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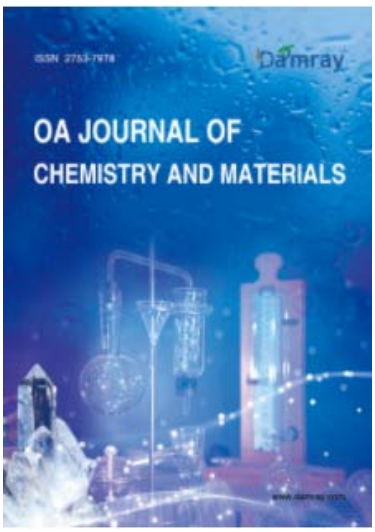
Study on Preparation of Poly (Lactic-Co-Glycolic Acid) and Methods to Relieve Acid Accumulation in PLGA Degradation Process



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Abstract

In recent years, Poly (lactic-co-glycolic acid), PLGA, one of the biodegradable polyesters with favorable biocompatibility and biodegradability, has found a wide range of applications in the drug delivery system, tissue engineering scaffold, medical devices, and so forth. However, acid degradation products of PLGA, may accumulate around the tissue to give rise to the well-known aseptic inflammation phenomenon. In this study, the basic amino acid Lysine was introduced into the PLGA to alleviate acid accumulation and the study is mainly divided into four parts. Preparation of PLGA and PLGA/Lysine composite nanofibers through electro-spinning; Lysine release behaviour of the PLGA-based composite nanofibers; in vitro degradation experiments of the PLGA and PLGA/Lysine nanofiber mats, and cell culture of vascular smooth cells with degradable fluid to evaluate cytotoxicity. Observing the morphology of composite nanofibers in each part of the study using SEM indicates that the addition of Lysine is capable of neutralizing PLGA acid product generated in the procedure of degradation.

Keywords

PLGA, Biocompatibility, Composite Nanofibers, Electro-Spinning, Biodegradation

1. Introduction

A variety of human tissue damage exists in our daily life, especially for bone, cartilage defects as well as tendons, ligaments and other connective tissue damage, and incalculable skin injuries. The foundation of tissue engineering undoubtedly brings hope to patients with tissue damage. The broad concept of tissue engineering was formally proposed in the U.S. National Science Foundation that it is a subject with the application of cell biology and engineering principles in order to research, restore and improve damaged tissue and its function of biological substitutes [1].

Organ or tissue loss is an enormous health problem and has been extensively studied in recent years. Bone damage

and soft tissue injury are so common that they need to be seriously taken into account. In the article, *Scaffold design for tissue engineering* (2002), G. Chen and Ushida discussed a promising method which is called "tissue engineering [2]" to treat loss or malfunction of tissue or organs. This method has been developed by scientific researchers and has been successfully applied to human bodies recently.

The scaffold, polymers produced in the form of nanofibers, can be considered as a vehicle on which cells are provided with desirable growing and proliferating conditions. Based on this function discussed above, a scaffold must be of biocompatibility, biodegradability, fitness for cell growth and proliferation and strong mechanical properties. Another article, *PLGA-based nanoparticles: An overview of biomedical applications* by Fabienne Danhier et al. [3] published in the *Journal of Controlled Release 2012* introduced a kind of copolymer---poly (lactic-co-glycolic acid), which is called PLGA for short. The author showed that due to some specific characteristics, PLGA-based nanoparticles was selected to be drug delivery system in biomedical applications, such as cancer, inflammation and other diseases [3, 9]. PLGA-based artificial bone-substitute materials [4] have obtained appealing results in bone repair, owing to their suitable properties, such as biocompatibility, degradability, processability, mechanical properties, and the ability to promote bone regeneration.

