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Review Article

Polycaprolactone as biomaterial for bone scaffolds: Review of literature

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ABSTRACT

Bone tissue engineering using polymer based scaffolds have been studied a lot in last decades. Considering the qualities of all the polymers desired to be used as scaffolds, Polycaprolactone (PCL) polyester apart from being biocompatible and biodegradable qualifies to an appreciable level due its easy availability, cost efficacy and suitability for modification. Its adjustable physio-chemical state, biological properties and mechanical strength renders it to withstand physical, chemical and mechanical, insults without significant loss of its properties. This review aims to critically analyse the efficacy of PCL as a biomaterial for bone scaffolds.

1. Introduction

Bone, the mineralized connective tissue of the body consists of macrostructures (such as cancellous and cortical bone), microstructures (like osteons, and single trabeculae), sub-microstructures (lamellae), nano-structures (fibrillar collagen), and sub-nanostructures (minerals, and collagen molecules).[1] The organic component of the bone comprises of collagen proteins predominantly type I collagen (90%) and non-collagenous proteins like, osteocalcin, osteopontin, bone sialoproteins and growth factors. The inorganic component consists mainly of calcium and phosphate ions which nucleate to form small crystals of hydroxyapatite.[2,3] The interactions between collagen, hydroxyapatite, and various organic and inorganic components of bone lead to its structural organisation and typical mechanical properties (compressive strength, fracture toughness and tensile strength).[4] Cortical porosity (average 10–30%) is due to its intracortical canals and spaces, while trabecular porosity (30–90%) is due to the intertrabecular marrow spaces. Porosity is reciprocally proportional to bone strength and stiffness. Cortical porosity of mandibular condyle is 3.53–3.98%, while trabecular porosity is 79.3%.[5]

Bone is a highly dynamic tissue wherein the old bone is replaced with the new bone continuously by bone remodeling. In the past few decades, bone tissue engineering has emerged as a promising strategy to overcome the shortcomings associated with the traditional techniques. It employs three components: cells, scaffolds and growth factors either alone or in combination with the aim to form neo-tissue at the site of bone loss. A bone scaffold is the 3D matrix framework that stimulates the attachment and proliferation of osteo-inductive cells on its surfaces.

The bone scaffold material can be divided into 3 generations, depending on the degree of integration by the recipient's bone.[6] The first generation includes pure metals (stainless steel, titanium), metal alloys (aluminium, zirconium) and polymers (silicone, polypropylene, polymethyl methacrylate). This group of grafts often develop a fibrous layer on the bone contact surface, leading to a lack of osteointegration and loosening of the graft. The second generation of substitutes is coated with an additional supporting layer to prevent the formation and deposition of connective tissue on the graft and thereby facilitating complete osteointegration. This group includes hydroxyapatite, calcium metaphosphate and bioactive glass. The third generation uses a material closely related to bone and is characterized by high osteo-conductivity, osteo-inductivity and biodegradable.

The aim of this study is to provide a comprehensive review on the efforts made to fabricate PCL or its composite scaffolds and methods employed to improve its mechanical properties as well as cellular activity in bone tissue engineering.

1.1. Polymers as biomaterial for scaffolds

The characteristics of a biomaterial that must be scrutinized thoroughly before considering it for bone tissue engineering applications includes chemical composition, biological and structural characteristics, degradation behavior and fabrication process. Polymeric scaffolds are of considerable interest due to their distinctive features, such

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as ability to harbour multiple spatio-temporal cues, provide osteo-inductive niche to the resident or transplanted cells at the injury site, degradation rate similar to the rate of osteogenesis and integrate with the host tissue through extensive chemical modifications. Polymers used as biomaterial for bone tissue engineering can be natural or synthetic, biodegradable or nonbiodegradable.

