Structural and mechanical characterisation of cellulose nanofibers (CNF) from Pennisetum Purpureum reinforced with polylactic acid (PLA)

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Abstract. This study aims to develop a composite scaffold based on polylactic acid reinforced with cellulose nanofibers from Pennisetum purpureum (PLA/CNF). The composite scaffolds were prepared via solvent casting and particulate leaching technique; sodium chloride (NaCl) was used as the porogen material. The influence of CNF on PLA is investigated; scaffolds were fabricated with different content of CNF (5%, 10% and 15%). The prepared composite scaffold was characterised using porosity measurements, Fourier-transform infrared spectroscopy (FTIR) and the compression strength and modulus were also evaluated in this study. The PLA/CNF scaffolds were highly porous with porosity higher than 80%. It was also shown that the porosity had a slight decrease with increasing CNF contents due to the compact arrangement of CNF within the scaffolds. Compression strength and modulus also show an increase in value as the CNF content increases. The results also show that introducing CNF to the PLA matrix can be considered beneficial for cartilage regeneration, cell attachment, and extracellular matrix (ECM) production.

Keyword: scaffolds, cellulose nanofiber, porosity, compression

1. Introduction
Recently, cellulose nanofibers (CNF) have gained demand, especially in the field of biomedical engineering. This is because it has excellent properties such as a high surface-area-to-volume ratio, good mechanical properties, and a low thermal coefficient. Cellulose nanofibers (CNF) mimics natural cellulose traits by having properties such as low density, easy biodegradability, reproducibility, and 100% environmental friendliness [1]. Scaffolding in tissue engineering is a promising method that can replace synthetic material with natural material. Cellulose nanofibers (CNF) have sound ability reinforcement and have a larger surface area. The term' tissue engineering' was created to mimic the extracellular matrix trait (ECM), focusing on tissue regeneration to support biomaterials and growth
factor in the cells [2]. Cellulose has a significant biomedical field application and has been used in scaffold fabrication with polylactic acid (PLA) [3].

This study represents the fabrication of PLA scaffolds reinforced with cellulose nanofiber from Pennisetum purpureum using solvent casting and particulate leaching technique [4]. Based on several research backgrounds, cellulose nanofibers’ usage can increase the material’s structural and mechanical properties [5]. PLA/CNF scaffolds’ characterisations were done using Fourier-transform infrared spectroscopy, porosity measurement, and compressive test to determine the nanofibers’ structural and physical properties.

2. Materials and method
2.1. Materials
CNF were prepared from Pennisetum purpureum (better known as elephant grass) using acid hydrolysis and ball milling technique, purchased from Bukit Kayu Hitam, Malaysia. Polylactic acid (PLA) was purchased from NatureWorks LLC. An organic solvent such as chloroform and dichloromethane were purchased from Fisher Chemical Co. Sodium chloride (NaCl) with analytical grade was used as a porogen.

2.2. Preparation of cellulose nanofibers (CNF) from Pennisetum purpureum
The technique used to produce composites scaffold is solvent casting and particulate leaching. Chloroform and dichloromethane were used in order to dissolve the PLA pellet until the solution becomes viscous. The desired amount of fibres (0, 5, 10, and 15wt%) were then dispersed into the PLA solution with a magnetic stirrer for about two hours whilst the temperature is kept at 70°C. After that, NaCl was added to the solution until the solution becomes homogenous. For sample preparation, the solution was then cast into an aluminium mould with a specific dimension. The dried PLA composites were then placed into the distilled water to leach out the salt for two days. The leached scaffold was then stored at room temperature overnight. After that, the samples were stored in a desiccator before characterisation [6].

2.3. Characterisation
In this research, scaffolds with different content filler were prepared. Five compositions were sampled, with four specimens representing each composite scaffold. The average value for four specimens was obtained for the overall result of each composite scaffold. Archimedes' principle [7] were used to calculate the porosity of the composites.

FTIR spectrometer (Perkin-Elmer RX1, UK) was used to study the prepared PLA/CNF scaffolds’ FTIR spectra. The Potassium Bromide (KBr) was used to mix the PLA/CNF scaffold after the samples were grounded, where the concentration KBr of the sample was 20%. The range of 400-4000 cm⁻¹ of the spectra was collected. The FTIR spectra were recorded in transmittance mode.

