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Collagen and the Effect of Poly-L-lactic Acid based Materials on its Synthesis

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Abstract

Collagen is an important protein in various biological functions such as providing elasticity and waterproofing to the skin, structural stability to the cells in connective tissues (e.g. tendons, and bone) as well as stabilisation of the atherosclerotic plaque. Collagen as a peptide with a peculiar triple helix structure is majorly composed of glycine, and proline amino acids and is synthesised by fibroblasts via intracellular and extracellular mechanisms. Collagen plays an important role in wound healing, bone repair and plaque build-up during atherosclerosis. Various factors such as interleukins, insulin-like growth factor-I, nicotine, and glucose have shown to influence collagen synthesis. This paper provides an overview on the collagen structure, synthesis mechanisms, and the effective parameters on its stimulation. Poly-L-lactic acid as a well-known biocompatible and biodegradable polymer has proved to stimulate collagen synthesis in various physical forms. As such, in this review a special emphasis is put on the effects of poly-L-lactic acid as well as its mechanism of action on collagen synthesis.

Keywords: Collagen, poly-L-lactic acid (PLLA), tissue engineering, chondrocytes

1. Introduction

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Collagen is the most abundant protein in the body and is found in almost every tissue including but not limited to skin, cornea, bone, tendon, cartilages, extracellular matrix (ECM), and blood vessels. Collagen forms an integral part of the ECM, providing structural support, and rigidity to the connective tissues such as bones, cartilage and ligaments. Structurally, collagen is made up of amino acids but the orientation varies according to the subtypes. There is a total of 28 types of collagen which have been discovered with type I comprising of 90% of the total collagen in the human body. Primarily, glycine (GLY) forms the backbone on any collagen molecule with the representation as GLY-proline-X or GLY-X-hydroxyproline forming the basic background for collagen. X represents any other 17 amino acids with every third position being GLY [1]. As shown in Fig. 1, collagen has a triple helical structure with three parallel polypeptide chains in a left-handed polyproline II-type helical coil [2]. Due to the tight packing of polyproline II-type, one of the residues creates a staggering effect forming a right helix. The continuity of GLY at every third position is disrupted at certain locations of nonfibrillar collagens [3].

Mechanical properties of collagen-containing tissues depend upon variety of factors such as hierarchical assembly, alignment, and length of collagen fibrils as well as contribution of other components [6]. The presence of calcium minerals for instance confers stiffness to bone and dentine, while their absence in soft tissues (e.g., blood arteries and skin) is the main cause of elasticity. In addition, hierarchical assembly of collagen which is highly dependent on its type and structure, primarily determines collagen function and its final mechanical strength [11]. For example, collagen in ECM organizes in sheet-like structures, thereby forming suitable substrates to support the cells. Specific collagen self-assemblies in bone and tendons however lead to strong shear and tensile strength, respectively [12]. The impact of the fibril length on tissue properties was also realized when a 30 times increase in tendon strength was observed

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for a 2-day-old chick compared to a 2-week-old embryonic chick [13, 14]. Importantly, collagen expression is modulated in accordance to the degree of mechanical loading that the specific tissue is exposed to [4, 5]. Fibre alignment in the tissue also influence the final properties greatly. Typically, fibrous tissues are much stronger when stress/strain is applied in the fiber direction than perpendicular to it [6]. Modulus of collagen Type II fibrils in articular cartilage was estimated to be 7 and 2.2 GPa when the applied force was parallel, and perpendicular to fibril alignment, respectively [11].

Collagen has a significant purpose in various disease implications mostly as a therapeutic boon. Collagen is integrated into atherosclerotic plaque buildup via the formation of fibrillar cap in the plaque buildup [15]. Wound healing and bone repair also require collagen by stimulating various factors in the process. The synthesis of collagen occurs primarily by fibroblasts that are present in the body. However, the collagen buildup occurs via the foreign body reaction.

Various biopolymers are able to stimulate the subclinical inflammatory response in the host which in turn increases the collagen content. Collagen production can be increased by various factors such as interleukins (ILs), nicotine, and other synthetic polymers [16-18]. Poly(L-lactic acid) (PLLA) is one such polymer which increases collagen production and various combinatorial therapy have proved to be beneficial in tissue engineering [18]. PLLA has been used as a volumizer for facial correction as “facial filling” of lipoatrophic HIV patients and studies have been conducted to show its effect on dermal thickness as discussed in later sections [19]. Commercially, PLLA is available as a lyophilised powder which includes PLLA microparticles (40-63 μm in diameter), carboxymethylcellulose (as an emulsifier), and nonpyrogenic mannitol (as a lyophilisation improver) and used as a hydrocolloid solution for facial filling purposes [20, 21]. Other polymers such as hyaluronic acid and poly (methyl methacrylate) also have bio-stimulatory effects. The performance of these polymers depends on physiological parameters (e.g., pH, charge, affinity for water) as well as physical properties

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(e.g., size, shape, texture, and surface area) [22]. In this review, we will discuss the cellular mechanism of collagen synthesis and the role of major amino acids present in the structure of collagen, its relation to various diseases such as atherosclerosis and bone repair, factors that stimulate collagen synthesis, and the effect of PLLA on collagen synthesis.

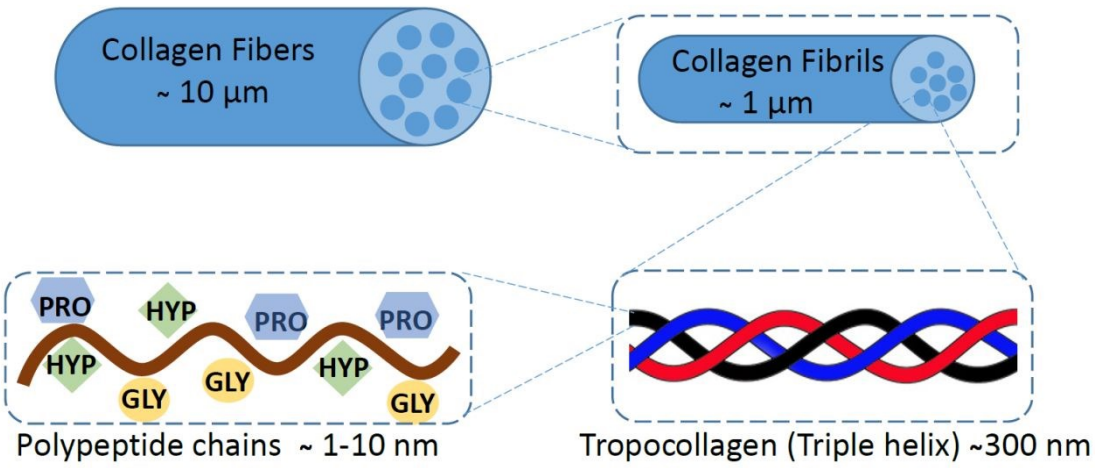

Fig. 1. Schematic representation of hierarchical structure of collagen, ranging from the fibers of collagen (length size 10 µm) to the fibrils, the polypeptide level at nanoscale. GLY, PRO and HYP stand for glycine, proline, and hydroxyproline, respectively.

2. Mechanism of collagen synthesis and production

Collagen is synthesised by fibroblast cells intracellularly and extracellularly. Various types of modifications such as post-translational, cleavage, and fibrillar assembly occur before complete collagen is formed [1]. Here, the natural mechanism of collagen synthesis and the crucial role of amino acids in its stabilisation are discussed.

Collagen synthesis starts by the transcription of pro-a1 and pro-a2 mRNA and then by the process of translation where these two mRNAs move into the cytoplasm from the nucleus. These mRNAs associate with the ribosomes to form pre-pro-polypeptide chain, the precursor of pro-polypeptide. The pre-pro-polypeptide then travels to the endoplasmic reticulum for post-translational modifications. In this step, the pre-pro-polypeptide is converted to pro-collagen by three major modifications. First, the N-terminal in the signal peptide is removed. Then, hydroxyl groups are added to the lysine and proline (PRO) residues by the hydroxylase enzyme

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with vitamin C as a co-factor. Finally, the glycosylation of the selected hydroxyl groups occurs with glucose b and galactose. Upon glycosylation, a triple helical zipper-like folding is formed with three left-handed helices turning into right-handed coil. The procollagen then moves to the Golgi apparatus where final modifications take place and then is assembled into the secretory vesicles to enter into the extracellular matrix. Procollagen then undergoes proteolytic cleavage by the collagen peptidases where the ends of the procollagen is removed to form tropocollagen (Fig. 2). Lysyl oxidases act on the lysines and hydroxylysines to form a covalent bond between the tropocollagen molecules to form a collagen fibril [1]. GLY (~27.6%) is present at every third position and constitutes the largest part in collagen. The next major and vital amino acids of collagen are PRO (~16%) and hydroxyproline (HYP) (~13.4%) [23]. Supplementation of the latter in the diet did not prove to be effective for collagen biosynthesis as it gets degraded after ingestion [24]. However, dietary supplementation with 2% GLY and 1% PRO improved collagen production, and led to enhanced intestinal villus height, nutrient absorption and whole-body weight gains [25]. PRO is hydroxylated by the action of prolyl hydroxylases during post-translational modifications with the help of oxygen, ascorbate, and iron as co-factors. This cyclic rotation creates and strengthens the helical molecule [1]. PRO is metabolically linked to two crucial pathways, i.e. the tricarboxylic pathway and the urea cycle with glutamate being the common element which is then reduced to PRO [26]. The presence of other amino acids such as glutamine, arginine, and ornithine in collagen has a significant role in accumulation, deposition, cell growth and proliferation of cells [27-29].