Apart from the advantages of PLGA listed above, a serious problem emerges in the process of tissue repair. Primary tissue composed of PLGA scaffold and cells will be implanted into the injured section to induce regeneration of new tissue; Simultaneously, the PLGA scaffold may degrade gradually and its degradation products (lactic acid and glycolic acid or their oligomers) may accumulate around local tissue giving rise to the well-known "aseptic inflammation" [2, 8] which will probably impede the effect of tissue repair. Therefore, how to find a way to relieve acid accumulation during PLGA degradation is an imminent issuetobeaddressed. Zhou [5] suggested a method to solve this problem by adding basic amino acid (Lysine) into PLGA producing a PLGA/Lysine composite film in which Lysine can neutralize the acid product generated by PLGA in the process of PLGA degradation in vitro.

As the morphology of a nanofiber mat is similar to the extracellular matrix (ECM), synthesis of a PLGA nanofiber mat has applicable potential in tissue engineering. Electro-spinning is, undoubtedly, considered to be an effective way to produce large quantities of nanofibers. Electrospun (bio) polymeric fibers have attracted widespread interest as functional materials with suitable morphology and properties for their use as tissue engineering scaffolds and/or wound dressings [10]. Polymeric nanofibers produced by electrospinning are frequently used to maintain a sustained drug release in the tumor site. Therefore, a magnetic polymeric nanofiber produced by electrospinning is an ideal nanosystem for cancer theranostics application [11].

The process of electro-spinning can be devided into five operational components: fluid charging, formation of the cone-jet, thinning of the steady jet, onset and growth of jet instabilities that lead to diameter reduction into the submicron regime, and collection of the fibers into useful forms [6]. Based on the fact that Lysine is hydrophilic (soluble in water) and PLGA is hydrophobic (tending to repel water), chemical engineers must come up with a method to mix them in order to maintain their stability in electro-spinning solution. Consequently, in the article, *Preparation, characterization, and encapsulation/release studies of a composite nanofiber mat electrospun from an emulsion containing poly (lactic-co-glycolic acid)* (2008), Liao et al. [7] proposed an effective way to synthesize the PLGA/Rhodamine B composite nanofiber mat (PLGA is hydrophobic, and Rhodamine B is hydrophilic). We can get some suggestions from this article on how to produce PLGA/Lysine composite nanofiber matsefficiently.

The introduction of basic amino acid into PLGA may possibly be an effective and reasonable method to solve the problem due to the reasons as follows. On the one hand, the basic amino acid can release from the composite nanofibers and then neutralize the acid product of PLGA during degradation. One the other hand, amino acid, one of the major components (must be innocuous) of the host organism, has the capability of participating in the process of metabolism leading to an excretion from the organismfinally.

2. Experiment in terms of PLGA nanofiber mats

In this paper, the basic amino acid Lysine was introduced into the PLGA to alleviate the acid accumulation and the study was mainly divided into four parts.

2.1. Preparation of PLGA and PLGA/Lysine composite nanofibers

In this step, we prepared PLGA and PLGA/Lysine composite nanofibers (Lysine with the mass percentage of 0.5%, 1.0%, 2.0%) through electro-spinning technology with the organic solution of Dimethyl Formamide (DMF) and trichloromethane. After preparing nanofibers, we characterized the composition, morphology, thermodynamic and mechanical properties of these nanofibers by means of FTIR, ATR, SEM, TA, DT, DSC and mechanicaltesting.

Electro-spinning technology aims at producing large quantity of nanofiber mats under restricted conditions including

high voltage (over 1kV), proper reception distance, temperature, humidity and so on. Fig.1 is a picture showing the process of electro-spinning. Since a similarity in morphology exists between nanofibers and extracellular matrix (ECM) which is fit for the growth and proliferation of cells, therefore the application of nanofibers as the substitute of ECM for tissue repair is of great importance in tissue engineering.

