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The return of a forgotten polymer – Polycaprolactone in the 21st century

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Abstract

During the resorbable-polymer-boom of the 1970s and 1980s, polycaprolactone (PCL) was used in the biomaterials field and a number of drug-delivery devices. Its popularity was soon superseded by faster resorbable polymers which had fewer perceived disadvantages associated with long term degradation (up to 3-4 years) and intracellular resorption pathways; consequently, PCL was almost forgotten for most of two decades. Recently, a resurgence of interest has propelled PCL back into the biomaterials-arena. The superior rheological and viscoelastic properties over many of its aliphatic polyester counterparts renders PCL easy to manufacture and manipulate into a large range of implants and devices. Coupled with relatively inexpensive production routes and FDA approval, this provides a promising platform for the production of longer-term degradable implants which may be manipulated physically, chemically and biologically to possess tailorable degradation kinetics to suit a specific anatomical site. This review will discuss the application of PCL as a biomaterial over the last two decades focusing on the advantages which have propagated its return into the spotlight with a particular focus on medical devices, drug delivery and tissue engineering.

1. Introduction

Polycaprolactone (PCL) was one of the earliest polymers synthesized by the Carothers group in the early 1930s [1]. It became commercially available following efforts to identify synthetic polymers that could be degraded by microorganisms [2]. PCL can be prepared by either ring-opening polymerisation of ε-caprolactone using a variety of anionic, cationic and coordination catalysts or via free radical ring-opening polymerisation of 2-methylene-1-3-dioxepane [3]. PCL is a hydrophobic, semicrystalline polymer; its crystallinity tends to decrease with increasing molecular weight. The good solubility of PCL, its low melting point (59-64 ^{o}C) and exceptional blend-compatibility has stimulated extensive research into its potential application in the biomedical field [4-6]. Consequently, during the resorbable-polymer-boom of the 1970s and 1980s, PCL and its copolymers were used in a number of drug-delivery devices. Attention was drawn to these biopolymers owing to their numerous advantages over other biopolymers in use at that time. These included tailorable degradation kinetics and mechanical properties, their ease of shaping and ease of manufacture enabling appropriate pore sizes conducive to tissue in-growth, and the controlled delivery of drugs contained within their matrix. Functional groups could also be added to render the polymer more hydrophilic, adhesive, or biocompatible which enabled favourable cell-responses. Due to the fact that PCL degrades at a slower rate than polyglycolide (PGA), polyD,L-lactide (PDLA) and its copolymers and was therefore originally used in drug delivery devices that remain active for over one year and in slow degrading suture materials (Maxon™).

Although initially attracting some research attention, PCL was soon overwhelmed by the popularity of other resorbable polymers such as polylactides and polyglycolides, which were studied in applications which demanded the polymer matrix to release encapsulated

drugs within days or weeks with a complete resorption 2-4 months after implantation. The medical device industry was interested in replacing metal devices (plates, screws, nails etc) by using biodegradable implants; however PCL did not have the mechanical properties to be applied in high load bearing applications. Furthermore, both the medical device and drug delivery community felt that faster resorbable polymers also had fewer perceived disadvantages associated with the long term degradation (up to 3-4 years for PCL) and intracellular resorption pathways; consequently, PCL was almost forgotten for most of two decades.

A resurgence of interest has propelled PCL back into the biomaterials/arena with the birth of a new field, namely *tissue engineering*; a trend which is depicted graphically in Figure 1. This huge resurgence of interest during the 1990s and 2000s has stemmed from the realization that PCL possesses superior rheological and viscoelastic properties over many of its resorbable-polymer counterparts which renders it easy to manufacture and manipulate into a large range of scaffolds, some of which are shown in Figure 2. In reality, PCL can be used in a wide range of scaffold fabrication technologies as described in section 8.1 and its relatively inexpensive production routes, compared with other aliphatic polyesters, is hugely advantageous. Furthermore, the fact that a number of drug-delivery devices fabricated with PCL already have FDA approval and CE Mark registration enables a faster avenue to market. Interestingly, in spite of these clear advantages, PCLs are not widely exploited by many research groups from a translational point of view. This review will discuss the applications of PCL as a biomaterial over the last two decades, including its relationship with other bioresorbable polymers. It will focus on the properties and advantages which have propagated PCL's return into the spotlight of drug delivery and especially the tissue engineering arena.

2. Synthesis and Physicochemical Properties of PCL

PCL is prepared by the ring opening polymerisation of the cyclic monomer ε-caprolactone and was studied as early as the 1930s [1]. Catalysts such as stannous octoate are used to catalyze the polymerisation and low molecular weight alcohols can be used to control the molecular weight of the polymer [7].

There are various mechanisms which affect the polymerisation of PCL and these are anionic, cationic, co-ordination and radical. Each method affects the resulting molecular weight, molecular weight distribution, end group composition and chemical structure of the copolymers [5]. PCL is a semi-crystalline polymer having a glass transition temperature (*T*g) of −60◦ C and melting point ranging between 59 and 64◦ C, dictated by the crystalline nature of PCL which enables easy formability at relatively low temperatures. The number average molecular weight of PCL samples may generally vary from 3,000 to 80,000 g/mol and can be graded according to the molecular weight [8].

PCL is soluble in chloroform, dichloromethane, carbon tetrachloride, benzene, toluene, cyclohexanone and 2-nitropropane at room temperature. It has a low solubility in acetone, 2-butanone, ethyl acetate, dimethylformamide and acetonitrile and is insoluble in alcohol, petroleum ether and diethyl ether [9]. PCL can be blended with other polymers to improve stress crack resistance, dyeability and adhesion and has used in combination with polymers such as cellulose propionate, cellulose acetate butyrate, polylactic acid and polylactic acid-co-glycolic acid for manipulating the rate of drug release from microcapsules. [4].

In the 1970's it had already been recognised that PCL is particularly amenable to blending and polymer blends based on PCL were thus categorized with three types of compatibility; firstly exhibiting only a single *T*g; secondly as mechanically compatible, exhibiting the *T*g values of each component but with superior mechanical properties and thirdly as incompatible, exhibiting the enhanced properties of phase-separated material [10]. Compatibility of PCL with other polymers depends on the ratios employed and is generally used to have better control over the permeability of the delivery systems. Copolymers (block and random) of PCL can be formed using many monomers, e.g. ethyleneoxide, polyvinylchloride, chloroprene, polyethylene glycol, polystyrene, diisocyanates(urethanes), tetrahydrofuran (THF), diglycolide, dilactide, δ-valerlactone, substituted caprolactones, 4-vinyl anisole, styrene, methyl methacrylate and vinyl acetate. [5].

Physico-mechanical properties of several degradable polymers, amongst them PCL, have been investigated and compared by Engelberg *et al* who investigated thermal properties (Tg, crystallization, melting and decomposition points) and tensile properties including Young's modulus, tensile strength and elongation at yield and break [11]. Some of these properties are listed in Table 1.

3. Biodegradation

When one considers biopolymers it is important to keep in mind that something which is biodegradable does not necessarily translate to being bioresorbable, that is, as it degrades and moves away from their site of action *in vivo* it is not necessarily removed from the body. In contrast, bioresorbability is a concept which reflects total elimination of the initial foreign materials and bulk degradation products by-products (low molecular weight compounds) with no residual side effects [12]. The definitions of *biodegradab*le, *bioresorbable, bioabsorbable*

and *bioerodable*, according to Vert, are detailed in Table 2, appropriate categorization of these properties are of fundamental importance in the discussion of polymer-based materials particularly in biomedical applications.

PCLs can be biodegraded by outdoor living organisms (bacteria and fungi) but they are not biodegradable in animal and human bodies because of the lack of suitable enzymes [13]. That is not to say they are not bioresorbable, but rather, the process takes much longer, propagating firstly via hydrolytic degradation. It is widely accepted that hydrolytic degradation of poly (α-hydroxy) esters can proceed via surface or bulk degradation pathways, depicted schematically in Figure 3. What determines the means by which this pathway proceeds is the diffusion-reaction phenomenon. Surface degradation or erosion involves the hydrolytic cleavage of the polymer backbone only at the surface [14]. This situation arises when the rate of hydrolytic chain scission and the production of oligomers and monomers, which diffuse into the surroundings, is faster than the rate of water intrusion into the polymer bulk. This typically results in thinning of the polymer over time without affecting the molecular weight of the internal bulk of the polymer which would generally remain unchanged over the degradation period (Figure 3a). The advantage to this type of erosion is the predictability of the process, giving desirable release vehicles for drugs as release-rates can be predetermined [15].

Bulk degradation occurs when water penetrates the entire polymer bulk, causing hydrolysis throughout the entire polymer matrix (Figure 3b). Random hydrolytic chain scission would take place and produce an overall reduction in molecular weight. If water molecules can diffuse into the polymer bulk, hydrolyse the chains enabling the monomers or oligomers to diffuse out, erosion will occur gradually and equilibrium for this diffusion-

reaction phenomenon would be achieved. If this equilibrium is disturbed, the degradation mechanism could provoke internal auto-catalysis, via the carboxyl and hydroxyl end group by-products. Whereas surface oligomers and carboxyl groups may freely diffuse into the surroundings (surface erosion situation), in the case of bulk degradation the internal concentration of autocatalysis products can produce an acidic gradient as each newly generated carboxyl end group formed during ester bond cleavage accumulates. This in turn accelerates the internal degradation compared to the surface leaving an outer layer of higher molecular weight skin with a lower molecular weight, degraded, interior (Figure 3c). The degradation mechanism thus becomes defined by bimodal molecular weight distribution. When the inner oligomers become small enough, they diffuse rapidly through the outer layer and this is accompanied by an onset of weight loss and a decrease in the rate of chain scission producing a higher molecular weight hollowed out structure. The rapid release of these oligomers and acid by-products can result in inflammatory reactions *in vivo* as reported in the bioresorbable device literature [16]. Furthermore, if the surrounding tissue is unable to buffer the pH change due to poor vascularisation or low metabolic activity then local, temporary disturbances may arise – an example of this has been observed from fibre reinforced PGA pins used during orthopedic surgery which led to increased osmotic pressure through local fluid accumulation at the time of rapid degradation [17].

The homopolymer PCL has a total degradation of two to four years (depending of the starting molecular weight of the device or implant) [18-20]. The rate of hydrolysis can be altered by copolymerisation with other lactones or glycolides/lactides [3]. Extensive studies by the authors group concerning *in vitro* and *in vivo* degradation of PCL scaffolds detected no evidence of internal catalysis evidenced by uniform molecular weight distribution over time and cross sectional examination of the scaffold struts over a 6 month [21] and 36 month (non-

published) period. Other degradation studies using PCL in separate *in vitro* (saline) and *in vivo* (rabbit) conditions reported that both hydrolytic degradation rates were similar and thus concluded that enzymatic involvement in the first stage of degradation phase (0-12 month) was not a significant factor in the degradation process [22, 23].

From degradation studies presented in the literature it can be concluded that PCL undergoes a two-stage degradation process: firstly the non-enzymatic hydrolytic cleavage of ester groups and secondly, when the polymer is more highly crystalline and of low molecular weight (less than 3000) the polymer is shown to undergo intracellular degradation as evidenced by observation of PCL fragments uptake in phagosomes of macrophages and giant cells and within fibroblasts [24] which supports the theory that PCL may be completely resorbed and degraded via an intracellular mechanism once the molecular weight was reduced to 3000 or less. It was also noted that in the first stage the degradation rate of PCL is essentially identical to the *in vitro* hydrolysis at 40°C and obeyed first-order kinetics. It was concluded that the mechanism of PCL degradation could be attributed to random hydrolytic chain scission of the ester linkages, which caused a decrease in molecular weight.

Ali *et al* studied the mechanism of PCL degradation *in vitro* by mean of gel permeation chromatography (GPC), differential scanning calorimetry (DSC) and scanning electron microscopy (SEM). It was hypothesized that the HO·radical was likely to be a significant cause of PCL degradation in implantable devices [25]. Chen *et al* studied the *in vitro* degradation behavior of the PCL microparticles and compared these with that of PCL film in PBS at 37±1°C at pH7.4. The shape of PCL was found to have no obvious effect on its degradation rate, which suggested that homogeneous degradation dominated the process.

Recently, accelerated degradation models for PCL have been investigated primarily by thermal methods by several groups [26]. Persenaire *et al*. proposed a two-stage thermal degradation mechanism of PCL [27] and found in the first stage there was a statistical rupture of the polyester chains via ester pyrolysis reaction. The second stage led to the formation of ε-caprolactone (cyclic monomer) as result of an unzipping depolymerisation process. Sivalingam *et al*. investigated the thermal degradation in bulk and solution [28, 29] and found that the polymer degraded by random chain scission and specific chain end scission in solution and bulk, respectively.

Pitt *et al* showed that the mechanism of *in vivo* degradation of PCL, PLA and their random copolymers was qualitatively the same. The degradation rate of random copolymers was much higher than those of the homopolymers under the same conditions [30]. On the other hand, the degradation rate of PCL/PLA block copolymers was found to be intermediate of PCL or PLA homopolymers and increased with PLA content in the 0-40% range [31]. However when the PLA content was greater than 40%, the degradation was found to exceed that of the homopolymers [32]. Degradation kinetics are highly dependent upon the molecular weight of the polymer(s). High molecular weight structures take much longer to degrade, as mediated through the chain length of the polymer. Higher molecular weight increases the chain length necessitating a greater number of ester bonds to be cleaved in order to generate water soluble monomers/oligomers to allow erosion to proceed; degradation consequently takes longer. Woodward and coworkers studied the *in vivo* (Dawley rats) and intracellular degradation of PCL and reported that degradation first proceeded with non-enzymatic bulk hydrolysis, and a transient initial inflammatory response occurred only for the first 2 weeks [24]. After 9 months, only when the molecular weight had reduced to approximately 5000 g/mol, did a loss in mass emerge and subsequently the PCL implants

fragmented. Figure 4 schematically depicts the interplay between the mass loss and molecular weight loss from a typical resorbable polymer scaffold *in vivo.*

For the study of intracellular degradation, low molecular weight PCL (Mn, 3000 g/mol) powders, 53-500 nm, were used. The authors reported that the powdered PCL was rapidly degraded and absorbed within 13 days inside the phagosomes of macrophage and giant cells, and that the sole metabolite was 6-hydroxyl caproic acid. Figure 5a illustrates the mechanism by which PCL degrades hydrolytically. Hydrolysis intermediates 6-hydroxyl caproic acid and acetyl coenzyme A are formed which in turn enter the citric acid cycle and are eliminated from the body [33].

More recently Sun *et al* designed a long-term study in which *in vivo* degradation of PCL was observed for 3 years in rats [23]. Distribution, absorption and excretion of PCL were traced in rats using radioactive labeling. The results showed that PCL capsules with initial Mw of 66 000 g/mol remained intact in shape during 2-year implantation, and broke into low Mw (8000 g/mol) pieces at the end of 30 months. The Mw of PCL deceased linearly with time. Tritium-labeled PCL (Mw 3000 g/mol) was subcutaneous implanted in rats to investigate its absorption and excretion and the radioactive tracer was first detected in plasma 15 days post-implantation. At the same time, radioactive excreta were recovered from feces and urine. An accumulative 92% of the implanted radioactive tracer was excreted from feces and urine at 135 days post-implantation. In parallel, the plasma radioactivity dropped to background level. Radioactivity in the organs was also close to background level confirming that the material did not accumulate in body tissue and could be completely excreted which was in accordance with early studies by Pitt *et al* [34].

Pulkkinen *et al* demonstrated that 2,2-bis(2-oxazoline) linked PCL (PCL-O) was degraded *in vitro* enzymatically by surface erosion which could enable the novel use of this material for drug delivery and other biomedical applications. The degradation, erosion (weight loss) and toxicity of PCL-O poly(ester-amide)s were evaluated *in vivo*. PCL and three PCL-O polymers with different PCL block lengths (Mn: 1500, 3900, 7500 g/mol) were melt-pressed in the form of discs and implanted subcutaneously in Wistar rats (dose approximately 340 mg/kg) for 1, 4 and 12 weeks. With an implantation of 12 weeks, up to 16.5% weight loss of polymer discs was measured for the most extensively linked PCL-O polymer (block length 1500 g/mol) whereas practically no weight loss was observed with the other polymers. Nuclear magnetic resonance (NMR), DSC and GPC studies as well as SEM micrographs before and after implantation and *in vitro* hydrolysis studies collectively indicated that enzyme based surface erosion of PCL-O polymers occurred *in vivo*. The *in vivo* evaluation based on results from hematology, clinical chemistry and histology of the implantation area and main organs (i.e. heart, lung, liver, kidney, spleen and brain) demonstrated that PCL-O polymers were biocompatible and safe, enzyme-sensitive biomaterials [35].

Surprisingly, despite more than 1000 papers being published during the last decade in the biomaterials and tissue engineering literature (Figure 1) which use PCL-based-scaffolds, only a small number of groups have included a study of the degradation and resorption kinetics of the PCL scaffolds.

The authors group have undertaken several long-term degradation studies of ordinary (Sigma) and medical grade (Birmingham Polymers) PCL scaffolds both *in vitro* and *in vivo* [21]. An accelerated degradation systems based on NaOH was also developed and validated

against a system based on simulated physiological conditions [36]. Figure 5b illustrates surface erosion of PCL and the associated changes in crystallinity over time (owing to its crystalline and amorphous components) which can lead to cyclic increasing and decreasing crystallinities throughout the degradation period. Microscopic and macroscopic views of an accelerated degradation system are shown in Figure 5c. PCL scaffolds were degraded from 0 to 5 weeks and were observed to degrade via a surface erosion pathway homogenously throughout the scaffold structure, through the thinning of the filament diameters.

