

## BIODEGRADABLE POLY(LACTIC ACID) SCAFFOLD FOR TISSUE ENGINEERING: A BRIEF REVIEW

**R. REVATI, M. S. ABDUL MAJID<sup>\*</sup>, M. NORMAHIRA**

*Universiti Malaysia Perlis (UniMAP), Kampus Tetap Pauh Putra, 02600, Pauh, Perlis*

*\* Corresponding author: shukry@unimap.edu.my*

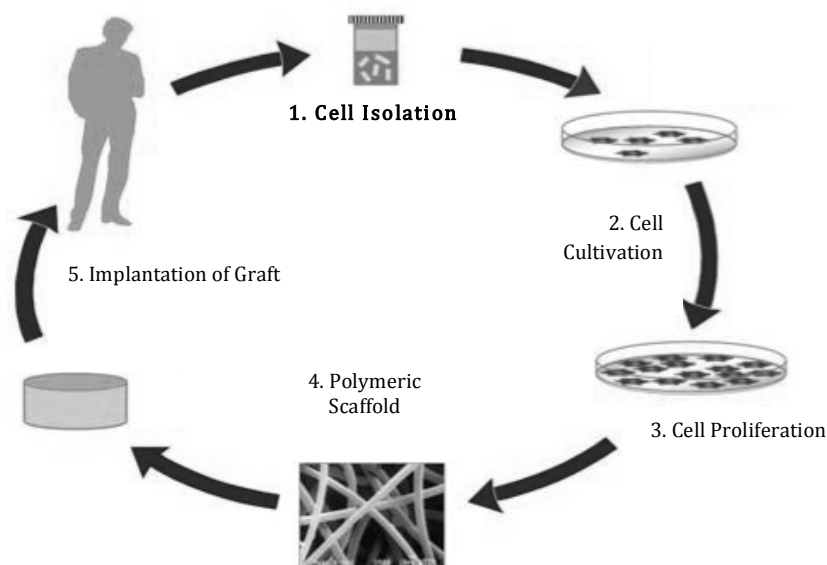
### ABSTRACT

Biodegradable polymers have been reorganized as alternative materials for tissue engineering applications, due to their ability to degrade through simple hydrolysis to products which can be accomplished through enzymatic activities in human body. Among various biomaterials used in the synthesis of biodegradable polymer scaffold, poly(lactic acid) (PLA) has recently received significant attention among researchers. PLA is at present one of the most preferred biodegradable polymers for this purpose and has convincingly demonstrated the proof of concept as biodegradable materials used in polymeric scaffold. This is due to their mechanical properties and biological properties such as biocompatibility and biodegradability. Most importantly incorporation of PLA with natural polymers such as gelatin and collagen enhanced the mechanical properties with controllable degradation time. In particular, PLA has been extensively studied for the development of scaffold for tissues such as bone tissue, drug delivery, stent and artificial organ in commercial use and in research. In this review paper, an overview of the scaffold design requirement and the effect of fabrication techniques on the pore size and physical properties were discussed in detailed.

**Keywords:** Poly(lactic acid), biodegradation, biocompatibility, scaffolds, mechanical properties

### INTRODUCTION

Tissue engineering is a multi-disciplinary field, which applies fundamentals from biological life science, organic chemistry and mechanics for the development of alternatives to maintain, restore and improve injured or diseased tissue [1]. Thousands of surgical procedures had been carried out every day in the United States (US) to repair or replace the damaged tissue and organs due to trauma, non-union healing fractures, or the use of bone grafts for resection requirement [2]. Thus, tissue engineering aims to develop alternative techniques to regenerate damaged tissues by the enhancement of biological substitutes or reconstruction of new tissues to overcome the limitations faced in this field [3, 4]. The brief explanation of tissue engineering can be seen in Fig. 1 which demonstrates the principles of tissue engineering.



