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Full Length Research Paper

Study of *in vitro* degradation of biodegradable polymer based thin films and tissue engineering scaffolds

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This paper reports the *in vitro* degradation of three-dimensional, highly porous tissue engineering scaffolds and non-porous thin films based on poly (L-lactide) (PLLA) biopolymers. The tissue engineering scaffolds were produced through the emulsion freezing/freeze-drying technique. The non-porous thin films were produced using solvent casting technique. *In vitro* degradation experiments in terms of water uptake, water diffusion and weight loss were performed for various period of time. Results show that the scaffolds had more water uptake than the thin films at the same experimental condition. After 6 weeks, the weight loss of scaffolds was much greater than the films.

Key words: Tissue engineering, biodegradable polymers, scaffolds, uptake, weight loss.

INTRODUCTION

The requirement for bone is a major clinical and socio-economic need, and it has been reported that the treatment of bone fracture costs over Ł 900 million annually in the UK (Rose and Oreffo, 2002). The conventional reconstruction for bone defects are autologous bone grafts, autogenous bone grafts or alternatively metals and ceramics. If the bone is taken from another part of the patient's own body, it is referred to as autologous bone grafts. For bone healing and regeneration, autologous bone provides osteogenic cells and essential osteoinductive factors (Rose and Oreffo, 2002). It also imparts relatively better chance of success. Nevertheless, the limitation of autograft tissues is that the availability of this type of tissues are inadequate for the required applications (Rose and Oreffo, 2002; Petite et al., 2000).Allografts which refer to tissues taken from some other's body, may introduce the risks of immunological rejection problem and transmission of pathogens from donor to host (Spitzer et al., 2002; Yaszemski, 2004). Another limitation of allograft is that the rate of incorporation of host tissue is commonly lower than that of autograft. Potential substitutes to bone grafts could be metals and ceramics (Yaszemski, 2004). Metals can offer immediate mechanical support at the defect site but as it exhibits poor overall integration with respect to the host tissue, may also fail due to fatigue loading. Ceramics have the disadvantage of brittleness and have low tensile strength and cannot be applied in the locations such as significant torsion, bending or shear stress. Bone tissue engineering, which is a new strategy, provides a prospective solution to regenerate bone in a reliable, economical and physiologically acceptable manner and has emerged as an alternative to bone-grafting procedures over the past decades in order to overcome the various limitations of current grafting procedures and bone substitute biomaterials. In order to regenerate bone tissue, there are three key elements: osteogenic progenitor cells, osteoinductive growth factors and osteoconductive scaffolds (Schieker et al., 2006). Scaffolds, which act as temporary substrate, facilitate necessary support for cells to proliferate and to maintain differentiated function of the cells, are major component among various strategies such as cell-based and factor-based strategies. In fact, the applicability and success of bone tissue engineering depends on the performance of the scaffolds. The aim of tissue engineering is to develop cell, construct living system technologies to restore the structures and functions of damaged or degenerated tissues (Lanza et al., 2007).

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Abbreviation: PLLA, Poly (L-lactide).

Tissue engineering provides a new solution to tissue loss. Scaffolds with porous microstructures are commonly used in tissue engineering. The use of temporary scaffold is envisaged in the situation where the natural tissue has been weakened by disease, injury or surgery and for this reason artificial support is required. Tissue engineering scaffolds should have high porosity, interconnectivity and correct pore sizes to facilitate cell adhesion, in-growth and mass transportation. The tissue engineering scaffolds should have proper degradation time and rate. They are particularly essential at the later stage of implantation when the cells start to migrate deep into the scaffold (Ma, 2004). Biodegradable materials are selected to fabricate tissue engineering scaffolds in order to eliminate the concerns of long-term biocompatibility and to avoid the second surgical operation (Ratner, 2004). By selecting proper materials and scaffold fabrication techniques, the degradation and mechanical properties of the scaffold can be controlled and the scaffolds can be used to meet the specific tissue requirements. A number of biodegradable polymers, natural or synthetic, have been widely used for constructing TE scaffolds. Poly (L-lactide) (PLLA) is a FDA approved synthetic polymer which is degraded by hydrolysis to lactic acid and is therefore suitable as a resorbable, non-toxic material for surgical use. PLLA is a widely used polymer for scaffold fabrication. The extra methyl group in the PLLA repeating unit (compared with PLGA) makes it more hydrophobic which reduces the molecular affinity to water and leads to a slower hydrolysis rate. PLLA is among the few synthetic polymers approved by the US Food and Drug Administration (FDA) for certain human clinical applications (Koegler and Griffith, 2004).

