Biodegradation of PLA-\textit{Pennisetum purpureum} based biocomposite scaffold

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Abstract. The \textit{in vitro} degradation and mechanical properties of a 3D porous \textit{Pennisetum purpureum} (PP)/polylactic acid (PLA) —based scaffold was investigated. In this study, composite scaffolds with PP to PLA ratio of 0\%, 10\%, 20\%, and 30\% were immersed in PBS solution at 37 °C for 40 days. Interestingly, the degradation rate was reduced for the PLA/PP\textsubscript{20} scaffold, though insignificantly, this could be attributed to the improved mechanical properties and stronger fibre-matrix interface. The FESEM results indicated that a sound fibre-matrix interface was formed in the PLA/PP\textsubscript{20} scaffold, which reflected the addition of \textit{P. purpureum} into PLA decreasing the degradation rate compared to in pure PLA scaffolds. The results suggest that the \textit{P. purpureum}/PLA scaffold degradation rate can be altered and controlled to meet the requirement imposed by a given tissue engineering application.

1. Introduction

Various biodegradable polymers have been utilised to produce scaffolds in tissue engineering. These microstructures maintain the extracellular matrix (ECM) production by cells and are presumed to evenly degrade, allowing the surrounding tissue to recover the supporting function of the scaffold [1]. Thus, biodegradable polymers are used to provide temporary support for cell growth, and the chemical properties of these polymers allow hydrolytic degradation through de-esterification [2]. Once degraded, the monomeric components of each polymer are removed by intrinsic pathways [3]. The biological performance of biomaterials highly depends on their degradation behaviour owing to the influence of degradation on cell performance and inflammatory response [4]–[6]. Therefore, it is crucial to investigate the degradation behaviour of biodegradable materials as the degradation rate is a major factor affecting cartilage tissue regeneration.

Utilisation of \textit{P. purpureum} as a reinforcement filler in a PLA matrix offers many advantages; it results in the production of biocomposites with favourable mechanical properties and controllable biodegradability. Ridzuan et al. determined that \textit{P. purpureum} fibres could potentially be used as reinforcement materials in polymer composites. They discovered that the application of alkaline treatment on fibres could effectively increase the surface roughness and minimises the hemicellulosases quantities of the fibre [22]. Hameem et al. studied the tensile and flexural properties of \textit{P. purpureum} and concluded that when polyester composites were reinforced with \textit{P. purpureum}, their mechanical properties improved [23]. Similarly, Ridzuan et al. reported that moisture absorption influences the mechanical properties of hybrid \textit{P. purpureum}/glass–epoxy composites [24]. They noticed that as the glass fibre content of the samples increased, the degree of moisture absorption decreased while the tensile and flexural strength of the composites increased. Based on several recent studies, it can be suggested that \textit{P. purpureum} fibres could be used effectively as reinforcement filler in polymer composites for cartilage tissue engineering applications [23]–[27].

In this review, the influence of fillers content on the \textit{in vitro} degradation behaviours of the PLA and \textit{P. purpureum}/PLA scaffolds will be described. Therefore, the composite scaffolds were immersed in PBS to study the scaffolds’ \textit{in vitro} degradation behaviour. The scaffold-immersed buffer solution was not changed until the experiment was ended to observe the effect of the PBS solution pH on the degradation behaviour of the scaffolds (pH was evenly lowered by the formation of acid due to the degradation of PLA). Finally, the surface morphology of \textit{P. purpureum}/PLA scaffolds influenced by
various fillers compositions will also be reviewed, implying the use of PBS medium to mimic conditions in the human body.

2. Materials and Methods

2.1. Materials

Pennisetum purpureum fillers were produced from Napier grass received from Bukit Kayu Hitam, Malaysia. Polylactic acid (PLA) was acquired from NatureWorks LLC. Organic solvents, chloroform and dichloromethane, were purchased from Fisher Chemical Co. and used as received. Sodium chloride (NaCl) with analytical grade was provided and used as a porogen.

2.2. Preparation of Pennisetum purpureum/PLA composite scaffolds

Composite scaffolds based on polylactic acid and Pennisetum purpureum fillers were prepared by solvent casting and particulate leaching methods. The first step is to prepare 60.0g/L PLA solution by dissolving PLA pellets in (1:1) dichloromethane and chloroform solution with stirring at 70˚C to get a clear solution. Then Pennisetum purpureum fillers were dispersed in PLA solution for 30 min by continuous stirring on a magnetic stirrer at medium speed. After the filler was evenly distributed, the sodium chloride particles were added to the composite with the ratio of 1 to 9. To eliminate sodium chloride, the obtained Pennisetum purpureum/PLA porous scaffolds were then soaked in distilled water for two days to leach out the sodium chloride by refreshing the medium every 4h. The filtered samples were dried overnight at room temperature and then kept in a desiccator for characterization.