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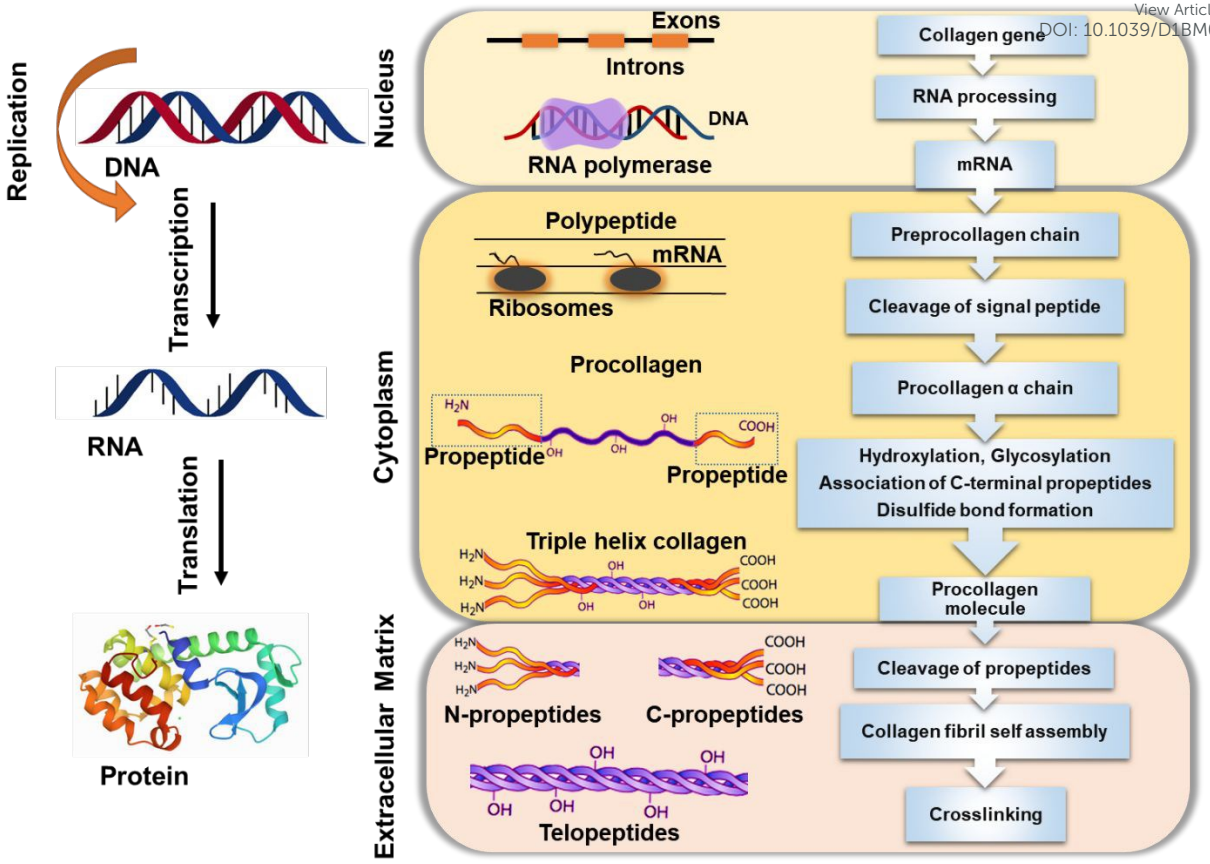

Fig. 2. Schematic representation of intracellular collagen synthesis.

3. Collagen and its implication in diseases/deformities

3.1 Atherosclerosis and plaque build-up

Atherosclerosis is a lipid driven inflammatory diseases and is initiated by the exposure of vascular cells to the excess lipids. By the action of various pathogenic factors, the low-density lipoprotein particles infiltrate via the permeable endothelium and accumulate into the extracellular matrix (ECM). Enzymatic modifications recruit other immune cells such as monocytes, macrophages, and T-cells to the site, leading to the differentiation of macrophages into foam cells. These low-density lipoprotein rich foam cells degrade the elastic lamina in the presence of cathepsin K and stimulate the migration of vascular smooth muscle cells to produce ECM components (Fig. 3) [30, 31]. Collagen level in the plaque reflects the plaque vulnerability or stability, and its loss is associated with inferior strength of the arterial wall

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[32]. A vulnerable plaque is defined as having a larger necrotic core which is infiltrated with macrophages overlying with a thin fibrous cap, whereas a stable plaque has a thicker cap, preventing the necrotic core from plaque rupture. The fibrous cap majorly consists of collagen I and III, proteoglycans, and interspersed smooth muscle cells. Therefore, the fibrous cap and collagen are of utmost importance for the stability and integrity of the lesion [33]. Matrix metalloproteins (MMP) (e.g., MMP-1, -3, and -8) that are mainly released by the macrophages can weaken the cap by collagen degradation [34]. As the unstable fibrous cap ruptures, the content of the necrotic core is released, triggering the coagulation cascade by activating the platelets in response to the released lipids and the tissue factors [33].

Internal factors in the atherosclerotic plaque also regulate the ECM levels and contribute to plaque stability. Transforming growth factor-β (TGF-β) has been reported to have an antiatherogenic effect. Apolipoprotein E knockout ($ApoE^{(-/-)}$) mice were tested with macrophage-specific TGFβ overexpression and they developed a smaller plaque, lesser macrophages, more smooth muscle cells, and significantly more collagen as compared to controls (untreated $ApoE^{(-/-)}$ mice) [35]. TGF-β is recorded to have a stabilising effect in atherosclerotic plaque by an IL-17 dependent pathway [36]. Enhanced TGF-β signalling in Smad7 (inhibitor of TGF-β signalling in T cells) deficiency leads to enhanced T helper 17 (TH17) differentiation in draining lymph nodes of atherosclerotic plaques. This, in turn, causes an IL-17A (IL-17A)–dependent increase in collagen fibre formation and fibrous cap development. ApoE on the other hand acts as a repressor for collagen type VIII as it was upregulated in $ApoE^{(-/-)}$ mice [37, 38]. The loss of ApoE upregulates the expression of type VIII collagen in the vessel wall. It should also be noted that the concentration of high-density lipoprotein (as a primary carrier of ApoE in plasma) is lowered as the plaque progresses resulting in collagen upregulation. The mechanism by which ApoE regulates type VIII collagen expression is still unknown [38].

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Deficiency of von Willebrand factor (vWF) -as a blood glycoprotein involved in hemostasis- is associated with a lower risk for atherosclerosis and atherothrombosis. vWF enables the binding of platelets along the damaged vessels, leading to thrombogenesis and plaque instability. The association of platelet glycoprotein Iba receptors to vWF promotes the adhesion of platelets to endothelial cells, whereas adhesion of collagen to vWF is mediated through α2β1 integrins [38]. Plaque stability can be enhanced by neutralizing the interaction of the platelet-vWF-collagen axis and reduce the thrombotic complications. Neutralisation of the interaction of the platelet-vWF-collagen axis could potentially enhance plaque stability and reduce thrombotic complications [39].

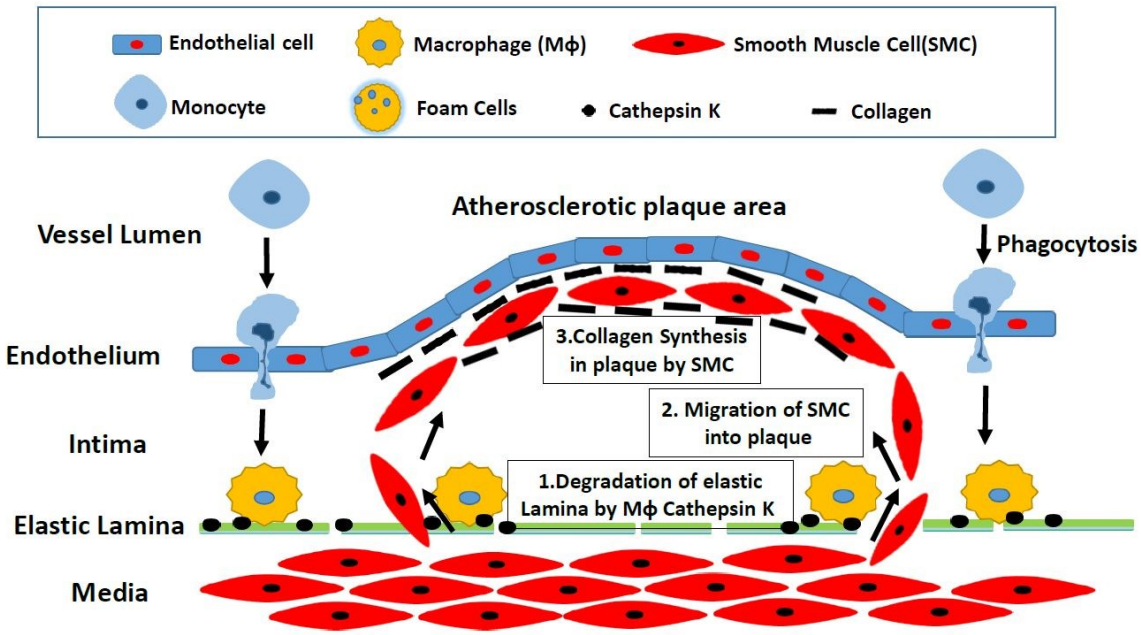

Fig. 3. Diagrammatic representation of collagen synthesis and deposition in atherosclerotic plaque by smooth muscle cells

3.2 Bone repair and wound healing

Bone is a composite tissue and structured with ECM with a prevalence of type I collagen (17-20%). Calcified bone contains about 70 % inorganic mineral (hydroxyapatite) (HAp), 25 % organic matrix, and 5 % water. Collagen 1 synthesised by osteoblasts is the main constituent (ca. 90–95%) of the organic matrix of bone. The synthesised collagen is deposited in parallel

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or concentric layers to produce mature (lamellar) bone [40]. Aside from collagen, osteoblasts secrete other proteins such as osteocalcin and osteopontin during the ossification process to transform minerals such as calcium phosphate into the crystalline form of HAp. Bone morphogenic factors (BMP) have a stimulatory effect on collagen synthesis. BMP-2 enhances the activity of osteoblasts by increasing the activity of alkaline phosphatase, which in turn stimulates collagen synthesis. BMP-2 has also been used for cartilage repair by the expression of specific isoform of type II procollagen and stimulate chondrocyte redifferentiation [41]. BMP-3 has also been reported to possess a direct stimulatory effect on osteoblast and collagen synthesis [42].

