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Poly (lactic acid) production for tissue engineering applications

M. Savioli Lopes[a,b] a*, A. L. Jardini[b], R. Maciel Filho[a,b]

[a]*Laboratory of Optimization, Design and Advanced Control, School of Chemical Engineering, State University of Campinas, Campinas (SP), P.O. Box 6066, 13083-970, Brazil*

[b]*Institute of Biofabrication, State University of Campinas, Campinas (SP), P.O. Box 6066, 13083-970, Brazil*

Abstract

Tissue engineering is the most fascinating domain of medical technology and has emerged as a promising alternative approach in the treatment of malfunctioning or lost organs where patients are treated by using their own cells, grown on a polymer support so that a tissue part is regenerated from the natural cells. This support is known as scaffold and is needed to serve as an adhesive substrate for the implanted cells and a physical support to guide the formation of the new organs. In addition to facilitating cell adhesion, promoting cell growth, and allowing the retention of differentiated cell functions, the scaffold should be biocompatible, biodegradable, highly porous with a large surface/volume ratio, mechanically strong, and malleable. The scaffold degrades while a new organ or tissue is formed. A number of three-dimensional porous scaffolds fabricated from various kinds of biodegradable materials have been developed. Bioabsorbable polymers have been identified as alternative materials for biomedical applications, since these polymers are degraded by simple hydrolysis to products that can be metabolized by the human body. With their excellent biocompatibility, poly-lactones such as poly-lactic acid (PLA), poly-glycolic acid (PGA), and poly-caprolactone (PCL), as well as their copolymers are becoming the most commonly used synthetic biodegradable polymers as fixation devices materials for biomedical devices. Among the biomaterials (biopolymers) used in the medical field, the poly (lactic acid) (PLA) has received significant attention. Poly-lactic acid (PLA) is at present one of the most promising biodegradable polymers for this purpose and has convincingly demonstrated the proof of concept for using in bioabsorbable polymer as bone fixation devices, owing to its mechanical property profile, thermoplastic possibility and biological properties, such as biocompatibility and biodegradability. It is produced from lactic acid, a naturally occurring organic acid that can be produced by fermentation. The objective of this study was to investigate the synthesis of PLA in a laboratory scale in order to characterize it in accordance with the needs for biomedical use.

* Corresponding author. Tel.:+55 19 98174500; fax: +55 19 35213909.
E-mail address: milena@feq.unicamp.br

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Introduction

Over the last century, biocompatible materials such as metals, ceramics and polymers have been extensively used for surgical implantation. Metals and ceramics have contributed to major advances in the medical field, particularly in orthopaedic tissue replacement. However, metals and ceramics are not biodegradable and their processability is very limited. Polymer materials have received increasing attention and been widely used for tissue engineering because of easy control over biodegradability and processability [1,2,3]. Biomaterials and biodegradable materials represent two of the most interesting areas of material science, in which chemical, medical, and environmental scientists are contributing to human health care, improving quality of life, protecting environment from white pollution, and reducing dependence on fossil fuels [4]. Bioabsorbable polymers are preferred candidates for developing therapeutic devices such as temporary prostheses, three-dimensional porous structures as scaffolds for tissue engineering and as controlled/sustained release drug delivery vehicles. Each of these applications demands materials with specific physical, chemical, biological, biomechanical and degradation properties to provide efficient therapy. Tissue engineering is the most recent innovative domain where these biodegradable materials provide surfaces that promote the regeneration and reconstruction of human organs. The constant efforts of cell biologists, materials scientists and engineers are creating a bright future for this polymer as a biomaterial [5]. Tissue engineering has emerged as a promising alternative approach in the treatment of malfunctioning or lost organs. Synthetic biodegradable poly-lactones such as poly-lactic acid (PLA), poly-glycolic acid (PGA), and poly-caprolactone (PCL) as well as their copolymers are now commonly used in biomedical devices [4] because of their excellent biocompatibility. Poly(L-lactic acid) (PLLA) is widely used in the biomedical field [6] due to its biodegradability, biocompatibility, thermal plasticity and suitable mechanical properties [5, 7]. Bioabsorbable fixation devices have been extensively used as dissolvable suture meshes and recently, by orthopedic surgeons [8,9]. Its main application includes surgical sutures, implants for bone fixation, drug delivery devices and materials for tissue engineering. In tissue engineering, cells can be grown in a PLLA scaffold that is inserted at the site of organ defect. When inserted in vivo, it is able to degrade simply by hydrolysis without any use of enzymes or catalysts, thus a second surgical removal of implant is deemed unnecessary [10]. PLA is obtained from lactic acid and converted back to the latter one when hydrolytically degraded. Lactic acid is a naturally occurring organic acid that can be produced by fermentation of sugars obtained from renewable resources such as sugarcane. Although there are multiple ways to fabricate PLA, none of them is simple or easy to execute. PLA synthesis requires rigorous control of conditions (temperature, pressure and pH), the use of catalysts and long polymerization times, which implies high energy consumption. The purpose of the present work is to provide information about the properties and the synthesis methods to obtain PLA bioabsorbable for biomedical devices applications.

