent removal steps, the process is extremely rapid. The PGSS process is particularly suitable for entrapment of delicate biopharmaceuticals such as proteins [63–65]. A significant challenge with the PGSS process is to produce drug encapsulated polymeric microparticles from a highly viscous liquefied polymer and with a rapid polymer solidification rate on depressurisation. Furthermore, residual CO2 may cause agglomeration of the final particulate product. To enable microparticle formation the solidification rate must be tightly controlled so that it is slow enough to allow for droplet formation and polymer chain rearrangement, but fast enough to prevent aggregation of newly formed particles when they arrive at the bottom of the collection vessel. Hao et al. used the PGSS process to produce microparticles from the crystalline polymer PEG [61]. Spherical particle production was favoured by reducing the pressure, increasing the temperature and reducing the nozzle size.

Production of particles from amorphous polymers is even more challenging than from crystalline polymers due to the increased. The increased viscosity of the polymer melt makes atomisation more difficult, and the greater solubility of CO2 in the polymer phase can result in plasticization and agglomeration of the final product. To overcome these problems Hao et al. produced PDLLA microparticles (6100 Da) by spraying the liquefied polymer/CO2 mixture into a collection chamber with a nitrogen back pressure [62]. The nitrogen suppressed the loss of CO2 from the liquefied CO2/polymer mixture thereby slowing down rate of polymer solidification allowing particle formation to occur. Cooling the collection chamber with liquid nitrogen prevented aggregation of the newly formed microparticles. A back pressure of >68 bar of nitrogen was required to produce fine particles. In the absence of back pressure a very fibrous product was formed. Whitaker et al. extended this work to produce drug entrapped PDLLA microparticles. Lysozyme, ribonuclease, insulin and calcitonin were successfully entrapped into the microparticles with minimal loss of structural integrity or functional activity.

3.4. Other drug delivery applications

More recently, scCO2 has been exploited to process a wider range of polymeric drug delivery systems. Moneghini et al. used GAS anti-solvent to co-precipitate the poorly soluble drug carbamazepine with the hydrophilic carrier PEG 4000 [66]. Co-precipitation with the PEG reduced drug particle size and improved crystal shape which markedly enhanced in vitro dissolution rate.

Duarte et al. produced microparticles from ethylcellulose/ methylcellulose blends using either a solvent evaporation technique or by a SAS process [67]. Microparticles were then impregnated with the NSAID naproxen by solubilising the drug in supercritical carbon dioxide using a RESS technique. Drug release was slower from particles impregnated using scCO2 when compared to those where drug was encapsulated by solvent evaporation. Following impregnation with naproxen using supercritical CO2, particles produced by SAS had faster and more controlled release than those prepared by solvent evaporation due to the smaller size of the particles and hence shorter diffusion path.

Reverchon et al. prepared amoxicillin loaded PMMA porous structures using a SCF phase inversion process in which CO2 acted as a non-solvent [68]. Amoxicillin was loaded into the structure by co-dissolving it in the solvent (DMSO) with the polymer, or suspending it in the polymer phase (PMMA dissolved in acetone). Pore size and morphology could be controlled by choice of solvent, temperature and polymer concentration. When amoxicillin was co-dissolved with the polymer, amoxicillin was uniformly distributed throughout matrix and the dissolution rate was much slower than when the drug was suspended in the polymer (3 h and 20 h respectively).

Kunastitchai et al. encapsulated micronozole into liposomes prepared with various compositions of phosphatidylchololine, cholesterol and poloxamer 407 [69]. At optimised conditions, partially crystalline, spherical and nonporous microparticle aggregates were prepared with size ca. 40 μm with residual content of methylene chloride and methanol of 80 ppm and 36 ppm respectively, well below regulatory limits (600 and 3000 ppm respectively). Increasing solute concentration increased yield but lead to larger less spherical particles and fibres due to increased viscosity and incomplete atomisation.

Another interesting application of supercritical carbon dioxide which has been investigated recently is the sterilisation of biodegradable polymers [70]. Sterilisation occurred without detectable chemical changes to the polymer, possibly as a result of the formation of carbonic acid, which lowers the intracellular pH.