2.3. In vitro degradation of P. purpureum/PLA composite scaffolds

2.3.1. Sample preparation

Before degradation, the P. purpureum/PLA scaffolds were pre-wetted to make sure that phosphate-buffered saline (PBS) solution penetrated through all the pores of the composite scaffolds [28]. The prepared scaffolds were pre-wetted by immersing the samples in ethanol for one h and then transferred into buffer (PBS) solution for one h; due to the hydrophobic character of PLA, the composite scaffolds cannot be directly immersed in the PBS solution [29]. Various P. purpureum/PLA composite scaffolds and pure PLA scaffold were prepared as a rectangle with a length of 13 mm, width of 13 mm and thickness of 25 mm. The samples were immersed in glass vials containing 20 ml of PBS solution and were placed in a 37˚C. The in vitro degradation of the scaffolds was further evaluated by pH changes, weight loss and water uptake test using PBS solution (0.1 M, pH 7.4) as degradation medium. All experiments were carried out in an incubator (37˚C) and the degradation period lasted for 40 days.

2.3.2. pH changes of degradation medium

The measurement of pH changes was performed by immersing the composite scaffolds in PBS solution (pH=7.4) for up to 40 days. The degradation medium was not refreshed in the whole degradation period. The pH value of the degradation medium was measured every five days using a pH meter (Model HI 2213 pH/ORD HANNA Instrument). Three samples were prepared for each composition, and the average pH of three samples was reported.

2.3.3. Water uptake and weight loss of P. purpureum/PLA scaffolds

The measurement of water uptake and weight loss was carried out by immersing the composite scaffolds in PBS solution at 37˚C for up to 40 days. At the end of each time point, the weight of the swollen samples was recorded after removing the excess surface water with filter paper. Water uptake percentage of the scaffolds was determined from the weight of the wet sample (w_w) and the initial weight of the dry sample (w_i) using the following equation (1). The reported water uptake was considered as the average value of three samples.

\[ \text{Water uptake} = \frac{(w_w - w_i)}{w_i} \times 100 \]  

(1)

The weight loss of the scaffolds was determined from the initial weight of dry sample (w_i) and weight of final dry sample (w_f) at the end of each time point using equation (2).
At the end of predetermined time intervals, all samples from each composition were removed and weighed for the calculation of the percentage of water uptake (equation 1). Samples then washed thoroughly with distilled water to eliminate any soluble inorganic salt and weighed after being completely vacuum dried at room temperature to constant weight for calculation of the percentage of weight loss (equation 2).

3. Result and Discussions

3.1. In vitro degradation of P. purpureum/PLA composite scaffolds

3.1.1. Effect of P. purpureum on the pH changes in degradation medium

The change in pH value over the degradation is presented in Figure 1. Acidic groups resulting from the PLA degradation may decrease the pH value of the degradation medium, while the dissolution of the P. purpureum fillers could alkalize the medium. Therefore, the pH of the PBS medium is highly dependent on both the degradation rate of PLA matrix and the dissolution of the P. purpureum fillers. As shown in Figure 1, the pH value of degradation medium for incubating pure PLA scaffold decreased with a sharp drop through the 0-10th day and gradually increased through the rest of the degradation period and reached around 7.43 at the end of 35th to 40th day of incubation. Also, the pH value of the medium for incubating PLA/PP10 scaffold decreased at a fast rate through the 0-10th day and reached around 7.32 at the end of 10th day of incubation, but after the 10th day, the pH began to increase up to 7.40 at the end of 40th day. Then, owed to the high molecular weight (Mw=200, 000) of PLA, it degraded quite fast. The pH of degradation medium for incubating PLA/PP20 and PLA/PP30 scaffolds were down sharply through the 0-15th day of incubation, and both scaffolds reached around 7.28 at the end of the 15th day of incubation. However, after the 15th day, the pH began to stable and exhibited an upward trend from the 20th day and reached 7.33 and 7.34 at the end of 40th day of incubation, respectively.

