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RESEARCH ARTICLE

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Characterization and *in-vitro* analysis of poly (ε-caprolactone)-"Jackfruit" Mucilage blends for tissue engineering applications

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Abstract

Jackfruit mucilage (JM) obtained from the fruit, *Artocarpus heterophyllus* was melt blended with poly(ε-caprolactone) (PCL). The physical properties of the blends with more than 60 wt% PCL were investigated. Depression in the equilibrium melting temperature (T_m^o) and the presence of extinction rings in the spherulites of PCL in the blends confirmed miscibility of the two components. The carbonyl and the C—O—C groups of PCL and JM were responsible for the interactions as identified by Fourier transform infrared spectroscopy. The thermal stability of PCL decreased marginally in the blends with increasing JM content. The scanning electron microscopy (SEM) images indicated that no phase separation occurred. Porosity and the mechanical strength of the blends decreased with increasing JM content. Blends exposed to porcine pancreatic lipase showed enzymatic degradation of JM but not PCL and SEM images showed holes in the sample indicative of selective degradation of JM. Cell growth inhibition technique using the L929 fibroblasts cells showed the degree of inhibition was dependent on the increasing JM content in the blends. Blends with low JM content have the potential to be used for increased cell viability while blends with high JM content can be used for the treatment of cancer.

KEYWORDS

blends, cell growth inhibition, enzymatic degradation, jackfruit mucilage, poly(ε-caprolactone)

1 | INTRODUCTION

Polymer blending using natural and synthetic polymers is an effective and economical route to develop new biomaterials with tailored end-use properties that cannot be obtained by individual polymers. A lot of interest is being generated in the use of biodegradable polymers, particularly in drug delivery and tissue engineering. Natural polymers have an added advantage over synthetic polymers because they possesses properties similar to human tissues as they have highly organized structure at both molecular and microscopic level, which if infused into biomaterials can result in favorable performance, such as the ability to induce tissue growth. However, natural polymers' low mechanical resistance, thermal and chemical stability, and difficulty to be produced on industrial scale limits its use on its own.[1-5] In comparison, synthetic polymers, such as poly(ε-caprolactone) (PCL), poly(butylene succinate) (PBS), poly(lactic acid), poly(anhydrides), and poly(hydroxyl alkonates) are more commonly used in the tissue engineering field due to their easily tailorable mechanical properties and degradation rates.[6,7] These

two groups of biodegradable polymers complement each other in their inherent properties, thus combining the two gives an opportunity to take benefits of each group's desired properties.

PCL is a synthetic biocompatible polyester with low melting point (60°C) and glass transition temperature (−60°C), which allows easy processing and gives the polymer a rubbery behavior at room temperature and as a consequence, a good permeability to low molecular weight drugs in drug delivery systems for biomedical use.[8] However, the degradation kinetics of PCL is slow due to its hydrophobic and semi-crystalline nature, which makes its resorption time longer than 2 years.[8]

PCL has been exhaustively studied in combination with a number of synthetic and natural polymers to explore its physical, mechanical, and biological properties.[8-10] Studies involving PCL and chitosan blends dominate the tissue engineering applications. Recently, PCL had been blended with pine resin, a plant extract to enhance the antibacterial property of PCL.[11] However, there are many underutilized natural compounds such as mucilage, a mucoadhesive material, from the fruit, *Artocarpus heterophyllos* that could be blended with synthetic polymers and used as a potential agent in tissue engineering.

Mucoadhesive materials are mostly used in the development of biomaterials for drug delivery systems and tissue engineering applications.[12] These materials are mainly water-insoluble but are capable of forming numerous hydrogen bonds through the carboxyl, sulfate, hydroxyl, and amino functional groups. Thus, the stronger the hydrogen bonding, the stronger the cell adhesion will be, which is one of the vital characteristic studied in the tissue engineering applications.[13] Mucilage, from the fruit, *A. heterophyllos*, commonly known as jackfruit, formed and retained within the cell wall of plants, is a white viscous liquid and is very tacky. Jackfruit mucilage consists mainly polysaccharide but also contains lignans, isoflavones, and saponins, which are termed as phytonutrients. Their health benefits are wide ranging from anticancer to antihypertensive, antiaging, antioxidant, and anti-ulcer.[14, 15] The potential use of jackfruit mucilage has been explored in pharmaceutical applications as tablet binders, excipients, and in drug delivery as it has a good swelling index of 12.66 and a good water uptake capacity.[9, 16] Even though there are studies that have reported the use of plant-based gums blended with PCL[11, 17-21] as a potential to be used as biomedical applications, from our literature survey it was found that there is no reported work on blends of jackfruit mucilage with polymers targeted toward tissue engineering. As PCL has been used extensively in tissue engineering, an opportunity arises by blending jackfruit mucilage with PCL to improve the properties of PCL for tissue engineering and other biomedical applications. To further explore the use of such blend system in tissue engineering applications, it is important to understand the miscibility and mechanical properties.