It was reported that the *in- vivo* and *vitro* degradation of aliphatic polyesters was catalyzed by carboxyl end groups formed by chain cleavage and the amorphous regions are preferentially degraded (Li, 2006). In general, the hydrolytic degradation of semi-crystalline, high molecular weight PLLA proceeds through random bulk hydrolysis in two distinct stages. The first stage is characterized by the preferential attack of the ester linkages in the more accessible amorphous regions, while the second stage is characterized by the attack of the less accessible crystalline regions. It was reported that the cleavage of an ester bond of PLGA polymers yielded a carboxyl end group and a hydroxyl one, and thus formed carboxyl end groups were able to catalyze the hydrolysis of other ester bonds (Li, 2006). This phenomenon is called autocatalysis. Autocatalysis rate equation is applicable when the extent of reaction is slow or before the specimen experiences significant weight loss. There are several methods to fabricate scaffolds which include non-designed manufacturing techniques and designed manufacturing techniques. Non-designed manufacturing techniques include emulsion freezing/freeze-drying, solvent casting/particulate leaching, phase separation, gas foaming/high pressure processing, melt moulding, electrospinning and combination of these techniques. Designed manufacturing techniques include solid free-form (SFF) or rapid prototyping (RP) technologies which have attracted much attention recently. To fabriccate polymer scaffold, the emulsion freezing/freeze-drying technique is potentially a very useful method (Whang et al., 1995).

MATERIALS AND METHODS

PLLA was purchased from Sigma-Aldrich with an inherent viscosity of 1. Solvents chloroform and acetic acid were of analytical grade. Phosphate buffer saline (PBS) tablet was also purchased from Sigma-Aldrich.

Production of polymer scaffolds consisted of making a polymer-solvent solution, addition of acetic acid, rapidly cooling the solution to lock up the liquid state structure, and removing the solvent and water phases by freeze-drying using a freeze-drying vessel (Whang et al., 1995). PLLA solutions of 10% (w/v) polymer concentrations were used. For comparison reason, non-porous thin films were produced by dissolving the polymers in chloroform and subsequently evaporating the solvent. Morphology of the scaffolds and the thin film were examined using a scanning electron microscope (SEM). The density and porosity of the polymer scaffolds were measured by liquid displacement method. Ethanol was used as the displacement liquid as it penetrated easily into the pores and at the same time no shrinkage or swelling was observed as a non-solvent of the polymers. Figures 1 and 2 show the schematic diagram of production of scaffold and thin film using PLLA polymer.

In order to assess the *in vitro* degradation study, polymer scaffolds samples were cut with sharp razor blade and weighed. Similarly, thin films were also cut and weighed. The specimens were placed in sealable vials in 10 ml of PBS solution (pH 7.4). At regular intervals, samples from buffer were removed, weighed to measure water uptake, dried and weighed to measure weight loss. Water uptake and weight loss was measured using the following equations:

$$Wateruptake(\%) = \left(W_w - W_d\right)/W_d \times 100 \quad (1)$$

Where, W_d and W_w are specimen weights before and after soaking in PBS.

$$Weightloss(\%) = \left(W_i - W_f\right)/W_i \times 100 \quad (2)$$

Where, W_i and W_f are specimen weights before and after soaking in PBS.

RESULTS

It has been found from previous research that using another biodegradable polymer with different polymer concentration could be fabricated into highly porous, three dimensionally interconnected structures under specific conditions. All the scaffolds are thick, homogeneous and physically manageable and are found to have less voids (pores >1 mm) (Sultana and Wang, 2008). Figures 3 and 4 represent the microstructure of the PLLA scaffold and PLLA thin film examined using SEM. A highly anisotropic, open porous and tubular morphology with an internal ladder and fibre-like network which is a characteristic features of the scaffolds prepared from the