Growth hormone secreted by pituitary, thyroid acts directly on the osteoblasts with hormone receptors, stimulating their activity, thus increasing the synthesis of collagen, osteocalcin, and alkaline phosphate. Bone remodelling is regulated by various local factors such as growth hormones and cytokines. Insulin-Like Growth Factor I and II (IGF I&II) are found in high concentrations in the osteoblastic matrix. IGF I &II increase the number and function of the osteoblasts and stimulate collagen synthesis. IGF-binding proteins are linked by IGF I&II which has a stimulatory effect on bone [40]. TGF-β, present in the bone matrix, is a potent stimulator of bone formation. TGF-β promotes osteoblastic differentiation, synthesis of the osteoid matrix, and inhibits the synthesis of the proteases, especially the matrix metalloproteinase (MMP), an enzyme that degrades it [44]. In osteoblasts, TGF-β induces proteolytic activation of CREB3L1 (cAMP response element binding protein 3-like 1). This activation results from inhibition of expression of TM4SF20 (transmembrane 4 L6 family member 20), which normally inhibits regulated intramembrane proteolysis of CREB3L1 [45]. Cleavage of CREB3L1 releases its NH_2-terminal domain from membranes, allowing it to enter the nucleus where it binds to Smad4 to activate transcription of genes encoding proteins required for assembly of collagen-containing extracellular matrix [46]. Platelet-derived growth

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factor functions in stimulating the protein synthesis in osteoblasts, favours bone resorption, stimulates the proliferation of fibroblasts and smooth muscle cells, improving neovascularisation, and collagen synthesis [47].

Wound healing is a process that involves the participation of various inflammatory cells until the formation of a fibrous clot. The initial phase begins with activation of thrombocytes to form a fibrin clot and maintain tissue homeostasis, followed by the secretion of cytokines by macrophages/neutrophils and finally keratinocyte stimulation to cover the wound [48]. Basement membrane protein collagen type VII alpha 1 chain (COL7A1) establishes an epidermal attachment to the basement layer. COL7A1 plays a dual role in wound healing including re-epithelisation of laminin-332 at the dermal-epidermal junction and supporting dermal fibroblast migration to regulates their cytokine production [49]. A study on human induced pluripotent stem cell-derived mesenchymal stem cells (hiPSC-MSCs) suggests that exosomes derived from hiPSC-MSCs promote collagen synthesis, and angiogenesis, thereby treating cutaneous wounds [50].

4. Factors that enhance collagen synthesis

Various factors have been examined to enhance the collagen synthesis in different *in vitro* and *in vivo* models [51, 52]. The level of collagen should be maintained at a balanced level for normal physiological functioning. A low level of collagen can cause osteoarthritis and bone weakening. Higher collagen levels lead to scleroderma and can damage internal organs such as kidney and heart [53]. Here, some important parameters affecting collagen synthesis are discussed.

Insulin-like growth factor I (IGF-I) was found to have a stimulatory effect on tendon collagen synthesis in non-smoking men. Local IGF-I administration could also increase the tendon collagen synthesis both in and around the tendon tissue [54]. IGF-1 has an effect on

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tendon construct formation and collagen fibrillogenesis when tested on isolated human tendon cells. Samples supplemented with IGF-1 showed a higher collagen content of 12.14% and 15.62% for 21 and 28 days, respectively as compared to samples without IGF-1. IGF-1 also had a stimulatory effect on fibrillar diameter showing a skewed distribution after 21 days with higher diameters up to 75 nm. After 28 days, the diameter range had a spread between 20- 95 nm [55]. The combination of TGF-β and IGF-1 in another study exhibited a positive effect on the maximal tensile load of on human anterior cruciate ligaments as well as their collagen content (2.9 fold increase) [56].

IL-13 was also found to promote collagen synthesis in Crohn's disease patients [57]. In this disease, intestinal fibrosis occurs as a result of the overproduction of tissue healing proteins such as collagen. It was found that infiltration of IL-13Rα1^{+}, KIR^{+} innate lymphoid cells, which produce IL-13, inhibits fibroblasts MMP synthesis and ultimately leads to collagen deposition [26]. **IL-6** also plays a major role in collagen synthesis especially during cardiac hypertrophy as tested *in vitro and in vivo* [58].

M-CSF (macrophage colony-stimulating factor) and IL-34 (IL−34) are macrophage differentiating factors that signal via M-CSF receptors and promote monocyte differentiation into macrophages. A higher level of M-CSF and IL-34 is observed in patients with liver fibrosis due to macrophage accumulation. Therefore, a study done on chronic hepatitis C virus patients revealed that macrophages generated with IL-34 (IL-34-Mφ) and M-CSF (M-CSF-Mφ) induce type I collagen synthesis by hepatic stellate cells, the main collagen-producing cells in liver fibrosis. This is a result of a decreasing collagenase and MMP1 expression by M-CSF and IL-34, thereby enhancing the collagen synthesis [59].

Vitamin C also has an important role in collagen synthesis. It stimulates lymphocyte proliferation and protects neutrophils from oxidative damage [45]. Furthermore, ascorbic acid

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has a role in the deposition of type IV collagen in the basement membrane, stimulating endothelial proliferation, inhibiting apoptosis, scavenging radical species, and sparing endothelial cell-derived nitric oxide to help modulate blood flow [60].

Studies also suggest that the **TGF-β** pathway induces collagen synthesis in palatal mesenchymal cells. miRNA-17-92 (miR-17-92) cluster was found to directly target TGF-β R2 (TGF-β receptor), Smad2, and Smad4 (main signal transducers of TGF-β receptors), which are involved in the TGF-β pathway, and inhibit collagen synthesis [61]. Similar observations were recorded with miR-133a in hepatic stellate cells and showed that it also decreases collagen expression by inhibition of the TGF-β pathway [28]. MicroRNA-214 (miR-214) mediates cardiac fibroblast proliferation and collagen synthesis via inhibition of mitofusin2, a key regulator of cell proliferation, and activation of ERK1/2 MAPK (extracellular signal-regulated kinase–mitogen-activated protein kinase) signaling pathway [62].

Nicotine, a stimulant and potent parasympathomimetic alkaloid, was also found to increase the chondrocyte proliferation in a time- and concentration-dependent manner. An increase in collagen II expression was observed in chondrocytes with a nicotine dosage of 25–100 ng/ml [16]. Another study on Windsor rats revealed that nicotine alone increases the collagen level in the aorta while the inclusion of melatonin to nicotine lowers the collagen content [63]. *In vitro* studies on atrial myocardial cells suggested that nicotine stimulates the collagen production 7 folds after 24 hours [64]. *In vivo* studies also demonstrated that nicotine activates fibroblast and therefore increases collagen production in various systems such as lungs, heart, and joints, confirming the *in vitro* results. The increase in collagen levels is due to altered PTHrP-receptor binding, resulting in decreased cyclic AMP (cAMP)/PKA (cyclic adenosine monophosphate/protein kinase A) signaling and causes a phenotypic change in the fibroblast. This lipogenic transdifferentiating is hypothesised to increase collagen production in fibroblasts.

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Soybean peptide and **collagen peptide** have different function to maintain tissue flexibility [65]. The former has antifungal and antioxidant properties which increases collagen I synthesis, while the latter increases corneal moisture content and viscoelasticity [65]. Tokudome et al. [66] showed that when dermal fibroblasts are supplemented with both soybean peptide and collagen peptide, there is an increase in collagen level due to a lower gene expression of MMP-1. Similarly, **asiaticoside**, extracted from *Centella asiatica* upregulates collagen 1 gene expression by enhancing the tissue inhibitor of metalloproteinase-1 mRNA expression [67].

Tumor necrosis factor-like weak inducer of apoptosis, a newly discovered tumor necrosis factor (TNF) superfamily of ligands is transmembrane homotrimer, stimulates collagen synthesis and improves the proliferation of cardiac fibroblasts by activating NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) pathway and increasing MMP-9 activity [68]. Aguilar et al. [17] found that **high glucose concentration** leads to a higher level in overall protein *O*-GlcNAcylation and overexpression of TGF-β1, SMADs2/3, and SMAD7 as the regulators for collagen synthesis, leading to an increase in collagen level.