The purpose of this research was to prepare blends of jackfruit mucilage and PCL as a potential agent in tissue engineering by investigating the miscibility, mechanical property, and the morphology. Enzymatic degradation of the blends was studied and the percentage weight loss monitored for a period of time. Furthermore, the biocompatibility of the blends was assessed through cell viability assays using L929 fibroblast cells to explore the potential for tissue engineering applications. The vision of this study is to incorporate underutilized natural compounds such as mucilage with PCL as a potential biomaterial developed for tissue engineering applications.

2 | EXPERIMENTAL

2.1 | Materials

PCL (Mw 80,000) was purchased from Sigma–Aldrich and used without further purification. The jackfruit mucilage (JM) extracted from the inner part of the fruit, *A. heterophyllos* was dissolved in dichloromethane and filtered to remove solid pieces of the fruit. The solvent was evaporated from the filtrate at room temperature in a fume cupboard and freeze-dried before use.

2.2 | Blend preparation-melt blending

PCL was melt blended with JM in a reaction holder connected to a temperature controller (Graseby Specac), which controlled the temperature precisely to ±0.5°C. The melt blends (wt%) were prepared in the composition range (PCL:JM) 80/20, 70/30, and 60/40 where the first value represents the PCL content, at 50 rpm for 20 min at 80°C. Blends with JM content >50% were very tacky so it was difficult for characterization. The neat samples (PCL and JM) were used as control. The blends were prepared as solid scaffolds using metal molds of dimensions of: 10 mm×10 mm×1 mm.

2.3 | Thermogravimetric analysis

Thermal degradation of JM, PCL, and the blends was measured using a Perkin-Elmer TGA 6 Thermogravimetric Analyzer. A total of 2 mg of the sample was

heated at a rate of 2°C min^{-1} from 25 to 500°C under a nitrogen atmosphere.

2.4 | Differential scanning calorimetry

Thermal analysis of JM, PCL, and the blends was carried out using an indium calibrated Perkin-Elmer DSC-6. Sample mass of ~10 mg was sealed in an aluminum pan and subjected to different thermal treatment. For isothermal annealing, the samples were heated to 80 °C at a heating rate of 10°C min^{-1} and kept for 2 min to remove thermal history. The melted samples were quench cooled to predetermined crystallization temperatures (T_c = 30, 35, 40, and 45°C) at a cooling rate of 40°C min^{-1} and isothermally crystallized for 24 h. The samples were then heated to 80°C at a rate of 10°C min^{-1} under nitrogen gas atmosphere at a flow rate of 20 ml/min. Melting temperature (T_m) was obtained at the maximum of the endothermic peak.

2.5 | Fourier transform infrared spectroscopy

Infrared spectra of JM, PCL, and the blends were obtained using a Fourier transform infrared spectroscopy (FTIR) (Perkin Elmer 1000 series Spectrophotometer) from 4000 to 400 cm^{-1} at a resolution of 2 cm^{-1}. Sixty-four scans were averaged and normalized. Spectrum manipulation was performed using the instrument's software. The difference spectrum was obtained by subtracting the spectrum of the mucilage from the blend spectrum and minimizing the peak at 2919 cm^{-1} to zero, to compensate for the difference in the sample thickness, to obtain the spectrum of the blended PCL.

2.6 | Polarized optical microscopy

The spherulite morphology of neat and blended PCL was examined under polarized optical microscopy (POM) equipped with a hot stage, cross polarizers, tint plate, and a Nikon camera. The samples were heated to 80°C on a microscope slide for 2 min to remove thermal history and then sandwiched between a cover glass to obtain a thin film. The slides containing the samples were quickly transferred to a hot stage preset at the following crystallization temperatures of 30, 35, 40, and 45°C for isothermal crystallization, which lies between the crystallization and melting temperatures of PCL. Isothermal crystallization was done for 24 h at the preset temperatures. Micrographs were taken using a Nikon camera.