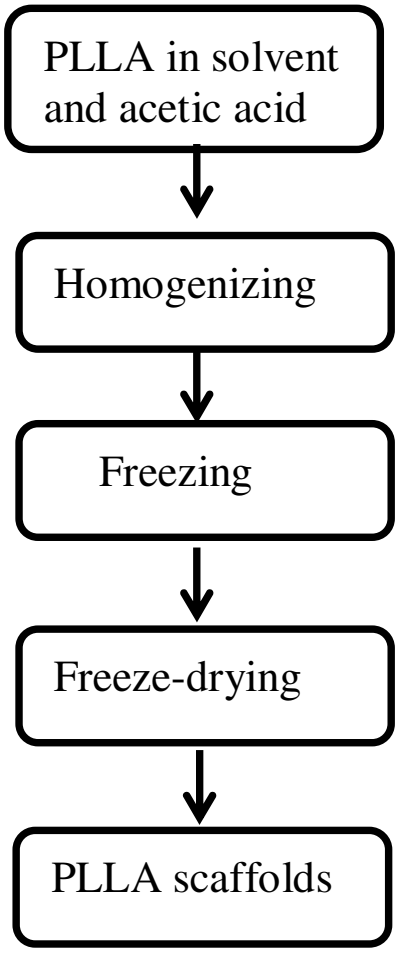

Figure 1. Schematic diagram of PLLA scaffold production.

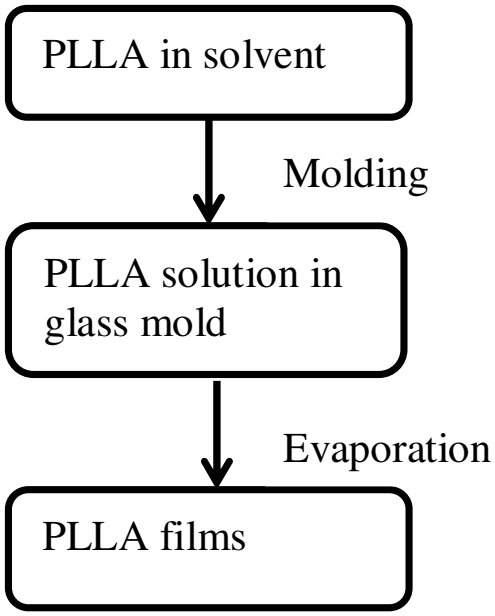

Figure 2. Schematic diagram of PLLA film fabrication.

manufacturing technique used was found in the PLLA scaffolds.

During freezing, the solvent, acetic acid crystallized and the polymer were expelled from the solvent crystallization front. Solvent crystals and acetic acid crystals became pores after subsequent sublimation. The characteristics of the pores were determined by the solvent crystal's morphology under the freezing conditions and the temperature gradient along the solvent crystallization direction led to anisotropic structure.

From the SEM micrograph in Figure 3, it was seen that the pores were irregular and the pore sizes ranged from ten to several hundred micrometer for PLLA scaffolds. It is expected that the pores of the scaffolds would open up on degradation and allow applications for bone regeneration. From the porosity measurement using liquid displacement method, the measured porosity of the scaffold was about 75%. Figure 5 shows the water uptake of PLLA scaffold and Figure 6 shows the water uptake of non-porous PLLA film. After several hours of immersion in PBS, water uptake of the film and scaffold reached equilibrium. Generally, the water uptake of PLLA scaffolds was much higher than that of thin films. For scaffold specimens, when all pores were filled with water, water uptake of polymer scaffolds was found to be 160% and for thin film, the uptake was only 1.4%. Figure 7 shows the weight losses of PLLA scaffold and thin film. After 2 week immersion, the PLLA scaffold exhibited higher weight loss than thin film, indicating faster degradation.

At 6 weeks, the scaffold's weight loss (~10%) was much higher than that of thin film (<2%). The accelerated weight loss of scaffolds could be the porosity. This property could cause physical disintegration and fragmentation of scaffolds. On the other hand, the hydrolytic scission of PLLA chains in the scaffolds could dominate the degradation process within the test period.

DISCUSSION

Figure 8 is the schematic representation of water uptake phenomena of non-porous and porous structures. Water uptake can occur in the materials in terms of 'absorbed water' which means the amount of water absorbed from media mainly depends on the hydrophilicity of material. Capillary water is termed as liquid water that is 'drawn in' through pores or capillaries of the materials. Moreover, the amount of water absorbed is related to the porosity and the amount of available liquid water at the surface of the material. For this reason, porous material can uptake and store more water, whereas the non-porous (dense) material can store a limited amount of water. The mechanism of initial stage of water absorption for polymeric and composite materials can be explained by classical diffusion theory (Fickian) (Braden and Clarke, 1984).

