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Mechanical, thermal and morphological characterization of 3D porous *Pennisetum purpureum*/PLA biocomposites scaffold

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Abstract

The mechanical, thermal, and morphological properties of a 3D porous *Pennisetum purpureum* (PP)/polylactic acid (PLA) based scaffold were investigated. In this study, a scaffold containing *P. purpureum* and PLA was produced using the solvent casting and particulate leaching method. *P. purpureum* fibre, also locally known as Napier grass, is composed of 46% cellulose, 34% hemicellulose, and 20% lignin. PLA composites with various *P. purpureum* contents (10%, 20%, and 30%) were prepared and subsequently characterised. The morphologies, structures and thermal behaviours of the prepared composite scaffolds were characterised using field-emission scanning electron microscopy (FESEM), Fourier transform infrared spectroscopy (FTIR), and thermogravimetric analysis (TGA). The morphology was studied using FESEM; the scaffold possessed 70-200 µm-sized pores with a high level of interconnectivity. The moisture content and mechanical properties of the developed porous scaffolds were further characterised. The *P. purpureum*/PLA scaffold had a greater porosity factor (99%) and compression modulus (5.25 MPa) than those of the pure PLA scaffold (1.73 MPa). From the results, it can be concluded that the properties of the highly porous *P. purpureum*/PLA scaffold developed in this study can be controlled and optimised. This can be used to facilitate the construction of implantable tissue-engineered cartilage.

Keywords: Polylactic acid; *Pennisetum purpureum*; scaffold; mechanical property; biocomposites

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1. Introduction

Recently, hyaline cartilage has been studied extensively. It functions as a surface covering for all the diarthrodial joints in the human body, protecting them from abrasion and wear [1]. Hyaline cartilage is also frequently known as articular cartilage. Articular cartilage is a heterogeneous and mechanically anisotropic tissue. Since cartilage does not contain nerves, it has very limited capacity to repair itself because of its low cellular content and lack of blood vessels [2]. In recent years, the development of new artificial cartilage, used to repair and restore defective cartilage, has been of considerable interest [3].

Biomedical composites can be used as a scaffold for tissue repair and regeneration; the growth of cells prior to implantation; and the deposition of engineered tissue. Their development offer significant market potential and attract continuous financial investment [4]. Concepts such as long-term sustainability, ecological awareness, and the emerging “green” economy have motivated the identification of green materials that are compatible with living human cells. Unlike synthetic polymers, natural fibre composites (NFCs) have increased in popularity among researchers because of their availability, carbon neutrality, low density, renewability, and biodegradability. In addition, they can be used without damaging the environment [5]. Moreover, the incorporation of fibre reinforcement within the polymer matrix of a composite is proven to increase its mechanical strength. It is also known to improve other crucial parameters, such as the degradation rate and structural properties of the composite, as well as the resultant cell proliferation, and viability. [6]. In addition, these natural fibre reinforced polymer composites have an extended lifespan, which could be used to improves the quality of life of human. They could be used for various potential applications, and are considered suitable for orthopaedic application and tissue engineering [7–12].

In tissue engineering, the most commonly used polysaccharides, such as starch, collagen, chitosan, alginate, and gelatin, have been widely studied with regard to various biomedical applications [13]. Several studies related to the application of 3D chitosan scaffold in xenotransplantation have been published by Steck [14] and Ehrlich [15]; where the human chondrocytes were seeded into chitosan scaffold before transplanted into mice for tissue regeneration. Owing to its biocompatibility, Chevallay and Herbage suggested that collagen could potentially be used as a material for scaffold synthesis; it can also be prepared in different

forms with varying physical properties [16]. Martino et al. reviewed the current application of chitosan in tissue engineering. They concluded that chitosan-based composite biomaterials are suitable for various tissue engineering applications because of their suitable degradation behaviour, and microstructures with desirable pore sizes [17]. Gong et al. studied the biological performance of polylactic acid (PLA)/gelatin scaffold materials. They determined that the gelatin particles increased the hydrophilicity and biocompatibility of the scaffolds [18]. Despite all these promising findings, it is difficult to utilise collagen, chitosan, and gelatin, as reinforcement material during the synthesis of scaffold. They are not ideal materials for tissue engineering because they promote the immune activation of cells, and inflammatory reactions occur due to their degradation behaviour [19]. However, these polysaccharides are still favoured as reinforcement materials in tissue engineering studies. The use of plant-based fibres as reinforcement in scaffolds is of novel interest with regard to tissue engineering applications. The development of new plant fibre-based composites for various applications in biomedical fields is of interest for future research.

Among all the synthetic polymers, PLA has been used widely in tissue engineering applications because of its superior biodegradability and biocompatibility [20–23]. In particular, PLA has been extensively studied to develop scaffold, which has been used to prepare bone tissues, drug delivery systems, stents and artificial organs, for both research and commercial use [24]. Chen et al. studied the morphology of a porous PLA scaffold prepared by thermally induced phase separation. The scaffolds were prepared via solid—liquid phase separation and liquid—liquid phase separation, where they exhibited anisotropic tubular morphologies and isotropic morphologies, respectively [25]. Cheung et al. studied the influence of silkworm silk/PLA on the mechanical and thermal behaviour of biocomposites. According to the authors, the tensile properties of the silk fibre/PLA composites were superior to those of pure PLA. The addition of the silk fibre further enhanced the thermal and physical properties of the composites, forming an ideal scaffold. The findings provide the impetus for this material to be potentially used as a material for tissue engineering [26].

A key challenge faced by the field of tissue engineering is the design of cartilage scaffold with favourable microstructure and mechanical properties that promote cell growth and attachment, and new tissue regeneration [27]. The use of *P. purpureum* as a reinforcement filler

in a PLA matrix offer many advantages; it results in the production of the biocomposites with favourable mechanical properties and controllable biodegradability. Ridzuan et al. determined that *P. purpureum* fibres could potentially be used as reinforcement materials in polymer composites. They determined that the application of alkaline treatment increases the surface roughness and minimises the hemicelluloses quantities of the fibre [28]. Hameem et al. studied the tensile and flexural properties of *P. purpureum* and concluded that when polyester composites were reinforced with *P. purpureum*, their mechanical properties improved [29]. Moreover, Ridzuan et al. studied the effect of elevated temperatures on the mechanical behaviour of *P. purpureum*/glass-reinforced composites. The tensile and flexural strength of the composites decreased as the temperature approached the glass transition temperature (T_g) at $>60^\circ\text{C}$; this was owed to the softening of the composite's matrix [30]. Similarly, Ridzuan et al. reported that moisture absorption influences the mechanical properties of hybrid *P. purpureum*/glass-epoxy composites [31]. They observed that as the glass fibre content of the samples is increased, the degree of moisture absorption decreased and the tensile and flexural strength of the composites increased. Recently, based on several studies, researchers suggested that *P. purpureum* fibres could effectively be used as reinforcement in polymer composites for tissue engineering applications [29–33].