3.5. Extraction of solvents with carbon dioxide

Organic solvents such as methylene chloride are generally used to solubilise biodegradable polymers during production of drug delivery systems. The final product will consequently contain residual amounts of solvent. The U.S.P limit for methylene chloride is 600 ppm. As methylene chloride is miscible with carbon dioxide, products produced by anti-solvent techniques generally have very low residual levels. However, products prepared using conventional emulsion techniques can have very high residual solvent levels, well in excess of the U.S.P limit.

Recently carbon dioxide has been used to extract residual solvent from microparticles and rods produced by Three Dimensional Printing™ (3D™) or emulsion techniques [71–73]. Solvent extraction utilises the plasticisation effect of the
carbon dioxide which increases the solvent diffusion coefficient and diffusion rate. Herberger et al. used CO2 as an extraction solvent to reduce the residual levels of methylene chloride in spray dried PdlLGA-darbepoetin alfa microparticles [71]. Whereas liquid CO2 was found to cause some protein aggregation and microparticle agglomeration, extraction with CO2 gas reduced residual solvent concentration and limited particle aggregation. Solvent extraction rate and degree of particle agglomeration were found to increase with CO2 pressure. Carbon dioxide extractions at constant pressure did not reduce residual solvent concentration to below target levels. Using extraction cycles, whereby CO2 pressure was increased as solvent was removed, a residual methylene chloride content of <600 ppm was achieved. In a similar study liquid CO2 was used to reduce the residual solvent in dense PdlLGA devices to <50 ppm [72]. Exposure to liquid CO2 plasticised the rods making them flexible and easily deformed for several hours after exposure.

Koushik and Kompella utilised scCO2 to not only reduce residual methylene chloride content in desorbed loaded PdlLGA (50:50 23.2 kDa) microparticles, but also to increase particle porosity [73]. Microparticles prepared by an o/w solvent evaporation technique were exposed to scCO2 (1200 psi, 33 °C, 30 min). Upon depressurisation, pore formation was induced by rapid expansion of entrapped CO2. Following scCO2 treatment, particle size increased from 2.2 to 13.8 μm, porosity increased from 39 to 92.4% and bulk density was reduced from 0.7 to 0.082 g/cc. Residual solvent content decreased from 4500 ppm to <25 ppm. Peptide stability was maintained following processing. Uptake of scCO2 treated particles was reduced in A549 cells and rat alveolar macrophages when compared to non-treated particles. Use of a large particle size, to reduce clearance by alveolar macrophages, and of a low mass density, to increase respirable fraction [74], may make the particles suitable candidates for sustained delivery to the respiratory tract. However, the toxicity of PdlLGA when administered via the inhaled route of delivery has yet to be fully elucidated.

More recently, Chattopadhyay et al. have evaluated a novel technique called supercritical fluid extraction of emulsions (SFEE) [75]. Indomethacin and ketoprofen were encapsulated into PdlLGA and PMMA microparticles using an oil-in-water emulsification technique. scCO2 was then used to extract the solvent from the emulsion. Drug entrapped particles (0.1 and 2 μm) with low residual solvent content (<50 ppm) and high loading efficiency (98%) were prepared. Particles were produced in either a batch fashion, by bubbling scCO2 through the emulsion, or in a continuous fashion by spraying the emulsion into an extraction column with supercritical CO2 flowing in the opposite direction. Coalescence was prevented by the presence of a stabilising surfactant in the aqueous phase.

4. Manufacture for tissue engineering scaffolds

Driven by the massive shortage of donor organs, and increasing frequency of age-related diseases with unsatisfactory treatments, the closely allied fields of tissue engineering and regenerative medicine aim to repair, restore or replace damaged organs. Bone marrow transplantation demonstrates that cell based therapies can have outstanding success in treating previously fatal and untreatable diseases. However, for most conditions the administration of cells alone does not result in integration into and repair of the host tissue.

To overcome this, a central strategy of tissue engineering is to try to provide the correct microenvironment for the cells (a niche) using biomaterials as a scaffold for tissue repair, regeneration or reconstitution in vivo or ex vivo. Typically, a therapeutic cell population is seeded onto a biodegradable scaffold which has been modified to provide the appropriate cues to promote cell proliferation, differentiation and organization into the desired tissue. Alternatively biomaterials loaded with the appropriate growth factors, or signalling domains may be administered to the site of injury to encourage infiltration of the patients’ own cells to promote healing.