Alpha hydroxy acids have also been found to strongly affect collagen synthesis. Lactic acid, for example, is used in over-the-counter skincare products as well as professional treatments. Lactic acid helps the older and dull cells on the skin surface to slough away by dissolving the bonds that hold them together, and greatly contribute to skin exfoliation. Lactic acid speeds up cell turnover by stimulating cell renewal and has shown signs of improvement when used regularly [69]. It also enhances collagen renewal and firms the skin texture. Fibroblasts are redirected to chondrogenic phenotype in the presence of lactate. During this process, lactate also improves the stimulation of aggrecan, TGF-β and collagen type II [70]. Studies on liver fibrosis caused by alcohol intake have also shed the light on the lactate effect rather than the direct impact of alcohol itself. The produced lactate and acetaldehyde as a result of alcohol oxidation were found to stimulate collagen synthesis on myofibroblasts [71].

Glycolic acid as another alpha hydroxy acid has been found to play the same role as lactic acid even with higher efficiency in collagen stimulation. In vitro cell proliferative as well as collagen upregulation effects of glycolic acid showed a steady increase in a dose-dependent manner. [72, 73]. Glycolic acid was also shown to improve the photo-damaged skin by chronic solar irradiation. The epidermal and dermal remodeling were observed upon treatment with glycolic acid. A larger deposition of collagen was achieved which was attributed to the longer treatment intervals [74]. Glycolic acid was also found to improve collagen production by modulation of matrix degradation, leading to the release of cytokines such as IL-1α which is regarded as the chief mediator for collagen synthesis [75].

5. Poly-L-lactic acid (PLLA) and collagen synthesis

PLLA is a polymer that benefits from high level of biocompatibility and biodegradability and thus has wide applications in biomedical area [76, 77]. It is obtained from lactic acid or lactide via poly-condensation or ring opening polymerisation, respectively (Fig. 4-a). It should be noted that L- and D- enantiomers of lactic acid result in different polymers of the monomer including PLLA, poly (D-lactic acid) (PDLA), poly (D- and L-lactic acid) PDLLA and meso-PLA. In 2014, PDLLA was also approved by the Korean FDA, and has been used for collagen stimulation and aesthetic applications [78]. PDLLA due to heterogeneity in chemical structure has lower crystallinity than PLLA and thus its degradation rate is faster [79]. Despite the existence of variety of poly(lactic acid) types, most studies related to collagen stimulation are still carried out on PLLA.

Regarding the synthesis of PLLA, as the polymerisation is regarded as a poly-esterification, high temperature together with continuous water removal (as the reaction by-product) is required for the reaction to proceed until a high yield is achieved. Despite developing new

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techniques, it is still challenging to synthesise PLLA with high molecular weight and yield as it needs strict control over the reaction parameters.

PLLA is susceptible to biodegradation due to the presence of hydrolytically unstable ester bonds in the backbone [80]. PLLA is hydrolysed by water molecules through cleavage of ester linkage, leading to reduction of molecular weight (Fig. 4-b). The produced oligomer are either directly metabolised or further degraded to lactate and finally to water and carbon dioxide [81, 82]. The degradation rate of PLLA is influenced by a variety of parameters such as molecular weight, crystallinity, object size as well as the location where it is implanted or injected. Regarding the latter for example, if the degradation products which are generally acidic compounds are not cleared away due to poor vascularisation, the pH in that specific place is then decreased, accelerating the degradation [83].

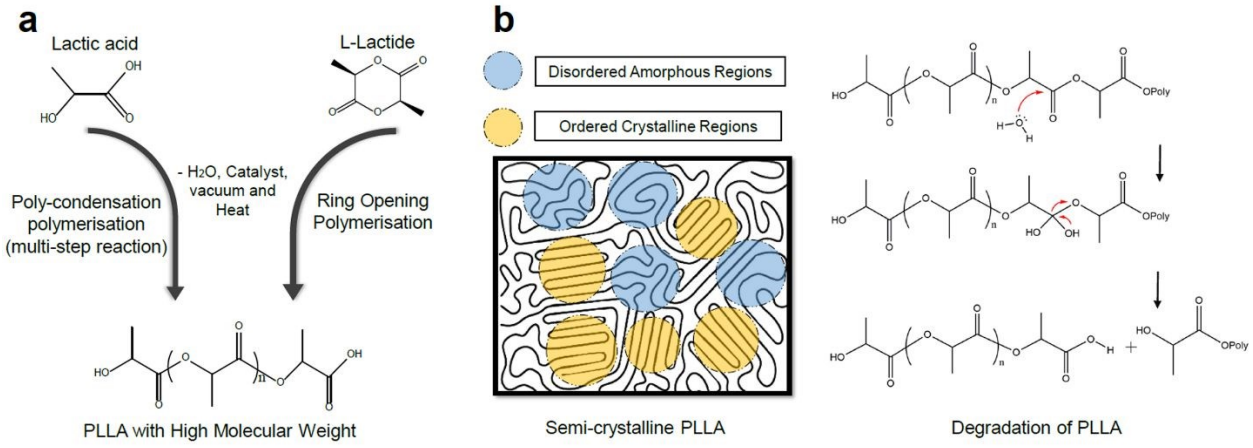

Fig. 4. Synthesis, chemical structure and degradation of PLLA. (a) Synthesis of PLLA through poly-condensation of lactic acid or ring-opening of L-lactide. (b) As a semi-crystalline polymer, PLLA degradation first occurs from the amorphous region as water can diffuse more easily in such parts. This lowers the molecular weight, and leaves acidic and hydroxyl functional groups, improving hydrophilicity. The water-soluble fragments are then attacked enzymatically which is accompanied by a big loss in the mass of PLLA and CO_2 release.

Variety of reports suggest that PLLA in various physical forms (e.g., particle and scaffold) contributes to enhancing collagen synthesis. Injectable PLLA particles as fillers have also been widely employed in cosmetic surgery specifically for facial volume restoration as well as facial

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lipoatrophy in HIV patients [84, 85]. In the following, the effect of PLLA alone or in combination with other agents on the collagen synthesis and applications in tissue engineering is discussed in Table 1.

5.1. PLLA mechanism of action on collagen synthesis

PLLA mechanism of action to increase collagen synthesis follows the principle of immunological response and foreign body reaction where PLLA is considered as a subclinical foreign body [86]. Foreign body reaction occurs in a successive manner starting with protein absorption followed by neutrophil and then macrophage infiltration and finally fibrotic encapsulation [87]. This principle was also proved in our recent study, where PLLA nanoparticles showed a significant stimulation in fibroblast macrophage cocultures compared with single line cell lines of either fibroblast and macrophage [88]. This also proved that fibroblasts are activated by the presence of macrophages to secrete collagen. The collagen stimulation mechanism was explored in a variety of animal models as well as human subjects [89, 90].

PLLA particles were suggested to become encapsulated and surrounded by mast cells, mononuclear macrophages, foreign body cells, and lymphocytes 3 weeks after the injection (Fig. 5). Collagen fibers increased in number and simultaneously cell numbers decreased one month after the injection, resulting in a gradual decrease of the inflammatory response (known as waning effect) [18]. Collagenesis continued and the synthesised collagen fibers were surrounded near the PLLA particles. After 9 months, there was no trace of PLLA as it was completely degraded and metabolised possibly by the lactic acid metabolic pathway [18]. Myofibroblasts and collagen were respectively observed after from the second, and fourth week of subdermal injection of poly (D,L- lactic acid) into rats [78]. It is worth mentioning that the degradation process of PLLA to lactic acid itself could potentially contribute to collagen

synthesis as lactate leads to chondrogenic trans-differentiation of fibroblast and expression of collagen type II, as stated above.

It is important to understand the molecular interaction between a biomaterial and the host's response. The response to the biomaterial depends on various physical and chemical parameters such as surface topography and availability of functional groups, chemical structure and bonding respectively. Foreign body giant cell is considered a distinctive feature in the foreign body reaction as a result of fusion of macrophages to form a multinucleated giant cell [91]. The final stages of the foreign body giant cell formation terminate by the formation of a fibrous cap around the giant cells. The fibrous cap consists majorly of collagen and other extracellular matrix components secreted by the fibroblasts surrounding the giant cell. The formation of fibrous cap starts with a loose net of collagen and develops gradually into a more stable form of collagen [92]. The fibrous cap thickness is directly proportional to the sturdiness and the mechanical support it can provide. Initially, the cells recruited to the foreign body site leads to a more cellular form but are replaced gradually into a more fibrous form due to the deposition of extracellular matrix [9, 93, 94].

Foreign body reaction includes four major stages: (1) blood-plasma proteins adsorption to the foreign body, (2) monocyte recruitment, differentiation to macrophages and macrophage adhesion, (3) formation of giant cells, (4) fibroblasts recruitment and fibrotic tissue formation (Fig. 5). It is well-understood that thrombus formation as the first reaction upon injury or biomaterial implantation leads to a series of cascades, including activation of the intrinsic and extrinsic coagulation system, platelet activation as well as complement system. Different blood proteins are deposited onto the biomaterial depending upon the morphology and physicochemical properties of the implant surface [97]. The main proteins such as IgG (immunoglobulin G), C3 (complement component 3), and factor XII get activated and

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deposited on the surface. The type of protein determines the class of inflammatory cells (macrophages/leucocytes/monocytes) that are adhered/deposited on the surface.

Inflammation is considered as the second stage of the foreign body reaction and occurs in acute and chronic stages. After protein absorption, toll like receptors present on membrane of mast cell initiate the immune response which attracts polymorphonuclear leukocytes (PMN) to the site. Upon adhesion, PMNs are activated, degranulated and secrete histamine, IL-4, IL-13, PMN-derived IL-8, MCP-1 and MIP1β in order to attract more lymphocytes [95]. In the chronic stages, circulating monocytes and lymphocytes respond to platelet, PMN- and mast cell-derived chemoattractant at the implantation site. The macrophages surrounding the site secrete cytokines, transforming growth factor-β (TGF-β), interleukin-1 (IL-1), IL-6, IL-8 and tumour necrosis factor α (TNF-α), degrading the biomaterial and inducing tissue remodelling [96]. The lymphocytes are mainly CD4+ lymphocytes and secrete IL4 and IL13 which induce macrophage phenotype switching and foreign body giant cell formation.