To benefit from the outstanding properties of *P. purpureum* and its derivatives, we have designed highly porous *P. purpureum*-PLA scaffolds, which are prepared using the solvent casting and particulate leaching technique. This preparation method was selected because it can be used to control the porosity and pore sizes of the scaffold. Furthermore, compared with other methods, this technique requires less energy and time, and it is also inexpensive and consumer friendly. The physical, morphological and mechanical properties of the resultant *P. purpureum*-PLA biocomposite scaffolds with various compositions are subsequently discussed.

2. Materials and Methods

2.1. Materials

P. purpureum powder was prepared from *P. purpureum* fibre also well known as Napier fibre which was purchased from a local plantation in Bukit Kayu Hitam, Kedah, in northern peninsular Malaysia. PLA of label 4032D was acquired from NatureWorks LLC. Chloroform and

dichloromethane were purchased from Fisher Chemical Co. and were used as organic solvents. Analytical-grade sodium chloride (NaCl) was purchased from Sigma-Aldrich, Germany and used as a porogen.

2.2 Extraction and preparation of Pennisetum purpureum powder

The *P. purpureum* grass leaves were removed from the internodes of each stem. Subsequently, the stems were crushed and then immersed in a tank filled with water. The stems were immersed in the water-filled tank for approximately two to three weeks for the retting process to occur. Prior to the extraction process, the soaked *P. purpureum* grasses were thoroughly washed with running water. The fibres were extracted manually from the stems, and the extracted raw fibres were then sun-dried to remove any excess moisture present within the fibres. To prepare the *P. purpureum* into powder form, the dried fibres were cut into small pieces, and then ground and sieved. The powder was ground once again and sieved using a 63 μm sieve to obtain fine *P. purpureum* powder.

2.3. Preparation of porous PLA scaffold reinforced with Pennisetum purpureum as a filler

The PLA and *P. purpureum* composite scaffolds with a porous microstructure were prepared by a salt-leaching and solvent casting method. The PLA pellets were dissolved in chloroform and dichloromethane (volume ratio of 1:1) to obtain a viscous solution (60.0 g/L). To produce a composite scaffold consisting of PLA and *P. purpureum*, the desired amount of fibre powder (composition listed in Table 1) was dispersed in a solution of PLA using a magnetic stirrer; the temperature was maintained at 70 °C for 2 h. Subsequently, NaCl was added to the solution, and the solution was stirred again for 20-30 min to obtain a homogenous composite. The PLA composite and NaCl were blended to a ratio of 1:9. The salt—PLA composite solution was then cast into a rectangular Teflon mould (width, length, and thickness of 13 mm, 25 mm, and 13 mm, respectively), to produce samples suitable for mechanical testing. The dried PLA composites were placed in distilled water for 48h to leach out the salts; every 4 h, the distilled water was refreshed to complete elimination of the salt. The leached scaffolds were stored overnight in a vacuum drier at room temperature. The resultant scaffolds were then stored in a desiccator prior to characterizations.

2.4. Characterization

2.4.1. Porosity measurement

In this study, a scaffold with four different compositions was prepared; therefore, the porosity of each composition had to be appropriately calculated. The four compositions were subsequently sampled, with three specimens representing each composition. The overall results were determined using the average value of the results of three specimens. The porosity of each scaffold was measured using Archimedes' principle [34].

The porosity of each scaffold was determined using the following equation:

$$P = \frac{(W_2 - W_3 - W_s)/\rho_e}{(W_1 - W_3)/\rho_e} \times 100\% \quad (1)$$

where W_s is the weight of the scaffold; W_1 is the initial weight of the glass vial filled with ethanol; W_2 is the weight of the glass vial following immersion of the specimen; W_3 is the weight of the glass vial on removal of the specimen; and ρ_e is the density of ethanol (0.789mg/mL).

2.4.2. Field-emission scanning electron microscope (FESEM) analysis

The morphology of the composite scaffolds was observed using a field-emission scanning electron microscopy (FESEM). All the samples were coated with a thin layer of platinum (Pt) via sputtering (Quorum, Q150R S). Subsequently, the coated samples were observed using a FESEM-NOVA NanoSEM 450 at an acceleration voltage of 3 kV.

Table 1. Composition, porosity and average pore size of the *P. purpureum*/PLA scaffold.

Samples	PLA (g)	<i>Pennisetum purpureum</i> (g)	Sample code	Porosity (%)	Average pore size (μm)
S1	2.4	0.00	PLA _C	99.6	105 $\pm$ 36
S2	2.4	0.24	PLA-PP ₁₀	99.4	170 $\pm$ 45
S3	2.4	0.48	PLA-PP ₂₀	99.1	103 $\pm$ 21
S4	2.4	0.72	PLA-PP ₃₀	99.2	158 $\pm$ 23

2.4.3. Fourier-transform infrared spectroscopy (FTIR) analysis

The FTIR spectra of the prepared *P. purpureum*/PLA scaffolds were studied using an FTIR spectrometer (Perkin-Elmer RX1, UK). The samples were ground and mixed thoroughly with potassium bromide (KBr), where the KBr concentration of the sample was 20%. The spectra were collected over the range of 400—4000 cm⁻¹. The FTIR spectra were recorded in transmittance mode.

2.4.4. Thermogravimetric analysis.

The thermal stabilities of the PLA, *P. purpureums*, and various composites blends were determined using a thermogravimetric analyser (TGA-50, Shimadzu, Japan); the results are shown in Table 1. The weights of the scaffolds were 10.0—12.5 mg. The fabricated samples (10–12.5 mg) were analysed over the temperature range of 30—600 °C at a heating rate of 20 °C/min. The TGA analysis was performed under a nitrogen atmosphere, with a nitrogen flow rate of 20 mL/min. The mass loss of the scaffolds was recorded and plotted as a function of temperature [35].