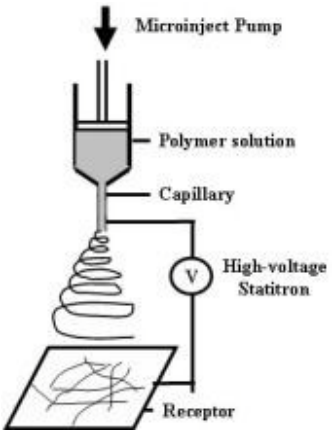

Figure 1. Process of electro-spinning.

In the preparation of PLGA and PLGA/Lysine nanofiber mat section, the stability of electro-spinning solution showed great importance, because it would probably effect the morphology and other qualities of nanofiber mats. Other conditions also needed taking into account seriously such as voltage, injection rate, reception distance, viscosity of electro-spinning solution, temperature, humidity and so on. The morphology of produced nanofiber mats could be clearly observed by scanning electron microscope (SEM). Fig. 2 showed the SEM pictures taken after the synthesis of PLGA and PLGA/Lysine composite nanofiber mats by electro-spinning method. Results indicated that the optimized parameters for preparing PLGA-based nanofibers were as follows: voltage 12 kV, injection rate 1.0 mL/h, collecting distance of 14 cm, ambient temperature 23°C and humidity of 20%.

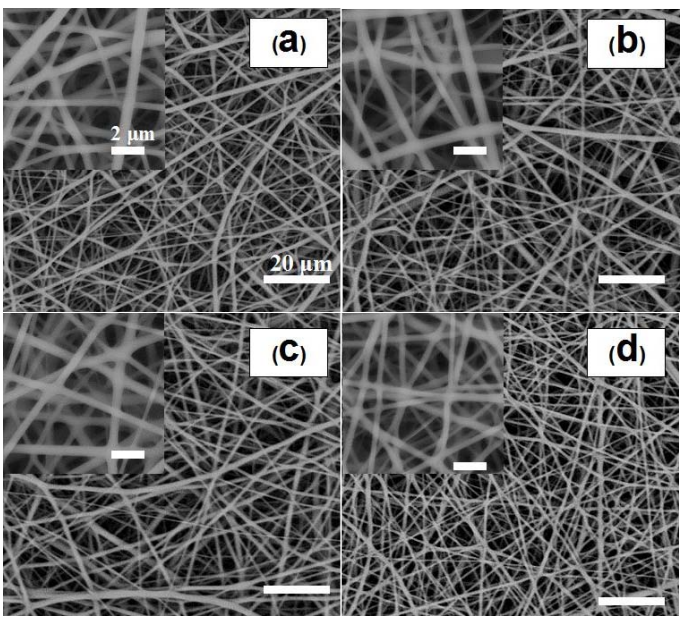

Figure 2. SEM photos of four kinds of PLGA nanofibers with Lysine of different concentration (a) PLGA (b) PLGA/0.5% Lysine (c) PLGA/1.0%Lysine (d) PLGA/2.0%Lysine.

Thermodynamic stabilities of these nanofiber mats could be also tested by means of TA (Thermogravimetric AnaLysineis), DTA (Differential Thermogravimetric AnaLysineis), and DSC (Differential Scanning Calorimetry).

2.2. Study of Lysine release behaviour of the PLGA-based composite nanofibers

Lysine encapsulated by PLGA nanofibers could release into the degradation liquid when researchers put different composite PLGA/Lysine nanofiber mats of the same size (1 cm×1 cm) into PBS (pH=7.4). The UV-VIS spectrophotometer was used to detect the concentration of Lysine released into PBS. It was expected that the release rate of Lysine could not be so rapid because the release rate accommodated to the rate of PLGA degradation made most sense in practical application.

As Lysine is hydrophilic, which means that Lysine will release from nanofiber mat and dissolve in water when PLGA/Lysine composite nanofiber mats are put into PBS (Phosphate Buffer Saline, a commonly used aqueous solution for degradation pH=7.4). Figure 3 showed Lysine release properties of three composite nanofiber with different Lysine concentration.