Natural polymers: These polymers are biocompatible and bioactive, thus, aid in enhancement of the cell performance (adhesion and proliferation). However, they are difficult to engineer due to their limited processing abilities, possess high risk of contamination and exhibit batch-to-batch variability. Eg. bioactive proteins (silk, collagen, gelatin, fibrinogen, elastin, keratin, actin, and myosin), polysaccharides (cellulose, amylose, dextran, chitin, and glycosaminoglycans), or polynucleotides (DNA, RNA).[7]

Synthetic polymers: These polymers are highly popular as scaffold material, as have defined chemistry, easy processing and tailoring ability, and can be modified to achieve desired properties for specific applications. Other merits include cost efficacy, ability to be produced in large quantity uniformly and a longer shelf time. Also the physico-chemical and mechanical properties such as tensile strength, elastic modulus, and degradation rate are comparable to bone. However, these polymers are not bioactive, hence, can elicit inflammatory responses inside the host. Polymers like, poly lactic acid (PLA), polyglycolic acid (PGA), poly-L-lactide (PLLA), poly ε-caprolactone (PCL), poly lactic-glycolic acid (PLGA) copolymers and poly hydroxy-alkanoates (PHA) are classified as synthetic polymers.[15,16] Within the class of synthetic materials, PCL has recently drawn much attention for biomedical applications including bone tissue engineering.[8–10]

1.2. Poly ε-caprolactone (PCL)

PCL is a member of the biodegradable polyesters (others being PGA, PLLA). It is an aliphatic semi-crystalline polymer, with melting temperature ranging between 59 and 64 °C (i.e. above body temperature), glass transition temperature of −60 °C. Hence, at physiological temperature, the semi crystalline PCL attains a rubbery state resulting in its high toughness[11] and superior mechanical properties (high strength, elasticity depending on its molecular weight). It is non-toxic and tissue compatible, hence widely used as resorbable sutures, as scaffolds in regenerative therapy and in drug delivery applications. PCL exhibits a longer degradation time (2–3 years) and is degraded by microorganisms or by hydrolysis of its aliphatic ester linkage under physiological conditions.[12] Due to the presence of five hydrophobic $–CH_2$ moieties in its repeating units, PCL degrades slowest among all the polyesters. The nano-fiber matrices made from polyesters follow an erosion rate of PGA > PLGA > PLLA > PCL.[13]

1.3. Fabrication of PCL scaffolds

The technique employed for fabricating PCL scaffolds depends upon the type of scaffold needed. For porous scaffolds, methods like: porogen leaching, saturation and release of CO_2, 3D printing, phase separation technique and freeze-drying have been employed. However, for fabrication of fibrous scaffolds techniques like electrospinning and melt electrospinning have been used.[14–18]

1.3.1. A. Solvent casting and porogen leaching

Porosity is the key factor in designing of scaffolds for bone tissue engineering since proper pores are needed to permit tissue growth and nutrient diffusion. The technique utilizes soluble porogen particles (water soluble salts such as sodium chloride and sodium bicarbonate) embedded in polymer that forms pores in the polymer matrix upon removal. The pore size can be tailored by using different sizes and concentration of porogens. For instance, 1:1 wt/wt NaCl and $NaHCO_3$ was used as a porogen in PCL solution to fabricate an interconnected scaffold having 70–80% porosities.[19] Recently, solvent casting has been used in combination with particulate leaching method to generate 3D scaffolds. Others fabricated PCL 3D scaffolds after casting the PCL solution containing salt and polyethylene glycol as porogens onto a Teflon mold. These porogens were then removed to generate scaffolds of varying pore sizes.[20,21]

1.3.2. b. Electrospinning (ES)

ES until now is the most favorable technique to fabricate highly controllable and ultra-fine polymer fibers and 3D scaffolds. The technique is versatile and has the ability to process various polymer solutions and their blends such as PCL and Aluminum Oxide, recombinant spider silk protein and gelatin, PLA, PLGA, silk fibroin and polyurethane etc. Ke et al. fabricated PCL/Gelatin nanofibrous membranes using electrospinning and demonstrated their significant potential for bone regeneration.[22] In another study, fibers using PCL/PLA blends were fabricated to prepare scaffolds from the nanofibrous structures. These scaffolds showed improved osteogenic differentiation of stem cells for cranial regeneration. The activation of BMP 2 signalling in these scaffolds further improved the osteogenic differentiation.[23,24]

1.3.3. c. Phase Separation technique

This includes either liquid or solid-liquid mixing generating polymer-poor and polymer-rich phases. On lowering the temperature freezing of solution occurs and removal of frozen solvent leads to pore formation. Phase separation results via varying the polymer concentration, using different solvents, or varying the cooling rate resulting in scaffolds with different morphology.[25] Grandi et al. demonstrated the separation phase technique using calcium alginate to process PCL into required dimensions, which resembled the porosity and the homogeneous pore size distribution of the bone.[26]