**Fig. 1** A representative demonstrating the principles of tissue engineering [3]

Biodegradable polymeric scaffolds have been receiving much attention among researchers in tissue engineering field. Among the entire biodegradable polymer, application of poly(lactic acid) (PLA) has been widely used as a scaffold in tissue engineering [5]. PLA products have been approved by the US Food and Drug Administration (FDA) as a biopolymer for clinical use due to their biocompatibility, biodegradation, good mechanical properties and processability [6]. Based on recent literature reviews, it has been shown that bioabsorbable synthetic polymers can stimulate isolated cells to regenerate tissues and act as a drug delivery carrier which has motivated many researchers to further explore scaffold research for cell transplantation [7]. Furthermore, PLA based scaffold can be produced by controlling the relevant parameters such as crystallinity, copolymer ratio, molecular weight, intrinsic viscosity and residual monomers to achieve the desired physical properties [8]. This paper review recent development in PLA as a biodegradable polymeric scaffold and the fabrication techniques that have been utilized in the past to develop a PLA based scaffold with desired properties especially the porosity and physical characterization.

### BIODEGRADABLE POLYMER

Biodegradable polymers can be either natural or synthetic polymers. These polymers are able to degrade by simple hydrolysis to products which can be accomplished through enzymatic activities in human body [9]. Over the past decades, the usage of such polymers has dramatically increased in the field of tissue engineering for the development of tissue scaffold. Biomaterials have been widely used for the fabrication of tissue engineering scaffolds, which can be classified as (1) naturally occurring polymers, such as proteins (silk, collagen, gelatin, fibrinogen, elastin, keratin, actin, and myosin), polysaccharides (cellulose, amylose, dextran, chitin, and glycosaminoglycans), or polynucleotides (DNA, RNA) [10], and (2) synthetic biodegradable polymers, which commonly used for biomedical purposes are hydrophobic polyester, such as polylactide (PLA), and polyglycolide (PGA), polyurethanes (PUs) and polyamides (PAs) [11].

Biocompatibility has been the primary consideration when selecting a material to be used in the development of scaffold, then it also should be non-toxic and non-inflammatory [12].

### **Poly(Lactic Acid)**

Poly(lactic acid) (PLA) is highly versatile, biodegradable, aliphatic polyester derived from 100% renewable resources. It has obtained much attention in biomedical fields, such as suture, bone fixation material, drug carrier and tissue engineering [13]. Since, lactic acid is a chiral molecules, PLA has stereoisomers, such as poly(L-lactide) (PLLA), poly(D-lactide) (PDLA), and poly(DL-lactide) (PDLLA). Relatively atactic and optically inactive PDLLA is amorphous whereas isotactic and optically active PLLA and PDLA are crystalline. Since the majority of lactic acid from biological resources exists in this form ( $\alpha$ ,  $\beta$ , and  $\gamma$ ), the L-isomer is a biological metabolite and constitutes the main fraction of PLA derived from renewable resource [14].

PLLA has gained great attention because of its excellent biocompatibility and mechanical properties. However, its long degradation times together with the high crystallinity of its fragments can cause inflammatory reactions in the body. In order to overcome these problems, 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 [15].

### **Poly(Lactic Acid) Properties**

Poly(lactide) is one of the most promising biodegradable polymers owing to its mechanical properties profile, thermoplastic processibility and biological properties, such as biocompatibility and biodegradability [16]. In order to satisfy engineering constraints, material should be able to tune their physical properties according to the requirement. PLAs properties have been the subject of extensive research.

Since dramatic changes in polymer chain mobility take place at and above the glass transition temperature ( $T_g$ ), the most important parameter for amorphous PLA is  $T_g$ . For semicrystalline PLA, the behavior of PLA can be predicted using important physical parameters which is both  $T_g$  and crystalline melting temperature ( $T_m$ ) [17]. Generally, PLA products are soluble in dioxane, acetonitrile, chloroform, methylene chloride, 1,1,2-trichloroethane and dichloroacetic acid. Lactic acid based polymers are not soluble in water, alcohols as ethanol, methanol and propylene glycol and unsubstituted hydrocarbons. Crystalline PLLA is not soluble in acetone, ethyl acetate or tetrahydrofuran [18].

Degradation of PLA is through hydrolysis, ie after a few months of exposure to moisture. Poly(lactide) degradation occurs in two stages. In the first stage, random non-enzymatic chain scission of the ester groups leads to a reduction in molecular weight. Secondly, 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 [19].