2.7 | Mechanical property

The tensile test was carried out in accordance with standards defined by International Standard IS0 527, for the determination of tensile properties of plastics (equivalent to ASTM D638),[22] using the EZ Texture Analyzer. The speed of the moving clamp was 5 mm min^{-1} and a static preload of 80 N was applied during the tests to keep the samples tight. The dumb-bell shaped specimens with the dimensions of 30 mm×10 mm and the thickness of 1.5 mm were tested for each blend. Five specimens were tested for each sample and the average values are reported. The stress and strain curves were plotted.

Stress was calculated using the following equation:

$$\text{Stress} = \frac{F}{A} \tag{1}$$

where stress is measured in Nm^{-2} or pascals (Pa), F is the force in Newtons (N) and A represents the cross-sectional area in m^2.

Strain was obtained using the following equation:

$$\text{Strain}\,(\%) = \frac{\Delta L}{L} \times 100 \tag{2}$$

where ΔL is the extension measured in mm and L is the original length measured in mm.

2.8 | Scanning electron microscopy

The surface and internal morphology of the blends were characterized using scanning electron microscopy (SEM) of the non-degraded and degraded samples. SEM tests were performed using the Nova Nano SEM 230. For internal morphology, the samples were broken in liquid nitrogen.

2.9 | Porosity test

The porsoity of the JM, PCL, and the blends was determined by the weighing method[23] according to the following equation:

$$\text{Porosity} = \frac{(W_2 - W_3 - W_s)}{(W_1 - W_3)} \tag{3}$$

Each sample was dry weighed (W_s) and then placed into a pycnometer filled with distilled deionized water. The combined weight of the pycnometer and water was taken as (W_1). The pycnometer was then placed into a vacuum oven to remove the air out of the sample. After

the vacuum process, the water level in the pycnometer decreased, therefore, the water was filled up again in the pycnometer and the entire weight was taken (W_2). Finally, the sample was taken out and the surface water was dripped back into the pycnometer to obtain the weight of the remaining water and the pycnometer (W_3).

2.10 | Enzymatic degradation studies

Enzymatic degradation was carried out on the blends using porcine pancreatic lipase using the enzymatic degradation method.[24] The method was slightly modified by changing the blends in the form of scaffolds with 10 mm x 10 mm × 1 mm dimensions, prepared by placing the samples in the mold of the above dimensions and hot-pressed under 50 kg cm^2 pressure at 80°C. The scaffolds were placed in small capped bottles containing 1 mL of phosphate buffer solution (PBS) (0.05 mol L^{-1}, pH = 7.2–7.4) with 1 mg lipase of concentration 1 mg ml^{-1}. Sodium azide was added as a bacteriostatic agent. The bottles were incubated at 37°C for 20 days while the buffer solution was changed daily. After a specified period of incubation, the scaffolds were removed from the flask, washed with sterilized water, and dried in vacuum oven at 25°C until constant weight. JM was immersed in 1 ml of buffer solution without any enzyme to act as control. The degree of degradation was calculated using the following equation:

$$\text{Weight loss}\% = \frac{m_i - m_f}{m_i} \times 100 \tag{4}$$

where m_i and m_f are the initial and final weights of the sample before and after degradation, respectively.

2.11 | Cell growth inhibition assay

L929 Cell Growth Inhibition (CGI) assay was carried out on PCL and the blends using the cell growth inhibition method.[25] Extraction of the scaffolds prepared through melt blending was carried out using the media (Eagle's minimum essential media [EMEM], Sigma-Aldrich, Australia), at 37°C for 24 h. The aqueous test samples were sterilized by passage through a 0.22 micrometer filter. Control solutions included in the study were silicone as the positive control, 4, 5, and 7.5% ethanol as negative control, and a null control in which only fresh media was applied (extraction media control). A fibroblast monolayer (L929 mouse fibroblasts, ATCC CCL-1)) was prepared by seeding 35 mm tissue culture plates with 5 x 104 cell/ml in complete media (EMEM) with 10 vol% fetal bovine serum (FBS, JRH, Biosciences, Sigma-Aldrich, Australia) supplemented with 1% penicillin/streptomycin (P/S, CSL Biosciences, Edwardstown, SA, Australia) and was incubated for 24 h at 37°C and 5% CO_2. Plates were washed with sterile PBS and test or control solutions were applied. After the 48 h exposure period, the cells were washed with Dulbecco's PBS, trypsinized and the number of viable cells in the test samples were analyzed by Vi-Cell counter (Beckman Coulter, Australasian analytical Systems, QLD, Australia). The number of cells in each test sample was compared with the control plate. The cell growth inhibition was calculated using the following equation:

$$\text{Percentage of cell growth inhibition} = \left(\frac{\text{test solution average} - 1}{\text{null average}}\right) \times -100 \tag{5}$$

Three independent samples of each aqueous test solutions were tested and each sample was run in triplicate.

2.12 | Statistical analysis

Statistical analysis was performed on the data from the CGI assay using analysis of variance (ANOVA) to determine whether there was significant difference ($p < 0.05$) in the cell growth of fibroblast monolayer (L929 mouse fibroblasts, ATCC CCL-1)) when exposed to blends containing different percentage of JM.

3 | RESULTS AND DISCUSSION

3.1 | Thermogravimetric analysis

Figure 1 shows the thermal degradation of PCL, JM, and the blends analyzed by measuring the weight loss with increasing temperature using thermogravimetric method. PCL showed high thermal stability. The curve displayed a sharp decrease in weight at around 400°C. However, the first derivative of the thermogravimetric analysis (DTGA) showed two peaks indicating thermal degradation happened via two-stage mechanism and is consistent with reported results.[24] The first step is due to the statistical rupture of the polyester chains via ester pyrolysis reaction and verified using MS and FTIR. The second step is the formation of ε-caprolactone (cyclic monomer) as result of an unzipping depolymerization process.[26] On the other hand, JM showed low thermal stability. The thermogravimetric analysis (TGA) curve showed a decrease starting around 100°C and gradually decreasing up to 250°C after which a sharp decrease was observed. The

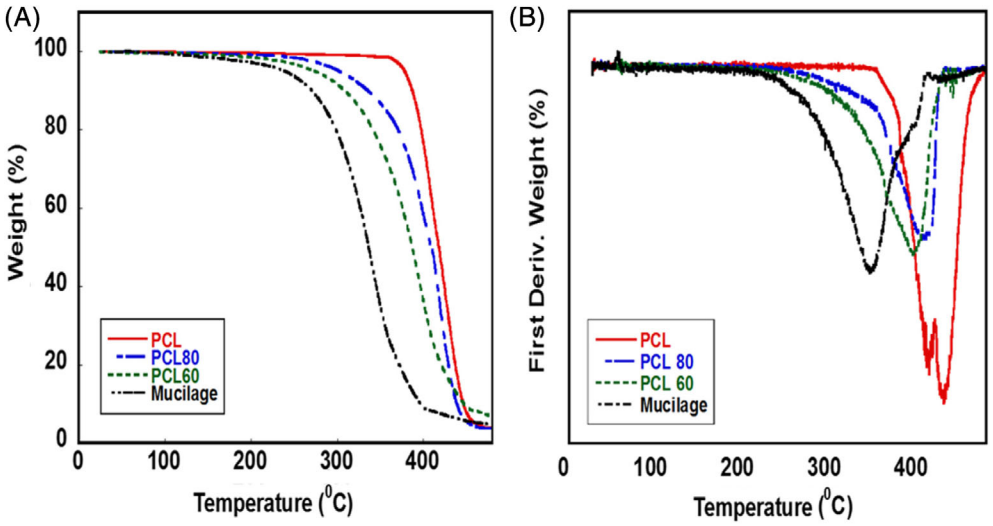

FIGURE 1 TGA thermogram of PCL, JM, PCL 80, PCL 60 (A); the first derivative curves (B). JM, jackfruit mucilage; PCL, poly(ε-caprolactone); TGA, thermogravimetric analysis [Color figure can be viewed at wileyonlinelibrary.com]

DTGA curve showed one peak suggesting a one-stage thermal degradation mechanism.