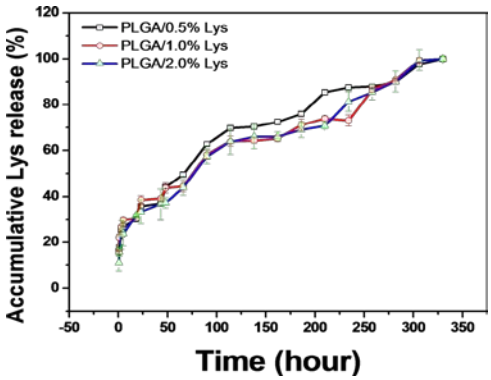

Figure 3. Change of accumulative Lysine release of three different kinds of composite nanofiber with respect to time.

The figure above indicated that Lysine released rapidly at the first 25 hours in PBS(pH=7.4), then Lysine of 0.5% held the highest capability of accumulative release from 50-275 hours compared to the other two nanofiber mats. So we could get to the conclusion that a higher concentration of Lysine in nanofiber mat might lead to impede the Lysine release process, which could possibly be explained as the effect of hydrogen bonding between Lysine and PLGA.

2.3. Vitro degradation experiment

In the process of vitro degradation, PLGA and PLGA/Lysine nanofiber mats were put in PBS at the temperature of 37 degree Celsius. Mass loss ratio, water absorption and the pH value of PBS solution were used as the major parameters to assess the degradation behaviour.

Fig.4 was SEM photos taken 20, 40, and 60 days after degradation process of PLGA and PLGA/Lysine nanofiber mats. Morphology changes of nanofiber mats could be easily discovered in different degradation periods. As the degradation process goes on, some breaks and holes appeared on the surface of nanofibers, and holes became larger with the increase of Lysine concentration in nanofiber, which was reasonable to say that the addition of Lysine promoted PLGA degradation process to some extent.

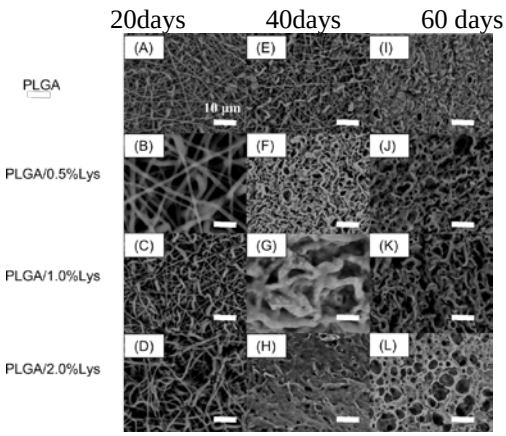

Figure 4. SEM photos taken 20, 40, and 60 days after PLGA and PLGA/Lysine nanofiber mats degradation.

Fig. 5 pointed out changes of pH value of distinct PLGA degradable liquid in an 8-week period. In Fig. 5, pH values of the degradable liquid of four kinds of nanofiber mats all reached to a declination, which attributed to the generation of acid product (lactic acid and glycolic acid); PLGA degradable liquid showed the most acidity (pH value is the lowest) in overall degradation process; pH values of PLGA/Lysine degradable liquid almoststayed above 7(neutral) in the degradation process, which indicated that the addition of Lysine indeed had the ability to neutralize PLGA acid product generated in the procedure of degradation.

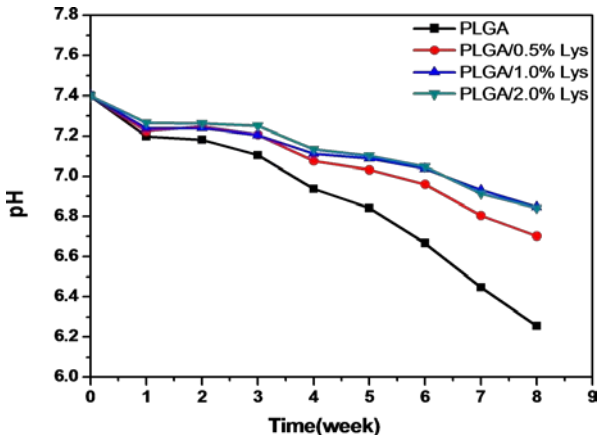

Figure 5. pH value curves of four kinds of nanofiber mats in degradation for an 8-week period.