2.4.5. Moisture content analysis

The prepared scaffolds with various compositions were kept in an exsiccator for one week, which was maintained at 20 °C with a relative humidity of 58%. Subsequently, they were weighed in petri dishes and dried in a vacuum oven at 40 °C for 24 h [36]. The moisture content is defined as the percentage weight loss of the initial sample that occurs during drying. The following formula is used to calculate the moisture content:

$$MC = \frac{(W_o - W_{od})}{W_o} \times 100\% \quad (2)$$

where, MC is the moisture content, W_o is the initial weight of the sample, and W_{od} is the weight of the scaffold after drying.

2.4.6. Compressive properties

The mechanical behaviours of the fabricated samples were determined by executing compression strength test according to ASTM F451-95. The cross-head speed was 1 mm/min and the load

capacity was 2 kN. Rectangular samples of appropriate dimensions (13mm (width) $\times$ 25mm (length) $\times$ 13mm (thickness)) were prepared. The length, width, and thickness of the samples were verified manually using a Vernier calliper. Three identical specimens were tested to represent composite; the mean compressive strengths of the various compositions were recorded, and the average values were reported. The compression modulus was determined from the slope of the initial linear portion of the stress—strain curve plotted using the test results.

3. Results and Discussion

3.1 Morphology and pore microstructure of scaffolds

The FESEM images were examined to elucidate the effect of the suspension composition as well as that of crosslinking on the scaffold morphology. The PLA_C scaffold had a wide pore-size distribution. When the *P. purpureum* content was increased, the overall homogeneity of the structure was slightly enhanced and there was a reduction in the average pore size (Figures 1b—d). The results obtained for the PLA-PP₁₀, PLA-PP₂₀, and PLA-PP₃₀ scaffolds were superior to those of the PLA_C scaffold.

The morphologies of the PLA_C, PLA-PP₁₀, PLA-PP₂₀ and PLA-PP₃₀ scaffolds, with various filler contents, are shown in Figures 1a—d. The surface of the pure PLA and PLA-PP₁₀ scaffolds were relatively smoother and uniform (Figures 1a and b). In contrast, the surface of the PLA-PP₂₀ and PLA-PP₃₀ scaffolds was rough and irregular (Figures 1c and d). As the *P. purpureum* content increased, the pore-size distribution of the material broadened, this is considered optimal for cartilage applications.

When the typical microstructures of the prepared scaffolds were compared, sample PLA-PP₃₀ showed the formation of a finely blended and interconnected fibrous structure (Figure 1d). Fibrous structures were also observed for the PLA-PP₁₀ and PLA-PP₂₀ scaffolds; however, the pore sizes were considerably larger and smaller, respectively than those observed for PLA-PP₃₀. The cell size and scaffold porosity can also be controlled by changing the filler content, as shown in previous studies [37,38].

The pore sizes measured using FESEM, of all the modified scaffolds are summarised in Table 1. Considering the scaffolds with various filler contents, all the samples exhibit high

standard deviations value with regards to the pore sizes. The maximum average pore size (135-181 μm) was determined in the case of the PLA-PP₃₀ scaffold. The pure PLA, PLA-PP₁₀, and PLA-PP₂₀ scaffolds had average pore sizes of 69-141 μm , 125-215 μm , and 82-124 μm , respectively.

The PLA-PP₃₀ scaffold was considered suitable for cartilage applications based on its average pore size, pore microstructure, pore homogeneity, and the high level of interconnectivity between its pores. As shown in Figure 1d, the *P. purpureum* particles are well-dispersed throughout the PLA matrix, which enhances the strength and flexibility of the material. Moreover, pores with rough walls are necessary to aid cell migration and extracellular matrix (ECM) growth following implantation. Therefore, it seems crucial to develop pore walls with the rougher surface to enhance the diffusion of the oxygen/nutrients supply as well as waste removal [25][38,39].

All the scaffolds were highly porous (>99%) with low weights, as illustrated in Table 1. This fulfilled the basic requirements determined from literature [38,40,41]. These samples possessed relatively similar porosities irrespective of the *P. purpureum* content; this is likely to be attributed to the porogen content of the specimens, which was fixed throughout the fabrication process. The porogen (NaCl) to matrix ratio constant was kept (9:1); however, as the *P. Purpureum* content increased, the level of porosity decreased from 99.6% to 99.1%, which was owed to the compact arrangement of the fibre network. As expected, this also slightly reduced the degree of homogeneity of the structure.

Figure 1b—d show cross-sectional FESEM images of the porous scaffolds of PLA-NF₁₀, PLA-NF₂₀, and PLA-NF₃₀. It can be observed that the scaffolds exhibit optimum levels of porosity (as shown by the porosity data in Table 1); in addition, the filler is evenly distributed throughout the scaffolds. In addition, the FESEM images also verified the fibrous structure of the scaffolds as well as the degree of interconnectivity between the pores. Possibilities to make an interesting host system is high with the presence of open, interconnected pores created by the fibre network as reviewed by Ehrlich [42]. The PLA-PP₃₀ scaffold exhibited a microstructure with large pores and homogeneous pore structure. In addition, there was a high degree of interconnectivity between the pores (Figure 1d). These observations indicate that the scaffolds can highly be suitable for use in cartilage tissue engineering.

Figure 1: FESEM images showing micro-scaled pores of porous *P. purpureum*/PLA scaffolds: (a) PLAC, (b) PLA-PP₁₀, (c) PLA-PP₂₀ and (d) PLA-PP₃₀.

3.2 FTIR analysis

FTIR analysis is widely employed and was used to interpret and determine the functional groups and structural elements present within the prepared scaffolds [43]. For comparison, the pure component was also analysed. Among the various composites, it could be observed that there were slight differences between the FTIR absorbance spectra of the scaffolds with regard to the shapes and positions of the absorbance peaks.

In the case of the pure PLA scaffold (Figure 2a), characteristic bands at 2922 cm^{-1} , attributed to —CH bending vibration was observed in the spectrum. In the FTIR spectrum of the pure PLA scaffold, peaks were also observed at 754 and 870 cm^{-1} (—CH bending vibration);

1045 and 1184 cm^{-1} (C—O stretching vibration); 1455 cm^{-1} ($-\text{CH}_3$ bending vibration); and 1755 cm^{-1} (C=O stretching vibration on ester group) [44][45].