1.3.4. d. 3D printing

Three-dimensional printing has evolved as a promising technology to fabricate scaffolds and complex structures with micron size precision and accuracy with homogenous distribution of cells within it.[27] Recently, Cho et al. 3D printed a composite scaffold of PCL/hydroxyapatite (HA) melt with HA exposed onto the surface of scaffold for enhanced cellular response and bone regeneration.[28] Similarly, in another study, PCL/HA with varying content of mineralization was fabricated using multi-material extrusion 3D printing system for osteochondral tissue engineering. Such scaffolds have ability to address the structural and compositional gradients found in osteo-chondral region. Thus, 3D printing can be utilized to make versatile scaffolds imitating extracellular matrix of the tissue using both natural and synthetic polymers.[29]

1.3.5. e. Melt electrospinning

The melt electrospinning utilizes polymer melts when compared to the polymer solution used in electrospinning. The principle remains same, the only difference is in the physical states of the polymer used, polymer melts are more viscous than the polymer solution. In a recent study, PCL fibers were developed for an application in oral and maxillofacial surgery. The fibers of 20 μm diameter were fabricated by spinning the PCL melt (melted at 73 °C) at a pressure of 1.2 bars and an acceleration voltage of 6 KV.[30] Zaiss et al. employed melt electrospinning technique to fabricate 3D scaffolds on structured and curved metallic collectors. The scaffolds made had an average fiber diameter of 15 μm and pore size of 250 μm–300 μm. Melt electrospinning, unlike solution electrospinning can be used to fabricate fibers of diameter greater than sub microns.[31]

1.4. Surface modification of PCL

PCL is relatively hydrophobic in nature. For it to be used as scaffold, hydrophilicity is needed, and cellular compatibility needs to be enhanced. For maximum cell adhesion, optimal water contact angle

values have been reported to be in the range of 45–70 °C or in the region of 30–60 °C. At very low contact angles water uptake increases leading to decreased protein adsorption needed for cell recognition and attachment. However very high contact angles and low surface energy causes low cell-conductive behavior and protein denaturation.[32] Several attempts have been made to modify the surface of PCL, by coating the scaffold with composites of fibrin, fibronectin, gelatin, growth factors, and proteoglycans. Double protein-coated PCL scaffolds have demonstrated superior initial cell adhesion, proliferation, and colonization. While Arginine-glycine-aspartic acid (RGD) coating showed smoother surfaces, and early bone deposition onto scaffold surfaces, due to reorganization of the ECM matrix on the surface.[33] Hydrophilicity of PCL surface can be enhanced by Plasma treatment (active screen plasma nitriding) and has shown improved cell attachment. However, this treatment leads to slower enzymatic degradation rate.[34] Zander et al. (2012) modified PCL electrospun fibers by air plasma followed by covalent attachment of laminin.[35] Gupta (2019) demonstrated that alkaline surface treatment (with controlled NaOH-concentration, reaction temperature, and treatment time) improves the surface morphology and cellular response of PCL. Increase intensity of treatment resulted in enhanced surface porosity (~60%), increased surface roughness (~700 nm), and improved cellular response till surface porosity reached ~35%.[36]

Rotbaum et al. (2019) studied the effect of pore geometry and size in 3D printed PCL scaffolds on their mechanical properties. Square pores (with dimensions 150–650 μm) had 59–79% porosity while quadrangular, hexagonal, triangular and complex pores had a constant porosity of approx. 70%. The mechanical properties were reported to be insensitive to strain rate and were strongly dependant on the pore size (porosity) rather than pore geometry.[37]

1.5. Blends of PCL

PCL scaffolds used earlier were unable to provide optimal mechanical strength and biocompatibility. Hence PCL blending with natural or synthetic polymer or ceramic was attempted. Incorporation of calcium phosphate-based ceramics, bioactive glasses and polymers into PCL led to improved biomaterials with better mechanical properties, controllable degradation rates, and enhanced bioactivity (Table 1). Lino (2019), blend PCL and poly-di-isopropyl fumarate enriched with Sr2+. *In vitro*, Blend +5% Sr2+ was pro-inflammatory and anti-osteogenic, Blend +1% Sr2+ released low quantities of cation; but was not cytotoxic for cultured macrophages; and demonstrated better osteo-compatibility. *In vivo*, Blend +1% Sr2+ significantly increased bone tissue regeneration without inducing any local inflammation.[38] Hwang (2019) blend Cellulose acetate solutions and calcium lactate with PCL nanofibrous scaffold by electrospinning to enhance the bio-physio-chemical properties of the composite fiber.[39]