Lactic acid

Lactic acid (2-hydroxypropionic acid), CH3–CHOHCOOH, is a simple chiral molecule which exists as two enantiomers, L- and D-lactic acid, differing in their effect on polarized light. The optically inactive D, L or meso form is an equimolar (racemic) mixture of D(-) and L(+) isomers [5]. Three stereoforms of lactide are possible: L-lactide, D-lactide, and meso-lactide (see Fig. 1).

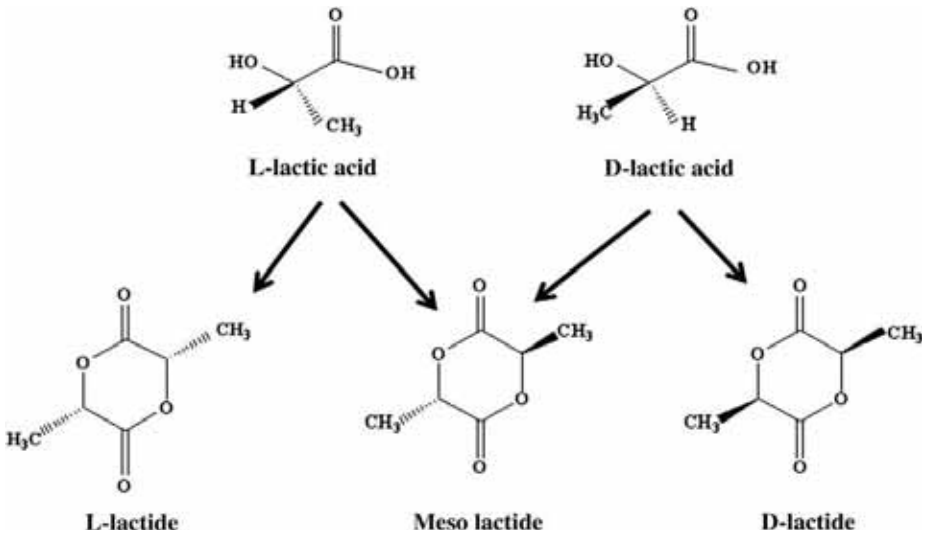

Fig. 1. Stereoforms of lactides [11]

Lactic acid is the most widely occurring hydroxycarboxylic acid, having a prime position due to its versatile applications in food, pharmaceutical, textile, leather and chemical industries [12, 13] and as monomer in the production of biodegradable polymers (PLA) [14].

Lactic acid is a naturally occurring organic acid that can be produced by chemical synthesis or fermentation. Chemical synthesis of lactic acid is mainly based on the hydrolysis of lactonitrile by strong acids, which provide only the racemic mixture of D-and L-lactic acid. The interest in the fermentative production of lactic acid has increased due to the prospects of environmental friendliness and of using renewable resources instead of petrochemicals [11]. Besides high product specificity, as it produces a desired optically pure L-(+)- or D-(-)-lactic acid, the biotechnological production of lactic acid offers several advantages compared to chemical synthesis like low cost of substrates, low production temperature, and low energy consumption [14,15].