Compression strength was used to determine fabricated samples' mechanical behaviour (following the standard ASTM F451-95). Load capacity was set at 1 kN while cross-head speed was 1 mm/min. Appropriates dimension of the composites scaffold 13mm (width) × 25mm (length) × 13mm (thickness) were prepared. A Vernier calliper was used to confirm the dimension of the samples. Four replicate specimens were tested to represent the composites; the average values of the various compositions were recorded, and the mean compressive strength was 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. Porosity measurement
As shown in Figure 1, all of the scaffolds were highly porous (>80%), and this satisfied the fundamental requirements defined in the literature [8]. These samples shared many characteristics regardless of the presence of CNF from P. purpureum. The porosities increased which most likely due to the porogen content of the specimens. The ratio of the porogen (NaCl) was kept constant (9:1). Nonetheless, the
level of porosity decreased from 85.9% to 80.63% as the content of *P. purpureum* increases. This is expected due to the compact arrangement of the fibre network in the scaffold. Therefore, the degree of homogeneity of the structure was expected to reduce.

![Figure 1: Porosity measurement of PLA/CNF scaffolds.](image)

### 3.2. Fourier-transform infrared spectroscopy

FTIR analysis was used to interpret or identify unknown elements' characterisation, primarily to determine the structural and functional groups of prepared scaffolds [9]. In this research, the pure PLA scaffold was also used to analyse. Among the variety of composites scaffold, it could be observed that the FTIR absorbance has a slight difference concerning the positions and shape of the absorbance peaks.

FTIR analysis was used to determine the presence of *P. purpureum* in the scaffold. FTIR spectra of pure PLA and PLA/CNF scaffolds are shown in Figure 2. The stretching vibration of the carbonyl groups of the PLA indicates the characteristic of peaks at 1757 cm\(^{-1}\). The peaks at 2985.15 cm\(^{-1}\) (-CH\(_3\) bending vibration); 1757 cm\(^{-1}\) (C=O stretching vibration); 1464 cm\(^{-1}\) (-CH\(_3\) bending vibration); 1381.06 cm\(^{-1}\) (-CH bending vibration); 1190.08 cm\(^{-1}\) and 1381.06 cm\(^{-1}\) (C-O stretching vibration) evidence exists of PLA within *P. purpureum*-PLA scaffold. The peak at 2909 cm\(^{-1}\) and 939.07 cm\(^{-1}\) is a typical band that is owed to *P. purpureum*.

As to compare the result of pure PLA and PLA/CNF scaffolds, two noticeable changes occur towards the scaffold. The presence of –CH\(_3\) was not detected in the pure PLA but can be detected on the blended composites scaffold. Besides, at peaks, 1757 cm\(^{-1}\) indicates the intensity of the stretching band attributes to the ester group where it becomes weaker as the content of CNF content of the PLA matrix blend increase. This difference indicates the interaction between the ester and the carboxyl group of composites. This means that as the CNF content of the PLA matrix increases, the degree of crystallinity of the material increases. According to the findings for PLA/CNF composites, certain characteristic bands' intensity decreased as the filler content increased, indicating that cross-linking occurs between the CNF and PLA of the composites scaffold.

### 3.3. Compressive test

A compression test is the most critical aspect in order to determine the mechanical behaviour of composites scaffold. The scaffold's ability under the load-bearing compression depends on their characteristics of natural fibre itself [10]. Cellulose nanofiber has a very tangle fibre bonding that could exhibit strong adhesion due to the interconnection of CNF in the scaffold [11]. However, the presence of filler will enhance the matrix compared to the PLA alone.
Figure 2: FTIR spectrums of PLA/CNF scaffolds.