1.6. Effect of scaffold chemistry or surface modification of PCL scaffolds on bone regeneration

PCL due to its hydrophobic nature and absence of functional groups that enable cell growth and proliferation, restricts any cellular interactions. Therefore, there is a need to modify the surface of tissue engineered PCL scaffolds to enhance their cellular compatibility and bone regeneration ability.[40] Several attempts have been made in this regard using techniques such as plasma deposition, starch-blending or attachment of mussel inspired material.[41–43]

The most commonly used technique to improve cellular interaction is to modify the substrate with the extracellular components or with mimicking synthetic peptides for example, arginine-glycine-aspartic acid (RGD), fibronectin, gelatin, fibrin. In a study, PCL scaffold modified with RGD peptide demonstrated improved BMSC attachment and cellular distribution, with increase in cell survival and growth.[44] Attempts made to functionalize PCL fibers with RGD peptide showed improved cell attachment, proliferation and osteogenic activity of Saos-2 cells.[45] When RGD was coated on PCL, the irradiated PCL surfaces were observed to be smoother, and promoted initial bone deposition onto scaffold surfaces, due to reorganization of the ECM matrix on the surface.[46] Coating of PCL scaffold with composite of fibrin, fibronectin, gelatin, growth factors, and proteoglycans have also been reported. When PCL scaffolds were first coated with gelatin type B and then with fibronectin, it was observed that the double protein coating showed higher colonization of pre-osteoblast cells and influenced both the seeding density and subsequent differentiation into osteoblasts.[47]

Plasma treatment and plasma polymer deposition technique produces the surface of amine, carboxy, hydroxy and aldehyde groups on materials depending upon the gas that is fed. Amine surfaces have been coated mainly by using NH_3, N_2 or N_2/H_2 plasma treatments or alkyl amine plasma depositions.[48–50] These groups increase the hydrophilicity of PCL surface and allow better cell adhesion. However, after the treatment the enzymatic degradation rate becomes slower.[40]

When PCL 3D scaffolds fabricated by additive manufacturing were coated with ethylene/nitrogen and hydrogen plasma treatment, it led to uniform distribution and penetration of plasma particles on it. This coating imparted uniform attachment and proliferation of Saos-2 cells.[41] Zander et al. modified PCL Electrospun fibers by air plasma treatment, followed by the covalent attachment of laminin.[35] Mussel inspired materials such as dopamine can be coated on the surface of a material by raising the pH without using solvents. Dopamine has abundant catechol and amine groups that can enhance cellular bioactivity of the synthetic polymers. PCL scaffolds when coated with polydopamine and functionalized with rhBMP2, showed improved surface wettability, cell proliferation and osteogenic ability.[51] Polydopamine coating on electrospun PCL fibers can tune the loading and release rate from these fibers, and hence can be used to improve the delivery of growth factors required for bone tissue formation in scaffolds.43 As described earlier alkaline surface treatment improves the surface morphology and cellular response of PCL. Also, increased treatment intensity, leads to increased surface porosity, surface roughness and cellular response till surface porosity is ~35%.[35] Hydroxyl functionalized PCL had 70% porosity and compressive modulus comparable to bone, and demonstrated enhanced cell adherence, better metabolic activity and osteogenic potential than PCL scaffolds.[52]

Altogether, these studies suggest that surface modification of PCL scaffolds enhances the cellular activity and their osteogenic potential, thus, overcoming the drawbacks of the synthetic polymer.