The presence of the *P. purpureum* and PLA was determined via the FTIR analysis of the scaffolds. The FTIR spectra of the pure PLA and *P. purpureum*-PLA scaffolds are shown in Figures 2 and 3. The characteristic peak at 1756 cm^{-1} (C=O of PLA) indicates the stretching vibration of the carbonyl groups of the PLA. The peaks at 2995 cm^{-1} ($-\text{CH}_3$ bending vibration); 1755 cm^{-1} (C=O stretching vibration); 1455 cm^{-1} ($-\text{CH}_3$ bending vibration); 1360 cm^{-1} ($-\text{CH}$ bending vibration); and 1132 and 1184 cm^{-1} (C—O stretching vibration) confirm the presence of the PLA within the *P. purpureum*-PLA scaffolds. Typical bands, such as those at 1088 and 1045 cm^{-1} , are owed to the presence of the *P. purpureum*.

Comparing the results of the pure PLA and *P. purpureum*-PLA scaffolds, it can be determined that two noticeable changes occur in each scaffold. The presence of $-\text{CH}_3$, with bending vibration, was detected in the blended composites but not in the pure PLA scaffold. Furthermore, the intensity of the stretching bands attributed to the ester group, located at 1755 cm^{-1} , became significantly weaker as the *P. purpureum* content of the PLA matrix blend increased. All these differences indicate that obvious interactions occur amongst the ester and carboxyl groups of the composites.

This indicates that the degree of crystallinity of the material increases as the *P. purpureum* content of the PLA matrix increases. Based on the results obtained for *P. purpureum*-PLA composites, it could be observed that the intensity of several characteristic bands decreased when the fillers content was increased, which indicates that cross-linking occurs between the *P. purpureum* and PLA of the composite scaffolds.

3.3 Thermogravimetric analysis (TGA)

Thermogravimetric analysis (TGA) measures the weight change of a material as a function of temperature or time in a controlled atmosphere. Derivative thermogravimetric (DTG) analysis was used to determine the decomposition temperature of the biocomposites. Following the TGA, the thermogravimetric (TG) curves and derivative thermograms (DTG) of the pure PLA and *P. purpureum*/PLA scaffolds were obtained; these are represented in Figures 4 and 5, respectively.

Figure 2: FTIR spectrum of porous *Pennisetum purpureum*/PLA scaffolds with microscale pores: (a) PLAC and (b) PLA-PP₁₀.

Figure 3: FTIR spectrum of porous *Pennisetum purpureum*/PLA scaffolds with microscale pores: (a) PLA-PP₂₀ and (b) PLA-PP₃₀.

Based on the DTG curves, the thermal degradation of the PLA and *P. purpureum*/PLA scaffolds occurred in a single weight-loss step. The incorporation of the *P. purpureum* within the PLA matrix influenced the thermal degradation temperature by enhancing the thermal stability of the composite scaffolds. Based on the thermal degradation temperature determined from the TGA curves, it seems that at temperatures below 300 °C, the *P. purpureum*/PLA scaffolds are more thermally stable than the PLA_C scaffolds. The *P. purpureum*/PLA scaffolds lose approximately 2.7% of their mass at approximately 250 °C. This effect is due to the loss of moisture and is a well-known phenomenon with regard to scaffolds with high porosity. Between 300—500 °C, the scaffolds decompose almost entirely; specifically, they undergo approximately 93% degradation.

Table 2 shows the thermal stability values of the synthesised pure PLA and *P. purpureum*/PLA scaffolds. The PLA_C scaffolds have an onset temperature of 301.6 °C, which decreases to 296.6, 298.0, and 295.0 °C when the blends are reinforced with *P. Purpureum* fillers at contents of 10, 20, and 30 wt%, respectively. For the PLA-PP₁₀, PLA-PP₂₀, and PLA-PP₃₀ scaffolds, there is little difference between the curves obtained. The final degradation temperature (FDT) of the *P. purpureum*/PLA scaffolds was approximately 42% greater than that of the PLA_C scaffold. The FDT also increased when the *P. Purpureum* filler was incorporated into the PLA matrix. In the case of the PLA-PP₁₀, PLA-PP₂₀, and PLA-PP₃₀ scaffolds, the FDT were 336.56, 353.07 and 362.97 °C. These values are greater than that obtained for the PLA_C scaffold (339.72 °C).

The interaction between the PLA matrix and *P. purpureum* fillers consequently increased the molecular weight or extended the molecular chain of the neat PLA which contributed to the higher FDT. Several researchers reported that when *P. purpureum* homogenously dispersed in a PLA matrix, it functions as a barrier, which provides heat insulation and prevents oxidation. It also lowers the permeability of volatile degradation products within the biocomposites, which helps to extend the thermal degradation process [46–48].

Figure 4: TGA results of porous *Pennisetum purpureum*/PLA scaffolds: (a) PLA_C, (b) PLA-PP₁₀, (c) PLA-PP₂₀ and (d) PLA-PP₃₀.

Figure 5: DTG results of porous *Pennisetum purpureum*/PLA scaffolds: (a) PLA_C, (b) PLA-PP₁₀, (c) PLA-PP₂₀ and (d) PLA-PP₃₀.

As expected, the *P. purpureum*-reinforced scaffolds behave very similarly. However, the behaviour is influenced by the filler content. The PLA-PP₁₀, PLA-PP₂₀, and PLA-PP₃₀ scaffolds had final residue percentages of 6.01, 5.98, and 7.00%, respectively. This indicates that the final residue percentage increases as the amount of *P. purpureum* that is blended into the PLA matrix increases. The polymer matrix undergoes complete degradation under the experimental conditions used; however, the *P. purpureum* filler does not decompose and, remains as a residue. The PLA_C scaffold has the lowest final residue percentage of 0.66% due to the absence of the *P. purpureum* filler.

Table 2. Thermogravimetric analysis (TGA) results obtained for the PLA_C, PLA-PP₁₀, PLA-PP₂₀ and PLA-PP₃₀ scaffolds.