Figure 3: Compressive strength

The addition of CNF consequently increases the compressive strength, as shown in Figure 3. The scaffold with PLA alone had the lowest compressive strength, and PLA/CNF-PP15 had the greatest. There are significant increases to the samples PLA/CNF-PP5 and PLA/CNF-PP10 compared to PLA alone. However, when the P. purpureum content increases to 15%, a further increase of the compressive strength is 5.12 MPa to 9.79 MPa. This indicates high adhesion interaction when the CNF with 15% and PLA matrix blend is used in this study. This is due to the linkage and interconnectivity of the fibre which give a good properties towards the composites scaffold.
The compressive modulus was also investigated to the different content filler (5, 10, and 15wt %), as shown in Figure 4. Due to the reinforcement, the highest compression modulus achieved was 9.03 MPa. This is due to the linkage and interconnectivity of the fibre. The pure PLA has a compressive modulus of 1.15 MPa, which increase to 9.03 MPa at a sample of PLA/CNF-PP15. The higher content filler adequate, the higher mechanical strength where the characteristic of PLA is brittle [12]. In this study, the sample PLA/CNF-PP15 had shown a promising characteristic that can be potentially used in a wide variety of application, especially in medical application.

4. Conclusion
This study's main objective is to fabricate the PLA/CNF scaffolds with different filler contents using solvent casting particulate leaching. The porosity measurement of the scaffold was highly porous (>80%). FTIR analysis indicates that as the content filler of P. purpureum increase, the crystallinity of material will also increase as the intensity of certain characteristic bands decreased as the filler content increased, indicating that cross-linking occurs between the P. purpureum and PLA of the composites scaffold. The compression test consists of two types which are compressive modulus and compressive strength, where the results favour the scaffold with a content filler of 15%. The PLA is brittle, and with the reinforcement of CNF, the scaffolds' strength was enhanced.

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References
[1] Dzobo, K., Thomford, N. E., Senthabelle, D. A., Shipanga, H., Rowe, A., Dandara, C., … Motaung, K. S. C. M. (2018). Advances in Regenerative Medicine and Tissue Engineering: Innovation and Transformation of Medicine. Stem Cells International, 2018, 1–24.
[2] R. Radakisinin, M. S. A. Majid, M. R. M. Jamir, M. Jawaid, M. T. H. Sultan, and M. F. M. Tahir, "Structural, morphological and thermal properties of cellulose nanofibers from Napier fibre (Pennisetum purpureum)," Materials (Basel), 2020.
[3] Zhang Y, Nypelö T, Salas C, Arboleda J, Hoeger I C and Rojas O J 2013 Cellulose nanofibrils J Renew Mater 1 195–211.
[4] M.S. Fartini, M.S. Abdul Majid*, M.J.M. Ridzuan • N.A.M. Amin, Geoff Gibson, Compressive properties of Napier (Pennisetum Purpureum) filled polyester composites. Plastics, Rubber and
Composites: Macromolecular Engineering, Plast. Rubber and Composites, vol. 45, no. 3, pp. 136-146, April. 2016.

[5] Xie H, Du H, Yang X and Si C 2018 Recent strategies in preparation of cellulose nanocrystals and cellulose nanofibrils derived from raw cellulose materials Int J Polym Sci 2018 1–25.

[6] Revati, R., Abdul Majid, M. S., Ridzuan, M. J. M., Normahira, M., Mohd Nasir, N. F., Rahman Y., M. N., & Gibson, A. G. (2017). Mechanical, thermal and morphological characterisation of 3D porous Pennisetum purpureum/ PLA biocomposites scaffold. Materials Science and Engineering: C, 75, 752–759.

[7] A. Amera, A.M.A. Abudalazez, A. Rashid Ismail, N. Hayati Abd Razak, S. Malik Masudi, S. Rizal Kasim, Z. Arifin Ahmad, Synthesis and characterisation of porous biphasic calcium phosphate scaffold from different porogens for possible bone tissue engineering applications, Sci. Sinter. 43 (2011) 183–19210.2298/SOS1102183A.

[8] 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.

[9] 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. 10815–10837 (2000).

[10] 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.

[11] M. E. Hassan, J. Bai, and D. Q. Dou, "Biopolymers; Definition, classification and applications," Egyptian Journal of Chemistry. 2019.

[12] Revati R., M.S. Abdul Majid*, Ridzuan M.J.M.; K.S. Basaruddin, M.N. Rahman Y., Cheng E.M., A.G. Gibson, In vitro degradation of a 3D porous Pennisetum purpureum/PLA biocomposite scaffold, Journal of the Mechanical Behavior of Biomedical Materials, Volume 74, October 2017, Pages 383-391.