Lactic acid can influence the metabolic function of cells in a variety of ways, as it can serve as an energy substrate and given its uncharged character and small size, it can permeate through the lipid membrane. Also, lactate is capable of entering cells via the monocarboxylate transporter protein shuttle system [16]. Once inside the cell, lactate is converted to glucose, serving as an energy source in the Cori cycle. In addition to its role as an energy substrate for cells, lactic acid has been shown to have antioxidant properties that may serve to protect cells from damage due to free radicals that are naturally produced throughout the cell life cycle .Addition to its role as an energy substrate for cells, lactic acid has

been shown to have antioxidant properties that may serve to protect cells from damage due to free radicals that are naturally produced throughout the cell life cycle [17].

Approximately 90% of the total lactic acid produced worldwide is made by bacterial fermentation and the remaining portion is produced synthetically by the hydrolysis of lactonitrile [5, 14]. The fermentation processes to obtain lactic acid can be classified according to the type of bacteria used [7, 18]. The carbon source for microbial production of lactic acid can be either sugar in pure form such as glucose, sucrose, lactose or sugar containing materials such as molasses, whey, sugarcane bagasse, cassava bagasse, and starchy materials from potato, tapioca, wheat and barley. Sucrose-containing materials such as molasses are commonly exploited raw materials for lactic acid production because they represent cheaper alternatives [15, 19]. Sugarcane bagasse is reported to be used as support for lactic acid production by Rhizopus oryzae and Lactobacillus in solid-state fermentation by supplementing sugars or starch hydrolysates as carbon source [20]. Brazil is the world's largest sugarcane producer with 648.921.280 million tons per year in 2008, which generated about 130 million tons of bagasse on dry weight basis, according to FAO Statistics Division [21], what may be an extra incentive to have a competitive lactic acid industry.

Poly-lactic acid

Polylactic acid (PLA) is a highly versatile, biodegradable, aliphatic polyester derived from 100% renewable resources [22]. It has extensive applications in biomedical fields, including suture, bone fixation material, drug delivery microsphere, and tissue engineering [11]. Because of these properties the PLA has been widely studied for use in medical applications.

PLA was discovered in 1932 by Carothers (DuPont) who produced a low molecular weight product by heating lactic acid under vacuum. In 1954 Du Pont produced the polymer with a molecular weight greater and patented. In 1968 Santis and Kovacs reported on the pseudo orthorhombic crystal structure of PLLA. The crystal structure was reported to be a left-handed helix conformation for the α-form [23].

The chemistry of PLA involves the processing and polymerization of lactic acid monomer. Since, lactic acid is a chiral molecule, PLA has stereoisomers, such as poly(L-lactide) (PLLA), poly(D-lactide) (PDLA), and poly(DL-lactide) (PDLLA). Isotactic and optically active PLLA and PDLA are crystalline, whereas relatively atactic and optically inactive PDLLA is amorphous [24,25]. The L-isomer is a biological metabolite and constitutes the main fraction of PLA derived from renewable sources since the majority of lactic acid from biological sources exists in this form (α, β, and γ) [26].

PLLA has gained great attention because of its excellent biocompatibility and mechanical properties. However, its long degradation times coupled with the high crystallinity of its fragments can cause inflammatory reactions in the body. In order to overcome this, PLLA can be used as a material combination of L-lactic and D, L-lactic acid monomers, being the latter rapidly degraded without formation of crystalline fragments during this process [27].

Companies, e.g. Cargill Dow Polymer LLC, Shimadzu Corp, Mitsui Chemicals, Musashino Co. are now producing PLA-targeting markets for packaging materials, films, textile fibers, along with pharmaceutical products [5]. The US Food and Drug Administration (FDA) and European regulatory authorities have approved the PLA resins for all food type applications and some chirurgical applications such as drug releasing systems [7,17].

Poly-lactic acid synthesis

PLA can be prepared by different polymerization process from lactic acid including: polycondensation, ring opening polymerization and by direct methods like azeotopic dehydration and

enzymatic polymerization [28]. Currently, direct polymerization and ring opening polymerization are the most used production techniques. Fig. 2 shows the main methods for PLA synthesis.