As previously described, PCL is an excellent candidate for co-polymerisation or blending to engineer desired mechanical properties and degradation kinetics of a medical device or scaffold. A Dutch group were amongst the first to use PCL based copolymers to design and commercialize nerve guides device. The degradation and the tissue response evoked by poly(1,3-trimethylene carbonate) [poly(TMC)] and copolymers of TMC with either 52 mol % D,L-lactide (DLLA) or 89 mol % ε-caprolactone (CL) were evaluated in vivo by subcutaneous implantation of polymer films in rats for periods up to one year [37]. Poly(TMC) specimens were extensively degraded after 3 weeks and, as confirmed by histology, totally resorbed in less than a year. A fast linear decrease in thickness and mass without a change in molecular weight was observed. Initially an acute sterile inflammatory tissue reaction, caused by the implantation procedure, was observed, followed by a mild macrophage-mediated foreign body reaction that lasted during the resorption period of the polymer. It was concluded that *in vivo*, poly(TMC) degraded via surface erosion involving cellular-mediated processes. The degradation of the copolymers was slower than that of poly(TMC), taking place via autocatalyzed bulk hydrolysis, preferentially of ester bonds. The TMC-DLLA copolymer degraded 20 times faster than the TMC-CL one. In both cases, the tissue reaction upon implantation resembled a so called sterile inflammatory reaction

followed by a foreign body reaction that was defined by a fibrous encapsulation. Significant mass loss was only observed for the TMC-DLLA copolymer, which underwent 96% mass loss in 1 year. When extensive mass loss started, a mild-to-moderate secondary foreign body reaction, related to clearance of the polymer fragments, was triggered. The results presented in this study demonstrate that poly(TMC) and both TMC copolymers (TMC-DLLA and TMC-CL) are biodegradable and biocompatible materials, making these polymers attractive for the preparation of short and long-term degradable devices for soft tissue engineering [37].

In conclusion, the degradation of PCL compared to PLA, PGA, copolymers thereof and many other resorbable polymers is slow, making it much more suitable for long-term degradation applications such as delivery of encapsulated molecules extending over a period of more than one year, which will be discussed in section 5.

4. Biocompatibility

Originally biocompatibility referred to the ability of a material to perform with an appropriate host response in a specific application [38]. However, more recent definitions are aiming to describe the biological mechanism in more detail (Williams 2007 Trends in biotechnology). *In vitro* biocompatibility or cytotoxicity is generally evaluated through cell culture systems. *In vivo* experimental, histological and pathological examination of the peri-implant and host responses - such as immunogenic, carcinogenic and thrombogenic responses are also studied. The complexity of these host responses is a result of a series of temporal and spatial processes involving numerous closely interdependent mechanisms of material-tissue interactions. It is these interactions that control the ultimate performance of a material within a biological environment. If we consider the field of biostable materials and permanently implanted devices/implants, the primary goal is minimizing and adjusting material-tissue

interactions. The interaction of the living environment and the material should be acceptable and stable for long-term therapies and performances. Conversely, in the field of biodegradable and bioresorbable polymers, the situation is quite the opposite with an added dimension of complexity afforded by the degradation and resorption by-products of the implants, which are able to strongly interact with living systems. From this point of view, biodegradable and bioresorbable polymers must be regarded as much closer to pharmacology than to material science. Hence, biocompatibility is a factor that must be considered before the selection of biodegradable polymers to be used in medical devices, scaffolds and drug delivery systems.

In general, bioresorbable polymers and devices are well tolerated by living tissue [39], with their biocompatibilities depending primarily on the factors briefly discussed below. The leaching of low molecular mass compounds either through degradation or because of the presence of leachable impurities is the mayor cause of triggering inflammation. Release of acidic degradation products from bioresorbable polymers and implants is also a large contributor to the observed secondary inflammatory reactions. Another important factor which influences inflammation responses is the site of implantation. If the capacity of the surrounding tissues to eliminate the by-products is low, due to poor vascularization or low metabolic activity, the chemical composition of the by-products may lead to local temporary disturbances. One example of this is the increase of osmotic pressure or change in pH manifested by local fluid accumulation or transient sinus formation [17]. Hence, problems of biocompatibility of bioresorbable polymers such as aliphatic polyesters are unquestionably related to biodegradability and bioresorbability.

The determination of both the degradation rate of the polymer and the local tissue

clearance is crucial in predicting the concentration of by-products present in the tissue and resultant host response. Originally, the inflammatory response of copolymers PCL and PLA after implantation in male wistar rats was studied in detail by by Pitt *et al* [24]. The injection of microspheres into the body resulted in the activation of neutrophils and causes mild localized inflammation. The rapid activation of neutrophils by PCL microspheres was confirmed by measurement of superoxide anion generation as measured by chemiluminescence. Neutrophils activation released chemotactic factors leading to influx of massive number of neutrophils into the affected site and causing inflammation. Phagocytosis of drug loaded polymeric microspheres by white blood cells was shown to be the main clearance mechanism by which foreign material was eliminated from the body [24]. Inflammatory reactions in bones were less pronounced than in muscles. Even so the investigators do not discuss this observation in great detail one might hypothesize that the pronounced primary inflammatory reaction in muscle might be due to a better vascularization of muscle tissue and a greater amount of implanted material.

The tissue reaction of implantable microspheres comprising PCL prepared by solvent evaporation methods was studied by implantation in the brain of wistar rats [40]. Results indicated no necrosis was observed which implied good biocompatibility of microspheres within the brain tissue. To prevent the phagocytosis of microspheres, modification of microspheres surface can be undertaken by steric stabilization. Flow cytometry has been used to study the effect of PCL microspheres on apoptosis and cell cycle of fibroblasts. Results revealed that PCL microspheres purified in different ways showed different cytocompatibility; with well-purified microspheres having supeior cytocompatibility [41].

Both PCL and PLLA are slow degrading polymers but their biocompatibility resulting

from degradation is quite different. Bergsma *et al* reported foreign body reactions to PLLA bone plates and screws [16]. Six out of ten patients had to be reoperated after postoperative periods between 35 and 44 months due to swelling at the implantation site. The authors reported no discoloration of the overlaying tissue, with no signs of acute or subcutaneous inflammation, or an increase in temperature or pain on palpation. Light microscopic analysis of the soft tissue showed a foreign body reaction without signs of inflammation around the PLLA. On the outer part of the PLLA a few polymorphonuclear leucocytes were present whereas the inner part was surrounded by dense connective tissue lying within macrophages, foreign body giant cells, and fibrocytes. The authors hypothesized that the observed foreign body reaction was a combination of a biochemical and biomechanical reaction of the crystal-like PLLA fragments [16].

Pistner *et al* (1993) and Gutwald *et al* (1994) studied two amorphous and one crystalline PLA in the paravertebral muscle of rats. The crystalline PLA remained almost stable in form and structure over a period of 116 weeks. No signs of inflammation and a mild foreign body reaction were observed. After 116 weeks, the amorphous PLA of higher molecular weight almost completely resorbed, whereas the amorphous PLA of lower molecular weight was metabolized. During the degradation and resorption period a mild to moderate histiocytic inflammation was found [42-44].

As discussed above it is crucially important to study biocompatibility not only from a short-term point of view but also in the long-term. Unfortunately most *in vivo* studies in the tissue engineering field suffer from being prematurely ended in order to extract histological and/or biomechanical data before the PCL scaffold itself has been cleared from the implantation site. It's accepted that long term in *vivo* data is costly to acquire but this does not

negate the need to have robust information pertaining to long term degradation of the microspheres and scaffold, the biocompatibility, the mechanical properties of the scaffold/new tissue and the ultimate outcome of implantation after months or even years.

Meek *et al* (2009) recognised a scarcity in literature on long-term nerve guide studies of Neurolac$^{(R)}$, after having shown that small fragments of the nerve guide comprising poly(DL-lactide-ε-caprolactone) [PDLLA-PCL] (which were assumed to fully resorb) could still be found on the edge of the epineurium of the regenerated nerve after implantation. Consequently they studied the 2-year degradation and possible long-term foreign body reaction against the nerve guides after implantation in the sciatic nerve of the rat. They demonstrated that nerve regeneration took place through the scaffold, and after 2-years of implantation no remains of the implant could be found macroscopically. However, microscopically, the polymer fragments along with multinucleated giant cells and macrophages were found along the regenerated nerve tissue. Hence, the authors concluded that a 3-year study was warranted to capture the nerve tissue after complete clearance of the polymer by the system [45].

Several studies by Hutmacher *et al* have looked at both the short term and long term biocompatibility of PCL scaffolds using different animal models and some this work is summarized in Figure 6 [21, 46-49]. Figure 6a shows the surgical implantation of the 5mm diameter PCL scaffolds into the parietal bone of the rabbit/rabbit. Figure 6b shows the explanted rabbit skull. Upper insets depict micro computed-topography (μCT) images of the mineralised bone within the critical sized skull defects/scaffold sites after 2-years implantation (empty defects showed incomplete bridging of the defects). Furthermore the histology (lower insets) demonstrated some new bone formation in the centre of the PCL

scaffolds/defect sites, as detected using von kossa staining which binds to calcium salts and turns black. Further development of these scaffolds by the authors has led to the production of second-generation scaffolds which are composite by nature and contained PCL with 20wt% tricalcium phosphate (TCP). Figure 6c depicts histology from a rat calvarial critical-sized models used to study the effect of PCL-TCP composite scaffolds implanted for 15 weeks, with some scaffold groups containing 5 μg rhBMP2 (recombinant human bone morphogenetic proteins, the primary interest in this growth factor family arises from their effective use in clinical bone regeneration). By 15 weeks, PCL–TCP/rhBMP2 defects exhibited complete healing of the calvarium as shown by histological staining in Figure 6c. The scaffold alone also stimulated some bone formation at this relatively early stage, compared with no healing observed for empty defects. These studies compound the biocompatibility of the PCL and PCL composites with no adverse biocompatibility effects found at short-term time points of 15 week up to long-term implantations of 2 years.

5. PCL Applied in Drug Delivery Systems.

PCL is suitable for controlled drug delivery due to a high permeability to many drugs excellent biocompatibility and its ability to be fully excreted from the body once bioresorbed. Biodegradation of PCL is slow in comparison to other polymers, so it is most suitable for long-term delivery extending over a period of more than one year. PCL also has the ability to form compatible blends with other polymers which can affect the degradation kinetics which in turn can be tailored to fulfill desired release profiles [50-52].

The delivery of therapeutic compounds can be hindered by their poor water solubility, however, recent advances in drug formulation have obviated the potential of colloidal vectors

to act as efficient solubilising agents in such cases [53]. The capacity of block copolymer micelles to increase the solubility of hydrophobic molecules stems from their structural composition, which is characterized by a hydrophobic core sterically stabilized by a hydrophilic corona. The former serves as a reservoir in which the drug molecules can be incorporated by means of chemical, physical or electrostatic interactions, depending on their physicochemical properties [53]. Drug release rates from PCL depends on type of formulation, method of preparation, PCL content, size and percent of drug loaded in the microcapsules. Due to a higher permeability of PCL it is blended with other polymers to improve stress, crack resistance, dyeability and control over release rate of drugs. Within the last decades, PCL polymers have been major area of interest to develop controlled delivery systems especially for peptides and proteins [52].

Lemmouchi *et al* have investigated the *in vitro* and *in vivo* release of the selected drugs, isometamidium chloride and ethidium bromide from PCL-PLLA, PCL-DLLA and PCL-TMC rods [54].

5.1. PCL Microspheres

Much research has been focused on degradable polymer microspheres for drug delivery. Administration of medication via such systems is advantageous because microspheres can be ingested or injected; they can be tailored for desired release profiles and in some cases can even provide organ-targeted release [51].

A microencapsulated drug is a promising drug delivery system with obvious advantages, such as improving the therapeutic efficiency and efficacy, prolonging the biological activity, controlling the drug release rate and decreasing the administration

frequency. As drug microparticles, besides biocompatibility, one of the most important requirements is that the matrix material should be biodegraded within a suitable period which is compatible with the drug release rate. Hence, biodegradable polymers have been the major focus of attempts to develop improved delivery systems for pharmaceutical research. There has been extensive research into drug delivery using biodegradable polymeric devices ever since bioresorbable surgical sutures entered the market two decades ago. Amongst the different classes of biodegradable polymers, the thermoplastic aliphatic poly(esters) such as PLA, PGA and especially their copolymers such as poly(lactide-co-glycolide) (PLGA) have generated tremendous interest because of their excellent biocompatibility, biodegradability, and mechanical strength, most of which can be tailored via the copolymerisation of different amounts of each respective polymer. They are easy to formulate into various devices for carrying a variety of drug classes such as vaccines, peptides, proteins, and micromolecules. Most importantly, they have been approved by the FDA for drug delivery [10].

The matrix material of bioresorbable microparticles can be decomposed into non-toxic and low molecular weight species concomitant with release of the drug which are then metabolized or absorbed by the organism. It is no surprise that a huge research interest is now focused on the application of biodegradable microparticles for controlled drugs release. Amongst them, polycaprolactone is one of the more widely utilised. The advantages of PCL include its high permeability to small drug molecules, and their negligible tendency to generate an acidic environment during degradation as compared to PLA and PGAs. The degradation of PCL homopolymer is very slow as compared to other polyesters making it more suitable for long term delivery systems extending to a period of more than one year, and with appropriate blending the delivery can be increased/decreased as desired [10].

PCL microspheres can be prepared by several different methods, some of which are reviewed by Freiberg *et al* [51]. Colloidal monomers dispersed in a liquid with opposite solubilities can be polymerised [55]. Spherical droplets are formed by oil-soluble organic monomers dispersed in aqueous media (oil in water, O/W) or by water-soluble monomers dissolved in water dispersed in an organic medium (water in oil, W/O) [56]. The polymerisation of dispersed monomers is achievable by various methods including emulsion, suspension, and dispersion techniques [57]. Emulsions are typically used to form uniform spheres on nanometer scales (10–100nm). The resulting polymer beads can be so uniform on the nano-scale that they may diffract visible light [58]. Dispersion polymerisation results in microspheres of the range 0.5-10μm. The reagents (including monomer, initiator, and stabilizer) are dissolved in an organic medium and since the initiator is soluble inside the monomer, polymerisation takes place inside the monomer droplets. The polymer beads, insoluble in the organic solvent, precipitate, and the stabilizer prevents bead flocculation [59]. Significant work on dispersion polymerisation in supercritical CO_2 has been undertaken in recent years which may be beneficial to medical applications since no toxic solvents are involved [60] [61].

Suspension polymerisation typically gives microspheres in the range of 50-500 μm. In suspension polymerisation the monomer is dispersed in a water phase with a stabilizer; the initiator is soluble in the monomer phase where polymerisation occurs. The size and quantity of the particles is determined by the size and quantity of dispersed monomer droplets and by the speed of mechanical stirring [57].

Solvent evaporation (also known as the double emulsion technique) and spray drying techniques are common techniques for producing microspheres from linear polymers and

have been reviewed by Vasir *et al* (2003)[62]. Briefly, microspheres can be produced by the evaporation of an organic solvent from dispersed oil droplets containing both polymer and biomolecule [63]. Often a double emulsion is employed whereby the biomolecule is first dissolved in water; this aqueous phase is dispersed in an organic solvent (usually dichloromethane, DCM), which contains the degradable polymer and the first W/O emulsion is formed. Dispersion of the first emulsion in a stabilized aqueous medium (usually using poly vinyl alcohol as stabilizer) forms the final O/W emulsion; microspheres are formed as the DCM evaporates and the polymer hardens, trapping the encapsulated drug [64]. A major obstacle in the entrapment of drugs into microspheres is attaining a high yield via good entrapment efficiencies. Many groups fail to achieve a high enough entrapment to warrant further production of the microspheres at the risk of losing too much drug in the process which is very costly. It is also surprising how few details are provided in many studies which detail the method of quantifying drug entrapment efficiencies.

5.2. PCL nanospheres

Nanospheres are colloidal drug delivery systems, which act as transport carrier compartments for drugs or other active molecules, with size ranging between 10-1000nm. Drug particles may be encapsulated, dispersed or absorbed in the nanospheres. They may also be termed as nanoparticles or nanocapsules depending upon whether the drug is in a polymeric matrix or encapsulated in the shell. Nanospheres and nanocapsules can be prepared by the same methods as those described for microparticles, except that manufacturing parameters are adjusted to obtain nanometer size droplets. This can be obtained by using a relatively small ratio of the dispersed phase to the dispersion medium and a substantially higher stirring speed [65].

Nanospheres can be used for selective targeting via the reticuloendothelial system to

the liver and to cells that are phagocytically active. The size of nanospheres allows them to be administered intravenously via injection unlike many other colloidal systems which occlude both needles and capillaries. Injectable nanoparticulate carriers have good applicability for specific drug delivery and medical imaging. But they cannot generally be used due to their elimination within seconds after intravenous injection by the reticuloendothelial system. To overcome this limitation, monodisperse biodegradable nanospheres have been developed from amphiphilic copolymers. These nanospheres were shown to exhibit increased blood circulation time and reduced drug accumulation in the liver of mice [65].The efficacy of these colloidal particles as drug carriers is closely related to their interaction with proteins and enzymes in different body fluids. The interaction phenomenon between lysozyme, a positively charged enzyme that is highly concentrated in mucosa and two different drug carriers: nanocapsules made of an oily core coated by PCL and nanoparticles made solely of PCL were analyzed. Results showed that the interaction of lysozyme with these colloidal drug carriers was highly affected by their surface charge [66]. Gref *et al* analysed plasma protein adsorption zeta potential and the particle uptake by polymorphonuclear cells by biodegradable PEG-coated PLA, PLGA and PCL nanoparticles. The influence of the PEG corona thickness and density, as well as the influence of the nature of the core was studied [67]. The conditions to stabilize PLGA and the PCL nanoparticles by freeze drying with several cryoprotective agents were identified. Studies indicated the necessity of adding sucrose, glucose, trehalose or gelatin to preserve the properties of nanoparticles regardless of the freezing procedure [68].