The degradation mechanism and rate of biodegradable polymers can be affected by numerous factors. Among

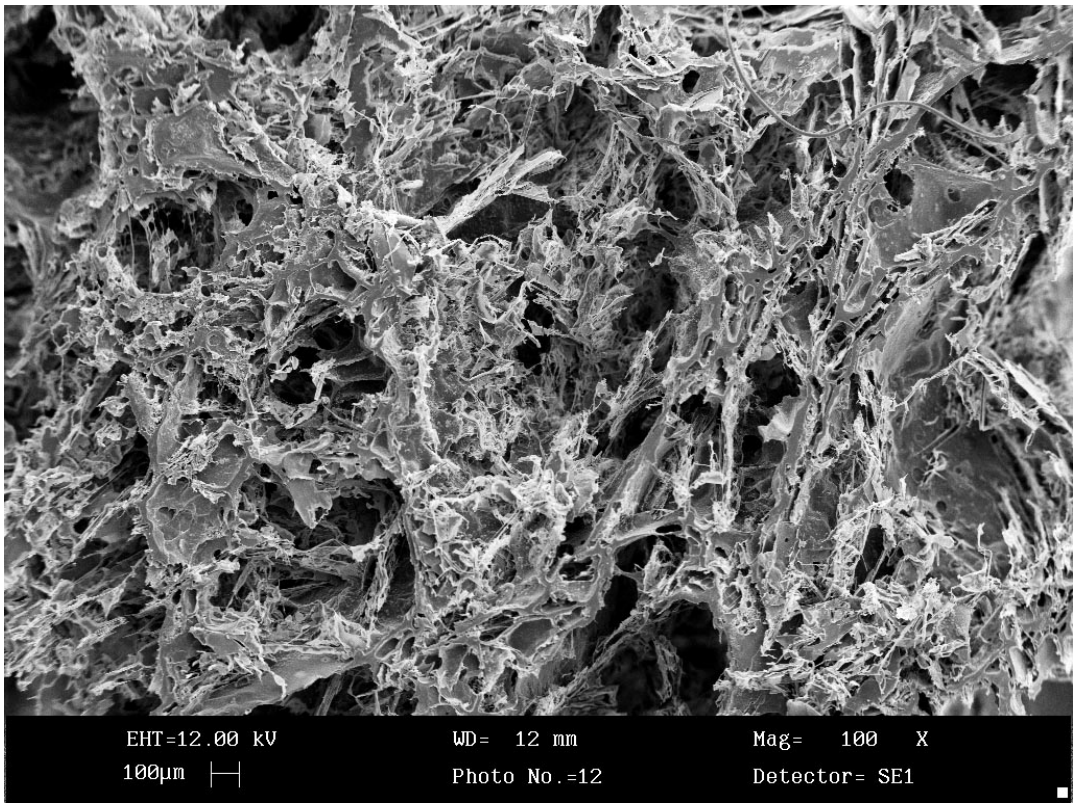

Figure 3. Scanning electron micrograph (SEM) of PLLA scaffold.