Foreign body giant cell formed by the fusion of macrophages is due to the response of IL4 and IL13. Various adherence receptors present on macrophages such as CD44, CD47 and E-cadherin are responsible for the macrophage fusion. Collectively, the formation of foreign body giant cell is due to the secretion of several membrane proteins by macrophages including CD11, CD45 and CD31 and expression of receptors for IL-1 IL-2, IL-4 and IL-8 by T lymphocytes.

Fibrous cap formation is the final stage in the process of foreign body reaction and leads to a fibrotic, collagenous capsule around the biomaterial. Fibroblasts are recruited and differentiate into myofibroblasts under the influence of TGF-β, which results in the synthesis and secretion of collagen [92, 95]. Capsule formation is affected by an assortment of pro-fibrotic and angiogenic development variables like PDGF, VEGF and TGF-β, which are emitted by M2 macrophages but moreover by a few other cell types counting other immune cells,

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keratinocytes, fibroblasts, endothelial cells. Matrix metalloproteinases secreted by macrophages also play a part in fibrous cap remodelling [9].

Integrins as a class of receptors on the cell surface also play a key role in moderating cell-to-cell interaction and cell adhesion to various proteins such as fibronectin and IgG. Integrin binding to macrophages has a significant function in the adhesion of macrophages to, and interaction with fibroblast for the formation of foreign giant cell. Cytoskeleton remodeling occurs over time in macrophages to allow their spreading over the biomaterial [98]. IL-3, IL-4, DAP12 (a 12KDa transmembrane protein), and STAT6 (signal transducer and activator of transcription 6) induce the fusion process of macrophages to giant cells which is dependent on the phenotype and biomaterial shape as well [99]. The biomaterial then undergoes degradation via a process known as frustrated phagocytosis by the mediators such as reactive oxygen species, and enzymes secreted by foreign body giant cells as well as macrophage, resulting in accelerated degradation of the biomaterial. Succeeding the foreign giant cell formation, the ECM formation, and fibrotic encapsulation takes place. This process separates the granuloma from the surrounding tissue and initiates the infiltration of fibroblasts, macrophages, and neovascularisation. The cells surrounding the foreign giant cell secrete angiogenic factors, such as TGF-β which is particularly important in the transactivation of Smad2 and secretion of collagen in the fibrotic cap by the fibroblasts differentiation [100]. Additionally, other cell types such as neutrophils, monocytes, and macrophages involved in the fibrotic reaction secrete TGF-β, as well. Cytokines such as IL-13, IL-10 are also secreted at a later phase of fibrosis but they have a minimal role in the earlier stages of fibrosis.

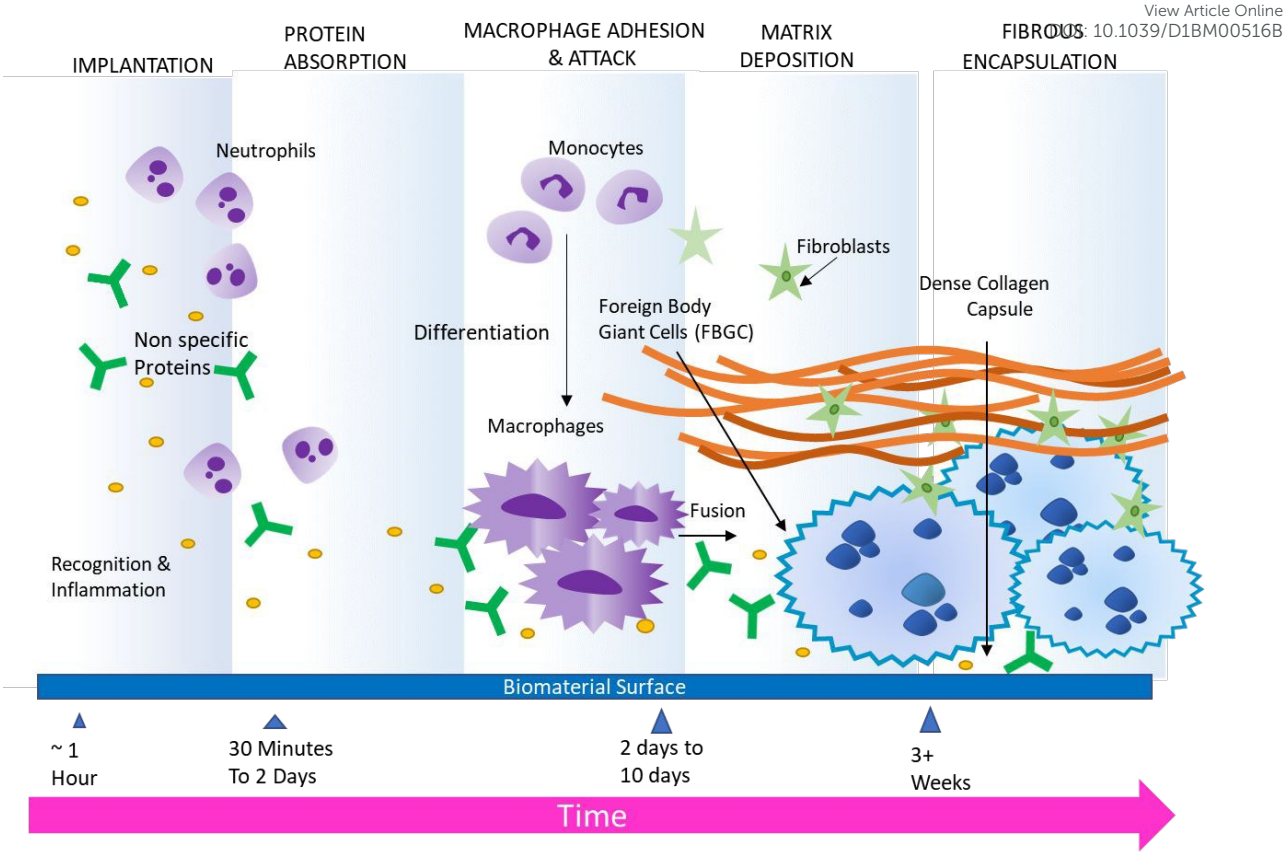

Fig. 5. Schematic representation of foreign body reaction to a biomaterial.

5.2. PLLA treatment with modifications to increase collagen synthesis

PLLA particles

PLLA particles are used in cosmetic surgery and act as a facial volumiser in HIV-related facial lipoatrophy patients. Sculptra is the brand name for injectable PLLA which was commercialised for its use in volume restoration. They are supplied in the form of powder and mixed with carboxymethylcellulose as an emulsifier, and nonpyrogenic mannitol (as a lyophilisation improver [101]. After injection carboxymethylcellulose and mannitol are removed due to water solubility and small size. The PLLA particles have irregular shape with the size ranging from 40 to 63 μm in diameter. The relatively large size prevents the particles from phagocytosis [101].

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PLLA particles can be used for deep tissue filling or in the dermal subcutaneous junction to firm the skin and soften lines and wrinkles. The collagen builds in and improves the skin thickness [102, 103]. PLLA particles does not take up space and does not create immediate results. However, it causes a gradual growth of fibrous tissues, resulting in volume restoration. A series of at least three sessions per treatment area is necessary for an optimal result [102]. A study on the PLLA injection to 568 patients with different injection sites reached the conclusion that the particles are effective for volume correction [104]. The main side effect, though with very low frequency of 1%, was reported to be collagen late nodules and was attributed to the incorrect technique. A clinical trial on 58 subjects for 24 weeks follow-up compared the efficacy of PLLA with that of hyaluronic acid for correction of nasolabial fold [105]. The safety of PLLA injection was further verified. It was also concluded that the efficacy of PLLA is in general comparable to that hyaluronic acid (Fig. 6). However, in young patients, PLLA was more effective as compared to hyaluronic acid. Another clinical trial on 23 patients after 18 months follow-up showed that polylactic acid is effective for penile augmentation [106].