6. Techniques of nanosphere preparation

Different methods have been reported in literature for the preparation of drug entrapped nanoparticles including, emulsion polymerisation method in continuous aqueous phase,

emulsion polymerisation method in continuous organic phase, interfacial polymerisation, interfacial disposition, solvent evaporation, desolvation of macromolecules and dialysis methods [69]. Select methods for preparing PCL nanospheres are discussed below:

6.1.1.1. Interfacial polymer disposition method

Interfacial polymer disposition is a procedure for preparing biodegradable nanospheres following displacement of a semi-polar solvent, miscible with water from a lipophilic solution. In this method, the polymer is first dissolved in an organic solvent, usually acetone. Similarly the mixture of phospholipid is prepared in acetone by increasing the temperature to near boiling point, the drug is then dissolved in benzyl benzoate and is added to the acetonic solution. The resulting organic solution is poured under stirring into water containing the surfactant poloxamer, with the aqueous phase immediately turning into a milky solution; indicating the formation of nanocapsules. Acetone is then removed under reduced pressure. The colloidal suspension thus formed is concentrated to the desired volume by removal of water [70]. Spray-dried polymeric nanocapsules and nanospheres have also been prepared from PCL suspensions containing diclofenac using interfacial deposition of the polymer [71].

6.1.1.2. Dialysis method

Indomethacin loaded nanospheres of PCL have been prepared by dialysis methods. The polymer was dissolved in organic solvent (dimethylformamide) and the drug was added to the solution under constant stirring at room temperature. After removing the organic solvent dialysis was undertaken for 24h using cellulose membrane bag. The miceller solution was collected from the bag, sonicated and centrifuged to remove aggregated particles and unloaded drug. Lyophilization was then performed to obtain nanospheres [72].

6.1.1.3. Emulsion polymerisation method

The earliest nanoparticles prepared by the polymerisation of a monomer were those proposed by Birrenbach and Speiser in the 1970s [73]. In emulsion polymerisation, droplets of water insoluble monomers are emulsified in an external aqueous and acidic phase containing a stabilizer. The monomers polymerise relatively fast by an anionic polymerisation mechanism, the polymerisation rate being dependent on the pH of the medium. At neutral pH, the monomer polymerises extremely fast, leading to the formation of aggregates. However, at acidic pH, between pH 2 and 4, the reaction is slowed, yielding nanospheres (frequently 200 nm) with a narrow-size distribution. The system is maintained under magnetic agitation while the polymerisation reaction takes place. Finally the colloidal suspension is neutralized and lyophilized following the incorporation of glucose as a cryoprotectant [52].

Water soluble drugs may be associated with nanospheres either by dissolving the drug in the aqueous polymerisation medium or by incubating blank nanospheres in an aqueous solution of the drug. High speed mixing or sonication is a critical step in emulsification of a drug or monomer solution into the external phase as it determines the size distribution, of the nanoparticles. In order to achieve narrow particle size distribution ultrasonication or high speed homogenization is required; hence these parameters should be carefully monitored during processing.

PCL nanospheres encapsulating numerous drugs have been investigated by various researchers and have been comprehensively reviewed by Sinha *et al* [52] who detail the use of PCL as a favorable ocular penetration carrier in nanosphere form compared with microspheres when used to deliver indomethacin [74]. Calvo *et al* further concluded that the

colloidal nature of PCL nanosphere and nanocapsule carriers was the main factor responsible for favorable corneal transport and that cornea penetration was not increased by variation of the inner structure or composition of the carriers [66, 75]. This finding was also observed by Marchal-Heussler *et al* in their use of PCL as a colloidal nanoparticle suspension containing cartelol, finding the inner oily core of the carrier provided better cartelol entrapment and provided a more pronounced effect on intraocular pressure compared with cartelol eye drops [76].

Other drug encapsulations for ophthalmic applications using PCL nanospheres/capsules include fluribrofen [77, 78], aceclofenac [79], cyclosporine A [75, 80] and Metipranol [81, 82]. Several orally admininstrated PCL nanosphere/nanocapsules have been investigated to deliver antihypertensive agents such as isradipine [83]. Many groups have utilised PCL copolymers in cancer-related nanoparticle delivery systems. A ligand-mediated nanoparticulate drug carrier was designed by Kim *et al*, which could identify a specific receptor on the surfaces of tumor cells. Biodegradable poly(ethylene oxide)/PCL (PEG/PCL) amphiphilic block copolymers coupled to biotin ligands were synthesized harboring the anticancer drug paclitaxel, prepared via micelle formation in aqueous solution. Results showed that the biotin-conjugated nanoparticles could improve the selective delivery of paclitaxel into cancer cells via interactions with over-expressed biotin receptors on the surfaces of cancer cells [84]. Tamoxifen-loaded PEO-PCL nanoparticles were also prepared using solvent a displacement process by Shenoy et al. The use of pluronic surfactants (F-68 and F-108) increased the stabilisation of the particles and achieved preferential tumor targeting and a circulating drug reservoir [85].

7. PCL Applied in Medical devices

7.1. Sutures

In the past four decades, several studies have been published relating to the biocompatibility of sutures made from aliphatic polyesters [86]. The material composition of the commercially available sutures are PGA (Dexon™), PLLGA 10/90 (Vicryl®), poly (glycolid-co-trimethylencarbonat) 67.5/32.5 (Maxon®), and polydioxanone (PDS). In the case of suture materials, inflammatory response is more pronounced for Dexon™ and Vicryl® (mononuclear cells, polymorphonuclear leucocytes and lymphocytes, histiocytes and multinucleated giant cells) than for Maxon® and PDS (mononuclear macrophages, a few neutrophils, multinucleated giant cells, organized collagenous capsule).

A block copolymer of PCL with glycolide, offering reduced stiffness compared with pure polyglycolide, is being sold as a monofilament suture by Ethicon, Inc. (Somerville, NJ), under the trade name Monacryl® [19].

7.2. Wound Dressings

A major pioneer in the characterization and application of resorbable polymers, amongst them PCL, was Pitt during the early 1980's [3]. Pitt and co-workers undertook several studies including degradation studies both *in vitro* [87, 88] and *in vivo* [30]. Later studies involved sub dermal delivery of L-methadone from PCL microspheres [89]. Since then, PCL has been utilised as an ultra thin film for dressing cutaneous wounds [90] and has also been used as a release vehicle for the chemical antiseptic chlorohexidine [91]. Blending PCL with the polymeric antimicrobial complex, poly (vinylpyrrolidone)-iodine to provide a ureteral biomaterial with reduced encrustation was investigated by Jones *et al*. Interestingly, they demonstrated a relationship between the degradation rate of the polymer and the

resistance to encrustation (the degradation was tailored via altering the high to low molecular weight ratio of PCL in the polymer blends) [92].

7.3. Contraceptive devices

Almost 10 million women have used the subdermal contraceptive implants including Norplant® Jadelle® and Implanon® which have often-traumatic retrieval operations associated with their end point. Consequently, the last two decades have seen substantial research into developing a biodegradable matrix implant for controlled release of contraceptives to circumvent the need for device-retrieval surgery. PCL is a highly desirable candidate for this role owing to its slow degradation, biocompatability and FDA approval. Dhanaraju *et al* have prepared and characterised PCL microspheres as an injectable implant system for the controlled delivery of contraceptive steroids [93-95].

Sun *et al* have developed a 2-year contraceptive device comprising PCL/Pluronic F68 compounds filled with levonorgestral powder which was approved by the SFDA to conduct phase II human clinical trials in China [23]. Preclinical studies using rats and dogs demonstrated good release kinetics of levonorgestral from this device, with no adverse effects. After implant retrieval at 2 years the implant was physically stable with an associated drop in the molecular weight of the polymer from 66,000 to 15,000 Da [96]. Recently, multi component biomaterials comprising a hydrogel matrix (2-hydroxyethyl methacrylate cross-linked by ethylene glycol dimethacrylate) with levonorgestral-containing PCL microspheres dispersed within were developed by Zalfen *et al.* Such assemblies show promise owing to the combination of several release mechanisms which can tailor the release of encapsulated drugs, potential applications include the design of implantable devices with long-term activity, as required by contraceptive and hormone replacement treatments [97].

7.4. Fixation Devices

Kulkarni *et al* [98, 99] and Cutright *et al* [100] were amongst the first to report preliminary experiments on the use of aliphatic polyesters in the design of internal fixation devices. Kulkarni *et al* (1971) used extruded pins of L- and D-PLA for the reduction of mandibular fractures in dogs and confirmed minimal inflammatory responses for both polymers [98]. Cutright *et al* (1971) reported data on mandibular fracture reduction in monkeys using transosseous ligatures with PLA suture materials [101]. Animals were sacrificed from 2 to 12 weeks. At 12 weeks, early features of bony union appeared and the sutures became infiltrated by cellular connective tissue with fibroblasts, endothelial cells, mononuclear phagocytes and giant cells. Sutures were progressively replaced by bands of new collagen and vascular connective tissue. The tissue reaction was limited to the immediate perisutural area.

Studies using pure PCL in orthopaedic applications are rare in the current literature. One study involved the fixation of rabbit humeri osteotomies with PCL reinforced with glass fibres versus stainless steel, the outcome demonstrating that although the PCL caused less stress shielding than stainless steel, the mechanical strength of the PCL was not sufficient for load bearing applications owing to high mal-union rates [102]. It should be noted however that several studies exist which exploit the positive properties of PCL and blend these with other materials producing superior copolymers and composites which may have the desirable properties for use in mechanically challenging applications where a more resilient material is needed [103].

Rudd *et al* have studied PCL for application as a resorbable composite implant during the last decade, with an aim at craniofacial repair. The PCL was polymerised *in situ* and has been reinforced with several different fibres including knitted vicryl mesh [104], phosphate glass fibres [105] and sodium- and calcium-phosphate glass fibres [106]. The studies have also included several cell biocompatibility studies [107, 108].

7.5. Dentistry

The filling material used in root canal systems during the common dental procedure; root canal treatment, has popularly involved gutta-percha in one of its many forms for almost 100 years. An optimal root filling material should provide a predictable seal, inhibit or kill residual bacteria, prevent re-contamination and facilitate periapical healing. The creation of a "seal" can be complicated and the final result is often deemed suspect. Alani *et al* aimed to develop a novel PCL/phosphate glass composite deliverable as a root filling and capable of releasing ionic species to enable a predictable seal in an aqueous environment. Different compositions of PCL-iron phosphate glass composites were produced and delivered into an ex vivo root canal model. Standardized root canals were prepared in extracted human teeth. The teeth were examined for root filling adaptation and precipitate formation (SEM), ion release (Na^{+}, Ca^{2+}, PO_4^{3-}, $P_2O_7^{4-}$, $P_3O_9^{3-,}$ and $P_5O_{10}^{5-}$), and sealing ability. The experiments were controlled with teeth obturated with contemporary gutta-percha and a conventional zinc-oxide/eugenol sealer. The adaptation of the PCL composite was statistically significantly better than the control groups. Precipitate formation was noted in some specimens but all released various ionic species in an inverse proportion to the iron oxide concentration. The experimental material exhibited significantly less leakage after 7 days immersion in saline compared with those not immersed, or the control GP group. PCL-phosphate glass composites showed good potential as a root filling material capable of producing a seal in an

aqueous environment without a sealer [109].

PCL is also used as the thermoplastic synthetic polymer-based root canal filling material recently introduced to the market as part of the composite Resilon™, which also contains bioactive glass, bismuth oxychloride and barium sulphate. This material replaces gutta-percha cones and is available in the Resilon®/Epiphany System. According to the manufacturer, the PCL polymer in Resilon™ provides the material with thermoplastic properties that enable its application in techniques that rely on thermoplasticity. According to Miner *et al* [110], the melting point of Resilon™ is the same as that of gutta-percha (60°C).

Traditionally, the augmentation of bony defects is carried out using allografts, xenografts, autogenous bone, and synthetic biomaterials with the transplantation of autogenous bone being regarded as the gold standard. Hutmacher [111] and Zein [112] have presented a suitable three dimensional PCL scaffold that can be used for mandible augmentation purposes. In a clinical study in dogs, Rai *et al* have regenerated critical-sized defects of the mandible with PCL and 20% TCP scaffolds in combination with platelet rich plasma [113]. Schuckert *et al* (2009) reported the first successful clinical case of the reconstruction of the anterior mandible on an osteoporotic patient using the 3D PCL scaffold in combination with platelet rich plasma, and rhBMP2 in a 71-year-old human female patient. A bacterial infection had caused a peri-implantitis in two dental implants leading to a large destruction in the anterior mandible. Both implants were removed under antibiotic prophylaxis and a PCL scaffold which had been specifically prepared for this clinical case containing platelet rich plasma and rhBMP2 (1.2 mg) was inserted. After complication-free wound healing, the radiological control demonstrated *de novo*-grown bone in the anterior mandible 6 months postoperatively. Dental implants were inserted in a third operation. A

bone biopsy of the newly grown bone, as well as of the bordering local bone, was taken and histologically examined. The bone samples were identical and presented vital laminar bone demonstrating the success of the procedure [114].

8. PCL Applied in Tissue Engineering

Tissue engineering can be defined as: "*an interdisciplinary field that applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function or a whole organ*"[115]. Tissue engineering was once categorized as a subfield of "Biomaterials", but having grown in scope and importance it can now be considered as a field in its own right. It is the use of a combination of cells, engineering and materials methods, and suitable biochemical and physio-chemical factors to improve or replace biological functions. Tissue engineering is closely associated with applications that repair or replace portions of or whole tissues (i.e., bone, cartilage, blood vessels, bladder, etc.). Often, the tissues involved require certain mechanical and structural properties for proper functioning. The term has also been applied to efforts to perform specific biochemical functions using cells within an artificially-created support system (e.g. an artificial pancreas, or a bioartificial liver).

Powerful developments in the multidisciplinary field of tissue engineering have yielded a novel set of tissue replacement parts and implementation strategies. Scientific advances in biomaterials, stem cells, growth and differentiation factors, and biomimetic environments have created unique opportunities to fabricate tissues in the laboratory from combinations of engineered extracellular matrices ("scaffolds"), cells, and biologically active molecules. A schematic showing this type of approach is depicted in Figure 7 which shows the combination of cells and biomolecules with a scaffold. The scaffold must be capable of

supporting cell attachment, proliferation and differentiation *in vitro* and may then be transplanted *in vivo*. Amongst the major challenges now facing tissue engineering is the need for more complex functionality, as well as both functional and biomechanical stability in laboratory-grown tissues destined for transplantation. The continued success of tissue engineering, and the eventual development of true human replacement parts, will grow from the convergence of engineering and basic research advances in tissue, matrix, growth factor, stem cell, and developmental biology, as well as materials science and bio informatics.

There are a vast array of manufacturing techniques to create scaffolds for tissue engineering, but one must pay special attention to the scaffold specifications and understand the interplay of factors affecting the material composition and design criteria. The desirable feature of any implantable polymeric scaffold material would be synchronization of polymer degradation with the replacement by natural tissue produced from cells. Figure 8 gives a graphical illustration of this complex interplay showing the molecular weight loss of a resorbable scaffold and how this relates to its mass loss and also to the growth of tissue *in vitro* prior to implantation. The degradation and resorption kinetics of the scaffold are designed to allow the seeded cells to proliferate and secret their own extracellular matrix in the static and dynamic cell-seeding phase (weeks 1-12) as concomitantly the scaffold gradually resorbs leaving sufficient space for cell proliferation and new tissue growth. The physical support by the 3D scaffold is maintained until the engineered tissue has sufficient mechanical integrity to support itself.

As scaffold candidates, the following characteristics are desirable: (i) three dimensional and highly porous structures with an interconnected pore network for cell growth and flow transport of nutrients and metabolic waste; (ii) biocompatible and bioresorbable

with a controllable degradation and resorption rate to match cell/tissue growth in vitro and/or in vivo; (iii) suitable surface chemistry for cell attachment, proliferation and differentiation and (iv) mechanical properties to match those of the tissues at the site of implantation [111]. In addition, the demanding requirements dictated by orthopaedic scenarios require a certain degree of initial mechanical support and as such, polymeric devices alone are insufficient and must be combined with additional components such as cells, growth factors and appropriate environments [49]. The degradation properties of a scaffold are therefore of crucial importance for biomaterial selection and design but also the long-term success of a tissue-engineered construct.

Scaffolds for tissue engineering have become a large focus of research attention and can be fabricated in a wide variety of ways and a biomaterial which lends itself very well to scaffold fabrication is PCL. PCL is an incredibly versatile bioresorbable polymer and by way of its superior rheological properties it can be used by almost any polymer processing technologies to produce an enormous array of scaffolds. The major scaffold fabrication technologies in which PCL has been used extensively are summarized in Figure 9 [116] and will be discussed in detail in the next section

8.1. Scaffold Fabrication for Tissue Engineering

A number of fabrication technologies have been applied to process PCL into 3D polymeric scaffolds of high porosity and surface area. From a scaffold design and function view point each processing methodology has its relative pro and cons. This section aims to provide the reader with an overview of the fabrication methods and is by no means an exhaustive list but rather aims to comprehensively discuss techniques which are most

pertinent to the fabrication of PCL scaffolds. The key rationale, characteristics, and process parameters of the currently used scaffold fabrication techniques are presented hereafter.

8.1.1. Conventional techniques

8.1.1.1. Porogen Leaching

Porogen-leaching consists of dispersing a template (particles etc.) within a polymeric or monomeric solution, gelling or fixing the structure, and removal of the template to result in a porous scaffold (Figure 7a). The specific methods to achieve such scaffolds are numerous, and porogen-leaching is an inexpensive technique to manufacture cell-invasive scaffolds. Scaffold manufacturing methods based on porogen-leaching are capable of producing structures with locally porous internal architectures from a diverse array of materials. Local pores are voids characteristically defined by small struts and plates or spherical and tubular cavities generally less than 300 μm in diameter. Highly interconnected voids are desired for tissue engineered constructs where local pores are interconnected within local regions of the scaffold microstructure.

Porogen leaching can also yield cavities with defined shape and solvent diffusion from emulsions can yield oriented pore structures. Although these methods yield interconnected pores that may comprise a continuous conduit throughout a scaffold, the pore connectivity is not an intentional result of a prior global design. Rather, the connectivity is a random product of variable, local void interconnections that are affected by polymer processing parameters. Such random connections may not provide optimal scaffold permeability *in vitro* for cell distribution and *in vivo* for vascular tissue in-growth [111].