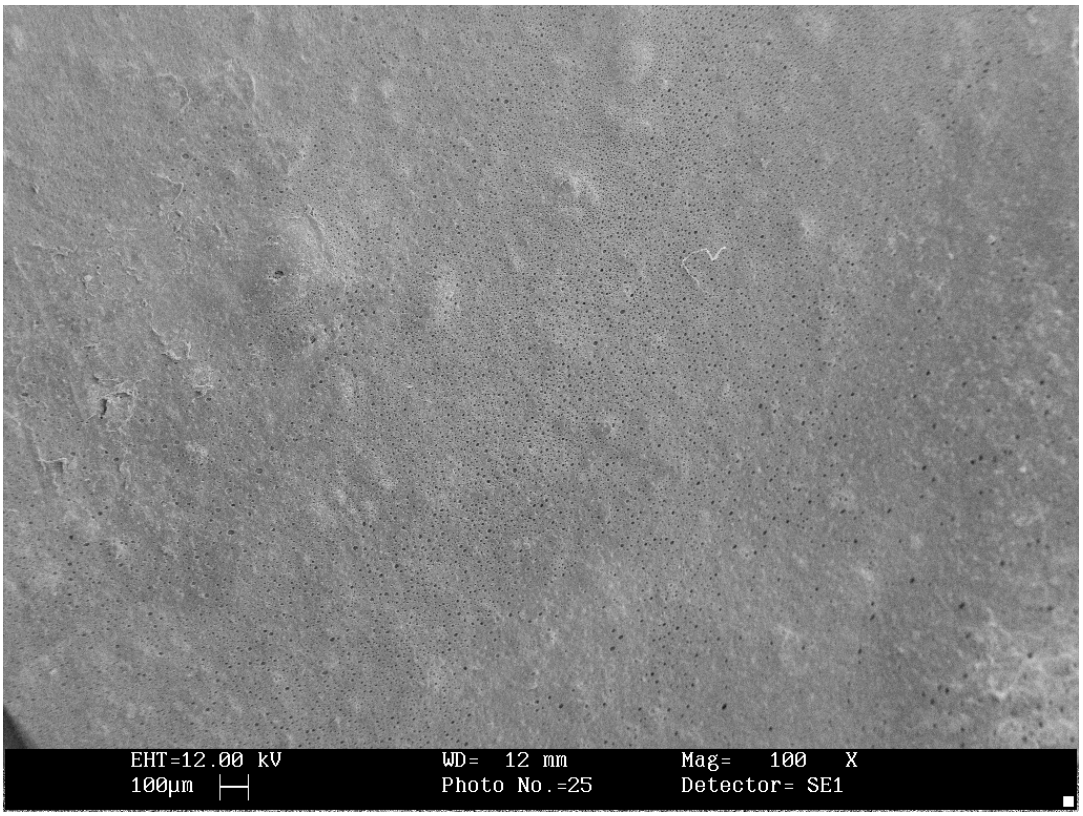

Figure 4. Scanning electron micrograph (SEM) of PLLA thin film.

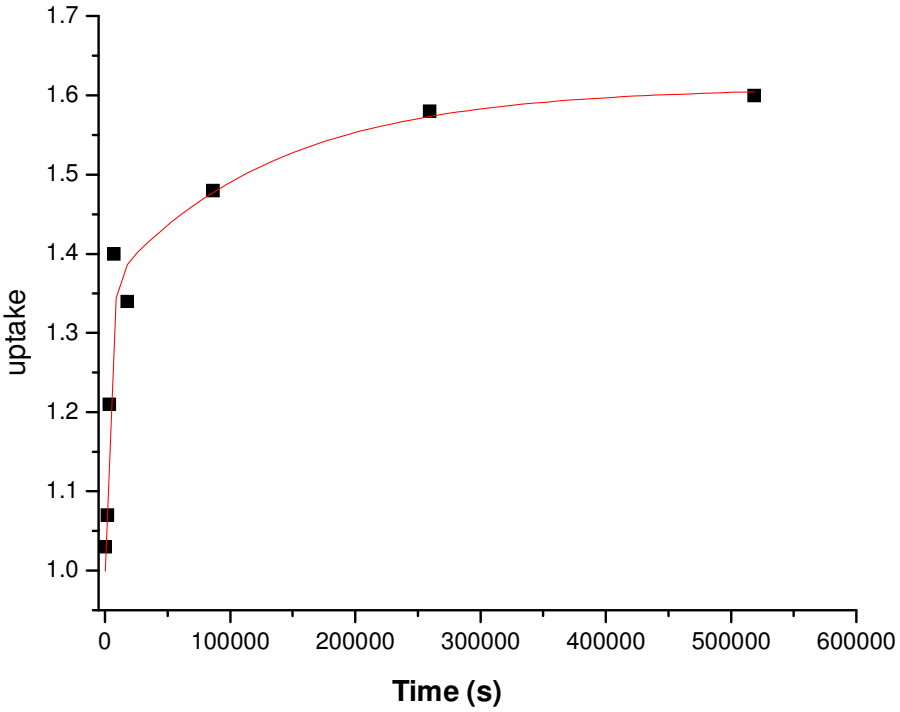

Figure 5. Water uptake profile of PLLA scaffold.