1.7. Effect of surface topology of PCL scaffolds on bone regeneration

Surface roughness is an easily tailorable and cost-effective factor that can influence cell behavior. The osteogenic ability of the hMSCs seeded on PCL scaffolds with surface roughness of R_a ~ 0.9–2.1 μm cultured in dexamethasone-deprived osteogenic induction medium and in basal growth medium was significantly superior to the cells cultured on tissue culture plate control in complete osteogenic induction medium with dexamethasone.[53] The surface having the highest roughness (R_a = 293–445 nm) achieved by homogenous distribution of apatite layer led to improved cell viability and osteogenic ability of pre-osteoblast cells when soaked in simulated body fluid for 7 days.[54]

Pore size is another important scaffold characteristic that regulates cell binding, migration, tissue regeneration and deposition. The larger pore size allows efficient nutrient diffusion, waste removal and minimal cell-cell contact.[55,56] Also, different pore sizes allow binding of different cell types on PCL scaffolds. Chondrocytes and osteoblasts showed higher cell proliferation in the PCL scaffold with the pore size of 380–405 μm and fibroblast proliferation was seen on scaffolds having pore size of 186–200 μm. Pore size of 290–310 μm demonstrated increased bone regeneration after implantation in rabbit cranial defect.[57] While, a pore size larger than 400 μm favors angiogenesis and enhances bone forming ability of the scaffolds.[58] The pore size of the PCL scaffold

Table 1
PCL Blend- Comparison of different materials used to blend with PCL along with technique employed and advantages/disadvantages.

S No.	Types of polymers	Blending with	Fabrication Technique	References	Advantages/Disadvantages
1	Natural	PCL- Chitosan (CS) composite structure as 3D hydrogel	Lyophilization	Zhong 2011, Thuaksuban 2011	Ratio is crucial. PCL/20%CS scaffolds showed good physical properties and cellular response. Increasing CS reduced the micro-groove pattern, surface roughness, tensile strength and elasticity of filaments, whilst compressive strength was not affected
		Adding 5–10% Nanofibrillated CS made PCL solution electrospinnable.	Electrospinning	Fadaiea 2018	Mechanical properties, wettability improved with enhanced tensile strength, Young's modulus, cell proliferation and adhesion.
		Silk	Lyophilization	Serrano 2009, Lim 2012, Hu 2012, Chen 2004.	Good cell adhesion, growth and proliferation, excellent biodegradability, biocompatibility but changes in silk content vary the results.
		Spider silk and Gelatin	Electrospinning	Xiang 2011	High porosity and good cytocompatibility.
		Alginate	Electrospinning	Hu 2019	Enriched cell adhesion, ECM interaction and angiogenesis
		Collagen	Electrospinning	Zhang YZ 2005	Linear increase in Human Dermal fibroblast density by 19.5% (2 days), 22.9% (4 days), and 31.8% (6 days).
2	Synthetic	PLLA and MWCNTs	Separation Phase	Amirian 2012	Degradation kinetics can be controlled by varying MWCNTs content
		PLA/HA	Electrospinning	Kareem 2019	Higher bioactivity with gradual reduction in mechanical properties
		Gravity spun PCL fibers with elastic electrospun Polyurethane (PU) fibers fabricated to PCL/PU	Solvent-Casting	Williamson 2006	For vascular tissue. Lumen of PCL scaffold supports formation of stable functional endothelial cells monolayer and controls release of bioactive molecules.
		PLGA	Electrospinning	Hiep 2010	Increased biocompatibility at increasing % of PLGA, good cell adhesion and proliferation of fibroblast cells on electro-spun mats
		PLLA-PCL in porous membranes	Freeze Extraction	. Haroosh 2012, Williamson 2006	Increased degradation time of PLLA, but with a loss in mechanical properties
		Polystyrene (PS)	Spin-coating	Ma 2012	PCL-Polystyrene thin films.
		PGA	Separation Phase	Grandi 2011	Comparable porosity and pore size with bone.
		PLA	Thermally induced nanofiber self-agglomeration (TISA), Electrospinning	Yao 2017	Higher mechanical properties *in vitro* bioactivity; enhanced cell viability of hMSCs and osteogenic differentiation.
3	Ceramic	Forsterite (Mg_2SiO_4)	Solvent-Casting and Particle Leaching	Diba 2012	Improves mechanical properties, bioactivity, biodegradability, non-cytotoxicity
		Calcium Alginate	Separation Phase	Grandi 2011	Resembled native bone porosity and homogeneous pore size
		Aluminum oxide PCL(AL_2O_3)	Electrospinning	Dong 2012	Mechanical properties improved
		Magnesium Phosphate (MP-PCL	Particulate Leaching	Wu 2012	Increased degradation time
		Calcium Phosphate	GF-SEDA (Gas Foaming and Spontaneous Emulsion Droplets Adherence)	Bao TQ, Franco RA, Byong T (2012)	In vitro scaffold demonstrated significant and better cell adhesion, growth, and proliferation over the course of 5 days in culture.
		Nanohydroxyapatite to form PCL/nHA	MM-M/LT (Modified Melt-Molding/ Leaching)	Klein 1983, Freed 1994, Rezwan 2006, Liu 2012	Excellent biocompatibility in-vitro and in-vivo. Shown potential for cartilage tissue engineering
		Bioactive glass microspheres and glass fibers from calcium phosphate ($50P_2O_5$ + 50CaO) glass	Solvent-Casting	Lei 2012, Ahmed 2008	Improved performance
		Polydiisopropyl fumarate (PDIPF)	Solvent –casting	Lino 2019, Fernandez JM et al., 2011	Sr^{2+} replaces Ca^{2+} in Hydroxyapatite. The blend scaffold exhibited Improved physical, mechanical and osteoinductive properties.