8.1.1.2. Scaffolds produced using phase separation methods

Phase separation has been employed for decades to produce hollow fibre membranes and other polymer membrane structures. Thermally induced phase separation (TIPS) in particular has produced a range of scaffolds for tissue engineering (Figure 9b). The morphological nature of the scaffold is variable and due to the self-assembling nature of the construct, may be combined with porogen leaching to generate macro-architecture within the scaffold. Phase separated scaffolds can additionally be moulded into a range of shapes and sizes, demonstrating applicability for many tissue engineered constructs. The TIPS technique for producing scaffolds is based upon the reduction of polymer solubility when the temperature is lowered, or when the polymer is frozen out of solution. These two types of TIPS are termed liquid-liquid and solid-liquid phase separation respectively.

Liquid-Liquid Phase Separation

Scaffold manufacture relies on the controlled phase separation of polymer solutions into high and low concentration regions upon cooling. The high concentration regions solidify (via crystallization or gelation), while the low polymer concentrations result in the pores, ultimately providing the space for penetrating cells. Binary phase diagrams are used to determine the polymer/solvent relationship for TIPS, by showing the phase boundaries as a function of temperature and composition. Such diagrams provide information on the type of liquid-liquid demixing when cooling below an upper critical solution temperature, and result in three distinct morphologies. When solutions with low polymer concentrations are cooled, the nucleation and growth of polymer rich phases predominates, while cooling high polymer concentrations results in gelation followed by nucleation and growth of polymer-poor regions. The nucleation and growth of polymer poor phases results in a non cell-invasive

material, while the nucleation and growth of polymer-rich phases results in a material without the structural integrity (or pore interconnectivity) required for cell invasive scaffolds.

Bicontinuous phases are of greatest importance for tissue engineered constructs, as both mechanical integrity and suitable interconnecting porosity for cell-invasion are possible. The liquid-liquid demixing mechanism that results in such bicontinuous phases is termed spinodal decomposition. Additional quenching routes passing through the meta-stable nucleation regions may still result in spinodal decomposition if cooling is rapid enough, since time is required to form nucleation-derived structures. Once the phase separated system is stabilized, then the solvent is typically removed by vacuum sublimation and a scaffold is attained. The combinations of liquid-liquid phase separation and quenching rates, crystallinity and vitrification of the polymer phase, and crystallization of the solvent can all influence the final morphology.

Solid-Liquid Phase Separation

If freezing can result prior to liquid-liquid demixing, then the polymer may be expelled to the boundaries of the solvent crystallites. A polymer solution separates into two phases, a polymer-rich phase and a polymer-lean phase. After the solvent is removed, the polymer-rich phase solidifies. Uniaxial freezing is an inexpensive process for manufacturing scaffolds with oriented pores, which are desired in applications where guided regeneration is desired, such as scaffolds for spinal cord injury and transplantation sheets for retina. The scaffold morphology obtained reflects the solvent crystal structure, and therefore crystal growth is important. The freezing rate therefore greatly affects the resulting morphology of the scaffold.

Polymerisation-Induced Phase Separation

Certain monomeric solutions, when polymerised in excess solvent, liquid-liquid phase separate due to an increasing insolubility of the growing polymeric radical. Unlike TIPS, such systems do not rely on temperature changes to induce phase separation but require the propagating polymeric radical to extract the remaining monomer out of the original solvent. When the two liquid phases are separated into a bicontinuous system, the propagating radicals in the polymer rich phase result in gelation.

Polymerisation-induced phase separation scaffolds, after formation, are immersed in excess water to exchange non-aqueous solvents or to remove any remaining monomer or initiator. Solvent removal by freezing, then sublimation, is not required. Such systems also have phase-separation boundaries with their formulations, where the morphological nature of the scaffold can be macroporous and cell-invasive, or result from solvent nucleation and growth mechanisms depending on the polymerisation conditions.

8.1.1.3. Supercritical Fluid Methods

Gas-foaming of biodegradable polymers found its original application in the biomedical sciences in drug delivery applications during the 1980s. It is a scaffold fabrication technique that permits solvent free formation of porous materials through generation of gas bubbles within a polymer. The use of supercritical fluid technology (typically CO_2) enables moulded polymers to be pressurized with a gas, until the polymer is saturated. The release of pressure results in nucleation and growth of the air bubbles within the material. These

bubbles reach up to 100 μm; however interconnectivity is required for cell-invasive structures and is not always attained in high levels.

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 supercritical CO_2 offer the possibility of novel processing routes for polymer drug composites, but there are still technical challenges to overcome, which have been reviewed by Howdle *et al* for the preparation of scaffolds in which the drug is dispersed throughout the polymer phase [117]. This technology has resultantly been used increasingly over the last 3 decades for the production of valuable biomaterial compounds [118, 119] and to include biomolecules such as proteins or DNA and may also be combined with particulate leaching to attain improved interconnectivity between pores.

8.1.2. Textile Technologies

Cell-invasive fibre-based scaffolds can be produced using methodologies developed for the textile industry, but with structures specifically designed for tissue engineering applications. Tissue engineering textiles have a relatively high surface area and their 'value-added' application, from an industry with established techniques, is an advantage. Additionally, textiles are typically formed into thin meshes and therefore the permeability is high, allowing the necessary nutrients to reach the seeded cells. More recently, electrospinning has attracted high interest to design and fabricate scaffolds with nanometre and submicron diameter fibres. PCL is used in a majority of these studies, and as such, it will be described in detail in the next section.

8.1.2.1. Classical non-woven textiles

Traditional fabrication of non-woven textiles is based on the production of continuous micron diameter; fibres by extruding a polymer melt or polymer solution through a spinneret which is then mechanically drawn onto a winder, or a series of winders, and collected onto a spool. The fibre of the diameter is determined by the extrusion rate and the speed(s) of the winder(s) with a constant drawing rate paramount for attaining uniform diameter, continuous fibres. The extrusion also will affect the crystallinity of the polymer, and therefore influence the mechanical strength and degradation behavior. Polymer solutions are also drawn onto winders, however, the fibre must pass through a coagulation bath containing a non-solvent for the polymer to precipitate. The collected fibres are then chopped into segments, processed and compressed into the final scaffolding shapes.

Langer & Vacanti (1993) used this type of scaffold made from PGA for their initial bone and cartilage tissue engineering work [115]. Numerous groups around the world since followed this route using such non-woven textiles in their experimental set ups. Even with significant research-efforts, only Wagner, Schmelzeisen and coworkers have been successful to-date in using non-woven PLGA to tissue engineer bone chips which have seen application bone grafts in low-load bearing areas [120, 121].

Oriented bundles of extruded fibres are of particular interest for neural tissue engineering. In this instance, the fibres are cut into segments and not air-blown into random orientations. It is well known that the shape of the fibre has a strong effect on the guidance of

regenerating axons. On flat surfaces, in the absence of chemical or surface bound gradients, neurites will grow in random directions. However, when fibres reach diameters below approximately 250 nm, the guidance of axons is favoured along the length of the fibre [122]. Due to this effect, oriented non-woven bundles of fibres might be promising scaffolds for neural tissue engineering. Van Lieshout *et al* produced multifilament double-bed knitted, fibrin- covered PCL scaffolds to potentially function as aortic valves. On testing, it demonstrated good durability, proper opening and it showed coaptation upon closing, but had higher associated leakage than those of tested porcine valves [123]. This valve is shown in Figure 2(g-i).

8.1.2.2. Electrospinning nanofibres

Electrospinning is a relatively inexpensive manufacturing technique for sub-micron and micron diameter fibres from polymer solutions or melts (Figure 9 c,d). Although it is a process known since the 1930s, it is a technique still in its relative developmental infancy in the commodity industry and recently is enjoying a massive surge of interest in the field of tissue engineering. Electrospinning is of great interest as a scaffold fabrication technique, since the resulting fibre diameters are in the size range (submicron to nanometer) of the extracellular matrix (ECM) microstructures, particularly the higher-ordered collagen microfibrils [124]. The flexibility of the electrospun fibres, due to the very high aspect ratio (length/diameter), is also beneficial, allowing seeded cells to remodel their surrounds. The size of scale is important in this instance; instead of many cells adhering to one fibre as is common with microfibres, one cell may adhere to multiple electrospun nanofibres. A plethora of research papers have focused on different natural and synthetic polymers but by far PCL is the most commonly used polymer in the electrospinning literature. Specific applications of

PCL scaffolds made by electrospinning in various tissue engineering applications will be discussed in section 8.2 - 8.8.

8.1.3. Solid Free-Form Fabrication

Solid free-form fabrication (SFF) and rapid prototyping (RP) [49] have been applied to fabricate complex shaped tissue engineered constructs. Unlike conventional machining which involves constant removal of materials, SFF is able to build scaffolds by selectively adding materials, layer-by-layer, as specified by a computer program (Figure 9f-j)). Each layer represents the shape of the cross-section of the computer-aided design (CAD) model at a specific level. Today, SFF is viewed as a highly potential fabrication technology for the generation of scaffold technology platforms. In addition, one of the potential benefits offered by SFF technology is the ability to create parts with highly reproducible architecture and compositional variation across the entire matrix due to its computer-controlled fabrication process.

8.1.3.1. Systems Based on Laser and UV Light Sources

Stereolithography

The stereolithography apparatus (SLA) (Figure 9g) is often considered the pioneer of the RP industry with the first commercial system introduced in 1988 by 3D Systems Inc. Stereolithography is based on the use of a focused ultra-violet (UV) laser which is vector scanned over the top of a liquid bath of a photopolymerizable material. The UV laser causes the bath to polymerise where the laser beam strikes the surface of the bath, resulting in the creation of a first solid plastic layer at and just below the surface. The solid layer is then

lowered into the bath and the laser generated polymerisation process is repeated for the generation of the next layer and so on, until a plurality of superimposed layers forming the desired scaffold architecture is obtained. The most recently created layer in each case is always lowered to a position for the creation of the next layer slightly below the surface of the liquid bath. Once the scaffold is complete, the platform rises out of the vat and the excess resin is drained. The scaffold is then removed from the platform, washed of excess resin, and then placed in a UV oven for a final curing [125].

For industrial applications the photopolymer resins are mixtures of simple low-molecular-weight monomers capable of chain-reacting to form solid long-chain polymers when activated by radiant energy within specific wavelength range. The commercial materials used by SLA equipment are epoxy-based or acrylate-based resins that offer strong, durable, and accurate parts/models. However, this material cannot be used as scaffold materials due to lack of biocompatibility and biodegradability. Hence, limited selection of photopolymerisable biomaterials is a major constraint for the use of the SLA technique in the design and fabrication of scaffolds for tissue engineering applications. However, biocompatible acrylic, anhydride and polyethylene oxide-based polymers may be explored in future research, as they are already in research or clinical stage typically as curable bioadhesives or injectable materials. The variation of the laser intensity or traversal speed may be used to vary the cross-link or polymer density within a layer so that the properties of the material can be varied from position to position within the scaffold. This would allow fabrication of so called biphasic or triphasic matrix systems. Microstereolithography in particular is thought to offer a great potential for the production of 3D polymeric structures with micrometer resolution.

Selective Laser Sintering

Selective laser sintering (SLS) (Figure 9h) also uses a focused laser beam, but to sinter areas of a loosely compacted powder. In this method, a thin layer of powder is spread evenly onto a flat surface with a roller mechanism. The powder is then raster-scanned with a high-power laser beam. The powder material that is struck by the laser beam is fused, while the other areas of powder remain dissociated. Successive layers of powder are deposited and raster-scanned, one on top of another, until an entire part is complete. Each layer is sintered deeply enough to bond it to the preceding layer. However, SLS poses significant material constraints for scaffold fabrication and is currently mainly used to make calcium phosphate based scaffolds for bone engineering. SLS has the disadvantage that incorporation of sensitive biomolecules is difficult because of the need to locally heat the powder layer so as to sinter it [111].

A novel 3D scaffold with branching and joining flow-channel network comprising multiple tetrahedral units has been developed as an implantable liver tissue replacement as shown in Figure 2j-o. PCL and 80% (w/w) NaCl salt particles serving as porogen were applied in a selective laser sintering process to obtain a scaffold with high (89%) porosity, a pore size of 100-200 μm and 3D flow channels. Cell biocompatibility studies showed promising results using Hep G2 cells and further optimisation of the scaffold is planned with different channel dimension and combination with human hepatocyte progenitors [126].

Solid Ground Curing (SGC)

Besides the classical laser-based SLA process, alternative processes using digital mask generators (e.g. liquid crystal displays or Digital Mirror Devices, DMD) have been used successfully to build structures out of polymers and ceramics. In the rapid prototyping literature, this process is also termed Solid Ground Curing (SGC). In contrast to traditional UV-laser based SLA machines, DMD systems are significantly cheaper and therefore more versatile in respect to material modifications. At the same time, DMD machines can expose a whole layer at once, whereas laser-based systems have to scan the contour of the object sequentially. DMD systems are based on a digital micro- mirror device. By projecting a bitmap onto the photosensitive resin, the liquid resin can be solidified selectively. Theoretically, DMD systems can be used to fabricate scaffolds with high resolution and geometric complexity. However, a prerequisite is the availability of a light curable biocompatible and bioresorbable polymer material.

Khetani and co-workers describe the development of a photopatterning technique that allows localized photoencapsulation of live mammalian cells to control the tissue architecture [127]. Cell viability was characterized using HepG2 cells, a human hepatoma cell line. The utility of this method was demonstrated by photopatterning hydrogels containing live cells in various single layer structures, patterns of multiple cellular domains in a single “hybrid” hydrogel layer and patterns of multiple cell types in multiple layers. The authors observed that UV exposure itself did not cause cell death over the doses and time scale studied, while the photoinitiator 2,2-dimethoxy-2-phenyl-acetophenone was itself cytotoxic in a dose-dependent manner. Furthermore, the combination of UV and photoinitiator was the least biocompatible condition presumably due to formation of toxic free radicals.

8.1.3.2. Three-dimensional Printing)

Three dimensional printing (3DP) technology (Figure 9f) was developed at the Massachusetts Institute of Technology [128]. 3DP is used to create a solid object by ink-jet printing a binder into selected areas of sequentially deposited layers of powder. Each layer is created by spreading a thin layer of powder over the surface of a powder bed. The powder bed is supported by a piston which descends upon powder spreading and printing of each layer (or, conversely, the ink jets and spreader are raised after printing of each layer and the bed remains stationary). Instructions for each layer are derived directly from a CAD representation of the component. The area to be printed is obtained by computing the area of intersection between the desired plane and the CAD representation of the object. The individual sliced segments or layers are joined to form the three dimensional structure. The unbound powder supports temporarily unconnected portions of the component as the scaffold is built but is removed after completion of printing.

The solvent drying rate is an important variable in the production of scaffolds by 3DP. Very rapid drying of the solvent tends to cause warping of the printed component. Much, if not all, of the warping can be eliminated by choosing a solvent with a low vapour pressure. Thus, PCL parts prepared by printing chloroform have nearly undetectable amounts of warpage, while large parts made with DCM exhibit significant warpage. It has been found that it is often an advantage to combine solvents to achieve minimal warping and adequate bonding between the biomaterials particles. Thus, an aggressive solvent can be mixed in small proportions with a solvent with lower vapour pressure. After the binder has dried in the powder bed, the finished component can be retrieved and unbound powder removed for post processing, if necessary.

The 3DP process is capable of overcoming the limitations of some SFF techniques in manufacturing certain designs, such as overhanging structures. The solution lies in the layering of powders. As the layers are spread, there is always a supporting platform of powder for printing and binding to take place. Thus, as long as the parts are connected together, overhanging structures are of no difficulty. However, one drawback of the powder supported and powder-filled structure is that the open pores must be able to allow the internal unbound powders to be removed, if the part is designed to be porous such as a scaffold for tissue engineering applications. The surface roughness and the aggregation of the powdered materials also affect the efficiency of removal of trapped materials. The resolution of the printer is limited by the specification of the nozzle size and position control of the position controller that defines the print head movement. Another factor is the particle size of the powder used, which simultaneously determines the layer thickness. A layer thickness between 100-400μm can be achieved depending on the printer. The versatility of using a powdered material is both an advantage and a constraint of the 3DP process. Most of the available biomaterials do not come in the powder form and need special processing conditions to get a powder which fulfils the requirements for 3DP. Despite the above discussed restrictions, 3DP has been explored by several tissue engineering groups for more than a decade and a spin-off from MIT (Therics Inc., MA, USA) has commercialized scaffolds made by 3DP.

More recently, several groups were able to print cells in combination with hydrogels by simple modification of office ink jet printers showing the proof-of-principle to one day have the capacity to build tissue engineered constructs in a fully automated system [129-131].

8.1.3.3. Systems Based on Extrusion/ Direct Writing

A number of groups have developed SFF machines that can perform extrusion of strands/filaments and/or plotting of dots in 3D with or without incorporation of cells [132, 133]. These systems are built to make use of a wide variety of polymer hot-melts as well as pastes/slurries (i.e. solutions and dispersions of polymers and reactive oligomers). Techniques such as FDM, 3D-Plotting, Multiphase Jet Solidification and Precise Extrusion Manufacturing all employ extrusion of a material in a layered fashion to build a scaffold. Depending on the type of machine, a variety of biomaterials can be used for scaffold fabrication.

Fused deposition modeling

A traditional fused deposition modeling (FDM) machine (Figure 9i) consists of a head-heated-liquefier attached to a carriage moving in the horizontal x-y plane. The function of the liquefier is to heat and pump the filament material through a nozzle to fabricate the scaffold following a programmed-path which is based on CAD model and the slice parameters. Once a layer is built, the platform moves down one step in the z direction to deposit the next layer. Parts are made layer by layer with the layer thickness varying in proportion to the nozzle diameter chosen. FDM is restricted to the use of thermoplastic materials with good melt viscosity properties; cells or other theromosensitive biological agents cannot be encapsulated into the scaffold matrix during the fabrication process.