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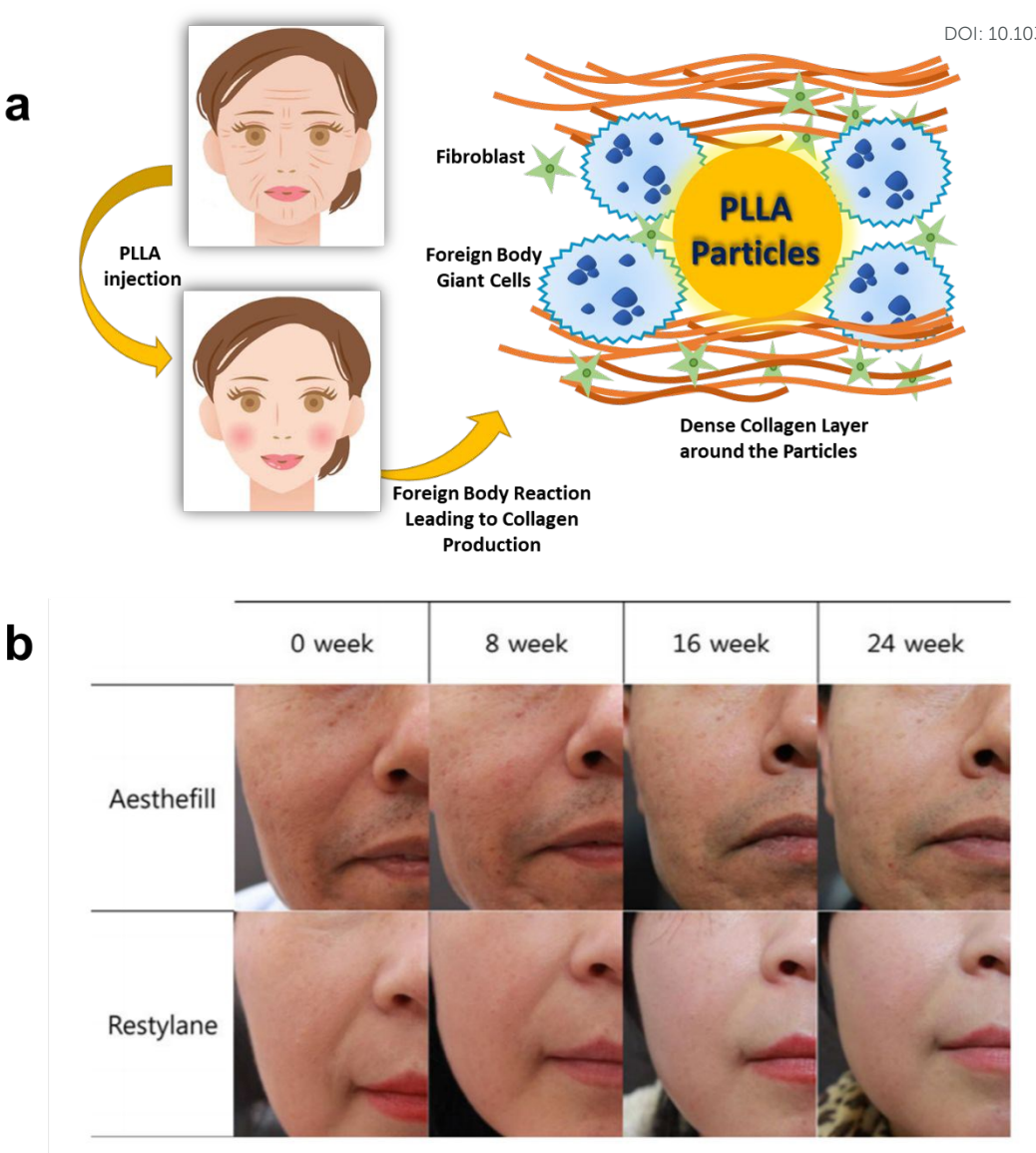

Fig. 6. PLLA particles for facial volumiser. (a) Working mechanism scheme. (b) Images of correction of the nasolabial folds of two patients over every two months. Aesthefill and Restylane are PLLA and hyaluronic acid, respectively. Reproduced from [105] with permission from John Wiley and Sons, copyright 2014.

A combination of ablative fractionated CO_2 laser and topical PLLA particles used for the treatment of atrophic scars was shown to be highly effective and have a synergistic effect in collagen synthesis [107]. The former increases the expression of heat shock protein which in turn activates transient amplifying epidermal stem cells to replace newly damaged cells [108]. Moreover, the expression of cytokines such as IL-1, tissue necrosis factor alpha, TGF-β, and

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MMP, produced by immune cells (leucocytes) remove the damaged collagen and participate in neocollagenesis by dermal fibroblasts, the process of making new collagen.

Ray et al. [88] also investigated the effect of PLLA nanoparticles (prepared by emulsion evaporation method) on the collagen synthesis in the macrophage fibroblast co-culture systems. Stimulation of collagen synthesis was observed in the co-culture system but failed in fibroblast single line culture, verifying the fact that an interaction between fibroblast and macrophage occurs which ultimately leads to an enhanced collagen level.

PLLA based fillers used in granulomatous reactions (chronic inflammation characterised by the accumulation of inflammatory cells) shows an increase in levels of collagen I and III, $CD68^+$ macrophages and $CD90^+$ fibroblasts. These types of cells are mostly found near the PLLA particles. The TGF-β1 and TIMP1 (tissue inhibitor of metalloproteinases 1) signaling pathways are also upregulated which stimulate collagen I &III synthesis [109]. Kim et al. [110] investigated the effect of PLLA particles on collagen gene expression in Hs68 (human dermal fibroblast) cell lines by reverse transcription polymerase chain reaction and western blotting techniques. When the cells were treated with 0.1% PLLA particles, a significant increase in collagen gene expression was observed which was attributed to the activation of the signaling proteins p38 (mitogen-activated protein kinases), Akt (protein kinase B), and JNK (c-Jun N-terminal kinases) [110].

Scaffolds of PLLA and its composites with other materials

Scaffolds are chiefly utilised for cell cultivation and its subsequent implantation where the damage has occurred in order to stimulate and accelerate tissue regeneration [111, 112]. The used material for the scaffold application should be biodegradable, non-toxic, strong, and porous. Porosity provides the cells with enough and suitable space to grow and for the nutrients to be transported homogenously through the scaffold. PLLA has shown to meet the key

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requirements for the use as scaffolds and thus has been the subject of numerous investigations for such purposes [113, 114].

Gundula et al. [115] prepared porous PLLA scaffolds by ternary thermally induced phase separation method and showed that the expression of type I and II collagen is significantly enhanced in the cultured chondrocytes. The differentiation of chondrocytes was more in scaffolds with smaller pores size (Fig. 7).

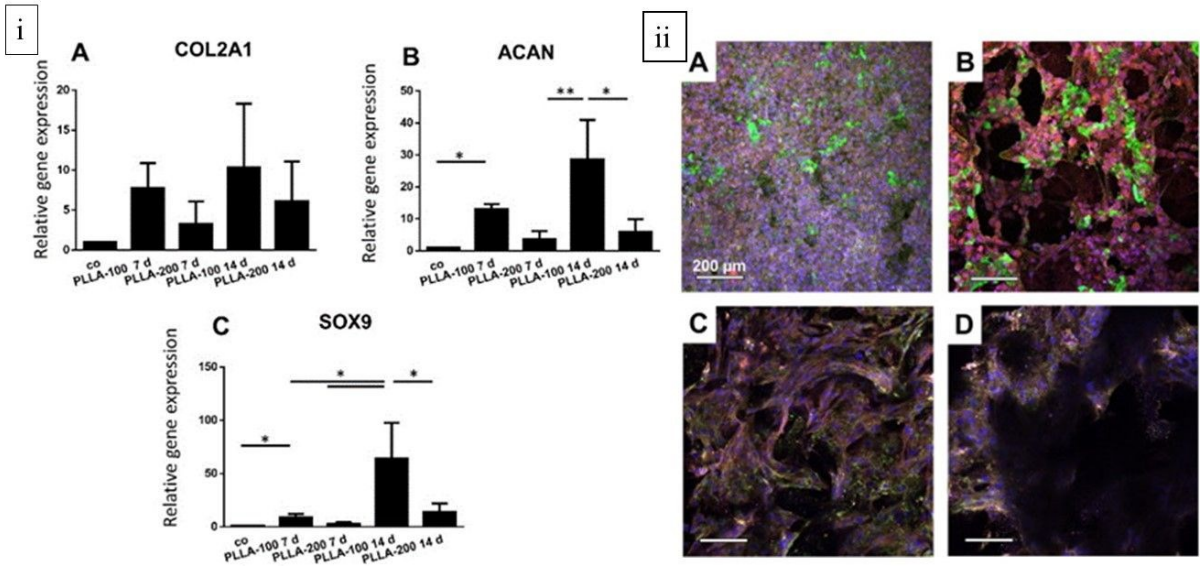

Fig. 7. ECM synthesis in PLLA cultured chondrocytes. (i) Gene expression profile of gene markers of type II collagen (COL2A1, A), aggrecan (ACAN, B) and SOX9 (C) in human nasoseptal chondrocytes cultures in PLLA of pore size 100 and 200 nm (PLLA 100 and PLLA 200, respectively) for 1 and 14 days. Significant differences: *p<0.05; **p<0.01; ***p<0.001. Scaffolds seeded under the same conditions and evaluated at the first day were treated as controls (co). **(ii)** Immunohistochemical analyses of ECM synthesis in: (A) articular chondrocytes after 7 days cultured on PLLA 100. (B) artilcular chondrocytes after 7 days cultured on PLLA 200. (C) nasoseptal chondrocytes after 7 days cultured on PLLA 100 (D) nasoseptal chondrocytes after 7 days culotured on PLLA 200. Red: type II collagen, green: type I collagen, blue: cell nuclei, grey: phalloidin-labeling for F-actin. Scale bars 200 µm Reproduced from [115] with permission from Elsevier, copyright 2017.

PLLA is also employed in combination with other materials including natural and synthetic polymers to enhance skin tissue regeneration and to take advantage of their features. When poly(glycerol sebacate)/PLLA blend scaffolds were tested for growth and proliferation of adipose-derived stem cells (ADSCs), an increase in collagen levels was clearly observed by Sirius red staining (Fig. 8) [116]. Bioactive glass (BG) in combination with porous PLLA

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scaffold was shown to induce chondrogenesis [117]. Type II collagen expression were significantly enhanced and ECM was synthesised in the inner parts of the scaffold. Furthermore, aggrecan gene expression was also stimulated in PLLA/BG scaffolds. Adult mesenchymal stem cells (MSCs) were grown on polyglycolic acid/PLLA (90/10) copolymer scaffolds and the collagen levels of type I, II and X were measured. Higher levels of collagen type I and X were observed but a negligible level of the type II were detected [118].