differentially regulates cell behavior *in vitro*, however, the scenario is different *in vivo*. A pore size of PCL scaffolds (350, 550, 800 μm) had limited influence on bone regeneration in immunocompromised mice model.[59]

1.8. Mechanical properties of PCL

The mechanical strength viz. compressive strength, elastic and tensile strength, fatigue of the scaffold should be sufficient to sustain and retain its properties even after implantation at high load bearing sites and perform appropriately until new tissue is able to restore the function. This would provide stable scaffold integration at the host site and subsequent reconstruction of the injured site while mechanically supporting it.[60] Calcium phosphate materials, although approved by FDA as bone fillers and explored as bone scaffolds for regeneration, their mechanical properties still fall short of the functional tissues. Two approaches are being used to increase their strength. PCL was either printed along with CaP (co-deposited) followed by sintering to form a scaffold or was coated on the surface of printed and sintered CaP scaffolds. Both approaches showed increased strength and modulus when compared to only CaP scaffolds.[61]

Interestingly, interpenetrating hydrogels of different concentrations of gelatin methacrylate and pectin-g-PCL led to formation of robust hydrogel with increased compressive and tensile moduli after double crosslinking by UV light and Ca^{2+} ions whereas crosslinking by UV light only led to reduction in mechanical properties. Further, these hydrogels showed increased growth of pre-osteoblasts cells *in vitro*, therefore, have great potential for bone regeneration.[62]

When Young's moduli of electrospun PCL fiber and PCL fiber scaffolds was measured using macro-tensile testing instrument, and atomic force microscopy, Young's modulus for fiber scaffold was 3.8 ± 0.8 MPa while for single fiber was 3.7 ± 0.7 GPa. The difference was due to the random structure of fiber scaffold.[63] Bulk PCL has tensile strength of about 25–43 MPa and elastic modulus of about 330–360 MPa. In comparison porous and fibrous PCL scaffolds have low tensile strength and elastic modulus, due to their pore structure.[64] Blending PCL with different type of ceramic materials improves the mechanical properties of PCL scaffold for bone tissue engineering (Table 2).

1.9. Degradation behavior of PCL

PCL exhibits slow degradation rate (3–4 years). It degrades through hydrolytic cleavage of ester groups.[65] Kulkarni (2008) studied the degradation of PCL and its copolymers and demonstrated enzymatic and hydrolytic degradation of PCL homopolymer.[66] Among the polyesters, PCL degradation is the slowest due to presence of 5-CH_2 moieties in its repeating units. In an attempt to accelerate the degradation time of PCL, Wu (2012) used magnesium phosphate and demonstrated a faster degradation rate.[67]

Bioactive Glass Microspheres reinforced PCL exhibited excellent mechanical properties, biocompatibility, bioactivity and faster degradation rate, but with increased weight loss and water absorption.[68] Diba (2011) accelerated the degradation time of PCL by fabricating PCL-forsterite- nano-composites, however that too increased weight loss and water absorption.[69] A blend with Chitosan has shown to cause an increase in water uptake, but decrease in the degradation rate.[70] Arginine-glycine-aspartic acid (RGD) has also been used to modify PCL to decrease the time degradation.[46] Spalthoff (2018) studied PCL-TCP scaffold pre-augmented scapula prior to a potential flap-raising procedure in a sheep and observed that a fair amount of the scaffold material was degraded and replaced by vital bone.[71]