Park *et al* have fabricated highly functionalized polymeric three-dimensional structures characterized by nano and microfibres for use as an extracellular matrix-like tissue engineering scaffold. A hybrid process utilizing direct polymer melt deposition (DPMD) and

an electrospinning method were employed together to obtain the structure. As depicted schematically in Figure 10. Each microfibrous layer of the scaffold was built using the DPMD process in accordance with computer-aided design modelling data considering some structural points such as pore size, pore interconnectivity and fibre diameter. Between the layers of the three-dimensional structure, PCL/collagen nanofibre matrices were deposited via an electrospinning process. To evaluate the fabricated scaffolds, chondrocytes were seeded and cultured within the developed scaffolds for 10 days, and the levels of cell adhesion and proliferation were monitored. The results showed that the polymeric scaffolds with nanofibre matrices fabricated using the proposed hybrid process provided favorable conditions for cell adhesion and proliferation. These conditions can be attributed to enhanced cytocompatibility of the scaffold due to surface nanotopography in the scaffold, chemical composition by use of a functional biocomposite, and an enlarged inner surface of the structure for cell attachment and growth [134].

A variation of FDM process, the so called “Precision Extruding Deposition” (PED) system, was developed and tested at Drexel University [135]. The major difference between PED and conventional FDM is that the scaffolding material can be directly deposited without filament preparation. Pellet formed PCL is fused by a liquefier temperature provided by two heating bands and respective thermal couples and is then extruded by the pressure created by a turning precision screw [136].

A design limitation of using an extrusion system in combination with thermoplastic polymers includes the fact that the pore openings for the scaffolds are not consistent in all three dimensions as observed. The pore openings facing the z-direction are formed in between the intercrossing of material struts/bars and are determined by user-defined

parameter settings. However, for pore openings facing both the x- and y-directions, these openings are formed from voids created by the stacking of material layers and hence, their sizes are restricted to the bar/strut thickness (diameter). As such, systems with a single extrusion head/liquefier do not exhibit variation in pore morphology in all 3 axes. A design variability exists by extruding one strut/bar directly on top of each other [136].

Direct writing

In the material science literature another term for extrusion based systems is used, namely "Direct-write techniques" (Figure 9j). The techniques employed in direct writing are pertinent to many other fields next to scaffold fabrication such as the capability of controlling small volumes of liquid accurately. Direct-write techniques involving colloidal ink containing PCL can be divided into two approaches: droplet-based including direct ink-jet printing, hot-melt printing and continuous (or filamentary) techniques.

8.1.4. Surface Modification of PCL

The purpose of surface modification is to retain the key bulk properties of the material while modifying the surface to improve biocompatibility. Typically, modifications can be either chemical or physical and can alter the compounds, or molecules on the existing surface, or may be achieved via coating the existing surface with a different material [137].

To functionalize PCL for bioconjugation, a chemical vapor deposition (CVD) polymerisation technique was utilized by Hu *et al* to modify material surfaces. Poly [(4-amino-p-xylylene)-co-(p-xylylene)] (PPX-NH2) was deposited on inert PCL surfaces to provide a reactive amine layer on the substrate surfaces. The biocompatibility of PPX-NH2 was evaluated with cells continuously proliferated on CVD treated PCL surfaces with high

survival rates. Biotin was conjugated on modified PCL surfaces to immobilize avidin for binding of biotinylated adenovirus. Scanning electron microscopy (SEM) examination illustrated that adenoviruses were evenly bound on both 2D films and 3D scaffolds; suggesting CVD was capable of modifying various substrates with different geometries. Using a wax masking technique, the biotin conjugation was controlled to immobilize avidin on specific sites. Due to the virus binding specificity on CVD-modified surfaces, cell transduction was restricted to the pattern of immobilized virus on biomaterials, by which transduced and non-transduced cells were controlled in different regions with a distinct interface. Because CVD was functional in different hierarchies, this surface modification should be able to custom-tailor bioconjugation for different applications [138].

Kim *et al* synthesised biocompatible photothermal agents using gold nanorod-embedded polymeric nanoparticles, which were synthesized using a nanoprecipitation method. Uniform gold nanorods which were sensitive to a photothermal effect by near-infrared were synthesized by a seed-mediated growth method. The hydroxyl groups of PCL diol were modified by esterification with mercaptopropionic acid to give PCL dithiol as a phase transfer and capping agent. Subsequently, hexadecyltrimethylammonium bromide was exchanged and/or removed by PCL dithiol. PCL dithiol-coated gold nanorods were further wrapped in a hydrophilic polymer, Pluronic F127, as a stabilizer. These newly formulated gold nanorods exhibited excellent stability in water and a maximum absorbance in the NIR region indicating a highly efficient surface plasmon resonance effect, phenomena useful for photothermal agents [139].

NaOH-treated PCL films are also investigated by Serrati *et al* for vascular tissue engineering by supporting the culture of primary vascular cells and endothelial-like EC2 cells

derived from endothelial progenitor cells. Results obtained demonstrated that EC2 seeded on NaOH-treated PCL films enhance the basal NO levels and showed a faster, more intense response to physiological stimuli such as VEGF, bradykinin and thrombin than vein endothelial cells. This could be indicative of a better capacity of EC2 cells to maintain their endothelial functionality when seeded on these polymers and reinforced the idea that endothelial-like EC2 cells derived from blood progenitors are an adequate source of endothelial cells to functionalize vascular grafts. Furthermore, NaOH-treated PCL films could be considered as a promising cellular NO production-inducing biomaterial for vascular tissue engineering applications [140].

PCL has been surface modified to possess a bone-like apatite layer bound to its surface as a scaffold for tissue engineering applications. The surface of PCL was treated with aqueous NaOH to introduce carboxylate groups onto the surface and was subsequently dipped in aqueous $CaCl_2$ and $K_2HPO_4{\cdot}3H_2O$ to deposit apatite nuclei on the surface. The surface-modified material successfully formed a dense and uniform bone-like surface apatite layer after incubation for 24 h in simulated body fluid with ion concentrations approximately equal to those of human blood plasma [141]. Yu *et al* also produced nanofibrous bone-like apatite-covered PCL using a similar technique. The surface of the mineralized PCL nanofibre was observed to be almost fully covered with nanocrystalline apatites and through mineralization, the wettability of the nanofibre matrix was greatly improved. Murine-derived osteoblastic cells were shown to attach and grow actively on the apatite-mineralized nanofibrous substrate. In particular, the mineralized PCL nanofibrous substrate significantly stimulated the expression of bone-associated genes, including Runx2, collagen type I, alkaline phosphatase, and osteocalcin, when compared with the pure PCL nanofibre substrate without mineralization [142].

Proteins that contain the Arg-Gly-Asp (RGD) attachment site, together with the integrins that serve as receptors for them, constitute a major recognition system for cell adhesion to surfaces. The RGD sequence is the cell attachment site of a large number of adhesive ECM, blood, and cell surface proteins and various groups have investigated the immobilization of this sequence onto potential implants/scaffolds. *Zhang et al* established a simple method to immobilize the RGD peptide on PCL film surfaces that significantly improved bone marrow stromal cell adhesion to these films. They extended their modification strategy to three-dimensional PCL scaffolds to investigate cell responses to the modified RGD-PCL scaffolds compared to responses to the untreated ones. The results demonstrated that treatment of 3D PCL scaffold surfaces with 1,6-hexanediamine introduced the amino functional groups onto the porous PCL scaffold homogenously and followed by the cross-linking reaction, RGD peptide was successfully immobilized on the PCL surface. Although the static seeding method used in this study caused heterogeneous cell distribution, the RGD-modified PCL scaffold still demonstrated the improved BMSC attachment and cellular distribution in the scaffold. More importantly, the integrin-mediated signal transduction FAK-PI3K-Akt pathway was significantly up-regulated by RGD modification and a subsequent increase in cell survival and growth was found in the modified scaffold. The study concluded that modification of 3D PCL scaffolds with RGD peptides elicits specific cellular responses and improves the final cell-biomaterial interaction [143].

Tissues engineered from biological cell sheets often lack substrate cues and possess poor mechanical strength hence Chong *et al* used micro-thin, biaxially-stretched PCL with surface modifications for layered tissue engineering. Polyacrylic acid was grafted onto PCL film surfaces by low-pressure plasma immobilisation which provided a surface suitable for

perivascular cells, forming the medial compartment of a biphasic cell-sheet. Subsequently, endothelial progenitor cell-selective CD34 antibody was conjugated onto the reverse surface (intimal compartment) to select and anchor endothelial progenitor cells for improved adhesion and proliferation. Using the blood vessel as a model, a biphasic culture system was then setup to represent a tunica intima (endothelial cells) and tunica media (smooth muscle cells). When suitable cell types were cultured in the corresponding compartments, confluent layers of the respective populations were achieved distinctively from each other. These results demonstrate the use of micro-thin, biaxially-stretched PCL films with cell-selective modifications for layered co-cultures towards the generation of stratified tissue [144].

Lu *et al* used coaxial electrospinning to immobilize bioactive agents into PCL fibres. Gelatin was cationized by derivation with N,N-dimethylethylenediamine and was used as a shell material for constructing a core-shell fibrous membrane. PCL formed the core section of the core-shell fibres thereby improving the mechanical properties of nanofibrous hydrogel. The outer layer was crosslinked by exposing the membranes in glutaraldehyde vapor. The adsorption behaviors of FITC-labeled bovine serum albumin (FITC-BSA) or FITC-heparin onto the fibres were then investigated. The core-shell fibres could effectively immobilize the two types of agents under mild conditions. Furthermore, vascular endothelial growth factor could be conveniently impregnated into the fibres through specific interactions with the adsorbed heparin in the outer CG layer. Sustained release of bioactive VEGF was also achieved for more than 15 days [145].

There are a vast array of manufacturing techniques to create scaffolds for tissue engineering, but one must pay special attention to the scaffold specifications and understand the huge interplay of factors affecting the design criteria. The desirable feature of any

implantable polymeric scaffold material would be synchronization of polymer degradation with the replacement by natural tissue produced from cells as previously described and illustrated graphically in Figure 8. In the following section the tissue engineering areas in which PCL scaffolds have been applied are comprehensively discussed.

8.2. Bone Engineering

Potential bone tissue scaffolds are under investigation by Thomas *et al* who have undertaken a mechanical and morphological study of electropun, aligned PCL nanofibre meshes produced at different collector rotation speeds (0, 3000, 6000 rpm). They noted higher alignments and tensile strengths with increasing rotation speeds, whereas, conversely, they showed lower hardness and young's modulus with increasing rotation speeds. These properties were attributed, microscopically and macroscopically to the crystallinity of the fibres and better alignment/tighter packing density. This study highlights the need to investigate individual parameters of a production process to gain optimised mechanical properties for a specific application [146].

Choi *et al* electrospun PCL/collagen nanofibres of different orientations, to engineer functional muscle tissue for restoring large skeletal muscle tissue defects. Human skeletal muscle cells were cultured on these substrates. Unidirectionally oriented nanofibres significantly induced muscle cell alignment and myotubule formation compared to random orientations of nanofibres [124].

PCL cylindrical scaffolds with gradually increasing pore size along the longitudinal direction were fabricated by a novel centrifugation method to investigate pore size effect on cell and tissue interactions. The scaffold was fabricated by the centrifugation of a cylindrical

mould containing fibril-like PCL and the following fibril bonding by heat treatment as depicted schematically in Figure 11 [147]. The scaffold showed gradually increasing pore size (from ~88 to ~405μm and porosity (from ~80% to ~94%) along the cylindrical axis by applying the centrifugal speed, 3000 rpm. The scaffold sections were examined for their *in vitro* cell interactions using different kinds of cells (chondrocytes, osteoblasts, and fibroblasts) and *in vivo* tissue interactions using a rabbit model (skull bone defects) in terms of scaffold pore sizes. It was observed that different kinds of cells and bone tissue were shown to have different pore size ranges in the scaffold for effective cell growth and tissue regeneration. The scaffold section with 380-405μm pore size showed better cell growth for chondrocytes and osteoblasts, while the scaffold section with 186-200μm pore size was better for fibroblasts growth. Also the scaffold section with 290-310 μm pore size showed faster new bone formation than those of other pore sizes. The pore size gradient scaffolds fabricated by this centrifugation method might be considered a good tool for the systematic studies of interactions between cells or tissues and scaffolds with different pore sizes [147].

Composite scaffolds with well-organized architecture and multi-scale porosity for achieving a tissue engineered construct to reproduce the middle and long-term behaviour of hierarchically complex tissues such as spongy bone has been investigated utilising fibre-reinforced composites. Scaffolds comprising PLLA fibres embedded in a porous PCL matrix were obtained by synergistic use of phase inversion/particulate leaching technique and filament winding technology. Porosities of up to 80% were achieved, with pore sizes of between 10 and 200 μm diameter. *In vitro* degradation was carried out in PBS without significant degradation of the scaffold after 35 days, whilst in NaOH solution, a linear increase of weight lost was observed with preferential degradation of the PLLA component. Upon cell seeding, marrow stromal cells and human osteoblasts reached a plateau at 3 weeks,

and at 5 weeks the number of cells was almost the same. Human marrow stromal cells and trabecular osteoblasts rapidly proliferated on the scaffold up to 3 weeks, promoting an oriented migration of bone cells along the fibre arrangement. The novel PCL/PLLA composite scaffold was concluded to show promise whenever tuneable porosity, controlled degradability and guided cell-material interaction are simultaneously required [148].

A bioactive and bioresorbable scaffold fabricated from medical grade PCL (mPCL) and incorporating 20% beta-tricalcium phosphate (mPCL-TCP) which has been well characterised studied by Hutmacher *et al*, has been further developed for bone regeneration at load bearing sites by Abbah *et al* [149]. Bone in-growth into mPCL-TCP in a large animal model of lumbar interbody fusion was evaluated using six pigs, each undergoing a 2-level (L3/4; L5/6) anterior lumbar interbody fusion and implanted with mPCL-TCP scaffolds +0.6mg rhBMP-2 as treatment group while four other pigs implanted with autogenous bone graft, which served as control. μCT scanning and histology revealed complete defect bridging in all (100%) specimens from the treatment group as early as 3 months. Histological evidence of continuing bone remodelling and maturation was observed at 6 months. In the control group, only partial bridging was observed at 3 months and only 50% of segments in this group showed complete defect bridging at 6 months. Furthermore, 25% of segments in the control group showed evidence of graft fracture, resorption and pseudoarthrosis. In contrast, no evidence of graft fractures, pseudoarthrosis or foreign body reaction was observed in the treatment group. These results reveal that mPCL-TCP scaffolds could act as bone graft substitutes by providing a suitable environment for bone regeneration in a dynamic load bearing setting such as in a porcine model of interbody spine fusion [149].

Bone research at the National University of Singapore based on medical grade PCL both *in vitro* and *in vivo* was commercialised after clinical approval in 2008 (Osteopore™).

Artlelon® is a unique patented biomaterial comprising polycaprolactone-based polyurethane which acts as a temporary support to the healing tissue; approximately half of the implant is comprised from PCL. Artelon® can be made to fibres, scaffolds and films and be used in a number of orthopedic applications and in other therapy areas. The hydrolysis results in a resorbable (PCL) and a non-resorbable poly(urethane-urea) fraction. The degradation products have been proven to be safe and tissue compatible and do not generate an acidic environment. When the hydrolysis of the ester bonds from the PCL component is completed about 50% of the initial mass remains at the implantation site and the remaining material is incorporated in the surrounding host tissue without eliciting any inflammatory or foreign body response [150]. The Artelon® CMC Spacer Arthro is a T-shaped device, where vertical portion separates the trapezial and the metacarpal bone of the carpometacarpal joint.

8.3. Cartilage Engineering

Cartilage degeneration caused by congenital abnormalities or disease and trauma is of great clinical consequence, given the limited intrinsic healing potential of the cartilage tissue. The lack of blood supply and subsequent wound-healing response, damage to cartilage alone, or chondral lesions, leads to an incomplete attempt at repair by local chondrocytes. Full-thickness articular cartilage damage, or osteochondral lesions, allow for the normal inflammatory response, but result in inferior fibrocartilage formation. To prevent progressive joint degeneration in diseases such as osteoarthritis, surgical intervention is often the only option. In spite of the success of total joint replacement, treatments for repair of cartilage damage are often less than satisfactory, and rarely restore full function or return the tissue to

its native normal state. Tissue engineering aims to address this challenge through development of a biocompatible, structurally and mechanically sound scaffold, with an appropriate cell source, which may be loaded with bioactive molecules that promote cellular differentiation and/or maturation[151]

Huang *et al* developed a biphasic implant made comprising PCL with TGF-β1-loaded fibrin glue to determine whether the implant could recruit mesenchymal cells and induce the process of cartilage formation when implanted in ectopic sites. PCL scaffolds loaded with various doses of TGF-β1 in fibrin glue were implanted subcutaneously, intramuscularly, and subperiosteally and assessed histologically 2, 4, and 6 weeks postoperatively. The entire pore spaces of the scaffolds were filled with various tissues in each group. The entire volume of the scaffolds in the groups loaded with TGF-β1 and implanted intramuscularly and subcutaneously was populated with mesenchymal cells surrounded with an abundant extracellular matrix and blood vessels. The scaffold loaded with TGF-β1 and implanted subperiosteally was found to be richly populated with chondrocytes at 2 and 4 weeks and immature bone formation was identified at 6 weeks. The study concluded that scaffolds loaded with TGF-β1 could successfully recruit mesenchymal cells and that chondrogenesis occurred when this construct was implanted sub-periosteally [152].

Nanofibrous materials, by virtue of their morphological similarities to natural extracellular matrix, have been considered as candidate scaffolds for cell delivery in tissue-engineering applications. Several studies have used electrospinning techniques to fabricate nanofibrous materials for cartilage tissue engineering applications [153] [154].