Since PLA with high molecular weight (e.g.  $10^6$  g mol<sup>-1</sup>) has a complete resorption time of 2 to 8 years, this may lead to inflammation and infection. Therefore, PLA production with low molecular weight (e.g. 60000 g mol<sup>-1</sup>) is desirable as it provides a short degradation period. Moreover, the degradation products of poly(lactides) are non-toxic which enhances practical applications in biomedicine. PLA is currently being commercialized for a wide spectrum of applications [16].

### **Poly(Lactic Acid) Applications in Tissue Engineering**

Poly(lactic acid) (PLA) is biodegradable, and it has been utilized as ecological material as well as surgical implant material and drug delivery systems. They have also been utilized as porous

scaffolds for the development of neo-tissue [20]. Moreover, PLA is a biomaterial which has been approved by the US Food and Drug Administration (FDA) to be used as suture material due to its features that offer decisive advantages [21].

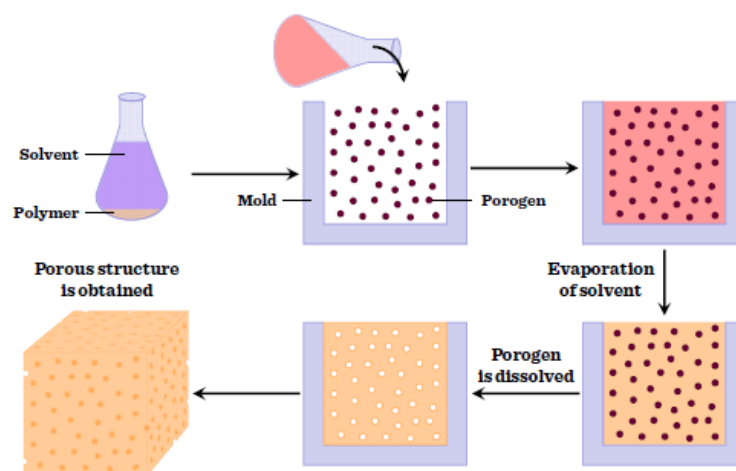
PLA is being widely used and is found to be one of the most preferred materials in tissue engineering. The use of PLA in these applications is not only because it is biocompatible and it is a renewable resource, but it works very well and gives excellent properties at a low price. PLLA fibres are the most favorable material in applications that require long retention of the strength, such as stent for vascular and urological surgery, and ligament and tendon reconstruction [22]. PLA based 3D porous scaffold have been developed using cell based gene therapy for culturing various cell types. The degradation rate of PLA highly depends on the microstructural factors such as porosity, chemical composition and crystallinity which may influence tensile strength for certain uses [23]. Injectable microsphere is one of the applications of PLLA, which has been used as temporary filling in facial reconstructive surgery and as embolic material in transcatheter arterial embolization. Among the various constructions of bioabsorbable scaffold, PLLA was used as scaffold for guided bone regeneration by free tibial periosteal grafts [24].

### **FABRICATION TECHNIQUES FOR PLA SCAFFOLDS**

Various methods exist for tailoring the properties of porous synthetic scaffolds. Polymeric scaffolds can be created using gas foaming process, solvent casting, freeze-drying, phase separation, solvent casting, membrane lamination, rapid prototyping, fiber bonding, phase inversion/particulate leaching and soft lithography [25]. Each fabrication technique has its own advantages and disadvantages, only the most commonly used techniques-solvent casting/particulate leaching, electrospinning, and soft lithography are elaborated in the following sections.

#### **Solvent Casting/Particulate Leaching**

Huang *et al.* carried out this method by dissolving PLA and polyethylene glycol (PEG) in dichloromethane with different weight ratio (e.g. 10/90, 20/80 and 30/70). The mixture solution is stirred at 600 rpm using magnetic stirrer for 0.5 h to get a colourless solution. NaCl powder with analytical grade is grounded and screened with a 109  $\mu\text{m}$  sieve to remove the smaller particles, and then screened with a 250  $\mu\text{m}$  sieve to retain the particles of sizes in between. According to the authors, the sieved salts are paved onto a petri dish and form a layer of approximately 1.5 mm thickness [26]. The salt-solution compound is placed in vacuum for 12 h to remove the solvent. The dried polymer-salt composite is placed in water for 48 h for leaching out the porogens as shown in Fig. 2. The leached samples are freeze-dried for 4 h and stored in vacuum ready for characterizations [27].