1.10. Cellular bioactivity on PCL scaffolds

Scaffolds can elicit host immune response leading to failure of implants. The biocompatibility can be improved by adding Aluminum oxide (Al_2O_3) and Hydroxyapatite (HA) to PCL nano-composite scaffolds.[72,73] Growth of cells has been reported to be significantly higher in blend scaffolds like recombinant spider silk protein/PCL/Gt, PCL/silk fibroin composite scaffolds, PCL/biphasic calcium phosphate hybrid composite scaffolds, and PCL/chitosan composite scaffold than pure PCL.[74–76] Addition of HA to PCL/SF composite scaffold allows increased cell proliferation, but decreased collagen- I production. Addition of bone morphogenetic protein 4-expressing bone marrow stromal cells strongly favours osteo-inductivity.[77,78] Lee et al. (2019) designed a- 3D PCL scaffold, which exhibited osteo-induction both *in-vitro* and *in-vivo* when isolated human tonsil-derived mesenchymal stem cells were cultured on the scaffolds. The cells rapidly differentiated into osteoblast-like cells with osteo-promoting capabilities. The scaffolds were then implanted in rabbit calvarial defect for enhanced vessel and bone regeneration.[79] Lin 2019 prepared PCL composite membrane containing 20 wt% cobalt-substituted hydroxyapatite (CoHA) powders by solvent casting for local release of cobalt ion to reduce infection and inflammation. Significantly increased cell proliferation (over 90% after 7 days of culture), calcium deposition, and good antibacterial effect was observed.[80] Park (2019) assessed the effectiveness of a tonsil-derived mesenchymal stem cell on a transplanted PCL/beta-tricalcium phosphate scaffold on rabbit mandible in a 10 × 8-mm bone defect at 12 weeks and observed extended dense new bone masses.[81] Ho (2018) studied the effects of Biodentine/PCL scaffold with odontogenesis properties on human dental pulp cells. The Biodentine/PCL scaffolds were fabricated with uniform macropores 550 μm and interconnections using an extrusion printer. The mechanical properties showed their compressive strength of 6.5 MPa, bioactivity, and proliferation and odontogenic differentiation of human dental pulp cells cultured on the scaffolds exhibited a good apatite-forming ability and capability to support proliferation and differentiation.[82]

Fedore (2017) fabricated PCL scaffolds using extrusion deposition and coated some with Polyethylene glycol (PEG) hydrogel that inhibits mineralization and cultured calvarial cells for osteoblastic differentiation and mineralization. Uncoated PCL scaffolds showed better differentiation of osteoblasts.[83] Wu (2019) fabricated 3D-printed calcium silicate, PCL, and decellularized extracellular matrix scaffolds and

Table 2
Changes in mechanical properties of PCL and blend PCL.

S N	Mechanical properties	Blending material	Mechanical properties (MPa)		References
			Pure PCL	Blend PCL	
1	. Compressive modulus	Magnesium Phosphate	4.32 ± 0.13	2.37 ± 0.15	WU 2012
2	Elastic modulus Compressive stress	Mg_2SiO_4	3.1(salt leaching) 0.0024 (solvent casting)	6.9 0.3	Diba 2011
3	Tensile Stress	Al_2O_3	3.4	7.3	Dong 2012
4	Tensile Stress	BGMs	14	17.5	McClure 2012
5	Young's Modulus Max Tensile strength	Fish Bone Extract (FBE)		9.18–9.42 82.3–97.4	Heo 2018