Three-dimensional, nanofibrous PCL scaffolds were assessed by Li *et al* for their ability to maintain chondrocytes in a mature functional state. Fetal bovine chondrocytes (FBCs) were seeded onto three-dimensional biodegradable PCL nanofibrous scaffolds or as monolayers on standard tissue culture polystyren as a control substrate. Gene expression analysis showed that chondrocytes seeded on the nanofibrous scaffold and maintained in serum-free medium supplemented with ITS+, ascorbate, and dexamethasone continuously maintained their chondrocytic phenotype by expressing cartilage-specific extracellular matrix genes, including collagen types II and IX, aggrecan, and cartilage oligomeric matrix protein. Specifically, expression of the collagen type IIB splice variant transcript, which is indicative of the mature chondrocyte phenotype, was up-regulated. FBCs exhibited either a spindle or round shape on the nanofibrous scaffolds, in contrast to a flat, well-spread morphology seen in monolayer cultures on tissue culture polystyrene. Histologically, nanofibrous cultures maintained in the supplemented serum-free medium produced more sulfated proteoglycan-rich, cartilaginous matrix than monolayer cultures. In addition to promoting phenotypic differentiation, the nanofibrous scaffold also supported cellular proliferation as evidenced by a 21-fold increase in cell growth over 21 days when the cultures were maintained in serum-containing medium. These results indicated that the biological activities of FBCs are crucially dependent on the architecture of the extracellular scaffolds as well as the composition of the culture medium, and that nanofibrous PCL acts as a biologically preferred scaffold/substrate for proliferation and maintenance of the chondrocytic phenotype thus being a suitable candidate scaffold for cartilage tissue engineering [155].

Wise *et al* electrospun oriented PCL scaffolds (500 or 3000nm fibre diameter) onto which they seeded human mesenchymal stem cells. Cell viability, morphology, and orientation on the fibrous scaffolds were quantitatively determined as a function of time.

While the fibre-guided initial cell orientation was maintained even after 5 weeks, cells cultured in the chondrogenic media proliferated and differentiated into the chondrogenic lineage, suggesting that cell orientation is controlled by the physical cues and minimally influenced by the soluble factors. Based on assessment by chondrogenic markers, they concluded that use of the nanofibrous scaffold (500 nm) enhanced chondrogenic differentiation and indicate that hMSCs seeded on a controllable PCL scaffold may lead to an alternate methodology to mimic the cell and ECM organization [156].

To evaluate the repair potential in large osteochondral defects on high load-bearing sites, Shao *et al* fabricated a hybrid scaffold system which comprised 3D porous PCL scaffold for the cartilage component and tricalcium phosphate-reinforced PCL scaffold for the bone portion. Osteochondral defects of 4-mm diameter X 5.5- mm depth were created in the medial femoral condyle of adult New Zealand White rabbits. The defects were treated with hybrid scaffolds without cells (control group) or seeded with allogenic bone marrow - derived mesenchymal stem cells (BMSC) in each part (experimental group) by press-fit implantation. Implanted cells were tracked using Adeno-LacZ labelling and tissues were evaluated at 3 and 6 months after implantation. Overall, the experimental group showed superior repair results as compared to the control group using gross examination, qualitative and quantitative histology, and biomechanical assessment. With BMSC implantation, the hybrid scaffolds provided sufficient support to new osteochondral tissue formation and the bone regeneration was consistently good from 3 to 6 months, with firm integration to the host tissue. Cartilage layer resurfacing was considered more complicated with all of the samples presenting cartilage tissues mixed with PCL scaffold filaments at 3 months. Histology at 6 months revealed some degradation phenomenon in several samples whereas others had a good appearance; however, the Young's moduli from the experimental group (0.72 MPa)

approached that of normal cartilage (0.81 MPa). *In vivo* viability of implanted cells was demonstrated by the retention for 6 weeks in the scaffolds. The authors concluded that this study indicated the promise of PCL-based hybrid scaffolds seeded with BMSC as an alternative treatment for large osteochondral defects in high-loading sites [157].

In another study, Li *et al* evaluated cell-seeded nanofibrous PCL scaffolds for cartilage repair *using* 7 mm full-thickness cartilage defects in a swine model. They utlised allogeneic chondrocytes or xenogeneic human mesenchymal stem cells (MSCs), with acellular PCL scaffolds and empty defects as control groups. Six months after implantation, MSC-seeded constructs showed the most complete repair in the defects compared to other groups. Macroscopically, the MSC-seeded constructs regenerated hyaline cartilage-like tissue and restored a smooth cartilage surface, while the chondrocyte-seeded constructs produced mostly fibrocartilage-like tissue with a discontinuous superficial cartilage contour. Incomplete repair containing fibrocartilage or fibrous tissue was found in the acellular constructs and the empty defects. Quantitative histological evaluation showed overall higher scores for the chondrocyte- and MSC-seeded constructs than the acellular construct and the no-implant groups. Mechanical testing showed the highest equilibrium compressive stress of 1.5 MPa in the regenerated cartilage produced by the MSC-seeded constructs, compared to 1.2 MPa in the chondrocyte-seeded constructs, 1.0 MPa in the acellular constructs and 0.2 MPa in the no-implant group. No evidence of immune reaction to the allogeneically- and xenogeneically-derived regenerated cartilage was observed, possibly related to the immunosuppressive activities of MSCs, suggesting the feasibility of allogeneic or xenogeneic transplantation of MSCs for cell-based therapy. The collective summary from this work was to show that biodegradable nanofibrous scaffolds seeded with MSCs could effectively repair cartilage defects in vivo, and that this approach is promising for cartilage repair [158].

A composite scaffold comprising a PCL stent and a type II collagen sponge for tissue-engineered trachea was developed by Lin *et al*. The PCL stent had surface grooves which were filled by the type II collagen with crosslinking treatment producing a ring-shaped collagen sponge. Rabbit chondrocytes ($3x10^6$ cells per ring) were seeded onto the collagen sponge of the scaffold and the cell-scaffold constructs were then implanted subcutaneously in the dorsum of nude mice. After 4 and 8 weeks, constructs were harvested and analysed for mechanical properties, histology, and biochemical assays. Constructs were strong enough to retain their tubular shape against extrinsic forces in the dorsum of nude mice and the gross appearance of the constructs revealed cartilage-like tissue at 8 weeks, with a modulus higher than that of native trachea. Histological and biochemical analyses of the tissue-engineered tracheal cartilage revealed evenly spaced lacunae embedded in the matrix, with abundant proteoglycans and type II collagen. The stent-sponge composite facilitated the proliferation of chondrocytes and was expected to provide adequate mechanical strength in possible future applications in trachea tissue engineering [159].

Meniscal tissue engineering has been investigated using hyaluronic acid/PCL to evaluate the tissue regeneration after the augmentation of the implant with expanded autologous chondrocytes. Twenty-four skeletally mature sheep were treated with total medial meniscus replacements, while two meniscectomies served as empty controls. The animals were divided into two groups: cell-free scaffold and scaffold seeded with autologous chondrocytes. Two different surgical techniques were compared: in 12 animals, the implant was sutured to the capsule and to the meniscal ligament; in the other 12 animals, also a trans-tibial fixation of the horns was used. The animals were euthanized after 4 months and specimens were assessed by gross inspection and histology. All implants showed excellent

capsular in-growth at the periphery. Macroscopically, no difference was observed between cell-seeded and cell-free groups. Better implant appearance and integrity was observed in the group without transosseous horns fixation. Using the latter implantation technique, lower joint degeneration was observed in the cell-seeded group with respect to cell-free implants. Histological analysis indicated cellular infiltration and vascularization throughout the implanted constructs with cartilaginous tissue formation being significantly more frequent in the cell-seeded constructs. The study supports the potential of a novel hyaluronic acid /PCL scaffold for total meniscal substitution, and furthermore, seeding of the scaffolds with autologous chondrocytes provided some benefit in the extent of fibrocartilaginous tissue repair [160].

8.4. Tendon & Ligament Engineering

Tendon reconstruction with PCL films using a rat model has been attempted with good functional recovery. PCL films were prepared by solvent casting and used for repair of gaps in Achilles tendons in a rat model. Five groups were studied; (i) sham operated (skin incision only); (ii) no repair (complete division of the Achilles tendon and plantaris tendon without repair); (iii) Achilles repair (with a modified Kessler type suture); and (iv) plasty of Achilles tendon defects with the biodegradable PCL films, and (v) animals subjected to 1cm, mid-substance defect with no repair. Functional performance was determined from the measurements of hindpaw prints utilizing the Achilles functional index. The animals were euthanized 8 weeks after surgery and histological and biomechanical evaluations were made. All groups subjected to Achilles tendon division had a significant functional impairment that gradually improved so that by day 28 there were no functional impairments in any group whereas animals with a defect remained impaired [146].

Artelon® Tissue Reinforcement/SportMesh™ are patches comprising PCL-based polyurethane which is sutured over ruptured tissue as reinforcement when repairing a torn tendon. The degradable Artelon® implant maintains its strength and elasticity over several years providing long term support of the soft tissue while being a scaffold for tissue in-growth and remodeling. The implant has been shown to partially degrade. The remaining material is incorporated into the patient's surrounding tissue, and intended to strengthen weak or repaired tissue [150].

Mesofol® is a transparent, absorbable film that can be inserted between muscles, muscles and tendons or nerves to prevent postoperative adhesion. The basic materials of the medical device are lactide and caprolactone. Mesofol® can also take over the fascia's sliding function, improving the physiologic free gliding and thus reducing postoperative pain and preventing negative effects due to tissue conglutination [161].

8.5. Cardiovascular engineering

The main failings of presently available mechanical or biological valve prostheses are thrombogenicity and poor durability. Tissue engineering has being used to provide a valve that does not have these disadvantages and is able to grow, repair, and remodel. Most of the replaced valves are mitral or aortic, so tissue engineering of the aortic valve is a challenge that, once met, will lead to a very useful product. To create a tissue-engineered aortic valve, a strong scaffold is a prerequisite. The scaffold on which cells are seeded is important in giving the specific valvular shape to the growing tissue, guiding the development of the tissue and providing support against mechanical forces. Since the opted tissue is ideally completely autologous, the scaffold should be biodegraded, leaving cells and extracellular matrix components that have developed into fully autologous tissue.

Van Lieshout *et al* developed two types of scaffolds for tissue engineering of the aortic valve; an electrospun valvular scaffold and a knitted valvular scaffold. These scaffolds were compared in a physiologic flow system and in a tissue-engineering process. In fibrin gel enclosed human myofibroblasts were seeded onto both types of scaffolds and cultured for 23 days under continuous medium perfusion. Tissue formation was evaluated by confocal laser scanning microscopy, histology and DNA quantification. Collagen formation was quantified by a hydroxyproline assay. When subjected to physiologic flow, the spun scaffold tore within 6 h, whereas the knitted scaffold remained intact. Cells proliferated well on both types of scaffolds, although the cellular penetration into the spun scaffold was poor. Collagen production, normalized to DNA content, was not significantly different for the two types of scaffolds, but seeding efficiency was higher for the spun scaffold, because it acted as a cell impermeable filter. The knitted tissue constructs showed complete cellular in-growth into the pores. They concluded that an optimal scaffold seems to be a combination of the strength of the knitted structure and the cell-filtering ability of the spun structure [123, 162]. Figure 12 shows the PCL knitted valve and a porcine stent-less valve prosthesis inside a physiological flow simulator.

Gene therapy approaches to treat heart conditions have also utlised PCL as a release reservoir in a similar fashion to micro/nanosphere drug delivery systems. Wang *et al* developed a gene therapy system to treat hypertension and/or congestive heart failure based on the release of atrial natriuretic peptide (ANP) from ANP-cDNA-transfected Chinese Hamster Ovary cells which had been encapsulated within PCL tubes. The encapsulated cells remained viable during culture and ANP secretion was maintained for at least 6 months [163].

Electrospun sheets comprising PCL with different fibre diameters (3-12 μm) were investigated for penetration depth using human venous myofibroblastsas as a means to optimize cell delivery during cardiovascular tissue engineering applications. Optimal cell delivery was observed using the largest diameter fibres (12 μm) highlighting the importance of optimizing the electrospun scaffold geometry for specific cell types [164].

Shape memory materials have been proposed for cardiovascular stents due to their self-expansion ability. The most ideal way to anchor a stent is using self-expansion in the range of body temperature. Ajili *et al* use have utilised a polyurethane/PCL blend as a proposed material for shape memory stents. Polyurethane copolymer based on PCL diol was melt blended with PCL in four different ratios of 20, 30, 40 and 50 wt.% and their shape memory behaviors were examined. All blends except for 80/20 showed shape memory effects with recovery temperatures of around the melting temperature of PCL in the blends. The melting behavior of the PCL in the blends was strongly influenced by composition. Changing the composition of the blend system and crystallization conditions adjusted shape recovery to the range of body temperature for PU/PCL 70/30 blend. The *in vitro* biocompatibility of 70/30 blend was further evaluated using human bone marrow mesenchymal stem cells and assessing cell adhesion, morphology and mitochondrial function. The results showed that the blend supported cell adhesion and proliferation, which indicated good biocompatibility in addition to shape memory properties, providing potential use as a stent implant [165].

8.6. Blood Vessel Engineering

There has been significant research regarding the effects of scaffold surface chemistry and degradation rate on tissue formation and the importance of these parameters is widely

recognised. In addition to these important factors are considerations of elastic properties. Several studies describing the role of mechanical stimuli during tissue development and function suggest that the mechanical properties of the scaffold are also important. Cyclic mechanical strain has been demonstrated to enhance the development and function of engineered smooth muscle tissues, and it would be necessary for the development of elastic scaffolds if one wishes to engineer smooth tissues under cyclic mechanical loading. Biodegradable polyesters, such as PGA, PLA and PLGA, although commonly used in tissue engineering, undergo plastic deformation and failure when exposed to long-term cyclic strain, limiting their use in engineering elastomeric tissues and thus composites and copolymers have been investigated to circumvent this issue [166].

Lee *et al* developed an elastic polymer fabricated from poly(glycolide-co-caprolactone) (PGCL) using solvent casting and particle-leaching techniques. The demonstrated superior elastic properties (extension and recovery, permanent deformation) compared with PLGA equivalents with good promise as smooth muscle-containing tissue candidates [167].

Jeong *et al* have developed tubular, elastic biodegradable poly(lactide-co-caprolactone) (PLCL) for mechano-active vascular tissue engineering. They demonstrated very flexible, rubber-like elastic with 200% elongation and over 85% recovery in tension. Moreover, under cyclic mechanical strain conditions in culture media,they maintained their high elasticity. Vascular smooth muscle cells showed good cell adhesion and proliferation on the scaffolds, responding optimally to the porosities of 200-250 μm, compared with the lower ranges between 50 and 200 μm. The *in vivo* biocompatibility was also established in a subcutaneous mouse model. The conclusion was a promising composite/copolymer elastic

material for the engineering of smooth muscle containing tissues such as blood vessels under mechanically dynamic environments [167, 168].

In vivo vascular smooth muscle cells typically reside in mechanically dynamic environments, align in a specific direction and exist in a contractile, differentiated phenotype which is critical for the contractile functions of smooth muscle cells [169]. A drawback of conventional *in vitro* tissue engineering is the possibility of smooth muscle reverting to a non-differentiated phenotype (non aligned and non contractile). A number of studies have demonstrated that the addition of cyclic strains and pulsatile flow in 2D or 3D culture systems gives superior smooth muscle cell responses with enhanced mechanical strength and collagen/elastin production [170, 171]. For such a mechano-active system, the scaffold used must be able to deliver mechanical stresses to adhered cells and materials such as PGA have non-elastic properties and are unsuitable for these applications. Initial studies by Kim *et al* using PLA and PGA scaffolds resulted in significant permanent deformation under cyclic mechanical strain conditions [172]. They next developed a mechano-active polymer comprising 50 wt% hard domains of L-lactide units with 50 wt% soft domain of caprolactone moeities (PLCL), this copolymer system exhibited rubber-like elasticity and could be fabricated into scaffolds via a variety of techniques [168, 173-177]. The superior elastic properties of these scaffolds are depicted in Figure 13. PLCL scaffolds with a 90% porosity showed 100% recovery at near 100% strain (Figure 13a-c). Furthermore, PLCL scaffolds could be easily twisted and bent (Figure 13c-e), in contrast the PLGA scaffolds largely deformed and were broken at low strains (20%) (Figure 13f). The study concluded the scaffolds to be highly flexible and elastic and suitable for vascular tissue engineering applications. This PLCL mechano-active scaffold has also shown potential use in cartilage engineering applications [178-180].

A three-layered robust and elastic artery was fabricated by Iwasaki *et al* using PGA and PCL sheets seeded with endothelial cells, smooth muscle cells and fibroblasts followed by culture in a novel hemodynamically-equivalent pulsatile bioreactor. The seeded-sheets were wrapped on a 6-mm diameter silicone tube and incubated in culture medium for 30 days after which the supporting tube was removed. The pulsatile bioreactor culture, under regulated gradual increase in flow and pressure from 0.2 (0.5/0) L/min and 20 (40/15) mm Hg to 0.6 (1.4/0.2) L/min and 100 (120/80) mm Hg, was performed for an additional 2 weeks (n=10). The engineered vessels acquired distinctly similar appearance and elasticity as native arteries. Scanning electron microscopic examination and Von Willebrand factor staining demonstrated the presence of endothelial cells spread over the lumen. Elastin and collagen were seen in in the engineered grafts and smooth muscle cells were detected. Tensile tests demonstrated that engineered vessels acquired equivalent ultimate strength and similar elastic characteristics as native arteries (Ultimate Strength of Native: 882+/-133 kPa, Engineered: 827+/-155 kPa, each n=8). The small-diameter arteries produced from three types of vascular cells using the physiological pulsatile bioreactor were concluded to be robust and elastic [181].