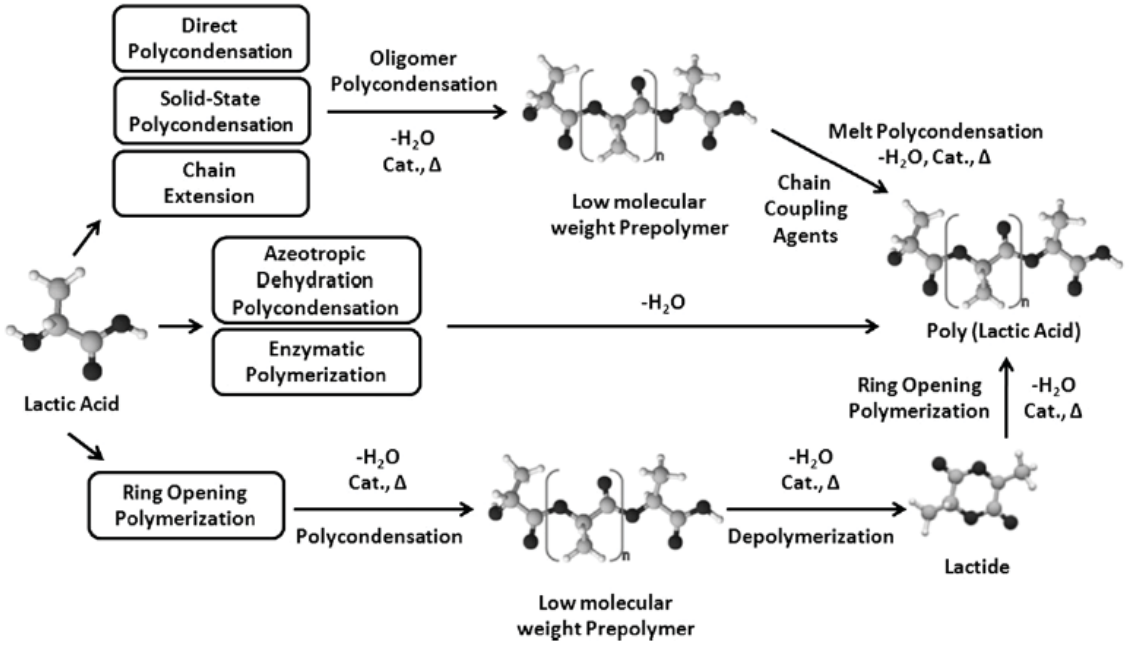

Fig. 2. Synthesis methods for Poly(Lactic Acid) [28]

The existence of both a hydroxyl and a carboxyl group in lactic acid enables it to be converted directly into polyester via a polycondensation reaction. However, the conventional condensation polymerization of lactic acid does not increase the molecular weight sufficiently [11]. The most common way to obtain high-molecular-weight poly lactic acid is through ring-opening polymerization of lactide [29]. Lactide can be prepared through a decompression method in which the water is separated from the system, and then, some catalysts are added into the reactor. After reacting for several hours, lactide is obtained. Then, the lactide opens its ring to polymerize [4].

Compared with ring opening polymerization, direct condensation polymerization has fewer manufacturing steps and lower cost, and is easier to manipulate and commercialize. The primary disadvantage of this method is the low molecular weight of the resultant polymer, which is due to the equilibrium among the free acid, the oligomers, and the water produced during the reaction or some special treatment. Thus, some conventional method-ring opening polymerization was developed.

Catalytic ring-opening polymerization of the lactide intermediate results in PLA with controlled molecular weight [30]. By controlling residence time and temperatures in combination with catalyst type and concentration, it is possible to control the ratio and sequence of D- and L-lactic acid units in the final polymer [5].

The ring-opening polymerization of lactide can be carried out in melt, bulk, or in solution and by cationic, anionic, and coordination-insertion mechanisms depending on the catalyst. Various types of initiators have been successfully tested, but among them, stannous octoate is usually preferred because it

provides high reaction rate, high conversion rate, and high molecular weights, even under rather mild polymerization conditions [31].

The Mitsui Toatsu Chemical Company polymerized poly-DL-lactic acid (PDLLA) using direct solution polycondensation, inwhich lactic acid, catalysts, and organic solvent with high boiling point were mixed in a reactor. The resultant product shows amolecular weight (MW) of about 300000 [4]. Achmad et al., report the synthesis of PLA by direct polymerization without catalysts, solvents and initiators by varying the temperature from 150 to 250 °C and the pressure from atmosphere pressure to vacuum for 96 h [32].