observed them to exhibit excellent biocompatibility, cellular adhesion, proliferation, and differentiation by increasing the expression of osteogenic-related genes.[84] da Cunha 2019 fabricated PCL-Biosilicate scaffold using extrusion printer, with 0°/90° pore sizes and pore interconnectivity, which led to 57% increase in the stiffness of scaffold, without increasing any toxicity.[85] González-Gil (2019) used a bone non-union model in Sprague-Dawley rats in six groups: control, live bone allograft, rhBMP-2 in collagen; acellular PCL; PCL with periosteum-derived MSCs, and PCL containing bone marrow-derived MSCs. Significant new bone formation was seen in LBA, CS^{BMP2} and PCL^{PMSCs} groups at 8 weeks. However, at 10 weeks, green fluorescent protein positive cells were detected only in LBA group in the outer cortical bone in close contact with the periosteum.[86] Nguyen 2019 conjugated a 'smart' Poly N-isopropyl acrylamide to fabricate PCL bead surfaces to serve as a thermo-responsive macrocarrier for non-invasive detachment of cells, and allowed human dermal fibroblasts and mesenchymal stem cells to adhere, and proliferate.[87]

Fuchs (2019) developed PCL scaffolds via melt electrospinning writing (MEW), and core membrane via film casting. PCL scaffolds and core membranes demonstrated good cytocompatibility for all cell lines, which even enhanced by fusing both components together.[88] Wei (2019) developed a gelatin collagen, PCL skin substitute using two different GC:PCL ratios (1:8 and 1:20). In vitro test results showed better cell proliferation in the scaffold with a lower collagen content. Hence a lower collagen and higher PCL scaffold was seeded with adipose-derived stem cells and studied for animal wound healing, which showed significant promotion in wound healing and skin regeneration.[89]

1.11. In vivo studies

Also several studies have been done and a few are summarized in Table 3.

Although it is a challenge to achieve the desired level of cell density, vascularisation and faster tissue maturation, successful bone constructs have been demonstrated using polymers specially PCL, which exhibited the greatest success among other polymers. However, a closer replication of cell's natural environment and stimuli can maximize osteogenesis.

Scaffold-induced cell homing by controlled release of chemokines and various other methods is being investigated. Several key chemokine receptors (CCR1, CXCR4, CXCR5 and CXCR6) are important factors in attracting stem cells.[90,91] Owing to the advancements in the 3D bioprinting technologies, a precise control over microarchitecture and spatial content of the construct is increasing credibly. The scope for creating complex PCL scaffolds tailored to patient-specific cost effective applications in the future is vast.

2. Conclusion

PCL polyester has easy availability, is relatively inexpensive and can be modified to adjust its chemical and biological properties, physiochemical state, degradability and mechanical strength and make it useable without significant loss of properties. Its longer degradation time makes it popular for use for replacement of hard tissues, load-bearing tissues by enhancing stiffness, and for soft tissues by decreasing its molecular weight and degradation time.

Declaration of competing interest

Authors have no conflict of interest to disclose.

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Indian Council of Medical Research (Bioengineering) :Biomaterial for designing of mandibular bony scaffold: Evaluation of polymer-

Table 3
In vivo studies using PCL based scaffolds.

S No.	Scaffold used	Experimented on	Inference	Reference
1	10% HA reinforced PCL manufactured by solvent casting and particulate leaching method	Rabbits with calvarial bone defect MG-63 pre-osteoblast-like cells were cultured	Increased cell proliferation and cell adhesion	Kim 2013
2	13%, 20%, 26% (v/v) uHA (urchin-like) dHA (MG2 +-doped) 10% chloride crystals reinforced PCL by Solvent casting and Particulate leaching method	Rabbits with distal femoral condyles defects MG-63 osteoblast-like cells were cultured	Increased Mechanical response, Higher ALP activity Higher proliferation, No inflammation. Good regeneration	Guarino 2016
3	PCL/HA scaffolds (60/40 w/w), realized via FDM immersed in unprocessed bone marrow blood (UBMB) taken from rabbits to create a coating and the formation of marrow clots (MC).	Rabbits with condyle and distal femur fractures	Better adhesion and proliferation and Improved osteogenic differentiation was observed.	Zheng 2017
4	PCL/PLGA/duck beak scaffold	New Zealand White rabbits with 5 mm critical defect in diaphysis of left radius	Scaffold promotes new bone formation	Lee 2016
5	3D electrospun PCL/PLA blend (mass ratio: 4/1) nanofibrous scaffolds	Critical-sized cranial bone defect in mouse	strongly favorable for hMSCs osteogenic differentiation and cranial bone formation.	Yao 2017

ceramic nano composite scaffold with BMP for critical size mandibular defect. (5/3/8/290/2015-ITR: 28,09,800)

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