Sample name	Initial degradation temperature (°C)	Degradation at 50% weight loss (°C)	Final degradation temperature (°C)	Final residue (%)
PLA _C	301.63	334.83	339.72	0.663
PLA-PP ₁₀	296.61	331.69	336.56	6.014
PLA-PP ₂₀	298.01	346.40	353.07	5.977
PLA-PP ₃₀	295.01	358.98	362.97	7.006

The results of the DTG analysis performed on the pure PLA and *P. purpureum*/PLA scaffolds with varying filler compositions are illustrated in Figure 5. The percent of derivative weight reduction reflects the highest thermal degradation rate. This was derived from the peak of DTG curves after the PLA_C, PLA-PP₁₀, PLA-PP₂₀, and PLA-PP₃₀ samples reached their maximum degradation temperatures ($T_{\max}$) of 339.72, 336.56, 353.07, and 362.97 °C, respectively. Furthermore, based on the DTG data, it is evident that all the scaffolds exhibited similar thermal behaviour (Figure 5); they demonstrate single-stage thermal degradation within a relatively narrow temperature range.

Each scaffold exhibited just one peak, the height of the peak of the PLA_C scaffold was greater than those of the scaffolds composed of *P. purpureum* fillers. There is hardly any difference between the heights of the peaks of the *P. purpureum*/PLA scaffolds. In addition, the $T_{\max}$ values of the scaffolds increased as the *P. purpureum* content increased. This may be owed

to the interactions between the PLA matrix and the fillers; the *P. purpureum* has a homogeneous nucleation effect on the crystallisation of PLA matrix.

The TGA/DTG results revealed that the thermal stability of the PLA scaffolds containing 10, 20, and 30 wt% *P. purpureum* increased as the *P. purpureum* content increased. Based on the obtained results, it was proven that the thermal stability of the *P. purpureum*/PLA scaffolds was superior to than that of the pure PLA scaffold.

3.4 Moisture content analysis

Moisture content analysis was used to determine the amount and percentage of moisture in the scaffolds. Owing to the hydrophilic nature of the natural fibres and their porous structure, it was almost impossible to synthesise the resultant biocomposites without the existence of voids. During moisture absorption, liquid moisture penetrates the free space of the micro-voids and then diffuses via the fibre-matrix interface. In this study, all the synthesised scaffolds retained a moisture content of 0.5—1.2%, as illustrated in Figure 6. However, the pure PLA scaffolds had a significantly lower moisture content compared with that of the other biocomposite scaffolds. This indicates that hardly any water was observed by the scaffold. The moisture absorption behaviour observed in this study are similar to that observed in a study by Hameem et al., in which Napier grass (*P. purpureum*) fibre polymer composites behaved in a similar manner [33].

Based on the graph presented in Figure 6, the moisture content percentage significantly increases as the *P. purpureum* content increases from 0 to 30 wt%. The maximum moisture content percentages determined for the PLA_C, PLA-PP₁₀, PLA-PP₂₀, and PLA-PP₃₀ scaffolds were 0.50, 1.01, 1.20, and 1.18%, respectively. Owing to the microstructure of the scaffolds, which are rough and uneven compared with those of the other scaffolds, the PLA-PP₂₀ and PLA-PP₃₀ absorbed a similar amount of moisture. The morphologies of the PLA-PP₂₀ and PLA-PP₃₀ scaffolds contributed to the increased void content of the scaffolds. In contrast, Chien et al. recently conducted moisture content tests on soy protein scaffolds; they determined that the moisture content of the scaffolds decreased when the level of enzymatic crosslinking increased [36]. This can be argued that the sorption from soy protein used is limited due to the presence of more hydrophobic residues than the cellulosic *P. purpureum* fibres [49].

Moreover, the hydrophilic nature of the *P. purpureum* fibres influenced the mechanical properties of the fabricated scaffolds. In this study, the mechanical strength of the scaffolds increased as the *P. purpureum* content of the PLA scaffolds increased. This indicates that the moisture content does not significantly influence the mechanical properties of the scaffolds. Therefore, for all the scaffolds, the percentage moisture content is considered to be within an acceptable range, since it had little influence on the physical and mechanical properties of the scaffolds.

Figure 6: Moisture content of porous *Pennisetum purpureum*/PLA scaffolds with microscale pores: (a) PLAC, (b) PLA-PP₁₀, (c) PLA-PP₂₀ and (d) PLA-PP₃₀.

3.5 Compressive properties analysis

Among all the types of mechanical testing, compression testing is preferred for cartilage material analysis. This is owed to the role of natural cartilage, which is load bearing under compression. As shown in Figures 7 and 8, clear trends can be observed when the *P. purpureum* content is varied. The *P. Purpureum* content does not influence the porosity or the pore sizes of the scaffolds. This could be attributed to the strong adhesion that exists at the interfaces of the PLA matrix and *P. purpureum* fillers, and its ability to bear loads and transfer them across the interface. However, the presence of the filler in the matrix enhances the mechanical properties of the composites compared with those of PLA alone. Similarly, the interaction between chitin and

the other matrix is often the key for the mechanical stability of the specific biomaterial as per reviewed by Ehrlich [15].

Naseri et al. determined that for a healthy cartilage, the compression modulus of an articular cartilage should be approximately 1.0 MPa [2]. This is dependent on the materials and testing conditions. However, the value of the compression modulus determined in this study was greater than that of natural cartilage. In addition, the moisture content of natural cartilage should be noted because it is slightly greater than that determined for the materials in this study.

The *P. purpureum* addition consequently increased the compressive strength, as shown in Table 3. The scaffold with PLA alone had the lowest compressive strength and the PLA-PP₃₀ scaffold had the greatest. In addition, there was a significant increase in the compressive strength of samples PLA-PP₁₀ and PLA-PP₂₀ compared with that of PLA alone. However, when the *P. purpureum* content increased to 30%, there was a further increase in the compressive strength from 7.49 to 9.32 MPa. Upon the addition of the *P. Purpureum*, there was an increase in the compressive strength of the samples; this indicates there was a high degree of adhesive interaction between the *P. purpureum* fillers and the PLA matrix of the blend used in this study. The influence of the *P. purpureum* on the compression modulus was investigated for samples PLA-PP₁₀, PLA-PP₂₀, and PLA-PP₃₀. Owing to the reinforcement, the mechanical properties of the materials significantly improved; the highest compression modulus achieved was 5.25 MPa. This pattern can be explained by the increase in the *P. purpureum* contents used for blending as well as the high levels of porosity and interconnectivity of the scaffolds.