To be effective such scaffolds must provide a large surface area for cell attachment and efficient gas and nutrient exchange (and are therefore usually porous) and release the appropriate growth factor(s) with the appropriate kinetics. Most conventional techniques to produce such polymer scaffolds require heat or solvents which can be deleterious to the cells, tissues and encapsulated biological molecules. Since it is solvent free and can operate at relatively low temperatures, supercritical fluid processing offers an attractive alternative.

In the field of regenerative medicine, supercritical fluid processing (principally scCO2) has been applied to:

1. The production of porous polymer scaffolds and composites.
2. Encapsulation and release of growth factors and cells for tissue engineering.
3. Extraction of residual solvents or other deleterious compounds from pre-fabricated scaffolds.

4.1. Production of porous polymer scaffolds

4.1.1. Gas foaming

Supercritical fluid processing lends itself to the production of porous matrices since alternative methods tend to be solvent and energy intensive and can lead to pore collapse in certain materials. Furthermore, their compressed state and ability to plasticize a wide range of polymers lends them to the formation of polymer foams [76]. Polymer foams are formed when a polymer, plasticized by saturation in the supercritical fluid is rapidly depressurized at a constant temperature. As pressure is released pockets of gas nucleate and grow in the polymer. As the supercritical fluid leaves the polymer, the \( T_g \) increases. At the point where the \( T_g \) for the polymer is higher than the foaming temperature, the porous structure is set [77,78].

This can even operate at sub-critical temperatures and pressures. Mooney et al. exposed compression moulded polymer pellets and solvent cast discs of the poly(α-hydroxy acids) to CO2 at a pressure of 5.5 MPa at room temperature. During an equilibration period of 72 h the polymers saturated with CO2...
before rapid depressurization over 15 s. Using this method, highly porous sponges could be formed (up to 97% for PLGA). Unlike the amorphous PLGA, PGA, which is crystalline, does not to foam due to the low amounts of CO₂ it absorbs. This fact was exploited to control porosity by mixing the crystalline and amorphous materials [79].

However a non-porous film was formed on the surface of the scaffolds, making them unsuitable for cell ingress, and presenting a barrier for cell nutrients and the waste products of cell metabolism. This surface skin is thought to be formed by the rapid diffusion of the CO₂ from the surface of the scaffold as the pressure is released [78,79]. Furthermore, it was found that the scaffolds produced had a relatively closed pore-network. To overcome this issue, an additional porogen (sodium chloride crystals of a defined size range) was incorporated into compression moulded PLGA discs before gas foaming. Upon production of the polymer foam, the porogen was leached out by incubation in water for 48 h. This created a scaffold with an interconnected pore network open to the surface, which was shown to be accessible to cultured smooth muscle cells [80].

Interconnectivity of the pores could be improved even further by partially fusing the salt porogen by exposure to 95% humidity [81].

The leaching of the porogen is a major disadvantage of the gas foaming/particulate leaching process since it results in loss of the majority of any incorporated growth factor. This can be overcome somewhat by encapsulating the active factors in alginate beads which are then incorporated into the compressed polymer together with the porogen before processing. This was able to increase the encapsulation efficiency to 55%±1% from 28%±18%. In this system the released VEGF was shown to retain >90% of its activity post-processing [82]. This study also investigated the use of alternative gases to foam the polymer — CO₂, N₂, and He. Carbon dioxide was found to be the most effective at producing porous scaffolds. The exact mechanism of this is not known, but was thought could be a result of an interaction between CO₂ and the carbonyl groups of PLGA [82].

In addition to the issues surrounding salt leaching, the gas foaming process suffers from relatively long manufacturing time. Whilst a 1 hour soak in high pressure CO₂ has been reported to be effective in foaming PLLGA, it produces particularly fragile scaffolds. Most studies using the technique report a gas equilibration period of 12–24 h or more, followed by the leaching step of at least 18 h to ensure complete removal of the porogen [79,80,82,83].

Under supercritical conditions, CO₂ foams polymers to create porous scaffolds suitable for tissue engineering applications. Since increasing pressure, increases the rate of gas diffusion into polymer systems, equilibration time is reduced compared to subcritical pressures [84]. Gas saturation equilibrium times as low as 10 min have been reported [85,86]. The effectiveness of this technique in foaming PLA and PLGA and use of the scaffolds for tissue engineering has been demonstrated both in vitro and in vivo [87–89].