2.4. Cell culture of vascular smooth cells

After eight weeks of degradation, we manipulated cell culture of vascular smooth cells with degradable fluid to evaluate cytotoxicity. This was the final step for this study and should be considered the most valuable part to the application of body tissue. The growth and proliferation condition (morphology) of the cells cultivated in different mediums containing degradation liquid of PLGA after eight weeks could be observed through the electronic microscope.

It could be clearly seen from Fig. 6 that cells cultured with PLGA degradable fluid all end up dying from first day to seventh day, while cells cultured with PLGA/Lysine degradable fluid grew and proliferated pretty well for the other three conditions. Nutrition for cell growth should be attributed to the addition of Lysine in nanofibermats.

Cell culture stands as such a significant procedure in tissue engineering that it is supposed to be taken into account seriously before being applied to human tissues.

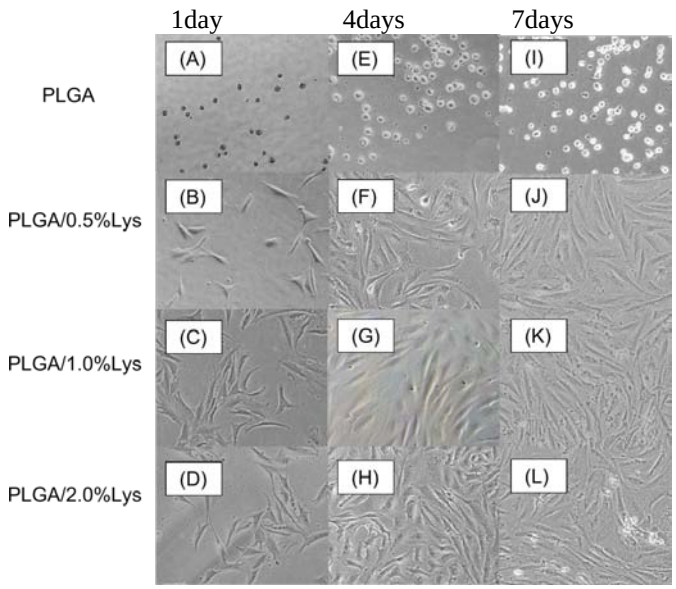

Figure 6. Morphology of vascular smooth cells cultured in four kinds of PLGA degradable liquid for 1 day, 4 days and 7 days respectively.

3. Discussion and Conclusion

Another solution to alleviate acid accumulation generated by PLGA degradation may introduce other basic chemical substances such as Arginine, Hydroxyapatite (HAP) which are likely to neutralize acid products as well.

In this paper, preparation of PLGA nanofiber mats by electro-spinning technology was discussed more in detail, since a similarity in morphology exists between EMC and nanofiber scaffold. Despite the advantage of nanofiber mats in the application of tissue engineering, a limit also embodies in the drug delivery system. According to this paper, Lysine release property is not so satisfactory as what we have expected. It is recommended that Lysine release into PBS (pH=7.4) with the same pace of PLGA degradation at which Lysine may have adequate interaction with acid products generated by PLGA degradation. In this case, an alternate for producing PLGA/Lysine composite nanofiber mat should be critically came up with. From my perspective, trying to encapsule Lysine (or other drugs) into PLGA forming a core-shell structure which can control drug release effectively in the process of synthesizing a nanosphere has much more possibilities to solve Lysine rapid release problem.

As the side effect resulted from acid accumulation is such a severe issue in tissue engineering that it needs to be resolved in an effective and economic way, research on this specific topic will certainly draw much attention and be further studied in the future.

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