The pH drops during the degradation time up to 10-15 days; releasing more acidic degradation products into the medium. After the initial 15 days of incubation, the pH value increases; owed to the P. purpureum degradation and OH release from the bio-composite scaffolds, which neutralise the acid degradation products of PLA. Hence, the results showed that the pH value of the degradation medium decreased at the beginning of the degradation and it has increased up to the end of incubation (figure 1). It could be concluded that the pH is decreased, by introducing P. purpureum fillers in PBS medium.

\[
\text{Weight loss} \, (\%) = \left( \frac{W_t - W_f}{W_t} \right) \times 100
\]

Figure 1: pH changes vs. incubation time in PBS for porous P. purpureum/PLA scaffolds: (a) PLA, (b) PLA/PP10, (c) PLA/PP20, and (d) PLA/PP30
3.1.2. Water uptake and weight loss of P. purpureum/PLA scaffolds

The scaffolds based on P. purpureum and PLA were prepared by solvent casting and particulate leaching methods. The main key point in the in vitro degradation studies was to use enzymes present in human serum for stimulating physiological conditions, which are responsible for the degradation of P. purpureum/PLA scaffolds. Degradation with PBS medium was performed under incubation. The water permeability of a scaffold and its ability to absorb water influence the absorption of cell culture medium or physiologically compatible buffers and the transportation of cell nutrients and metabolites throughout the scaffold. The PBS uptake of the scaffold material and that of the structure, give various measures of the ability of the scaffold to bind to the fluid.

Figure 2 shows the water uptake of the P. purpureum/PLA scaffolds with different P. purpureum content. According to the results, the percentage of water absorption is decreased throughout the degradation period. It can be observed that after the immersion of scaffolds in medium, compared to PLA/PP20 scaffold, the water content of P.LAC, PLA/PP10, and PLA/PP30 scaffolds decreased rapidly, though with the introduction of P. purpureum fillers which have a hydrophilic behaviour. However, the water uptake of the biocomposite scaffolds decreased remarkably in the presence of PBS medium (figure 2), which owed to the degradation of the scaffolds in the presence of these enzymes, which similar to the presence of the enzyme in human serum.

The PLA/PP20 scaffold shows that the percentage of water uptake decreased slowly and reached around 245%, compared to than that of the P.LAC, PLA/PP10, and PLA/PP30 scaffolds, which decreased significantly and reached around 175, 165, and 155%, respectively. PLA/PP20 composite scaffold exhibits the highest water absorption percentage throughout the whole incubation period. The water uptake ability of the P.purpureum/PLA scaffold gradually increases with the further addition of P. purpureum content due to the presence of P. purpureum fillers on the surface of the composite scaffold. P. purpureum is naturally hydrophilic because they are cellulose fibres. Thus the presence of P. purpureum within the scaffold can significantly enhance the water absorption ability of scaffold. In contrast, PLA/PP30 scaffold shows that the percentage of water uptake decreased rapidly, compared to PLA/PP20 scaffold, owing to the weakness of the fillers to protrude from the surface of the scaffolds. This result agrees with the result of the weight loss analysis (Figure 3).

![Figure 2: Water uptake vs. incubation time in PBS for porous P. purpureum/PLA scaffolds: (a) P.LAC, (b) PLA/PP10, (c) PLA/PP20, and (d) PLA/PP30](image)

In tissue engineering, it is expected that the degradation rate of P. purpureum/PLA scaffolds is controllable in the regeneration and repair process of tissue. This is highly achieved by modifying the composition of the polymer composites. However, it is compulsory to highlights the influence of P. purpureum on the in vitro degradation behaviour of scaffolds as degradation rate is a critical factor affecting the recovery of damaged cartilage. The degradation mechanism of biodegradable polymer is
chemical degradation, and it was observed that the chain ends cleavage resulted in weight loss, while the random scission influences the reduction in weight loss. Therefore, the water absorption is considered to be particularly important for the degradation of the scaffold (as illustrated in figure 2). The weight loss for the different porous *P. purpureum*/PLA scaffolds after incubation in PBS is shown in figure 3. It can be found that the weight loss of the PLA/PP_{20} scaffold proceeds slowly throughout the whole degradation period. After 40 days of incubation, the scaffold loses about 7.4% of its initial weight. The weight loss trend of PLA_{C}, PLA/PP_{10}, and PLA/PP_{30} scaffolds were similar, characterised by a rapid weight loss phase at initial 15 days, followed by a dramatic weight loss at the remaining weeks. As a result, 20.1, 28.8, and 27.4% of the original weight are lost after 40 days of incubation, respectively.