Barry et al. used scCO₂ to foam the non-degradable polymer poly(ethyl methacrylate)/tetrahydrofurfuryl methacrylate (PEMA/THFMA), which has been shown to be an excellent scaffold material for cartilage repair. An 8 hour equilibration time was required in order to ensure complete permeation of the polymer. Like the gas foaming technique, a surface skin was formed, however a greater degree of pore interconnectivity (up to 57%) was found compared to that reported for the gas foaming technique applied to PLGA. In vitro cell culture revealed enhanced phenotypic maintenance of chondrocytes cultured on the foamed material (as measured by extracellular matrix secretion), demonstrating the utility of a three-dimensional environment for promoting tissue regeneration [90].

Further optimisation of the processing conditions of PEMA/THFMA demonstrated that the degree of porosity and their interconnectivity could be tightly controlled simply by controlling the venting rate. Venting over 30 s reduced porosity, and the interconnectivity, whilst venting over 60 min allowed the pores to grow, and produced scaffolds with a porosity of around 89% with high connectivity (74% open pores). In this process, an additional porogen/leaching process is not therefore necessary. As might be expected, as porosity increases, mechanical strength decreases, an important factor in many biomedical applications e.g. weight bearing tissues such as bone. There is therefore an upper limit, above which pore size and low mechanical strength precludes their use in certain applications [91].

In attempting to improve the mechanical properties of the THFMA for soft prosthetic applications, the foaming of blends of THFMA with styrene—isoprene—styrene copolymer elastomer (SIS) has been investigated. These blends produce materials with properties predominantly plastic or elastomeric (depending upon their composition) whilst maintaining the hydrophilicity of the THFMA component. Exposure of the blends to scCO₂ at 40 °C/100 bar for 8 h and venting over 15 min foamed the polymers, which contracted upon cooling. There appeared to be some elastic forces present in the scaffolds which limited the degree of foaming. Indeed, mechanical testing revealed the blends to be elastomeric, and these systems may therefore lend themselves to use as scaffolds for particular tissues, such as cartilage. The degree of porosity and interconnectivity of the pores could be tuned by modifying the blend composition and processing temperature, with a reduction in the degree of foaming as temperature increased [92].

Similarly, Mathieu et al. have shown that the morphology of the foams can be controlled to mimic the structure of bone. Bone from different sites around the body is anisotropic, both morphologically and mechanically, to varying degrees. They found that similar anisotropic structures can be formed by controlling the cooling rate of the foamed polymer — in this case PLLA — and the density of the gas nucleation. Rapid cooling locked in large numbers of spherical pores, whilst slower cooling enabled pore elongation (provided no coalescence occurred). Incorporation of ceramic modifiers also influenced the process, reducing porosity and surface area, it was thought due to modification of the polymer viscosity [85]. Fig. 3 demonstrates the similarity of the polymer foams produced to cancellous bone from various sites around the body, simply by varying the processing parameters.
Alternatively, the interconnectivity of the pores can be improved by post-processing the scaffolds generated by gas foaming using ultrasound. Wang et al. produced polymer foams of PLA (using sub-critical pressures of CO$_2$) and exposed them to pulsed ultrasound at a frequency of 20 kHz and average power input of 100 W. This not only slightly increased pore size, but also improved their interconnectivity as a result of pore wall rupture [93].

4.1.2. Supercritical fluid emulsion templating

An alternative to the use of supercritical fluid in the gas foaming of polymers to create porous scaffolds is emulsion templating. Here, a variety of porous hydrophilic materials can be produced by reaction-induced phase separation of concentrated oil-in-water emulsions e.g. using sol-gel chemistry, or free radical polymerisation. Removal of the internal phase (i.e. the emulsion droplets) yields the porous product. Conventional methods require large amounts of water immiscible organic phases as the internal phase (usually >75%), which can be difficult to remove after the reaction. For example, for inorganic materials firing the products at temperatures >600 °C is often used, which would clearly not be appropriate for thermolabile materials or those containing growth factors for regenerative medicine.