Ring-opening polymerization was carried out most commonly by a stannous octoate catalyst, but for laboratory demonstrations tin (II) chloride is often employed. Stannous alkoxide, a reaction product between stannous octoate and alcohol, was proposed as the substance initiating the polymerization through coordinative insertion of lactide. Alcohol could affect the polymerization through reactions leading to initiator formation, chain transfer, and transesterification. Carboxylic acids affect the polymerization through a deactivation reaction. Experiments have shown that alcohol increases PLA production rate while carboxylic acid decreases it. The higher the alcohol concentration, the lower is the polymer molecular weight. However, the final molecular weight of PLA is not sensitive to the carboxylic acid concentration [11]. Gupta et al., made an effort to present an updated review on the various aspects of PLA synthesis. In this review, a collection of more than 100 catalysts for the synthesis of PLA are mentioned [5].

Polycondensation method produces oligomers with average molecular weights several tens of thousands and other side reactions also can occur, such transesterification, resulting in the formation of ring structures as lactide. These side reactions have a negative influence on properties of the final polymer [29]. That subproducts production cannot be excluded, but can be controlled by the use of different catalysts and functionalization agents, as well as by varying the polymerization conditions [33].

Enzymatic polymerization emerges as one of the most viable alternatives to avoid these difficulties. Enzymatic synthesis is an environmentally benign method that can be carried out under mild conditions and can provide adequate control of the polymerization process [4]. Chanfreau et al., reported the enzymatic synthesis of poly-L-lactide using a liquid ionic (1-hexyl-3-ethylimidazoliumhexafluorophosphate [HMIM][PF6]) mediated by the enzyme lipase B from Candida antarctica (Novozyme 435). The highest PLLA yield (63%) was attained at 90 °C with a molecular weight (Mn) of 37.8 9 103 g/mol [34]. Kim and Woo obtained PLA of Mv about 33000 through the azeotropic dehydration at 138 °C for 48–72 h using amolecular sieve as a drying agent and m-xylene as a solvent [35].

Poly-lactic acid properties

Polylactide is one of the most promising biodegradable polymers owing to its mechanical property profile, thermoplastic processability and biological properties, such as biocompatibility and biodegradability [5]. In order for biopolymers to be useful, it is necessary to be able to tune the material properties to satisfy engineering constraints [36]. PLAs properties have been the subject of extensive research [37].

Poly (lactic acid) exists as a polymeric helix, with an orthorhombic unit cell. Properties of PLA depend on the component isomers, processing temperature, annealing time and molecular weight [11]. The stereochemistry and thermal history have direct influence on PLA crystallinity, and therefore, on its roperties in general. PLA with PLLA content higher than 90% tends to be crystalline, while the lower optically pure is amorphous. The melting temperature (Tm), and the glass transition temperature (Tg) of PLA decrease with decreasing amounts of PLLA [29].

Polylactide is a clear, colorless thermoplastic when quenched from the melt and is similar in many respects to polystyrene. Polylactic acid can be processed like most thermoplastics into fiber and film. [11]. Physical characteristics such as density, heat capacity, and mechanical and rheological properties of PLA are dependent on its transition temperatures [38]

For amorphous PLA, the glass transition temperature (Tg) is one the most important parameters since dramatic changes in polymer chain mobility take place at and above Tg. For semicrystalline PLA, both Tg and melting temperature (Tm) are important physical parameter for predicting PLA behavior [7,24, 39]. The melt enthalpy estimated for an enantiopure PLA of 100% crystallinity (ΔH°m) is 93 J/g; it is the value most often referred to in the literature although higher values (up to 148 J/g) also have been reported. The melting temperature and degree of crystallinity are depended on the molar mass, thermal history and purity of the polymer [23].