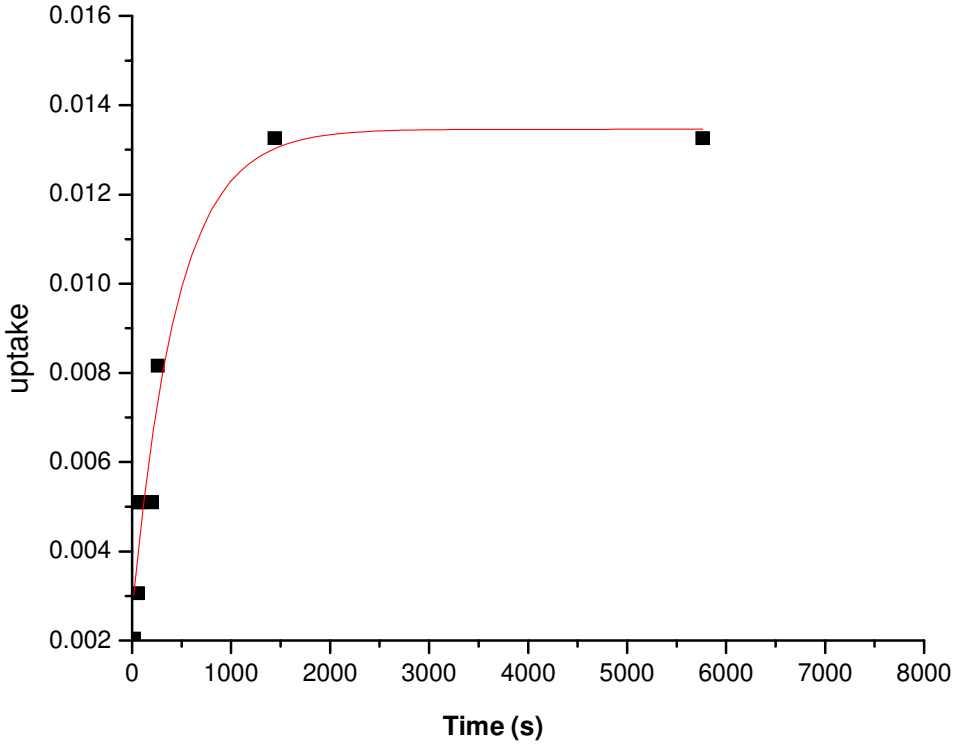

Figure 6. Water uptake profile of PLLA film.

the factors which affect degradation are: molecular weight, structure and content of co-monomer unit, crystallinity, orientation, blending, porosity, pH, temperature and catalytic molecules or ions (Tsuji, 2008). It was also

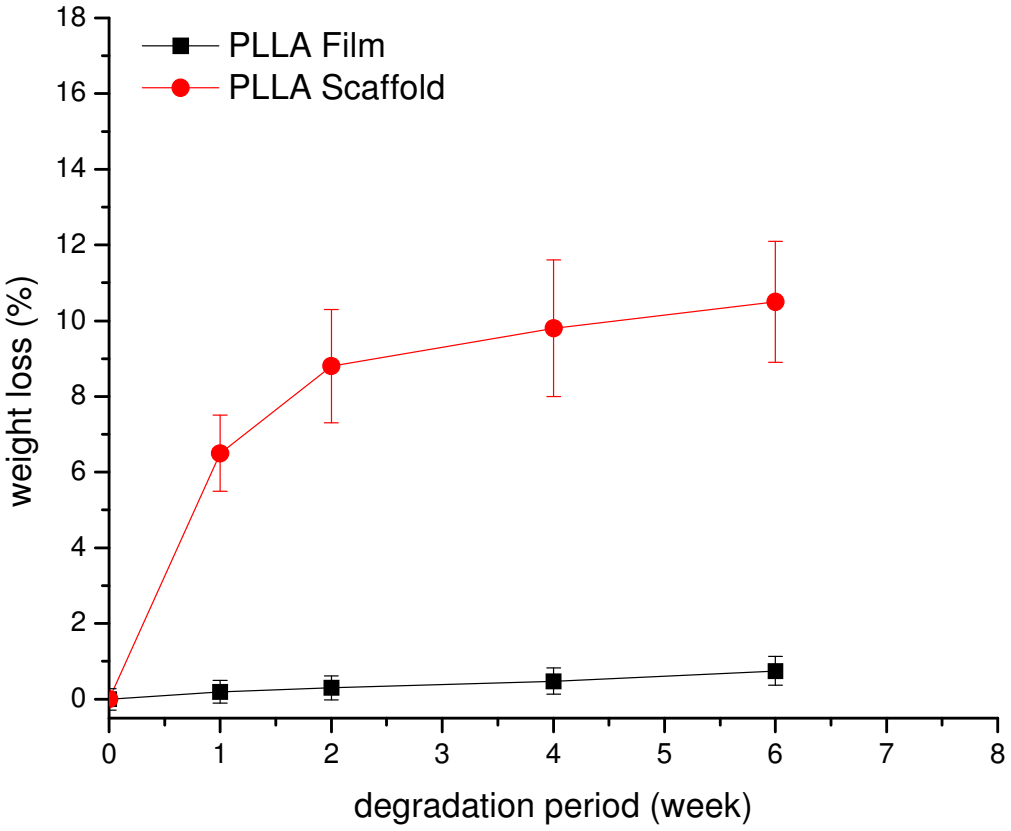

Figure 7. Weight loss profile of PLLA scaffold and thin film after *in vitro* degradation for 6 weeks.