The compressive moduli of samples PLA_C, PLA-PP₁₀, PLA-PP₂₀, and PLA-PP₃₀ are summarised in Figure 8. The pure PLA scaffold has a compressive modulus of 1.73 MPa, which increases to 5.25 MPa in the case of sample PLA-PP₃₀, which has the highest *P. purpureum* content. Compared with the results obtained for the pure PLA scaffold, the scaffolds become stronger as the *P. purpureum* content increases. The incorporation of fillers within the PLA renders the scaffolds less brittle. This observation is supported by the results of previous rheological studies, which showed that sufficient stress transfer occurs across the PLA-*P. Purpureum* interface when a load is applied to the blend. The length of the PLA polymer chain decreases when the *P. purpureum* content increases; therefore, samples, PLA-PP₂₀ and PLA-PP₃₀ exhibit solid-like behaviour.

Figure 7: Compressive strength of porous *P. purpureum*/PLA scaffolds with microscale pores: (a) PLAC, (b) PLA-PP₁₀, (c) PLA-PP₂₀, and (d) PLA-PP₃₀

Figure 8: Compressive moduli of porous *P. purpureum*/PLA scaffolds with microscale pores: (a) PLAC, (b) PLA-PP₁₀, (c) PLA-PP₂₀, and (d) PLA-PP₃₀.

In the current study, the PLA-PP₃₀ scaffolds have shown promising mechanical properties (1.73—5.25 MPa) that would enable chondrogenesis. In addition, the PLA-PP₃₀ scaffolds possess minimal moisture content as well as appropriate pore sizes and surface morphologies, which would promote cell adhesion. Therefore, following implantation, these synthesised scaffolds are likely to promote the regeneration of cartilage and the cultivation of ECM with appropriate mechanical properties. In the future, *in-vivo* testing will be conducted using the *P. purpureum*/PLA scaffolds. Following ECM growth, the scaffolds will be observed and evaluated. The findings from these studies will be reported.

Table 3. Mechanical properties of *Pennisetum purpureum*/PLA scaffolds

Scaffold	Compressive strength (MPa)	Compressive Modulus (MPa)
PLA _c	1.94 ± 0.09	1.73 ± 0.08
PLA-PP ₁₀	4.33 ± 0.21	2.75 ± 0.13
PLA-PP ₂₀	7.49 ± 0.37	3.72 ± 0.18
PLA-PP ₃₀	9.32 ± 0.46	5.25 ± 0.26

4. Conclusions

In summary, biocomposites consisting of cellulose fibres blended within a PLA matrix were prepared via the solvent casting and particulate leaching technique to produce highly porous ($\approx$ 99% porosity) 3D scaffolds. This scaffold exhibited excellent porosity, suitable pore sizes, and high levels of pore interconnectivity, as well as optimal mechanical properties and appropriate moisture contents. The *P. purpureum*/PLA scaffolds exhibited excellent morphologies and structures, and the filler was evenly distributed within them. The pore sizes of the scaffolds ranged from 69—215 μ m. The surface roughness of the scaffolds is increased with the incorporation of the *P. Purpureum* into the PLA matrix. The biocomposite scaffolds exhibited increased surface roughness compared with that of the pure PLA scaffold. Following implantation, this shall contribute to cell attachment, proliferation, and even ECM growth. The TGA performed on the synthesised scaffolds showed that both the decomposition temperature and the amount of final residue increased as the *P. purpureum* content of the scaffold increased. The mechanical properties of the PLA and *P. purpureum* biocomposite scaffolds are promising. The addition of *P. purpureum* enhanced the mechanical performance of the scaffolds; the

compression modulus of PLA-PP₃₀ increased from 1.73 to 5.25 MPa with an addition of 30 wt% *P. purpureum* filler. In tissue engineering, this value is considered the maximum required for natural cartilage. Moreover, the moisture content had little influence on the mechanical behaviour of the scaffolds. Amongst all the samples, the PLA-PP₃₀ scaffold had the highest moisture content, 1.20%. These results demonstrate that tissue engineering scaffolds could potentially be developed via the incorporation of *P. purpureum* fibres into PLA matrix hence be used as biocompatible scaffolds for cartilage tissue engineering.

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References

- [1] Z. Izadifar, X. Chen, W. Kulyk, Strategic design and fabrication of engineered scaffolds for articular cartilage repair., *J. Funct. Biomater.* 3 (2012) 799–838. doi:10.3390/jfb3040799.
- [2] N. Naseri, G. Lenart, O. Kristiina, 3-Dimensional porous nanocomposite scaffolds based on cellulose nanofibers for cartilage tissue engineering : tailoring of porosity and mechanical performance, *R. Soc. Chem.* 6 (2016) 5999–6007. doi:10.1039/C5RA27246G.
- [3] B. Johnstone, M. Alini, M. Cucchiaroni, G.R. Dodge, D. Eglin, F. Guilak, H. Madry, A. Mata, R.L. Mauck, C.E. Semino, M.J. Stoddart, Tissue engineering for articular cartilage repair--the state of the art., *Eur. Cell. Mater.* 25 (2013) 248–67. doi:vol025a18 [pii].
- [4] K.L. Pickering, M.G.A. Efendy, T.M. Le, A review of recent developments in natural fibre composites and their mechanical performance, *Compos. Part A.* 83 (2016) 98–112. doi:10.1016/j.compositesa.2015.08.038.
- [5] H. yan Cheung, M. po Ho, K. tak Lau, F. Cardona, D. Hui, Natural fibre-reinforced composites for bioengineering and environmental engineering applications, *Compos. Part B Eng.* 40 (2009) 655–663. doi:10.1016/j.compositesb.2009.04.014.
- [6] C.J. Chirayil, L. Mathew, S. Thomas, Review of recent research in nano cellulose preparation from different lignocellulosic fibers, *Rev. Adv. Mater. Sci.* 37 (2014) 20–28.
- [7] M.I. Sabir, X. Xu, L. Li, A review on biodegradable polymeric materials for bone tissue engineering applications, *J. Mater. Sci.* 44 (2009) 5713–5724. doi:10.1007/s10853-009-3770-7.