The density of amorphous and crystalline PLLA has been reported as 1.248 g ml-1 and 1.290 g ml−1, respectively. The density of solid polylactide was reported as 1.36 g cm−3 for l-lactide, 1.33 g cm−3 for meso-lactide, 1.36 g cm−3 for crystalline polylactide and 1.25 g cm−3 for amorphous polylactide [7]. In general, PLA products are soluble in dioxane, acetonitrile, chloroform, methylene chloride, 1,1,2-trichloroethane and dichloroacetic acid. Ethyl benzene, toluene, acetone and tetrahydrofuran only partly dissolve polylactides when cold, though they are readily soluble in these solvents when heated to boiling temperatures. Lactid acid based polymers are not soluble in water, alcohols as methanol, ethanol and propylene glycol and unsubtituted hydrocarbons (e.g. hexane and heptane). Crystalline PLLA is not soluble in acetone, ethyl acetate or tetrahydrofuran [11].

PLA also can be tailored by formulation involving co-polymerizing of the lactide with other lactones-type monomers, a hydrophilic macromonomers (polyethylene glycol (PEG)), or other monomers with functional groups (such as amino and carboxylic groups, etc.), and blending PLA with other materials [4]. The PLA properties may be controlled through the use of special catalysts isotactic and syndiotactic content with different enantiometric units [5]. Broz et al., prepared a series of blends of the biodegradable polymers poly (D,L-lactic acid) and poly(ε-caprolactone) by varying mass fraction across the range of compositions. Polymers made from ε-caprolactone are excellent drug permeation products [36].

PLA degrades primarily by hydrolysis, after several months exposure to moisture. Polylactide degradation occurs in two stages. First, random non-enzymatic chain scission of the ester groups leads to a reduction in molecular weight. In the second stage, the molecular weight is reduced until the lactic acid and low molecular weight oligomers are naturally metabolized by microorganisms to yield carbon dioxide and water [7, 40].

The polymer degradation rate is mainly determined by polymer reactivity with water and catalysts. Any factor which affects the reactivity and the accessibility, such as particle size and shape, temperature, moisture, crystallinity, % isomer, residual lactic acid concentration, molecular weight, water diffusion and metal impurities from the catalyst, will affect the polymer degradation rate [7, 22, 41].

The molecular weight has a significant impact on the properties of polymers such as degradation, mechanical strength and solubility. High molecular weight PLA (e.g. 106 g mol^{-1}) has a complete resorption time of 2 to 8 years. This prolonged existence in vivo in some organs may lead to inflammation and infection [42]. Therefore, production of low molecular weight PLA is desirable as it provides a shorter degradation rate. Mainil-Varlet et al., studied the degradation rate of low molecular weight PLLA (60000 g mol^{-1}) and found that the implants were able to maintain mechanical properties for a period of time usually required for bone fracture healing. Low molecular weight PLAs that are used for drug delivery have a weak retarding effect. They degrade by hydrolysis relatively fast into lactic acid, which reduces the risk of material accumulation in tissue [43, 44] Wichert and Rohdewald., 1993 used PLA with a molecular weight of 2000 g mol^{-1} as a matrix polymer for the microencapsulation of an inhalable steroid [45]. PLA with Mw between 2000 and 20000 g mol^{-1} was used by Andreopoulos et al., 2000 as an

implantable antibiotic release system. They found that the sustained release of antibiotics in low and high Mw implants lasted 33 days and more than 3 months, respectively [46]. Jabbari and He, developed an injectable and bioresorbable macromer using PLLA (Mn 1200 g mol−1) as a starting material. It is reported that an injectable hydrogel can be prepared by the addition of acrylate or fumarate units to low molecular weight PLLA. This particular functionalized PLA has a favourable biodegradation rate [47].

Tissue engineering applications

Organ failure and tissue loss are devastating problems in human beings. The current approach to treatment is based on transplantation. However, tissue engineering is the most fascinating domain of medical technology where patients with organ defects and malfunctions are treated by using their own cells, grown on a polymer support so that a tissue part is regenerated from the natural cells. The great advantages of tissue engineering are that a donor is not required and there is no problem of transplant rejection [5]. Tissue engineering is an interdisciplinary field that applies the principles of life science and engineering to the development of biological substitutes that aim to maintain, restore or improve tissue function.