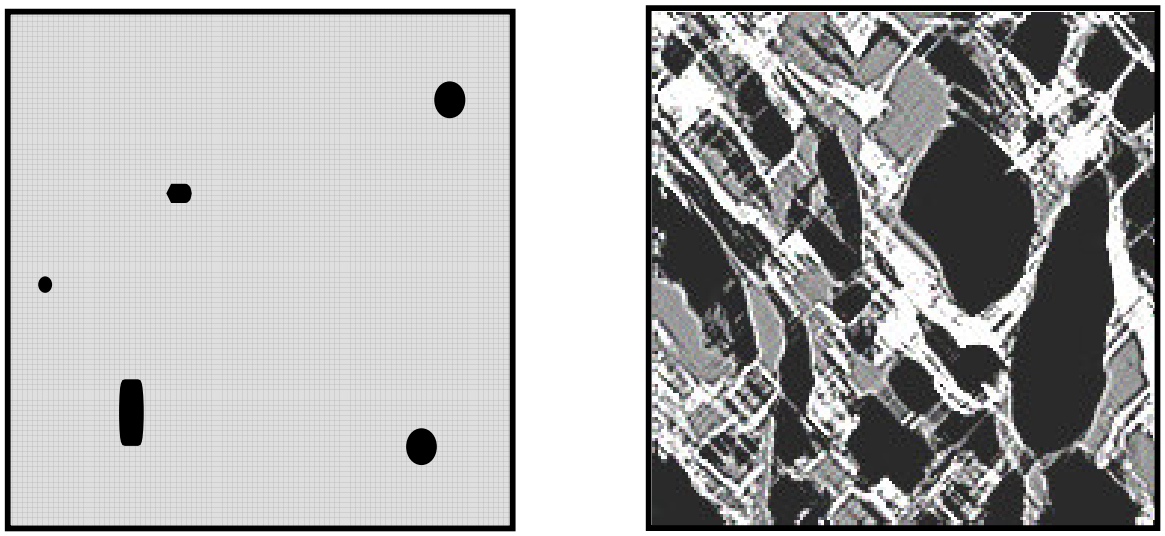

Figure 8. Schematic diagram of water uptake of non-porous and porous material.

reported that when catalytic molecules or substances such as enzymes and alkalis are present in the degradation media or environment, the degradation of polymer-based materials proceeds via a surface erosion mechanism. In the surface erosion mechanism, catalytic molecules or ions act only on the surface of materials and will not diffuse into the material. As a result, the material is eroded from the surface while the core part of the

material remains unchanged. On the other hand, the degradation of biodegradable polymers takes place via a bulk erosion mechanism in the absence of catalytic molecules or ions as in a phosphate-buffered solution. It was also reported that the hydrolytic degradation mechanisms depends on the thickness of biodegradable materials and the critical thickness above which the degradation mechanism changes from bulk erosion to surface erosion depends on the molecular structure of biodegradable or hydrolysable polymers. A significant weight loss can be observed at an early stage of degradation for a surface erosion mechanism. On the other hand, the weight loss occurs only at a late stage of degradation for a bulk erosion mechanism when a large decrease in molecular weight takes place and when water soluble oligomers and monomers are formed. In order to trace bulk erosion, molecular weight change is most effective. On the other hand, it is quite ineffective in the case of surface erosion (Tsuji, 2008). From the above discussion and the obtained results, it can be anticipated that the scaffolds degradation in PBS at 37°C were mainly controlled by bulk erosion rather than surface erosion.

Conclusions

Water uptake of the scaffolds was much greater than the thin films. This process can increase the hydrolytic attack of the polymer matrix. PLLA thin films exhibited slow weight loss in the *in vitro* physiological environment, whereas PLLA scaffolds showed faster weight loss at the same condition.

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