In the blends, the TGA curves were intermediate between PCL and JM. The initial degradation temperature in the blends increased with increasing PCL content when compared to JM. The decrease in mass in the blends started at ~280 and 250°C for PCL80 and PCL60, respectively. The DTGA curves of the blends showed one peak suggesting a one-step degradation mechanism. These blends showed degradation temperatures suitable for tissue engineering purposes.

3.2 | Differential scanning calorimetry

The normalized differential scanning calorimetry (DSC) heating profiles of isothermally crystallized PCL and the blends at 45°C are shown in Figure 2(A). The melting temperature (T_m) and the enthalpy of the endothermic peak were found to decrease in blends with increasing JM content. The glass transition temperature (T_g) of PCL was found to be −62°C similar to the reported value[27] while no T_g was detected for the JM. The miscibility of this blend system was determined by observing the depression in the T_m of PCL in the isothermally crystallized blend samples and the decrease in the degree of crystallinity rather than the T_g method. The T_m of PCL was found to decrease in the blends with increasing JM content and decreasing crystallization temperatures (T_c).

Depression in the T_m of the crystalline component in the crystalline/amorphous blends is an indication of miscibility. However, depression in T_m can be due to both thermodynamic and morphological[28] reasons. To eliminate morphological effects, depression in the equilibrium melting temperature (T_m^o) is considered the accurate indication of miscibility. T_m^o is generally determined by the

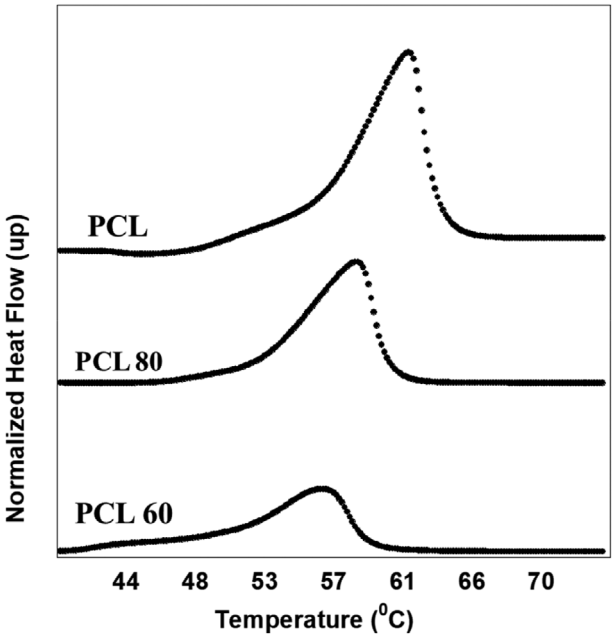

FIGURE 2 Normalized DSC thermogram showing the depression in melting temperature (T_m) of PCL and the blends isothermally crystallized at 45°C. DSC, differential scanning calorimetry; PCL, poly(ε-caprolactone)

Hoffmann and Weeks extrapolation plot where the T_m of the crystals grown at different T_c's are plotted. The plotted T_m's are extrapolated linearly to meet the line $T_m = T_c$. The point of intersection is the T_m^o according to the following equation:

$$T_m = \frac{T_c}{\beta} + T_m^o\left[1 - \frac{1}{\beta}\right] \quad (6)$$

where β is the crystal thickening factor, which is assumed to be constant between the different samples crystallized at different T_c's.

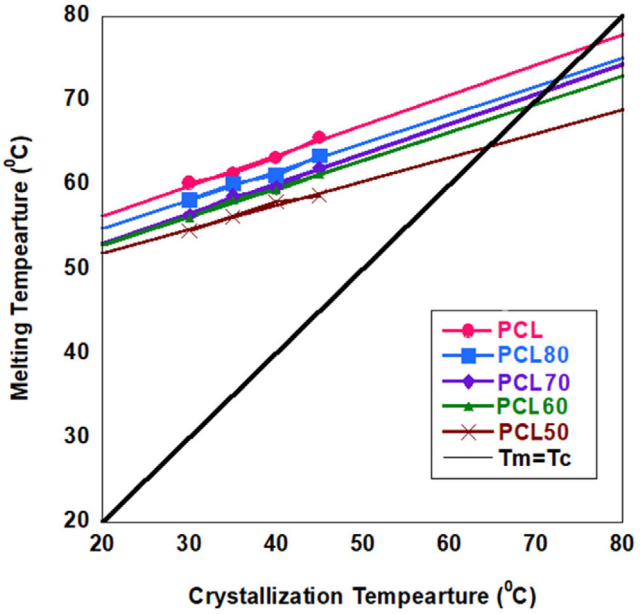

FIGURE 3 Linear Hoffmann and weeks plot showing the T_m^o of poly(ε-caprolactone) (PCL) and PCL in the blends [Color figure can be viewed at wileyonlinelibrary.com]