Butler et al. demonstrated that the emulsion templating principle can be applied to supercritical CO$_2$-in-water(C/W) emulsions. Using perfluoropolyether (PFPE) surfactants and poly(vinyl alcohol) to stabilize the C/W emulsions of acrylamide polymers, they vented the CO$_2$ following polymerization, leaving polymer scaffolds with interconnected pores (see Fig. 4). Increasing the volume fraction of the CO$_2$ internal phase increased porosity, and increasing the surfactant concentration led to more open interconnected structures [94]. A disadvantage of this protocol for bioengineering applications is the use of a non-degradable surfactant, and the mean pore sizes (~50 μm) produced may not be suitable for all applications — it has been reported that scaffolds for bone regeneration for example require pore sizes between 200 and 400 μm [95].

A variation on the emulsion templating technique has recently been reported by Partap et al., who produced emulsion templated alginate hydrogels in which the scCO$_2$ not only served as the templating “oil” phase, but also as a source of acidity used to release Ca$^{2+}$ from its chelated form, freeing it to cross-link the alginate and form the porous hydrogel. Calling the technique “reactive emulsion templating (RET)”, they were able to produce 3D hydrogels with an open and interconnected porous network, with a mean pore size similar to the emulsion templating technique reported by Butler et al., and with mechanical stability demonstrated in cell culture media at 37 °C over at least 4 weeks [96].
4.2. Encapsulation and release of growth factors and cells using supercritical fluids

Supercritical fluid polymer processing has been shown to be promising technique for the fabrication of porous scaffolds suitable for tissue regeneration. However whilst a number of studies have demonstrated the biocompatibility of unmodified materials in vitro and in vivo, implantation of unmodified scaffolds is unlikely to be sufficient to promote cell infiltration, proliferation and differentiation into the desired tissue. To provide the necessary signals for this to occur, research has focused upon the incorporation of growth factors, genes or cell signalling moieties into the scaffold materials, and surface modification of the polymers in order to promote cell infiltration and attachment.

Taking advantage of the plasticisation of biocompatible and biodegradable polymers such as PLA, PLGA and polycaprolactone (PCL) when exposed scCO₂, Howdle et al. have demonstrated that substances can be encapsulated within the liquified polymers. Since the polymers can be processed under relatively low temperatures and modest pressures, and in the total absence of organic solvents, the activity of labile molecules such as proteins can be maintained.

In this process the solid bioactive material is homogeneously mixed into the supercritical fluid plasticized polymer using an impeller [64]. Alternatively, to promote mixing of low loadings of active material, solutions can be freeze-dried onto the polymer before processing [65]. Following release of the pressure, porous scaffolds, microparticles or fibres containing the bioactive material can be produced.

Yang et al. demonstrated the utility of this technique in encapsulating bone morphogenetic protein 2 (BMP-2) into PLA scaffolds for bone tissue engineering in an ex vivo model. Growth factor released from the scaffolds promoted adhesion, migration, expansion and differentiation of human osteoprogenitor cells on the scaffolds [97].

Alternatively, the bioactive factor can be homogeneously mixed together with the polymer powder or solution before exposure to scCO₂, and fabrication of the porous scaffold [82,98–101]. Recently, Mathieu compared three different mixing strategies prior to supercritical fluid foaming with the aim of efficiently incorporating ceramic fillers for use in orthopaedic tissue engineering. They assessed pre-mixing the ceramic powder with polymer pellets before a compression moulding step, dispersion of the ceramic materials into a polymer solution before evaporation of the solvent, and melt extrusion. They found that in their system the solvent and melt processing conditions provided the most homogeneous distribution of the filler, with the melt extrusion technique producing scaffolds with the best mechanical properties. Since the ceramic fillers are stable to high temperatures, and was solvent free they favoured the latter approach [101].

Kim et al. used the gas foaming particulate leaching method to encapsulate hydroxyapatite (commonly used in bone tissue engineering) into porous PLGA scaffolds. The hydroxyapatite (HA) was mixed with the polymer and salt particles prior to foaming under high pressure CO₂, forming scaffolds with an interconnected porous network, devoid of the surface skin often seen using this technique. In vitro and in vivo studies revealed that the gas foamed scaffolds showed significantly higher cell growth, alkaline phosphatase activity and mineralization compared to similar scaffolds produced by the solvent casting/particulate leaching method, possibly due to higher exposure of HA at the scaffold surface [99].