PLLA/silk fibroin blend nanofibrous scaffolds were fabricated and assessed in terms of interactions with chondrocytes and chondrogenicity. It was revealed that the expression of cartilage-related genes (collagen II, aggrecan, sox9, collagen I, and collagen X) were significantly higher in the blend scaffolds compared to pure PLLA scaffold and supported chondrocyte adhesion and spread. A greatest collagen content was observed in PLLA/ silk fibroin scaffold compared to pure PLLA and TCPS (tissue culture polystyrene) as the control, for all the time points. At days 3, 7, and 14, the collagen content for the PLLA scaffold was less than that of the TCPS, but exceeded at day 21 [119]. The collagen content normalised with total DNA however was not statistically significant between pure and blend scaffolds. In another study of blend scaffold, the levels of collagen II and aggrecan in human primary chondrocyte were also higher in PLLA/gelatin blend scaffold (50/50, and 70/30 w/w) as compared to pure PLLA and polystyrene coated cell culture plates as controls [120].

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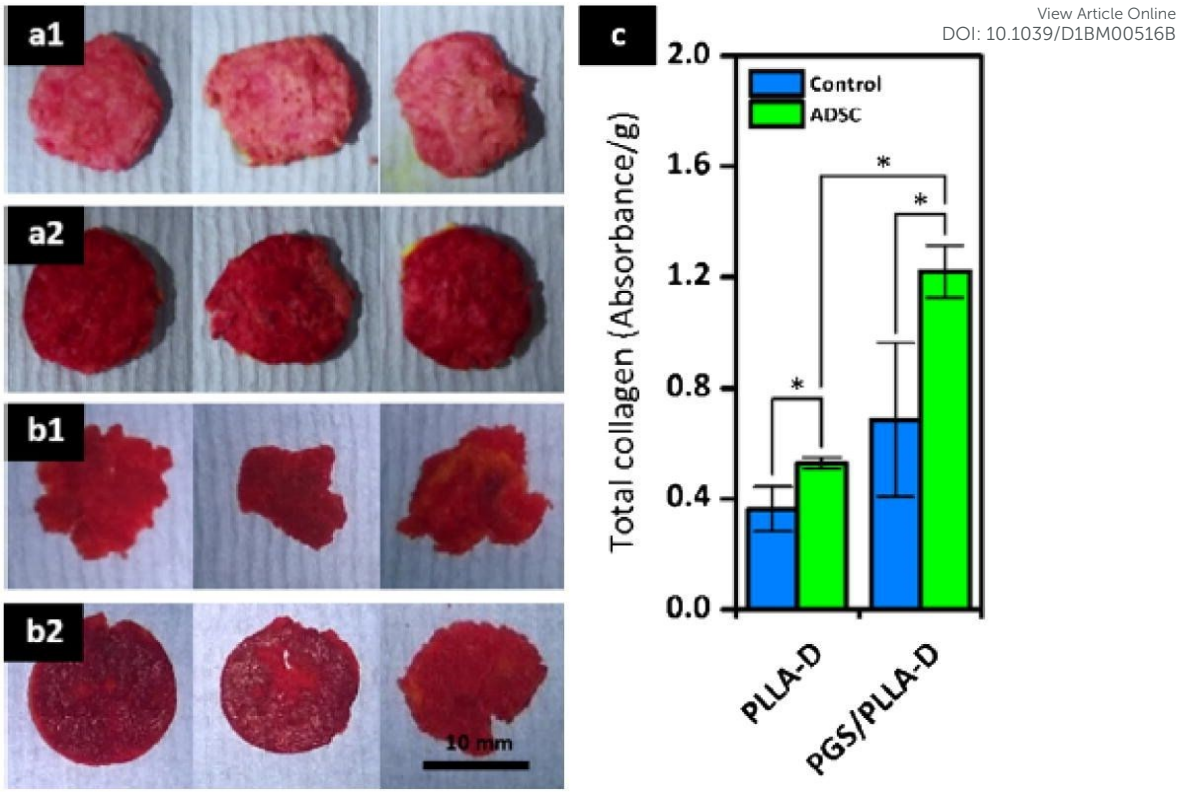

Fig. 8. Effect of incorporation of an effective component on collagen synthesis (i) ECM deposition of ADSC in PLLA scaffolds followed by Sirius red staining. Visualised Sirius red staining of (a1) cell free PLLA-D control samples and (a2) ADSC-seeded PLLA-D samples, as well as (b1) cell free poly(glycerol sebacate)/PLLA-D control samples and (b2) ADSC-seeded poly(glycerol sebacate) /PLLA-D samples. (c) Determination of total collagen amounts by Sirius red staining shown as mean ± SD (n = 3; ∗P < 0.05). Reproduced from [116] with permission from Elsevier, copyright 2015.

Wound healing process can be accelerated by employing wound dressings stimulating epithelialisation as well as collagen expression. Jouybar et al. [121] investigated wound healing of full-thickness skin defect mice (adult male BALB/c mice) by PLLA nanofibrous scaffolds coated with Aloe Vera. To compare the healing efficiency, pure PLLA, Vaseline (negative control) and a commercial wound dressing band (positive control) were used. The healing rate of the latter was found comparable to the Aloe Vera-coated PLLA scaffold. In addition, faster wound healing was observed in the Aloe Vera-PLLA compared to pure PLLA. The Vaseline treatment group had the lowest healing rate. The accelerated healing process was attributed to higher collagen level (~66% after 17 days) in the groups treated with the Aloe Vera-PLLA

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scaffold. As assessed by histological images 17 days after surgery, the scaffold-gel had more collagen, lower inflammation depth, and more neutrophil removal, compared to other treatment groups (Fig. 9). Such an increment in collagen level was not seen 7 and 12 days after surgery, suggesting long-term recovery process, and stimulation of collagen synthesis.

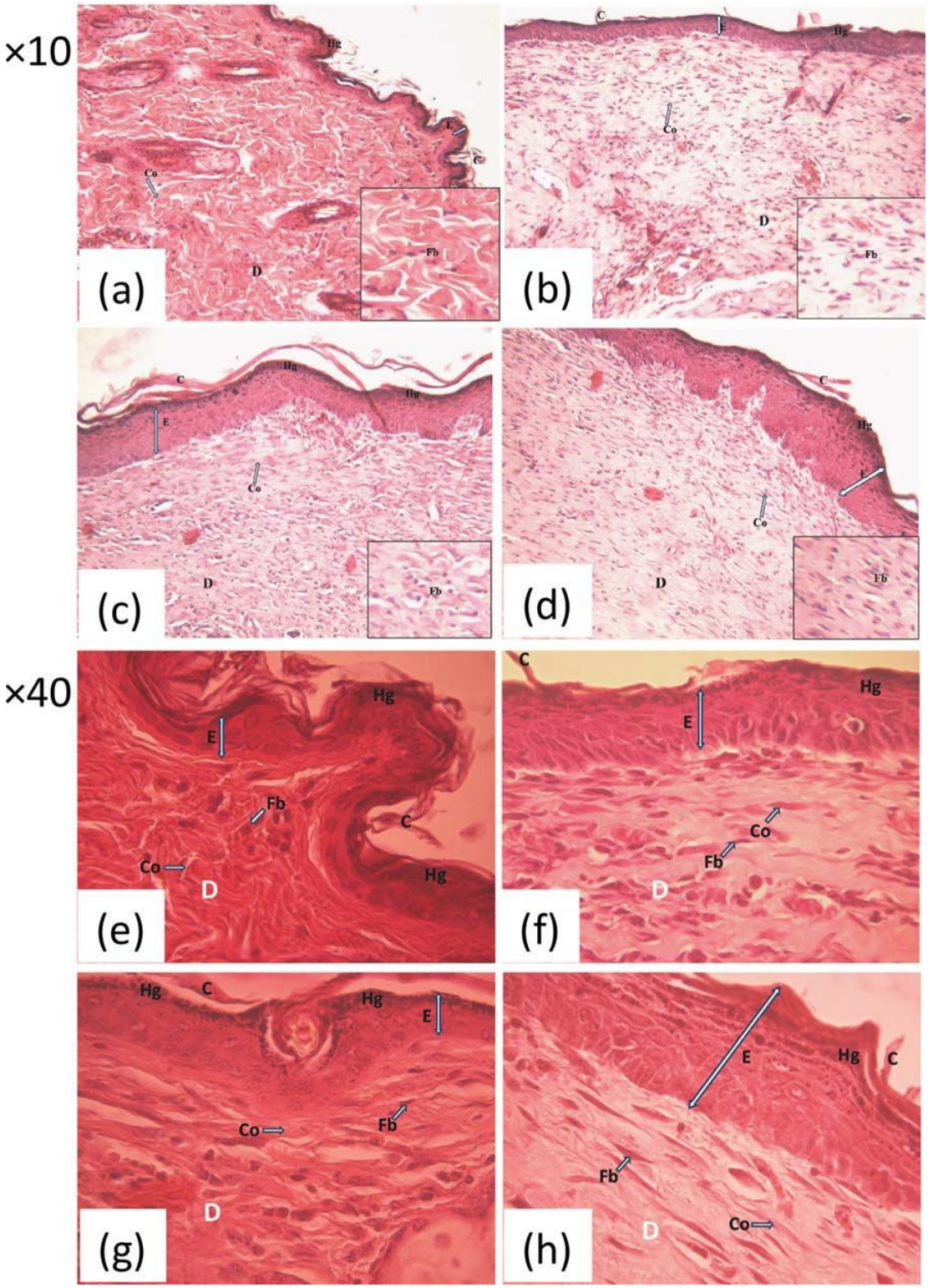

Fig. 9. Pathological (H&E staining) microscopic images of the wound 17 days after surgery in two magnifications: (a,e) scaffold with gel, (b,f) scaffold without gel, (c,g) Vaseline gauze, (d,h) commercial product. E, D, C, Fb, Hg, and Co stand for: epidermis, dermis, crust, fibroblasts, hypergranulosis, and collagen, respectively. Reproduced from [121] with permission from John Wiley and Sons, copyright 2017.