![Figure 3: Weight loss vs. incubation time in PBS for porous *P. purpureum*/PLA scaffolds: (a) PLA_{C}, (b) PLA/PP_{10}, (c) PLA/PP_{20}, and (d) PLA/PP_{30}](image)

In our study, the weight loss of PLA_{C} and PLA/PP_{10} scaffolds increased faster from the early period than the PLA/PP_{20}. Based on FESEM results, we can conclude that the micro-structure of PLA/PP_{10} and PLA_{C} scaffold is similar. This increase in weight loss is possibly because the medium could diffuse more readily into the scaffolds than into PLA/PP_{20} as the pore size of the PLA_{C} and PLA/PP_{10} scaffolds are larger and wider compared to that of PLA/PP_{20}. Therefore, it is deduced that the PLA_{C} and PLA/PP_{10} scaffolds displayed a faster degradation than PLA/PP_{20} in good accordance with the literature [28]–[30]. Wu et al. reported that the scaffold with larger pore size tends to degrade more quickly (i.e., are less resistant to hydrolysis) than those with smaller pore size. The reason is that they possess thicker pore walls and therefore a smaller surface area to volume ratio, which delay the diffusion of small chains (acidic-by-products), resulting in a stronger acid-catalyzed (auto-catalyzed) hydrolysis [31]. Additionally, if the adhesion is weak, the PLA/PP_{30} scaffold has poor strength and fatigue properties. In this study, PBS medium can diffuse quickly along the interface of *p. purpureum*/PLA scaffold is owing to insufficient adhesion and disturb the interface which leads to rapid strength loss of the scaffolds.

The development of excellent adhesion between matrix and reinforcement is still a challenge in composite science. PLA/PP_{20} scaffold caused reduction of degradation when comparing to PLA_{C}, PLA/PP_{10}, and PLA/PP_{30} scaffolds. Moreover, the addition of 20 wt% of *P. purpureum* into PLA composite scaffold increased the water absorption from 175 to 245 % demonstrating the moisture effect of the *P. purpureum* additives. It is well known that fibre-matrix bonding generated on the scaffold surface can act as a load bearer and improve thermal stability. It has been described that the hydrolytic chain cleavage preceded preferentially in the semi-crystalline regions, and thus leading to the increase in composite crystallinity. Therefore, the well dispersed of *P. purpureum* particles helped in the easy breach of the medium into the inner of the PLA/PP_{20} scaffold from the interface between *P. purpureum* particles and PLA matrix. Afterwards, the degradation of semi-crystalline regions of PLA matrix in the PLA/PP_{20} scaffold happened before the crystalline regions. When the polymer chains in semi-crystalline regions degrade, the number of semi-crystalline regions decrease, the percentage of crystalline to semi-crystalline increased. Thus, the increased *P. purpureum* is expected to reduce the
degradation of the PLA/PP20 scaffold. This might be owing to small molecules produced by the first degradation of grafted PLA molecules onto *P. purpureum* particles played a role of plasticiser and thus improved the toughness of the PLA/PP20 scaffold. Small molecules produced by the slower degradation of PLA/PP20 scaffold also played a role of plasticiser and enhanced the toughness of the PLA/PP20 scaffold to some extent.

However, in this study, after 40 days of incubation, it was found that overloading *P. purpureum* fillers could increase the degradation of PLA/PP30 scaffold. The weight loss of the PLA/PP30 scaffold gradually increases with the further addition of *P. purpureum* content due to the tendency of fillers to agglomerate; some of the fillers would not have been fully enclosed within the composite scaffolds, hence, some of will have protruded from the surface of the scaffolds. With the *in vitro* degradation results, we can conclude that the interface between *P. purpureum* and PLA matrix in PLA/PP20 scaffold was more susceptible to erosion by the PBS medium. Based on the mechanical, water absorption, weight loss and FESEM morphology data obtained from *in vitro* study which was performed in a controlled environment using PBS as a medium. These results are in agreement with the behaviour of the same *in vivo* in cartilage tissue repair, as found in few studies suggesting a low regenerative capacity of cartilage. Therefore, chondrocytes need to be expanded in culture and seeded onto a scaffold that can degrade slowly and resorb as the new tissue structures grow.

**4. Conclusions**

*P. purpureum*/PLA composite scaffolds were successfully prepared via solvent casting and particulate leaching technique. The pH value showed that the PLA/PP20 and PLA/PP30 scaffolds were kept nearly 7.33 and 7.34 at the end of 40 days. According to the *in vitro* degradation test showed that of the three composites and pure PLA scaffold, the PLA/PP20 scaffold exhibited the aspected bioactivity for cartilage regeneration. Biodegradation results show that the reinforcement of *P. purpureum* could increase the water absorption of PLA scaffolds and significantly influence the degradation rate of the PLA matrix. The results suggest that the *in vitro* degradation of PLA/PP20 scaffold more suitable for culture of chondrocyte-like cells and facilitates their application in cartilage tissue engineering applications.

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