- [8] N. Saba, P. Tahir, M. Jawaid, A review on potentiality of nano filler/natural fiber filled polymer hybrid composites, *Polymers (Basel)*. 6 (2014) 2247–2273. doi:10.3390/polym6082247.
- [9] H. Ku, H. Wang, N. Pattarachaiyakoo, M. Trada, A review on the tensile properties of natural fiber reinforced polymer composites, *Compos. Part B Eng.* 42 (2011) 856–873. doi:10.1016/j.compositesb.2011.01.010.
- [10] D.W. Hutmacher, J.C.H. Goh, S.H. Teoh, An introduction to biodegradable materials for tissue engineering applications, *Ann. Acad. Med.* 30 (2001) 183–191.
- [11] O.D. Samuel, S. Agbo, T.A. Adekanye, Assessing mechanical properties of natural fibre reinforced composites for engineering applications, *J. Miner. Mater. Charact. Eng.* (2012) 780–784.
- [12] O. Faruk, A.K. Bledzki, H.P. Fink, M. Sain, Biocomposites reinforced with natural fibers: 2000-2010, *Prog. Polym. Sci.* 37 (2012) 1552–1596. doi:10.1016/j.progpolymsci.2012.04.003.
- [13] H.-F. Ko, C. Sfeir, P.N. Kumta, Novel synthesis strategies for natural polymer and composite biomaterials as potential scaffolds for tissue engineering., *Philos. Trans. R. Soc. A*. 368 (2010) 1981–1997. doi:10.1098/rsta.2010.0009.
- [14] E. Steck, M. Burkhardt, H. Ehrlich, W. Richter, Discrimination between cells of murine and human origin in xenotransplants by species specific genomic in situ hybridization, *Xenotransplantation*. 17 (2010) 153–159. doi:10.1111/j.1399-3089.2010.00577.x.
- [15] H. Ehrlich, M. Ilan, M. Maldonado, G. Muricy, G. Bavestrello, Z. Kljajic, J.L. Carballo, S. Schiaparelli, A. Ereskovsky, P. Schupp, R. Born, H. Worch, V.V. Bazhenov, D. Kurek, V. Varlamov, D. Vyalikh, K. Kummer, V.V. Sivkov, S.L. Molodtsov, H. Meissner, G. Richter, E. Steck, W. Richter, S. Hunoldt, M. Kammer, S. Paasch, V. Krasokhin, G. Patzke, E. Brunner, Three-dimensional chitin-based scaffolds from *Verongida* sponges (*Demospongiae*: *Porifera*). Part I. Isolation and identification of chitin, *Int. J. Biol. Macromol.* 47 (2010) 132–140. doi:10.1016/j.ijbiomac.2010.05.007.
- [16] B. Chevallay, D. Herbage, Collagen-based biomaterials as 3D scaffold for cell cultures: applications for tissue engineering and gene therapy., *Med. Biol. Eng. Comput.* 38 (2000) 211–218. doi:10.1007/BF02344779.
- [17] A. Di Martino, M. Sittinger, M. V. Risbud, Chitosan: A versatile biopolymer for orthopaedic tissue-engineering, *Biomaterials*. 26 (2005) 5983–5990. doi:10.1016/j.biomaterials.2005.03.016.
- [18] Y. Gong, Z. Ma, Q. Zhou, J. Li, C. Gao, J. Shen, Poly(lactic acid) scaffold fabricated by gelatin particle leaching has good biocompatibility for chondrogenesis., *J. Biomater. Sci. Polym. Ed.* 19 (2008) 207–221. doi:10.1163/156856208783432453.
- [19] L. Bačáková, K. Novotná, M. Pařízek, Polysaccharides as cell carriers for tissue engineering: The use of cellulose in vascular wall reconstruction, *Physiol. Res.* 63 (2014)

S29–S47.

- [20] D. Garlotta, A literature review of poly(lactic acid), *J. Polym. Environ.* 9 (2001) 63–84. doi:10.1023/A:1020200822435.
- [21] P. Gentile, V. Chiono, I. Carmagnola, P. V. Hatton, An overview of poly(lactic-co-glycolic) acid (PLGA)-based biomaterials for bone tissue engineering, *Int. J. Mol. Sci.* 15 (2014) 3640–3659. doi:10.3390/ijms15033640.
- [22] K. Madhavan Nampoothiri, N.R. Nair, R.P. John, An overview of the recent developments in polylactide (PLA) research, *Bioresour. Technol.* 101 (2010) 8493–8501. doi:10.1016/j.biortech.2010.05.092.
- [23] V. Srebrekoska, G. Bogoeva-Gaceva, Biocomposites based on poly (lactic acid) and their recyclability, *Qual. Life.* 2 (2000) 21–32.
- [24] R.P. Pawar, S.U. Tekale, S.U. Shisodia, J.T. Totre, A.J. Domb, Biomedical applications of poly (lactic acid), *Recent Patents Regenerative Med.* 4 (2014) 40–51.
- [25] J.S. Chen, S.L. Tu, R.Y. Tsay, A morphological study of porous polylactide scaffolds prepared by thermally induced phase separation, *J. Taiwan Inst. Chem. Eng.* 41 (2010) 229–238. doi:10.1016/j.jtice.2009.08.008.
- [26] H.Y. Cheung, K.T. Lau, X.M. Tao, D. Hui, A potential material for tissue engineering: Silkworm silk/PLA biocomposite, *Compos. Part B Eng.* 39 (2008) 1026–1033. doi:10.1016/j.compositesb.2007.11.009.
- [27] G. Portocarrero, A.T. Livingston, Challenges in cartilage tissue engineering, *J. Tissue Sci. Eng.* 4 (2013) 1–2. doi:10.4172/2157-7552.1000e120.
- [28] M.J.M. Ridzuan, M.S. Abdul Majid, M. Afendi, S.N. Aqmariah Kanafiah, J.M. Zahri, A.G. Gibson, Characterisation of natural cellulosic fibre from *Pennisetum purpureum* stem as potential reinforcement of polymer composites, *Mater. Des.* 89 (2016) 839–847. doi:10.1016/j.matdes.2015.10.052.
- [29] M. Haameem J.A., M.S. Abdul Majid, M. Afendi, H.F.A. Marzuki, I. Fahmi, A.G. Gibson, Mechanical properties of Napier grass fibre/polyester composites, *Compos. Struct.* 136 (2016) 1–10. doi:10.1016/j.compstruct.2015.09.051.
- [30] M.J.M. Ridzuan, M.S.A. Majid, M. Afendi, M.N. Mazlee, A.G. Gibson, Thermal behaviour and dynamic mechanical analysis of *Pennisetum purpureum*/glass-reinforced epoxy hybrid composites, *Compos. Struct.* 152 (2016) 850–859. doi:10.1016/j.compstruct.2016.06.026.
- [31] M.J.M. Ridzuan, M.S. Abdul Majid, M. Afendi, K. Azduwin, N.A.M. Amin, J.M. Zahri, A.G. Gibson, Moisture absorption and mechanical degradation of hybrid *Pennisetum purpureum*/glass-epoxy composites, *Compos. Struct.* 141 (2016) 110–116. doi:10.1016/j.compstruct.2016.01.030.
- [32] M.J.M. Ridzuan, M.S. Abdul Majid, M. Afendi, S.N. Aqmariah Kanafiah, J.M. Zahri,