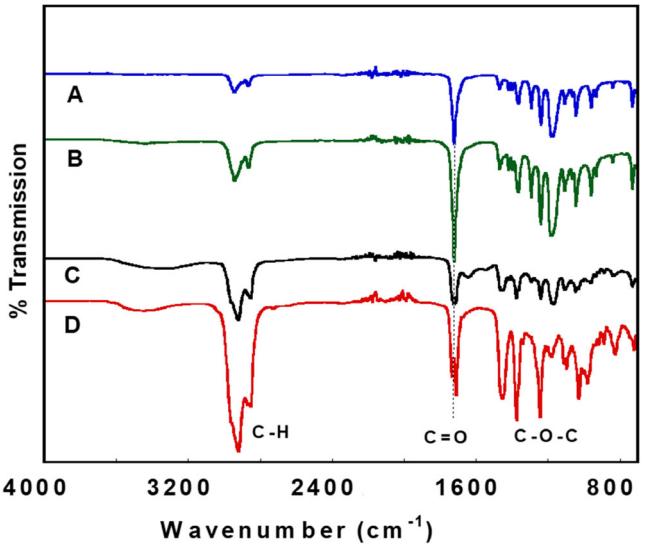

FIGURE 4 Fourier transform infrared spectroscopy (FTIR) spectra of poly(ε-caprolactone) (PCL) (A), PCL 80 (B), PCL 60 (C), and jackfruit mucilage (JM) (D) [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Equilibrium melting temperatures (T_m^o) and percentage crystallinity for PCL and the blends

Sample	T_m^o (°C)	Crystallinity (Xc%)
PCL	76.6	56.3
PCL 80	72.5	42.6
PCL 70	71.1	36.6
PCL 60	69.4	30.6
PCL 50	67.5	24.8

Abbreviation: PCL, poly(ε-caprolactone).

Figure 3 shows the linear Hoffmann and Weeks plot of PCL and the blends. The T_m^o of PCL was estimated to be 76.6°C, comparable to the reported value of 77°C.[29] However, this value is higher than some reported values of 72°C.[30] The difference can be attributed to the difference in the molecular weight of PCL. The T_m^o for PCL and the different blends are given in Table 1. The T_m^o was found to decrease with increasing JM content in the blend indicating miscibility between the two components.

Percentage crystallinity (Xc %) of PCL in the blends was determined using the following equation:

$$\text{Xc}\,(\%) = \frac{\Delta H_m}{H_X^o} \times \text{weight fraction of PCL} \times 100\% \qquad (7)$$

where ΔH_m is the experimental melting enthalpy of the blends determined from the area of the endothermic peak in the DSC heating scan and ΔH_X^o is the enthalpy of fusion for the hypothetical perfect crystal of PCL with the value of 136 J/g.[31, 32] Percentage crystallinity and the T_m^o are given in Table 1.

The percentage crystallinity of PCL was calculated to be 56.3%. The blends showed a decrease in crystallinity as the JM content was increased and is attributed to the dilution effect of the JM on the crystallization of PCL.

3.3 | Fourier transform infrared spectroscopy

To identify which group(s) were involved in the interactions between the two components, infrared analysis was performed. The interactions between the two components of the blends were determined by observing band shifts in the FTIR difference spectrum. Figure 4 shows the FTIR spectra of PCL, JM, and the blends. For PCL, the band at 1721 cm^{-1} is attributed to the stretching of the C═O, the band at 1175 cm^{-1} is due to the vibration of the C—O—C group.

The JM spectrum exhibited bands analogous to PCL indicating the presence of compounds having similar functional groups in the JM. A few FTIR studies conducted on JM has classified it to contain polysaccharide.[33] The band at 3346 cm^{-1} is due to the O—H stretching, 1710 cm^{-1} is due to the stretching of the C═O and 1179–1027 cm^{-1} are attributed to the vibrations of the C—O—C groups.