Recently there has been research focus on sutureless tissue fusion, to handle the limits of conventional suturing such as vascular wall damage due to the penetrating needle, intraluminal foreign body reactions caused by non-absorbable suture material and thrombocyte aggregation, and impaired endothelial function. Sutured wounds also have greater and longer duration inflammatory response than laser soldered wounds. Furthermore suturing does not create a watertight connection, which can, for example, in visceral surgery lead to an entry for pathogens resulting in severe complications such as infections or death

Bregy *et al* investigated the use of fused diode laser soldering of vascular tissue using PCL scaffolds doped with bovine serum albumin (BSA) and Indocyanine green (ICG) which concluded with strong and reproducible tissue bonds, with vessel damage limited to the adventitia [182]. Other approaches to tissue adhesives include the development of biocompatible tissue adhesives that do not involve any chemical or biochemical reactions, during their application *in vivo*; their use being based exclusively on their temperature-dependent rheological properties. Branched oligomers consisting of a core molecule with biodegradable chains bound, and the relationship between their composition and their adhesive properties under *in vitro* conditions, was investigated by *Cohn* et al. The oligomers comprised trimethylolpropane as the trifunctional central molecule, while lactoyl and caprolactone units formed the biodegradable segments. Oligomers with glass transition temperatures, in the 20-25ºC range, were found to perform better. A strong connection was found between the length of the PLA blocks, the glass transition temperature of the different materials and their Adhesive Failure Strength at 37 ºC. The remarkable flexibilizing effect of the caprolactone units incorporated along the PLA blocks, allowed to generate longer biodegradable chains, and to improve, therefore, the adhesive strength of the oligomers, while keeping their Tg within the appropriate temperature interval [183].

Bennan *et al* evaluated the growth potential of a tissue-engineered autologous vascular graft for pediatric cardiothoracic surgery in a juvenile animal model. PGA nonwoven mesh tubes (3-cm length, 1.3-cm id; Concordia Fibres) coated with a 10% copolymer solution of 50:50 PLLA and PCL were statically seeded with 1 x 10^6 cells/cm^2 autologous bone marrow derived mononuclear cells and were implanted as inferior vena cava interposition grafts in juvenile lambs. After 6 months implantation, neotissue was characterized using qualitative histological and immunohistochemical staining and

quantitative biochemical analysis. All grafts were patent and increased in volume as measured by difference in pixel summation in magnetic resonance angiography. The volume of seeded grafts at explant averaged 126.9% +/- 9.9% of their volume at 1 month. Magnetic resonance imaging demonstrated no evidence of aneurysmal dilation. The tissue-engineered vascular graft resembled the native inferior vena cava histologically and had comparable collagen and glycosaminoglycan contents. Immunohistochemical staining and Western blot analysis showed that Ephrin-B4, a determinant of normal venous development, was acquired in the seeded grafts 6 months after implantation, collectively providing good evidence of growth and venous development when implanted in a juvenile lamb model [184].

8.6.1. Electrospinning in Blood Vessel Engineering

Multi-layering electrospinning techniques have been employed to develop a scaffold architecture mimicking morphological and mechanical features of a blood vessel (Figure 14). Bi-layered tubes comprising PLA/PCL were investigated and shown to support 3T3 mouse fibroblasts and human venous myofibroblasts which attached, proliferated and produced extracellular matrix over a 4 week culture period. The scaffolds also demonstrated desirable pliability (elastic up to 10% strain) [185].

Pektok and co-workers investigated the degradation and healing characteristics of small diameter PCL vascular grafts, produced via electrospinning, in the rat systemic arterial circulation. 2mm internal diameter grafts were produced and implanted for 24 weeks. Results concluded faster endothelialization and extracellular matrix formation, accompanied by degradation of graft fibres, compared with polytetrafluoroethylene grafts. The study concluded that healing characteristics of PCL may potentially lead to the clinical use of such grafts for revascularization procedures [186].

Scaffolds for vascular tissue engineering are limited by issues of inconsistency, poor adherence of vascular cells, or inadequate biomechanical properties. Studies using electrospun PCL/collagen scaffolds were shown to support adherence and growth of vascular cells under physiologic conditions and that endothelialized grafts resisted adherence of platelets when exposed to blood whilst maintaining their structural integrity and patency in a rabbit aortoiliac bypass model over 1 month of implantation. Further, at retrieval, these scaffolds continued to maintain biomechanical strength that was comparable to native artery, which indicates that electrospun PCL/collagen scaffolds combined with vascular cells may become an alternative to prosthetic vascular grafts for vascular reconstruction [187].

Electrospinning permits fabrication of biodegradable elastomers into matrices that can resemble similar mechanical properties as the native extracellular matrix. However, achieving high-cellular density and infiltration of scaffolds made from this technique remains challenging and time consuming. Stankus *et al* have overcome this limitation by electrospraying vascular smooth muscle cells (SMCs) concurrently with electrospinning a biodegradable, elastomeric poly(ester urethane)urea (PEUU). Trypan blue staining revealed no significant decrease in cell viability from the fabrication process and electrosprayed SMCs spread and proliferated similar to control unprocessed SMCs. PEUU was strong, flexible and anisotropic with tensile strengths ranging from 2.0 to 6.5 MPa and breaking strains from 850 to 1700% dependent on the material axis. The ability to microintegrate smooth muscle or other cell types into a biodegradable elastomer fibre matrix embodies a novel tissue engineering approach that could be applied to fabricate high cell density elastic tissue mimetics, blood vessels or other cardiovascular tissues [188].

8.7. Skin Engineering

Engineered human skin is often fabricated using collagen scaffolds which are associated with poor mechanical properties. Powell *et al* blended PCL with collagen before electrospinning the composite to form submicron fibres. Tensile testing indicated that the inclusion of PCL from 10-100% significantly improved the strength and stiffness of the acellular scaffold. However, epidermal formation and reduced cell viability was evident at when the PCL content was increased beyond 10% and there was an associated loss of engineered skin construct strength indicating that high cell viability and proper development of the epidermis and important factors for developing engineered skin with high strength [189].

Studies by Dai *et al* produced PCL/collagen composites for tissue engineered skin substitutes and demonstrated good cell attachment and proliferation of fibroblasts and keratinocytes [190]. They next utilised PCL/collagen blends as skin substitutes through seeding human single-donor keratinocytes and fibroblasts alone on both sides of the 1:20 biocomposite to allow for separation of two cell types and preserving cell signals transmission via micro-pores with a porosity of 28.8 ± 16.1 [mu]m. The bi-layered skin substitute exhibited both differentiated epidermis and fibrous dermis *in vitro*. Less Keratinocyte Growth Factor production was measured in the co-cultured skin model compared to fibroblast-alone condition indicating a favorable microenvironment for epidermal homeostasis. Moreover, fast wound closure, epidermal differentiation, and abundant dermal collagen deposition were observed in composite skin *in vivo* [191].

Composite tissue engineering has been investigated by Reed *et al*, using several cell-types (human foreskin fibroblasts, murine keratinocytes and periosteal cells) cultured

together on PCL nanofibres. The aim was to produce trilaminar constructs, to reflect a compound tissue, although clear obstacles exist in maintaining an appropriate interface between the tissue types and neovascularisaion of the composite structure [192].

A major challenge encountered in using electrospun scaffolds for tissue engineering is the non-uniform cellular distribution in the scaffold with increasing depth under normal passive seeding conditions. Because of the small surface pores, typically few microns in diameter, cells tend to congregate and proliferate on the surface much faster compared to penetrating the scaffold interior. In order to overcome this problem, Chen *et al* used a vacuum seeding technique on PCL electrospun scaffolds while using NIH 3T3 fibroblasts as the model cell system. This serves as a precursor to the bilayer skin model where the fibroblasts would be residing at an intermediate layer and the keratinocytes would be on the top. Vacuum seeding was used in this study to enhance fibroblasts seeding and proliferation at different depths. Results showed that the kinetics of cell attachment and proliferation were a function of varying vacuum pressure as well as fibre diameter. Cell attachment reached a maximum between 2-8 in. Hg vacuum pressure and fell for lower vacuum pressures presumably because of cell loss through the filtration process. Cell proliferation and collagen secretion over five days indicated that vacuum pressure did not affect cellular function adversely. The combined impact of scaffold architecture (400 nm versus 1 μm average fibre diameter of scaffolds) and vacuum pressure were also compared. At a given pressure, more cells were retained in the 400 nm scaffolds compared to 1μm scaffolds. In addition, the cell intensity profile shows cell intensity peak shift from the top to the inner layers of the scaffold by lowering the vacuum pressure from 0 to 20 in. Hg. For a given vacuum pressure the cells were seeded deeper within the 1100 nm scaffold. The results indicated that cells can be seeded in PCL electrospun scaffolds at various depths in a controlled manner using a simple

vacuum seeding technique. The depth of seeding was a function of both pressure and scaffold fibre diameter [193].

Ananta *et al* developed a biodegradable hybrid scaffold consisting of a PLA-co-PCL (PLCL), and collagen, which was constructed by plastic compressing hyperhydrated collagen gels onto a flat warp-knitted PLCL mesh. Neonatal (foreskin) fibroblasts were seeded inside and on top of the collagen component. The collagen compaction process was characterized, and it was found that the duration, rather than the applied load under the test conditions in the plastic compression, was the determining factor of the collagen and cell density in the cell-carrying component. Cells were spatially distributed in three different setups and statically cultured for a period of 7 days. Short-term biocompatibility of the hybrid construct was quantitatively assessed with AlamarBlue and qualitatively with fluorescence staining and confocal microscopy. No significant cell death was observed after the plastic compression of the interstitial equivalents, confirming previous reports of good cell viability retention. The interstitial, epithelial, and composite tissue equivalents showed no macroscopic signs of contraction and good cell proliferation was observed with a two- to threefold increase in cell number over 7 days. Quantitative analysis showed a homogenous cell distribution and good biocompatibility. The results indicate that viable and proliferating multilayered tissue equivalents can be engineered using the PLCL-collagen hybrid construct in the space of several hours. By suspending the cells homegenously in rapidly polymerising collagen gels, this allowed for the formation of a isotropic construct within the space of one hour as as opposed to weeks and has potential in *in vitro* tissue engineering of complex tissues such as skin, bladder, ureter and blood vessels [194].

8.8. Nerve engineering

During the 1990s Dendunnen and co-workers began evaluating PCL as a composite,

combined with PLLA in guided nerve regeneration [195]. Cytotoxicity tests, subcutaneous biodegradation and an *in situ* implantation studies in the sciatic nerve of the rat were undertaken. The nerve guide copolymer was found to be non-toxic, according to ISO/EN standards, and it showed a mild foreign body reaction and complete fibrous encapsulation after implantation. Onset of biodegradation of the inner layer was seen after one month of implantation. After 18 months of implantation complete fragmentation was observed, as well as a secondary inflammatory response characterized by foreign body giant cell activity and phagocytosis of polymer debris. Recovery of both motor and sensory nerve function was observed in all nerve guides. Further studies compared the speed and quality of nerve regeneration after reconstruction using the biodegradable nerve guide compared with an autologous nerve graft. A short nerve gap (1cm) was concluded to be better reconstructed using this composite compared with autologous nerve grafts, as evaluated using light microscopy, transmission electron microscopy and morphometric analysis [196, 197].

Electrospinning provides constructs with high surface to volume ratios which is attractive to cell attachment. Fibres may also be aligned to provide directionality cues to cells; it's no surprise that this technique has a large research focus in neuroscience. Kim *et al* looked at the role of aligned polymer fibre-based constructs in the bridging of long peripheral nerve gaps, demonstrating a significant role of sub-micron scale topographical cues in stimulating endogenous nerve repair mechanisms, depicted in Figure 15. Nisbet *et al* assessed axonal infiltration and guidance within neural tissue engineering scaffolds, made from PCL, and characterised of the inflammatory response. The extent of microglial and astrocytic response was measured following implantation of electrospun PCL scaffolds into the caudate putamen of the adult rat brain. No evidence of microglial encapsulation was found and neurites infiltrated the implants, evidence of scaffold-neural integration. Whilst the

inflammatory response was not influenced by the degree of PCL fibre alignment, the extent of neurite entry was. Large porosity, as was the case with the randomly orientated polymer fibres, enabled neurite infiltration and growth within the scaffold. However, neuronal processes could not penetrate scaffolds when fibres were partially aligned and instead, preferentially grew perpendicular to the direction of PCL fibre alignment at the implant-tissue interface i.e. perpendicular, not parallel, contact guidance was provided. This investigation highlighted that electrospun PCL fibres are compatible with brain tissue and provided preliminary insights regarding the influence of microglia and astrocytes in neural integration within such scaffolds [198].

PCL/collagen blends were investigated by Schnell *et al* as a conduit for axonal nerve regeneration after peripheral nerve injury. Aligned PCL and collagen/PCL nanofibres designed as guidance structures were produced by electrospinning and tested in cell culture assays. 100% PCL fibres were compared with 25:75% collagen/PCL blends. Both types of eletrospun fibres gave good Schwann cell migration, neurite orientation and process outgrowth, however Schwann cell migration, neurite orientation, and process formation of Schwann cells, fibroblasts and olfactory ensheathing cells were improved on collagen/PCL fibres compared to pure PCL fibres. While the velocity of neurite elongation from dorsal root ganglia explants was higher on PCL fibres, analysis of isolated sensory neurons showed significantly better axonal guidance by the collagen/PCL material. This study indicated that electrospun fibres comprising a collagen and PCL blend represents a suitable substrate for supporting cell proliferation, process outgrowth and migration and as such would be a good material for artificial nerve implants [199].

The Neurolac® Nerve Guide comprising PDDLA-co-PCL (65/35) is indicated for reconstruction of a peripheral nerve discontinuity and provides guidance and protection to regenerated axons and prevents ingrowth of fibrous tissue into the nerve gap during nerve regeneration from the proximal to the distal nerve stump of the transected nerve. Neurolac® is designed to prevent kinking and collapse with associated patient comfort and early flexion of joints is feasible. Degradation of the Neurolac® Nerve Guide occurs through hydrolysis leading to gradual reduction of molecular weight, initial mechanical properties are reported to retain up to 10 weeks providing support and protection to the healing nerve, whereafter, rapid loss of mechanical strength and gradual mass loss occurs. The final degradation products, lactic acid and hydroxyhexanoic acid, are resorbed, metabolized and excreted by the body and studies show that the Neurolac® Nerve Guide has been resorbed within 16 months [200]. An interesting follow up study challenged the 16 month Neurolac® resorption claim. Meek *et al* studied the 2-year degradation and possible long-term foreign body reaction against the nerve guides after implantation in the sciatic nerve of the rat. After 2 years of implantation, the biomaterial could not be found macroscopically, however biomaterial fragments in company of multinucleated giant cells and macrophages were found along the regenerated nerve tissue. Although sufficient nerve regeneration was obtained after long-term implantation in the rat sciatic nerve, biomaterial fragments and foreign body reactions against these fragments, even after 24 months of implantation, could still be found. Nerve guides were shown to resorb, albeit not completely up to 2 years of implantation. It is still not known whether the remaining biomaterial fragments and foreign body reactions may cause granulomas or other complications after longer implantation periods [45].

9. Sterilisation of PCL-Based Drug Delivery Systems, Medical devices and Scaffolds

Sterilization of the final formulation containing the lactide and/or glycolide polymers

is an important issue often overlooked in the early stages of medical devices or drug delivery system development. Terminal sterilization and aseptic processing are two main methods reported for sterilization of PCL-based products. Steam sterilization usually involves subjecting the product to steam at 121°C for at least 20 min or 115°C for 30 min. This method cannot be used with PLGA systems because at higher temperature/pressure condition the polymer softens, melts and leads to deformation of the matrix form, and undergoes hydrolysis. Heat sterilization involves exposing the product to higher temperatures for longer periods of time which are destructive to both the polymer and the entrapped drug.

Sterilization using a gas such as ethylene oxide can be achieved when heat steam sterilization is harmful to the formulation. However, ethylene oxide is known to soften and plasticise these polymers. Also, the residual gas vapors left in these devices were found to be mutagenic, carcinogenic and allergic. Radiation sterilization (60Co*γ* rays) has been used in several cases to sterilize formulations containing lactide and/or glycolide polymers [201]. The effect of radiation on PLGA has been a subject of various investigations [202]. Subjection of PCL to gamma irradiation produced dose-dependent polymer chain breakdown, molecular weight loss (decrease in inherent viscosity), increased in vitro and in vivo bioerosion rates and increased drug release kinetics. Aseptic processing is an effective but somewhat expensive technique for formulations containing PCL polymers. Because of the excellent solubility of the polymers in a number of organic solvents, they can be filter-sterilized. The drug delivery system can then be formulated in a clean room environment using Good Manufacturing Practice (GMP) protocols. PCL microparticles and other devices have already been introduced into the market and many more are undergoing clinical trials.

The effect of PCL-sterilisation by gamma irradiation (dose 2.5 Mrad) on: (1) degradation rate (catalysed by lipase), (2) mechanical properties, (3) the ability of cells to attach and subsequently grow on its surface was investigated by Cottam *et al*. Gel permeation chromatography (GPC) was used to determine the effects of gamma irradiation of weight average and number average molecular weights. Gamma irradiation significantly decreased the rate of degradation, although the rates depended on the initial mass of polymer; it also affected the appearance of the degraded specimens when they were examined by scanning electron microscopy. Irradiation also significantly increased the mechanical yield stress but not the failure stress of PCL. It caused a significant increase in molecular weight and decrease in molecular number which could be attributed to chain scission and cross-linking. Chondrocyte attachment and growth on PCL was not significantly affected by gamma irradiation [203].

10. Medical Grade Polycaprolactone: From Bench to Bedside

Despite the plethora of excellent research utilizing PCL and its composites for tissue engineering purposes, most studies still use PCL with impurities, from sources such as Sigma Aldrich, Solvay, and Union Carbide, often leading to cell culture and *in vivo* studies being unsuitable for translation into the clinical arena. Groups presently using non-clinically relevant PCL should consider switching to medical grade PCL (mPCL) (Birmingham polymers, Boehringer Ingelheim) after proof-of-principle studies have been undertaken; an approach which would enable a faster translation of technology from the laboratory through clinical trials and into the clinic.

Over the last decade an intensive study into the biocompatibility, degradation behavior, mechanical properties and formability has resulted in the production of FDA-

approved PCL implants, [48] [204, 205]. This work was initiated using Sigma Aldrich PCL for proof-of-principle studies before switching to mPCL and culminating in the production of first generation scaffolds using FDM. *In vitro* studies show clear cell attachment and spreading over the scaffold struts encircling and beginning to bridge across the pores as demonstrated by confocal laser scanning microscopy with clear cell sheet formation produced by cells generating their own extra cellular matrix as shown by SEM (Figure 7a,b).