A variation of these techniques is to process pre-encapsulated materials with supercritical fluids to generate porous scaffolds impregnated with bioactive factors [83,102]. Hile et al. encapsulated basic fibroblast growth factor (bFGF) in a water-in-oil emulsion with the protein in the aqueous phase and the polymer (PLGA) in the oil phase, followed by saturation with supercritical CO₂. The CO₂ extracts the organic solvent, and upon pressure release porous polymer foam is produced. This successfully produced polymer foams which released bFGF in a controlled fashion, although residual solvent levels remained higher than accepted pharmacopoeial limits [102]. The ability of supercritical CO₂ to extract organic solvents has been exploited by others to remove solvents from PLGA scaffolds fabricated using a three-dimensional printing process. It was found that chloroform could be efficiently removed from such scaffolds to within pharmacopoeial limits relatively quickly (over a matter of hours) using both a batch and continuous process [72].
Richardson et al. took pre-encapsulation a stage further by demonstrating dual growth factor delivery each with different release kinetics. Platelet derived growth factor (PDGF) was pre-encapsulated into PLGA microspheres using a double emulsion process. These microspheres were then mixed with a different PLGA together with vascular endothelial growth factor (VEGF) and alginate (previously shown to improve protein encapsulation efficiency) before gas foaming. Following foaming the particulate and microsphere polymers had fused, creating a continuous homogeneous matrix.

The PDGF was released more slowly, and could be controlled by altering the polymer molecular weight, whilst the VEGF was release more rapidly, it was thought because it was more closely associated with the surface of the polymer. Dual delivery of these growth factors with distinct release kinetics was shown to promote angiogenesis in vivo to a greater extent than either growth factor given alone [103]. Such systems, employing the delivery of numerous biologically active factors, are likely to be essential to control many tissue regeneration processes which tend to be the result of a complex temporal interplay between a multitude of growth factors and cell signals such as interactions with the extracellular matrix. An alternative approach to particulate fusing involves the use of laser sintering to create precision and custom shaped biodegradable materials containing bioactive materials [104].

Recently it has been shown that a variety of cell types can actually survive supercritical fluid mixing into polymer scaffolds. Ginty et al. mixed the myoblastic C2C12 cell line, 3T3 fibroblasts, and primary hepatocytes and chondrocytes into scaffolds. Ginty et al. mixed the myoblastic C2C12 cell line, 3T3 fibroblasts, and primary hepatocytes and chondrocytes into scaffolds. The plasma treated surfaces were shown to promote cell attachment onto the scaffolds, however the cells tended to preferentially attach to the periphery of the scaffolds due to the greater probability of cell contact during culture[106]. Indeed, encouraging cells to colonise the entire scaffold, throughout the porous matrix, is a particular challenge. By using plasma gas Barry et al. were able to modify the surface throughout porous PtsLLA scaffolds generated using supercritical fluid. The plasma treated surfaces were shown to promote cell attachment onto the scaffolds, however the cells tended to preferentially attach to the periphery of the scaffolds due to the greater probability of cell contact during culture[106].

5. Conclusions and future challenges

Supercritical fluids have shown promise in a number of areas. In anti-solvent applications, there has been considerable commercialisation activity around the controlled preparation of controlled drug particles, particularly with respect to control of particle size and morphology. Some work has been carried out with respect to encapsulation of drugs into polymeric hosts, but the requirement of large volumes of both conventional solvent and of scCO2 have proved limiting. Similarly, the RESS process showed initial promise for drug delivery devices. However, a significant limitation is the requirement for high solubility of both the drug and the polymer in scCO2; a relatively rare combination. A key challenge in this area will be the development of new highly scCO2 soluble polymers, and promising work in this area is beginning to be reported [108,109].

The PGSS technique does not require additional solvent, and in general the drug material should be insoluble in scCO2. Thus there are significant attractions to the technique. The particle sizes of the polymer drug composites are limited by the spray process and the viscosity of the scCO2 polymer melt. A key challenge in this area will be to demonstrate good product performance with high quality controlled release data, and perhaps even to drive down to submicron particle sizes, opening up potential routes to pulmonary delivery. Finally we have reviewed the applications to tissue engineering, and in particular the encapsulation of growth hormones into polymeric scaffolds. The technique has recently been extended to the encapsulation of mammalian cells, and the key challenge in this area is now likely to be the development of implantable tissues, and the introduction of mechanical strength for load bearing tissue applications. Overall, it is clear that SCFs have moved beyond the stage of scientific curiosity, but the key challenge now is to ensure that real commercial opportunities are developed in drug delivery and tissue engineering.

References


Key Papers