Due to the overlap of the IR bands from both the components, it was difficult to identify from the blend

spectrum, which group(s) were involved in the interactions. The difference technique was used to determine changes in the blended component. The difference spectrum was obtained by subtracting the spectrum of the JM from the blend spectrum and minimizing the band at 2919 cm^{-1} to zero to adjust for the difference in the thickness of the samples.

This band was unique to the JM. The difference spectrum obtained is that of the blended PCL. Bands that showed aberrations when compared to neat PCL spectrum were considered to be involved in the interactions. The C═O and the C—O—C bands were found to be involved in the interactions.

3.4 | Polarized optical microscopy

The spherulitic morphology of PCL and the blends annealed at different temperatures (30, 35, 40, 45°C) were obtained and representatives at 45°C are given in Figure 5. The micrograph of the JM is not shown as it was amorphous. The spherulite size of PCL in the neat and in the blend samples increased with increasing T_c. PCL spherulite showed negative birefringence without extinction rings (banding) when crystallized at the different temperatures. However, in the blends extinction rings were observed in the PCL spherulites. The extinction rings became regular and well defined with increasing JM content. It has been reported that the emergence of extinction rings in crystalline/amorphous blends is an indication of miscibility.[29, 34] With decreasing concentration of JM in the blends, the number of spherulites per unit area increased suggesting that JM had a nucleating effect.

According to Keith and Padden,[34] the extinction rings are the result of lamellar twisting that occurs to release the stress that is exerted on the lateral surface of the lamellar due to the anomalies present in the amorphous fraction between the interlamellar regions. When the distance between the lamellar twist is large, the banding pattern becomes well defined. For this blend system, the JM resided in the amorphous fraction of the interlamellar region of PCL resulting in thinner lamellar (depression in T_m). With increasing JM content, the force exerted on the PCL lamellar amplified, forcing the lamellar to twist to release the energy. Thus, the residence of the JM in the amorphous fraction in the interlamellar region confirms the blends to be miscible.

3.5 | Mechanical properties

The tensile properties of PCL and the blends are given in Figure 6. PCL shows a tensile strength of ~19 MPa, close