More recently, this work has matured to evaluate the parameters necessary to produce mPCL-tricalcium phosphate (mPCL-TCP) composites (so-called second generation scaffolds) by FDM techniques [112], with highly reproducible design and fabrication results for these 3D bioresorbable scaffolds, with the necessary properties for bone engineering [206] [207]. The inclusion of TCP increases hydrophilicity and osteoconductivity of the scaffold [208] [209].

A major concern with any work related to long-term resorbable polymers is the biocompatibility and mechanical behaviour of the implanted scaffold months down the line. In the present climate, insufficient long-term degradation studies have been undertaken looking at *in vivo and in vitro* degradation behavior of most resorbable polymers, particularly PCL [32]. Most groups look at the short term characteristics which are not necessarily extrapolated. Hutmacher and co-workers have systematically considered the long-term degradation kinetics of mPCL and mPCl-TCP *in vitro*, including an accelerated degradation system, utlising NaOH, and also long-term *in vivo* degradation studies studies [36] [46].

The same mPCL-TCP scaffolds have been studied i*n vivo* in several animal models and have been modified via the inclusion of fibrin glue and lyophilized collagen type I to incorporates biomolecules such as rhBMP2 [210] [47].

Most recently, long-term (2 year) *in vivo* implantation of mPCL-TCP scaffolds within a critical-sized pig cranial model has shown excellent bone regeneration, showing mineralisation throughout the constructs as demonstrated by histological staining and microcomputed tomography. It was notable that bone regeneration within mPCL-TCP scaffolds implanted alone and those implanted in combination with autologous bone marrow precursor cells was significantly different – with enhanced bone regeneration observed via the incorporation of cells. Figure 16a illustrates the implantation of an mPCL-TCP scaffold into a pig critical-sized cranial defect with near-complete mineralization observed within the scaffolds after 2 years as demonstrated using uCT scanning (Figure 16b). The corroborating histological analysis shows mineralized bone (black staining of calcium deposition using von kossa staining, Figure 16d) with histomorphometrical analysis giving an 86% mineralization value.

11. Future directions - the use of PCL in the 21st Century

A clear upward trend in PCL usage in research over the past decade signifies the recognition of this highly versatile resorbable polymer, particularly in the field of biomaterials and tissue engineering. The days of its utilization as a drug delivery device have been surpassed by the realization that PCL may be processed into composite structures with superior mechanical and biocompatible properties compared to the polymer on its own. Data analysed and discussed in this review allow us to conclude that PCL and its copolymers collectively provides a promising polymer platform for the production of longer-term

degradable implants which may be easily manipulated physically, chemically and biologically to possess tailorable degradation kinetics to suit a specific anatomical site.

Despite a number of drug delivery and medical devices fabricated with PCL having FDA approval and CE Mark registration it is surprising that more devices and scaffold based on PCL have not been commercialized. One might speculate that many research groups may not have the infrastructure and capacity to perform translational research, but rather focus their studies and efforts on shorter-term, more achievable goals, including increasing publication counts. The difficulties in performing long-term degradation and biocompatibility studies are evident, particularly when one considers the typical length of grant funding awards would not last beyond 3 years but emphasis should not be lost on the importance of this longer term data, especially in view of the changes already observed in the biocompatibilities of some resorbable devices as degradation proceeds. The use of PCL and the excellent translational research which is already well underway holds great promise for future medical applications. This review highlights the huge diversity of these applications; ranging from sutures to wound dressings, artificial blood vessels, nerve regeneration, drug-delivery devices and bone engineering applications. Finally, the return of PCL into the biomaterials and tissue engineering arena is a reflection of its tremendous promise as a scaffold candidate for tissue engineering, as we move forwards into the 21st century.

Competing interests' statement

The authors declare that they have no competing financial interests

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Figure Captions

Figure 1
Publications using PCL in the field of *Biomaterials* or *Tissue Engineering* during the last 20years. *Sourced from Web of Science.*

Figure 2
Structures made from PCL: Nanospheres (a,b). Nanofibres (c,d). Foams (e,f). Knitted textiles (g,h,i). Selective laser sintered scaffold (j-o). Fused deposition modeled scaffolds (p-u). *Copyright courtesy of Elsevier, Wiley and Mary Ann Liebert.*

Figure 3
Degradation modes for degradable polymers: Surface erosion (a). Bulk degradation (b). Bulk degradation with autocatalysis (c).

Figure 4
Graphical illustration of the mass and molecular weight loss over time for a resorbable polymer such as PCL. Initial hydration (0-6 months), through degradation and mass loss (6 12 months), resorption (post 12 months) and metabolisation (post 18 months).

Figure 5
The degradation of PCL via hydrolysis intermediates 6-hydroxyl caproic acid and acetyl coenzyme A, which are then eliminated from the body via the citric acid cycle (a). Schematic visualization of how crystalline fragmentation could have taken place(b). Accelerated degradation of PCL over 5 weeks in NaOH (c).

Figure 6
Rat and Rabbit short and long-term biocompatibility studies. Implantation of 5mm PCL scaffold into calvaria (a). Schematic showing explanted reconstructed rabbit calvarial after 2-year implantation. Center photo shows gross overview of reconstructed cranial defect site; from the top view, the remaining implanted PCL scaffolds are clearly visible along with bony mineralization observed within the scaffolds that has replaced the original struts. μ-CT three-dimensional reconstructed images indicate dense mineralization within the defect space. Dotted rings indicate location of original defect created, and the images display only those thresholds that correspond with new bone (top inset). Histological sections were stained with Macneal's tetrachrome with von kossa, which reflects mineralization by staining calcium depositions black. The section gross overview of both defects demonstrates scaffold well integrated into the defects (bottom left inset). There is also bone in-growth and remodeling occurring within the scaffold and around its periphery, shown at higher magnification

(bottom right inset) (b). Bone specific staining of rhBMP-2 treated mPCL–TCP/collagen scaffolds demonstrate marked bone repair at 15 weeks.Traverse and longitudinal sections exhibited extensive bone healing within defects treated with rhBMP-2. Staining consists of MacNeal/von Kossa (black = bone, blue = ECM), Goldner's Trichrome (green = bone, red = osteoid), and Masson's Trichrome (dark blue = bone, red = cortical bone). Arrows indicate edge of host bone. Percentage of bone formation (% BV/TV) is measured by image analysis from transverse MacNeal/von Kossa sections and longitudinal Masson's Trichrome sections.Images taken with an objective of 40× indicate new bone (NB) contacting the implant strut (S) surface within rhBMP-2 treated defects whereas non-treated scaffolds stimulate a fibrous tissue interface and empty defects consist of fibrous tissue directly adjacent to host bone (HB). Significant values are represented as $*P < 0.05$ indicating significantly different to time-matched empty defect (c).
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Figure 7
Scaffold-based tissue engineering aims to promote the repair and/or regeneration of tissues through the incorporation of cells and/or biomolecules within a 3D scaffold system which can be maintained in vitro culture conditions until implantation.

Figure 8
Graphical illustration of the complex interdependence of molecular weight loss and mass loss of a 3D scaffold plotted against the time frame for tissue engineering of a bone transplant. (A)Fabrication of a bioresorbable scaffold; (B) seeding of the osteoblast population into the scaffold in static culture (petri dish); (C) growth of premature tissue in a dynamic environment (spinner flask); (D) growth of mature tissue in a physiological environment (bioreactor); (E) surgical transplantation; (F) tissue engineered transplant assimilation/remodeling [111].*Copyright courtesy of Elsevier.*

Figure 9
Snapshot: Polymer scaffolds for tissue engineering. Fabrication routes for PCL scaffolds.

Figure 10
Schematic diagrams of: the process, direct polymer melt deposition. It has controllable parameters including control of the nozzle diameter, processing temperature, applied air pressure and movement velocity of the nozzle (a). The electrospinning process during which a drop of a polymer solution is ejected from the micro nozzle and spread onto a grounded substrate in the shape of a nanofibrous matrix (b). The developed hybrid process. Hybrid scaffolds containing microfibers and nanofiber matrices could be built via a combined process of continuous DPMD and electrospinning (c). Overall 3D woodpile structure with dimensions of 9 mm × 9 mm × 3.5 mm (d). The hybrid basic unit layer composed of microfibers and the electrospun nanofibers matrix (e). Magnified images (f and g). Microfiber prepared for the pre-testing experiment: Microfibers with lengths of ca. 10 mm and diameters of ca. 0.4 mm (h). A schematic diagram of three different types of microfibers: microfiber-only, surface-modified microfibers with PCL nanofibers and the PCL/collagen blend nanofibers (i), SEM image of a microfiber coated with PCL/collagen nanofibers (j) and magnified image of the PCL/collagen nanofibers deposited on the surface of a microfiber (k).
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Figure 11
Schematic diagram showing the fabrication process of pore size gradient PCL scaffold by a centrifugation method and SEM photographs of the top surfaces of the selected PCL cylindrical scaffold sections along the longitudinal direction (100; *, average pore size). *Copyright courtesy of Elsevier.*

Figure 12
Pictures taken of the knitted valve inside the physiological flow simulator (a). Pictures taken of the porcine stent-less valve prosthesis inside the physiological flow simulator (b). *Copyright courtesy of Mary Ann Liebert*

Figure 13
Elastic behaviors of PLCL scaffolds. PLCL scaffolds (a) were extended at 250% of initial length (b) with 3MPa for 5 sec and recovered (c) by releasing the load. The scaffolds were twisted (d) and folded (e) via a cyclic strain apparatus. PLGA (f) was broken at 20% strain. *Copyright courtesy of World Scientific.*

Figure 14
SEM micrographs of the bilayered tubular construct: Bilayered tube (entire view) (a). bilayered tube wall(b). Details of the interface (mixing zone) between inner and outer layers (c–(). Details of the outer layer(PLA)(e–f). Details of the inner layer (PCL) (g–h). SEM micrographs of electrospun PLA/PCL constructs after culture with 3T3 mouse fibroblasts for: 1 week (i); 2 weeks (j); and 4 weeks (k). *Copyright courtesy of Elsevier.*

Figure 15
DRGs on aligned and random fibre film in vitro. (A–D) Double immunostained DRG on the aligned fibre film: (A) representative montage of NF160 (a marker for axons) immunostained DRG neurons on the film and (B) montage of S-100 (a marker for Schwann cells) immunostained Schwann cells on the film. (C) Magnified NF160 (red, from box in A) and S-100 (green, from box in B) overlapped image. (D) Double immunostained aligned axons (NF160, red) and endogenously deposited laminin protein (laminin, green). (E–I) Fabrication of the fibre films and distribution of alignment of the films. (E) Schematic of aligned fibre film fabrication by electrospinning process. Random fibre film was deposited on a flat metal target instead of on a high-speed rotating metal drum. (F) and (H) Representative SEM image of the aligned fibres (F, magnified fibres below) and the random fibres
(H). Scale bar ¼ 1 mm and 30 mm, respectively. (G) and (I) Distribution of fibre alignment in aligned (G) and random fibre (I). (J) and (K) Double immunostained DRG on the random fibre film: (J) representative montage of NF160þ neurons and (K) S-100þ Schwann cells. Scale bar ¼ 500 mm. (L) Shows the quantitative comparison of orientation of neurite outgrowth on the aligned and random fibre film. Direction of arrows indicates the orientation of neurite outgrowth, and length of arrows indicates the rate of occurrence (percentage) (n ¼ 25 per DRG). (M) Shows the quantitative comparison of the extent of neurite outgrowth and Schwann cells migration on the films. The distance between the longest neurite

outgrowth (n ¼ 25 per DRG)/the furthest migrated Schwann cells (n ¼ 10 per DRG) and DRG was measured and averaged. *P < 0.05. Error bar ¼ s.e.m.
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Figure 16
Implantation of the mPCL-TCP scaffold into the pig cranium (a). SEM analysis using backscattered electron mode. Visualisation of the calcium content represented in grey (b). Histological analysis showing new bone formation (nb) scaffold struts (s) osteoblasts (ob) and osteocytes (oc). Extensive mineralization is evident throughout the scaffold (c).

Tables

Table 1 – Polymer properties

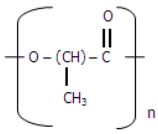
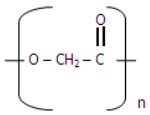
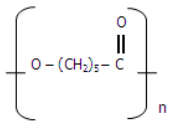
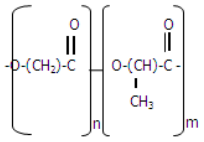
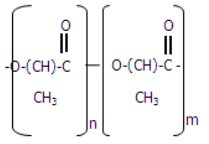
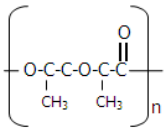
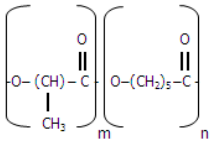
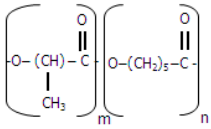
Polymer	Polymer repeat structure	Melting Point (°C)	Glass Transition (°C)	Processing Method	Approx. Deg time (months)	Area of application and references	Products with regulatory approval
Poly (lactide)		173 - 178	60 - 65	Extrusion. Inj. Moulding. Compression. moulding Solvent Casting.	6 to 12	Orthopedic surgery Oral and Maxillofacial surgery	Fixsorb® (screws, nails ,pins) Neofix® (screws, nails, pins) Arthrex: Bio-Tenodesis® interference screw, Bio-Corkscrew® suture anchor) Linvatec: SmartScrew® SmartNail® SmartTack® SmartPin® BioScrew® Zimmer : Bio-statak® (suture anchor) prostatic stent, suture anchor, bone cement plug
Poly (glycolide)		225 - 230	35 - 40	Extrusion. Inj. Moulding. Compression moulding. Solvent Casting.	>24	Orthopedic sugery General surgery Sutures	Bioscience : Biofix® screws Dexon™ sutures, mesh. Bondek® suture Valtrac™ anastromis ring, prostatic stent
Polycaprolactone		-65 - 60	-65 - 60	Extrusion. Inj. Moulding. Compression moulding. Solvent Casting. Electrospinning	>24	Drug Delivery Sutures	Capronor Ethicon : Monocryl Suture
Poly (D,L-lactide-co-glycolide) **Poly (D,L-lactide-co-glycolide) 85/15** **Poly (D,L-lactide-co-glycolide) 82/18** **Poly (L-lactide-co-glycolide) 1090**		Amorphous	50 – 55	Extrusion. Inj. moulding. Compression moulding. Solvent Casting.	5 to 6	Suture Drug Delivery Oral and maxillofacial surgery General surgery Suture, periodontal surgery, general surgery	Polysorb™ sutures Makar : Biologically Quiet™ Interference Screw, Staple, 85/15 Biomet : Lactosorb®, screw, plates, mesh, surgical clip, pins, anchor Vicryl suture, Vicryl Mesh
Poly (L-lactide-co-D,L-lactide) 98/2 **Poly (L-lactide-co-D,L-lactide) 50/50** **Poly (L-lactide-co-D,L-lactide) 70/30** **Poly (D-lactide-co-D,L-lactide-coL-lactide**		Amorphous	55 - 60	Extrusion. Inj. moulding, Compression moulding. Solvent Casting,	12 to 16 12	Orthopaedic surgery Oral and maxillofacial surgery BD Biosciences – not got clinical approval	Phusiline® Interference Screw Sulzer: Sysorb® Screw (50/50) Resor Pin® 70/30Geistlich biomaterials Macrosorb System (screws, plates, mesh, nails, pins 70/30 Protego FX screw no hits on net Polypin®, Zimmer : Leadfix BD™ 3D OPLA® (Open-cell Polylactic acid) scaffold
Polydioxanone		58 - 63	-65 - 60	Extrusion. Inj.moulding, Compression moulding. Solvent Casting,	>24	Orthopaedic surgery General surgery Sutures	Ethipin Orthosorb, Suture mesh foils Bone cement plug
Poly (D-L-lactide-co-caprolactone) 65/35		Amorphous		Dip coating from chloroform-	24*	Nerve Regeneration	Neurolac® Polyganics B.V., Groningen, The Netherlands.
A polycaprolactone-based composite containing dimethacylate monomers		60				Oral surgery Miner 2000 Shipper 6	Resilon™ Root canal filling
Polycaprolactone based polyurethane		amorphous				Tissue reinforcement. Torn tendon replacement patch. Interpositional spacer in osteoarthritis	Artelon® Sportmesh™ Arteleon® CMC spacer Arthro
Lactide co caprolactone					Klopp 2008	To prevent adhesion	Mesofol

Table 2
Definitions of *biodegradable, bioresorbable, bioabsorbable* and *bioerodable,* according to Vert (1992).

Biodegradables are solid polymeric materials and devices which break down due to macromolecular degradation with dispersion in vivo but no proof for the elimination from the body (this definition excludes environmental, fungi or bacterial degradation). Biodegradable polymeric systems or devices can be attacked by biological elements so that the integrity of the system, and in some cases but not necessarily, of the macromolecules themselves, is affected and gives fragments or other degradation by-products. Such fragments can move away from their site of action but not necessarily from the body.

Bioresorbables are solid polymeric materials and devices which show bulk degradation and further resorb in vivo; i.e. polymers which are eliminated through natural pathways either because of simple filtration of degradation by-products or after their metabolization. Bioresorption is thus a concept which reflects total elimination of the initial foreign material and of bulk degradation by-products (low molecular weight

compounds) with no residual side effects. The use of the word ‘bioresorbable ' assumes that elimination is shown conclusively.

Bioerodibles are solid polymeric materials or devices, which show surface degradation and further, resorb in vivo. Bioerosion is thus a concept, too, which reflects total elimination of the initial foreign material and of surface degradation by-products (low molecular weight compounds) with no residual side effects.

Bioabsorbables are solid polymeric materials or devices, which can dissolve in body fluids without any polymer chain cleavage or molecular mass decrease. For example, it is the case of slow dissolution of water-soluble implants in body fluids. A bioabsorbable polymer can be bioresorbable if the dispersed macromolecules are excreted.