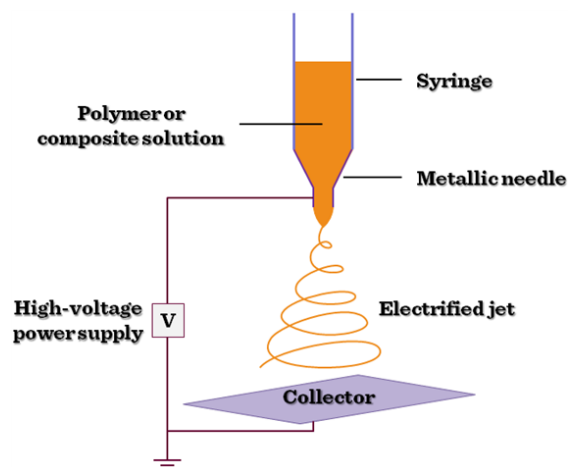


**Fig. 2** Solvent casting/particulate leaching [28]

The pore size of the scaffold obtained through this method was approximately 250  $\mu\text{m}$  which is similar to the size of sieved salt between 190  $\mu\text{m}$  and 250  $\mu\text{m}$ . The interconnected pore structure is being exhibited as critical to be justified in tissue engineering scaffold. When the PEG portion was low, the pores were not detected in the 10/90 sample whereas many salt particles are identified to be attached on the texture as the effect of PEG presence. Probably because PEG is commonly used as a plasticizer to PLA, it decreases the viscosity and make the materials less sticky. The salt residues are easier to leach out in pure PLA. Therefore, the salt particles are more easily leached out when the PEG portion increases.

### Electrospinning

Gómez-Pachón *et al.* reported that in order to obtain a 16% w/v of PLA solution, the PLA is dissolved in 2,2,2-trifluoroethanol, using magnetic stirrer. The PLA solution is electrospun to obtain both random and uniaxial oriented nanofiber scaffolds by using two different types of collector systems separately which are uniaxially aligned nanofibers (AN) and randomly oriented nanofibers (RON). As shown in Fig. 3, the electrospinning process is performed at room conditions, at a flow rate of 0.6 mL/h and at an applied voltage of 15 kV and a digitally controlled pump. The collector to obtain oriented nanofibers is an aluminium cylinder custom-designed that rotates at a predetermined velocity in the range of 1 to 10000 rpm, providing tangential velocities ranging between 1 to 3142.6 m/min. The nanofibers were collected at 1100 m/min and 1217 m/min. Subsequently, the electrospun scaffolds were kept in desiccators at room temperature. Some scaffolds were annealed and the temperature was selected based on the crystallization temperature of as-spun aligned nanofibers (around 70°C). In general, annealing takes place at any temperature between the crystallization and melting point [29].

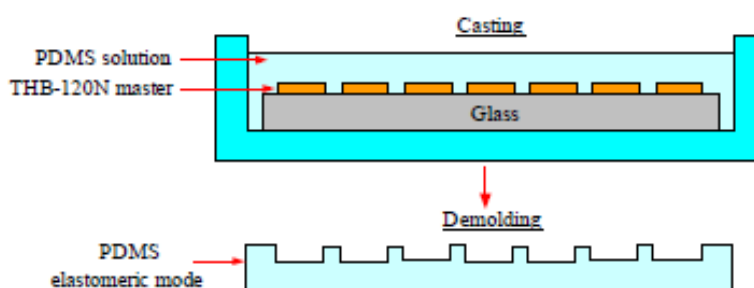


**Fig. 3** Electrospinning [28]

Even though the scaffold was created with two different velocities: 1100 m/min and 1217 m/min respectively, almost all nanofibers are aligned along the tangential direction of the cylindrical rotating collector. Uniaxially aligned nanofibers (AN) had an average diameter:  $643.8 \pm 50$  nm and the nanofibers showed high degree of nanofiber orientation with smooth and homogeneous surfaces. The average diameter of randomly oriented nanofibers (RON) was  $992 \pm 257$  nm and it showed a rough surface with considerable variation in the diameter along the longitudinal axis. It was proven that the quality of the uniaxially aligned nanofibers (AN) is better than randomly oriented nanofibers (RON). The rotational collector does not only stretch nanofibers to a regular diameter and surface but also orient the nanofibers.