In order to further improve the efficiency of PLLA scaffolds, carbonaceous nanomaterials (e.g., graphene and carbon nanotubes) can be incorporated into the scaffolds. By doing so, a significant enhancement was achieved in the osteogenic differentiation of bone marrow stem cells. Also, expression of type I collagen, as well as osteogenesis-related proteins, was found to be markedly high in bone mesenchymal stem cells (BMSCs) cultured on composite scaffolds compared to pure PLLA [122].

Blended scaffolds of PLLA, collagen, and HAp were tested for its osteogenic potential by culturing human mesenchymal stromal cells and supplementing platelet-derived growth factor-BB (PDGF-BB) [123]. High expression of collagen I, fibronectin and cadherin is observed in PLLA/collagen/HAp and PLLA/HAp scaffolds and thus have a superior osteoinductivity, the property of inducing the process of osteogenesis. Therefore, scaffolds of different materials prove to have a higher stimulatory effect in collagen synthesis as compared to simple scaffolds [123]. Apart from an enhanced collagen synthesis, the combinatorial approach has also proved to be beneficial in other prospects. Combination of PLLA, HAp, cellulose, and loofah provided a highly biocompatible scaffold for human chondrosarcoma cells [124]. Exclusion of cellulose led to stronger scaffolds in terms of mechanical properties. Loofah is extracted from *Luffa cylindrica* and has a ligneous netting system, which can potentially improve the scaffold attachment to the ECM components. The histological analysis showed production of ECM that defined proteoglycan and type I-II collagens.

Table 1. PLLA in various forms and combinations for collagen synthesis

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Physical form	Effect of collagen synthesis/ percentage increase	Signaling pathway	Cell lines /Animal model	Results and Observation	Ref
PLLA nanoparticles	The collagen synthesis is doubled when treated on fibroblast macrophage coculture		Fibroblast macrophage coculture	The collagen synthesis follows the foreign body reaction.	[88]
Injectable microparticles			Elderly patients with severe lipoatrophy and solar elastosis	Papule and nodule formation seen in patients during initial phases of PLLA trials	[84]
Scaffolds	Type II collagen expression increased by 650 % and 900 % after 7 and 14 days incubation on scaffolds, respectively	SOX9	Nasoseptal chondrocytes	Scaffolds with smaller pore sizes promotes chondrocyte differentiation, revealing the importance of scaffold porosity	[115]
Topical PLLA particles (Sculptra) + CO_2 laser	An improvement of 95% in scar recovery		Fresh human cadaver and treatment of ablative fractional laser in combination of PLLA and CO_2	The combined therapy had a synergistic effect on collagen synthesis and atropic scar improvement. Modified Manchester Scar Scale was used to evaluate atrophy, color and contour of scar.	[107]
PLLA particles (Sculptra)	Collagen level of fibroblasts increased by 330% when cultured with PLLA after 48 hours incubation	MAPK and Akt pathways	Hs68 (human dermal fibroblast)	SB203580 (p38/Akt inhibitor) and SP600125 (JNK inhibitor) inhibits collagen synthesis and therefore it proves the involvement of certain signaling proteins such as JNK,Akt and p38 in collagen synthesis)	[110]
Nanofibrous PLLA/poly-(α,β)-dl-aspartic acid/Collagen) scaffolds	Collagen gene expression increased (ca. 25%) and was used to confirm differentiation of adipose derived stem cells		Adipose derived stem cells (ADSCs)	The cell proliferation increased in the scaffolds.	[125]
Poly(glycerol sebacate)/PLLA scaffold	ADSC-seeded scaffolds showed that ADSCs produced significantly (P<0.05) more collagen (approximate 50%) per sample in Poly(glycerol sebacate)/PLLA-D than in PLLA-D specimens		Adipose derived stem cells	Poly(glycerol sebacate) /PLLA porous scaffolds enhances scaffold cell proliferation, penetration & tissue in-growth characteristics	[116]

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PLLA scaffolds + Bioactive glass1393(BG)	The expression of the type II collagen was significantly increased in 1% BG after 14 days (200% increase) and in 1% BG after 7 days (50%) compared with the controls (24 h of culture in neat PLLA100 scaffold)	SOX9	Nasoseptal chondrocytes.	The gene expression of Type II collagen and aggrecan increased significantly in cells cultured from 1% BG. The inner surface of the scaffold are colonised by chondrocytes synthesising an ECM	[117]
Polyglycolic acid/PLLA (90/10) copolymer scaffolds	Increase in collagen types I, II, and X.		Adult mesenchymal stem cells	Collagen type I, II and type X production increased in adult mesenchymal stem cells cultured on polyglycolic acid/PLLA copolymer scaffold. A more homogenous cell distribution profile and matrix accumulation between cell clusters	[118]
PLLA/silk fibroin nanofibrous scaffold	An increased collagen level (39% after 21 days incubation) observed in chondrocytes cultured on PLLA/ silk fibroin scaffold for all time points	SOX9	Chondrocytes	Collagen II, aggrecan, sox9, collagen I, and collagen X gene expressions were increased in chondrocytes cultured on PLLA/ silk fibroin scaffolds and also supporeds chondrocytes adhesion.	[119]
PLLA nanofibrous scaffolds + Aloe vera gel	Highest cell proliferation is detected on cells seeded on PLLA at days 3 and 5, while no difference was observed between TCPS (Control) and PLLA at day 3. Proliferation rate of fibroblast cultured on PLLA was also significantly more than TCPS at day 5. Collagen levels increased by 203% in scaffold-gel after 7 days		Adult male BALB/c mice	Gel coated scaffolds accelerated wound healing process and had the highest overall repair scores	[121]
PLLA/Collagen/HAp	Increased collagen type I was observed in PLLA/collagen/HAp as compared to PLLA/HAp and PLLA/collagen		Human mesenchymal stromal cells	Increased expression of collagen I, fibronectin and cadherin in PLLA/collagen/ HAp and PLLA/HAp scaffolds led to better osteoinductivity.	[123].
PLLA, HAp, cellulose, and loofah	The histological analysis showed production of ECM		Human chondrosarcoma cells	Although the addition of cellulose deteriorated mechanical properties of	[124]

	that defined proteoglycan and type I-II collagens			the scaffold, it improved biocompatibility. Immunohistochemical examination showed the production of collagen type I and IIqualitatively.	

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Conclusion and future prospects

Collagen synthesis occurs primarily in fibroblast starting from the transcription, translation, and post-translational modifications. The presence of collagen is beneficial during various conditions such as providing stability to the fibrous cap of the atherosclerotic plaque, wound healing, and bone repair. Various growth factors have a stimulatory effect on collagen synthesis such as BMP and IGF1, which have been summarised in this paper. Alpha hydroxy acids such as lactic acid have also been in the forefront of studies as they stimulate collagen synthesis. Interestingly, PLLA as the polymer of lactic acid has exhibited promising results on collagen synthesis, and thus has been utilised in various forms ranging from particles to scaffolds for this purpose. Although the PLLA mechanism of action is not fully understood, foreign body reaction is known to be the most widely-accepted mechanism. It seems there is a gap in the proposed mechanisms of the literature considering the ultimate degradation of PLLA to lactic acid as well as the stimulatory effect of the latter on collagen synthesis. In other words, future studies on the relationship of degradation product of PLLA and collagen synthesis can shed more light on the mechanism. As reviewed in the paper, growth factor and hormones such as interleukins, insulin growth factors have stimulatory effects on collagen synthesis and thus could be incorporated into PLLA for further improving collagen production in future investigations.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Abbreviations

ADSCs: Adipose derived stem cellsl; ApoE(-/-): Apolipoprotein E knockout; BG: Bioactive glass; BMSCs: Bone mesenchymal stem cells; JNK: c-Jun N-terminal kinases; COL7A1: Collagen type VII Alfa 1 chain; C3: Complement component 3; cAMP/PKA: Cyclic adenosine monophosphate / protein kinase A; TH17: Enhanced T helper 17; ECM: Extracellular matrix; ERK1/2 MAPK: Extracellular signal-regulated kinase / mitogen-activated protein kinase; GLY: Glycine; hiPSC-MSCs: Human induced pluripotent stem cell-derived mesenchymal stem cells; HAp: Hydroxyapatite; HYP: Hydroxyproline; IgG: Immunoglobulin G; IGF-I: Insulin-like growth factor I; ILs: interleukins; M-CSF: Macrophage colony-stimulating factor; MMP: Matrix metalloproteins; MSCs: Mesenchymal stem cells; NF-κB: Nuclear factor kappa-light-chain-enhancer of activated B cells; p38: mitogen-activated protein kinases; PLLA: Poly-L-lactic acid; PRO: Proline; Akt: Protein kinase B; STAT6: Signal transducer and activator of transcription 6; TGF-β R2: TGF-β receptor; TCPS: Tissue culture polystyrene; TIMP1: Tissue inhibitor of metalloproteinases 1; TGF-β: Transforming growth factor-β; TNF-α : Tumor necrosis factor; vWF: von Willebrand factor.

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