The last two decades have seen extraordinary achievements in human organ reconstruction based on tissue engineering. Initial developments were confined to the use of biostable materials as scaffolds culturing cells that were then harvested into tissue. More recently, biodegradable materials have found enormous interest as supports because of the fact that the support disappears from the transplantation site with the passage of time, leaving behind a perfect patch of the natural tissue [5]. The surface properties of materials play a critical role in determining their applications, especially for biomaterials in biocompatibility. Different surface modification strategies, such as physical, chemical, plasma, and radiation induced methods, have been employed to create desirable surface properties of PLA biomaterials.

As a kind of important aliphatic polyester, Poly lactic acid (PLA) is biodegradable, and it has extensive applications in biomedical fields, including suture, bone fixation material, drug delivery microsphere, and tissue engineering [11]. PLA has been utilized as ecological material as well as surgical implant material and drug delivery systems, and also as porous scaffolds for the growth of neo-tissue [5, 39]. PLA has been approved by the Federal Drug Administration (FDA, USA) for use as a suture material because of features that offer crucial advantages [48, 49].

The medical applications of this polymer arise from its biocompatibility: the degradation product, lactic acid, is metabolically innocuous. The fibers may be fabricated into various forms and may be used for implants and other surgical applications such as sutures. Tissue engineering is the most recent domain where poly (lactic acid) is being used and is found to be one of the most favorable matrix materials [5]. The use of poly-lactic acid in these applications is not based solely on its biodegradability nor because it is made from renewable resources. PLA is being used because it works very well and provides excellent properties at a low price [22]. It is difficult to obtain a material with all the properties required for an application, but the diversification of PLA applications is such that a single polymer may prove useful in many applications by simple modifications of its physical-chemical structure, resultant of chirality of lactic acid molecule with two asymmetric centers existing in four different forms [4].

In applications that require long retention of the strength, such as ligament and tendon reconstruction, and stents for vascular and urological surgery, PLLA fibers are the preferred material [50]. Three-dimensional porous scaffolds of PLA have been created for culturing different cell types, using in cell-based gene therapy for cardiovascular diseases;muscle tissues, bone and cartilage regeneration and other treatments of cardiovascular, neurological, and orthopedic conditions [51, 52, 53]. The PLA may take 10 months to 4 years to degrade, depending on the microstructural factors such as chemical composition,

porosity and crystallinity that may influence tensile strength for specific uses [54]. The polymer has already shown favorable results in the fixation of fractures and osteotomies [55, 56].

One application of PLLA in the form of injectable microspheres is temporary fillings in facial reconstructive surgery. PLLA microspheres have also been used as an embolic material in transcatheter arterial embolization, which is an effective method to manage arteriovenous fistula and malformations, massive hemorrhage, and tumors fistula and malformations, massive hemorrhage, and tumors [57, 58].

PLA microfibers have been evaluated for tissue response using a rat-subcutaneous implantation by Sanders et al., The fiber diameter ranged from 4 to 15 mm, and it was observed that the capsule thickness was much lower for the thin fiber [59]. Kellomaki et al., studied the design and manufacturing of different bioabsorbable scaffolds for guided bone regeneration and generation. Among the various constructions, self-reinforced PLLA rods were used as a scaffolds for bone formation in muscle by free tibial periosteal grafts. At 6 weeks after implantation, new, histologically mature, bone had been generated in predesigned cylindrical form [52].

Conclusion

The biodegradable and bioabsorbable polymer PLA synthesized from renewable resources for biomedical devices application has attracted much attention of researchers and industry. The diversification of PLA applications is such that a single polymer may prove useful in many applications by simple modifications of its physical–chemical structure, resultant of chirality of lactic acid molecule with two asymmetric centers existing in four different forms.Various devices have been prepared from different PLA types including degradable sutures, drug releasing microparticles, nanoparticles, and porous scaffolds for cellular applications. An exciting application, for which the PLA offer tremendous potential, is bone fixation devices, since the metallic fixations have several disadvantages. Recently, biodegradable materials have been replacing metallic ones for the fixation of fractured bones in the forms of plates, pins, screws, and wires. Since materials for bone fixation require high strength, similar to that of bone, PLA has a large application in this field. Indeed, PLA is the prime example of a ‘‘biomaterial’’ with emerging multidimensional applications, points to a promising future for their applications in medical science and particularly in tissue engineering and other human health care fields.

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