### Soft Lithography

Gou-Jen Wang *et al.* prepared the PLA solution using the PLA particles and dioxane with ratio 1:1. The authors used a magnetic stirrer to enable the PLA particles to completely dissolve into the dioxane at  $60^\circ\text{C}$ . JSR THB-120N spun on glass substrate was photo-patterned through standard UV lithography and developed to be used as a master. Fig. 4 describes the manufacturing procedures of the PDMS elastomeric mold. The PDMS solution is cast onto a vessel containing of photoresist master and the vessel then placed at  $70^\circ\text{C}$  for few hours. The PDMS mold is peeled off from the master after the solidification for micro molding the PLA microstructures.

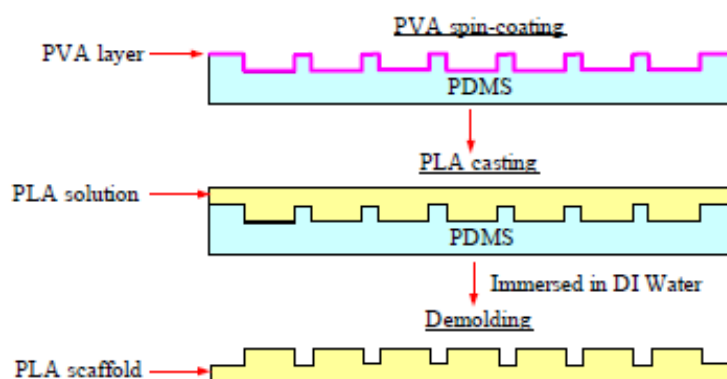


**Fig. 4:** Fabrication procedures of the PDMS elastomeric mold [30]

The procedures to fabricate the PLA scaffold are illustrated in Fig. 5. PVA (1%) solution is spun on the PDMS mold as the mode release such that the PLA scaffold can be easily peeled off from

the mold. The spin-coating parameters for a 6  $\mu\text{m}$  thick PVA film are 1000 rpm for 25 sec followed by 1500 rpm for 10 sec. The dimensions of the final product can be affected by the thickness of the PVA layer. Therefore, the dimensions of the PDMS mold should include the effect of the PVA layer.

The PLA solution is cast onto a vessel containing the PDMS mold and placed at room temperature for 180 min to evaporate the dioxane. The vessel is immersed into de-ionized water for a couple of hours to hydrolyze the PVA film and peel the PLA microstructure from the PDMS mold [30].



**Fig. 5:** Fabrication procedures of the PLA scaffold [30]

A micro-vessel scaffold is the object to be fabricated for tissue engineering: that the diameter ranges from 60  $\mu\text{m}$  to 120  $\mu\text{m}$  with respect to each micro-channel and the length of each segment is 800  $\mu\text{m}$ . A PLA scaffold for micro-vessels can be obtained by bonding the PLA microstructures with a flat PLA plate.

### CONCLUSION AND FUTURE WORKS

This paper reviews the potential of PLA to be widely used in tissue engineering due to its biological safety and physical properties which can be tailored by controlling several parameters. PLA can be fabricated using various techniques including solvent casting/particulate leaching, TIPS, electrospinning and soft lithography. Each technique gives out different porosity and physical structure of a scaffold. Finally, the review reports an alternative strategy to improve the physical properties of scaffold, involving the reinforcement of natural fillers such as Napier fibers, and kenaf fibers with PLA matrices using an effective doping method. Furthermore, the addition of natural fillers enhanced the biodegradability and mechanical properties of PLA scaffolds for their use for cell growth, allowing an increased success of tissue regeneration.

### ACKNOWLEDGEMENTS

Authors would like to thank the Research Acculturation Grant Scheme (RAGS) of Kementerian Pendidikan Malaysia for financially supporting this work under Grant No. 9018-00073. The School of Mechatronics at Universiti Malaysia Perlis, is appreciated for use of its facilities.

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