- A.G. Gibson, Characterisation of natural cellulosic fibre from *Pennisetum purpureum* stem as potential reinforcement of polymer composites, *Mater. Des.* 89 (2016) 839–847. doi:10.1016/j.matdes.2015.10.052.
- [33] M. Haameem, M.S. Abdul Majid, M. Afendi, H.F.A. Marzuki, E.A. Hilmi, I. Fahmi, A.G. Gibson, Effects of water absorption on Napier grass fibre/polyester composites, *Compos. Struct.* 144 (2016) 138–146. doi:10.1016/j.compstruct.2016.02.067.
- [34] A. Amara, A.M.A. Abudalazeh, A. Rashid Ismail, N. Hayati Abd Razak, S. Malik Masudi, S. Rizal Kasim, Z. Arifin Ahmad, Synthesis and characterization of porous biphasic calcium phosphate scaffold from different porogens for possible bone tissue engineering applications, *Sci. Sinter.* 43 (2011) 183–192. doi:10.2298/SOS1102183A.
- [35] F. Mustata, N. Tudorachi, D. Rosu, Thermal behavior of some organic/inorganic composites based on epoxy resin and calcium carbonate obtained from conch shell of *Rapana thomasiana*, *Compos. Part B Eng.* 43 (2012) 702–710. doi:10.1016/j.compositesb.2011.11.047.
- [36] K.B. Chien, R.N. Shah, Novel soy protein scaffolds for tissue regeneration: Material characterization and interaction with human mesenchymal stem cells, *Acta Biomater.* 8 (2012) 694–703. doi:10.1016/j.actbio.2011.09.036.
- [37] A.A. Kumar, K. Karthick, K.P. Arumugam, Properties of biodegradable polymers and degradation for sustainable development, *Int. J. Chem. Eng. Appl.* 2 (2011) 164–167.
- [38] E.J. Caterson, W.J. Li, L.J. Nesti, T. Albert, K. Danielson, R.S. Tuan, Polymer/alginate amalgam for cartilage-tissue engineering., *Ann. N. Y. Acad. Sci.* 961 (2002) 134–138.
- [39] B. Dhandayuthapani, Y. Yoshida, T. Maekawa, D.S. Kumar, Polymeric scaffolds in tissue engineering application: A review, *Int. J. Polym. Sci.* (2011) 1–19. doi:10.1155/2011/290602.
- [40] J. Ai, A. Kiasat-Dolatabadi, S. Ebrahimi-Barough, A. Ai, N. Lotfibakhshaiesh, A. Norouzi-Javidan, H. Saberi, B. Arjmand, H.R. Aghayan, Polymeric scaffolds in neural tissue engineering: A review, *Arch. Neurosci.* 1 (2014) 15–20. doi:10.5812/archneurosci.9144.
- [41] S.I.A. Razak, N.F.A. Sharif, W.A.A. Rahman, Biodegradable polymers and their bone applications : A review, *Int. J. Basic Appl. Sci.* 12 (2012) 31 – 49. <http://www.ijens.org/IJBAS Vol 12 Issue 01.html>.
- [42] H. Ehrlich, E. Steck, M. Ilan, M. Maldonado, G. Muricy, G. Bavestrello, Z. Kljajic, J.L. Carballo, S. Schiaparelli, A. Ereskovsky, P. Schupp, R. Born, H. Worch, V.V. Bazhenov, D. Kurek, V. Varlamov, D. Vyalikh, K. Kummer, V.V. Sivkov, S.L. Molodtsov, H. Meissner, G. Richter, S. Hunoldt, M. Kammer, S. Paasch, V. Krasokhin, G. Patzke, E. Brunner, W. Richter, Three-dimensional chitin-based scaffolds from *Verongida* sponges (*Demospongiae*: *Porifera*). Part II: Biomimetic potential and applications, *Int. J. Biol. Macromol.* 47 (2010) 141–145. doi:10.1016/j.ijbiomac.2010.05.009.

- [43] J. Coates, R.A.M. Ed, J. Coates, Interpretation of Infrared Spectra , A Practical Approach Interpretation of Infrared Spectra , A Practical Approach, Encycl. Anal. Chem. (2000) 10815–10837.
- [44] D. a S. Marques, S. Jarmelo, C.M.S.G. Baptista, M.H. Gil, Poly(lactic acid) synthesis in solution polymerization, Macromol. Symp. 296 (2010) 63–71. doi:10.1002/masy.201051010.
- [45] A.M. Haaparanta, E. Järvinen, I.F. Cengiz, V. Ellä, H.T. Kokkonen, I. Kiviranta, M. Kellomäki, Preparation and characterization of collagen/PLA, chitosan/PLA, and collagen/chitosan/PLA hybrid scaffolds for cartilage tissue engineering, J. Mater. Sci. Mater. Med. 25 (2014) 1129–1136. doi:10.1007/s10856-013-5129-5.
- [46] F. Wang, G. Guo, Q. Ma, M. Gu, X. Wu, S. Sheng, X. Wang, Investigation on the thermo-mechanical properties and thermal stability of polylactic acid tissue engineering scaffold material, J. Therm. Anal. Calorim. 113 (2013) 1113–1121. doi:10.1007/s10973-013-3221-1.
- [47] Z. Lei, W. Xing, J. Wu, G. Huang, X. Wang, L. Zhao, The proper glass transition temperature of amorphous polymers on dynamic mechanical spectra, J. Therm. Anal. Calorim. 116 (2014) 447–453. doi:10.1007/s10973-013-3526-0.
- [48] S.-J. Sheng, F. Wang, Q.-Y. Ma, X. Hu, Impact of foaming air on melting and crystallization behaviors of microporous PLA scaffolds, J. Therm. Anal. Calorim. 122 (2015) 1077–1088. doi:10.1007/s10973-015-4770-2.
- [49] J.C. Arboleda, M. Hughes, L.A. Lucia, J. Laine, K. Ekman, O.J. Rojas, Soy protein–nanocellulose composite aerogels, Cellulose. 20 (2013) 2417–2426. doi:10.1007/s10570-013-9993-4.