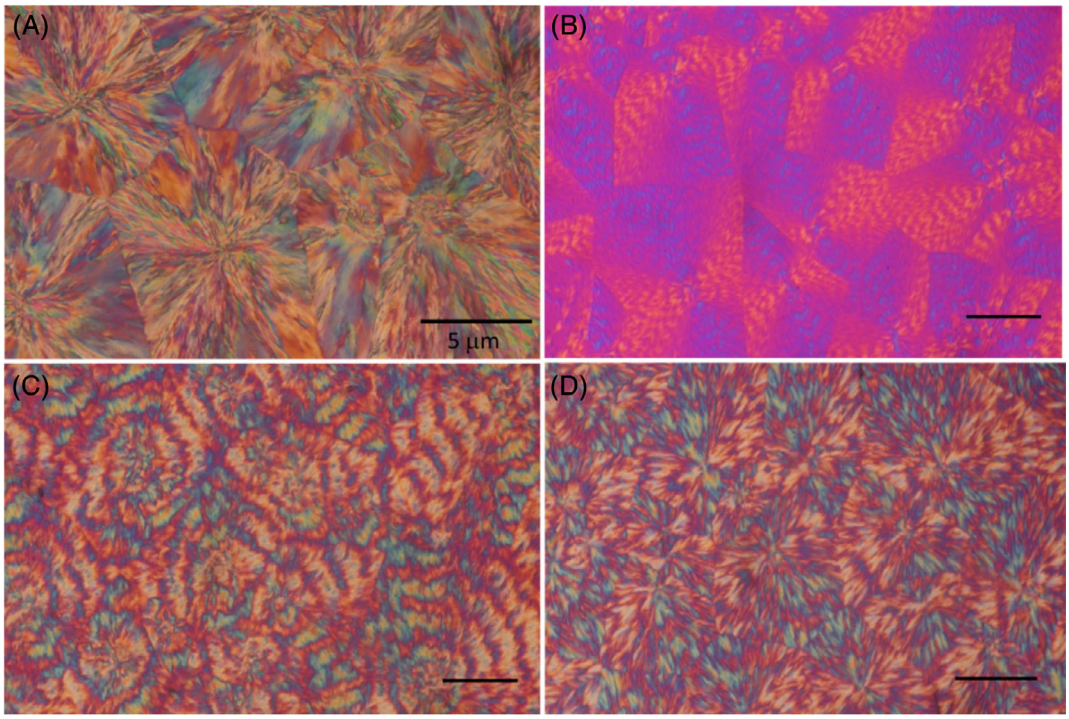

FIGURE 5 Polarized optical microscopy (POM) of pure poly(ε-caprolactone) (PCL) and its blends isothermally crystallized at 45°C. neat PCL (a), PCL 80 (B), PCL 60 (C), and PCL 50 (D) [Color figure can be viewed at wileyonlinelibrary.com]

to reported value.[35] The stress–strain curves showed that as JM content increased in the blend system, the flexibility of PCL decreased.

The tensile strength, strain, brittleness, and the toughness of the prepared blends decreased with increasing content of the mucilage. The tensile strength, strain, elastic modulus, and elongation at break are given in Table 2.

With increasing JM content the tensile strength of the blends decreased whereas the strength of PCL is notably higher than the blends. The elasticity of the blends (Elastic modulus), strain-hardening effect, and extended elongation also decreased with increasing JM content. With the addition of JM to PCL in increment of 20% of the blends, the strength and the strain decreased ca. two and three times, respectively. The blends became soft and this is due to JM acting as a diluent by disturbing the orderly nature of the PCL chains that allowed less flexibility and ductility in the blends.

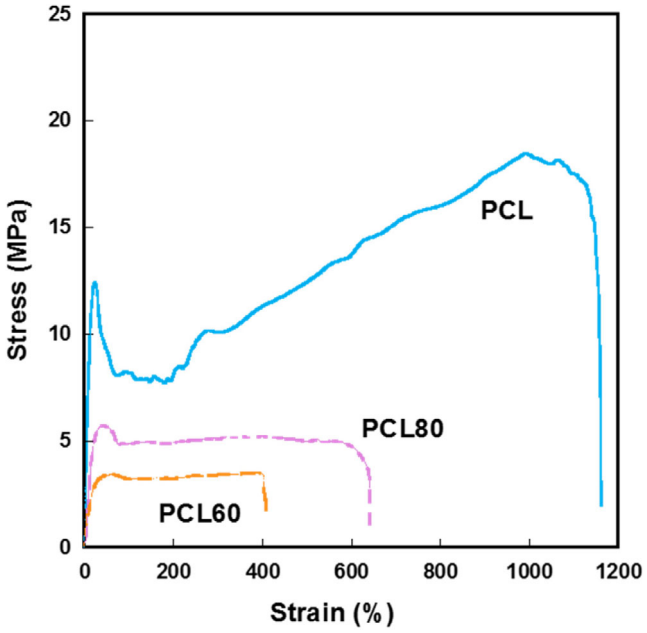

FIGURE 6 Stress–strain curves of poly(ε-caprolactone) (PCL) and the blends [Color figure can be viewed at wileyonlinelibrary.com]

3.6 | Enzymatic degradation studies

The percentage weight loss of the samples after enzymatic degradation in porcine pancreatic lipase over a period of 20 days is given in Figure 7. PCL did not show any degradation in this enzyme and is in agreement with reported results[36, 37] that suggested that porcine pancreatic lipase shows no degradation against PCL. This is because PCL is a hydrophobic polymer and would hinder water intrusions and thus limiting degradation by hydrolysis[38] and also due to the high crystallinity, degradation rate decreases.

On the other hand, JM showed the maximum degradation. The mass loss occurred gradually over the 20 day period because JM contains polysaccharides, proteins, and lipids and is susceptible to degradation easily. Enzymatic degradation caused by hydrolysis of ester bonds and the chemical scission of macromolecular chains breaking them into small fragments or oligomers.[39]

Blends, PCL 80 and PCL 60 showed an increasing degradation pattern with increase in JM mass content. This increase in the degradation is due to the water swelling ability of the JM and the reduced crystallinity in the blends. The blends tend to absorb more enzyme as JM content increased. Water and enzyme penetration is very difficult in a highly crystalline polymer.

3.7 | Scanning electron microscopy

Figure 8 shows the SEM micrographs of PCL, JM, and the blends. PCL appeared to be smooth while JM showed a rough and irregular appearance. The morphology of PCL 80 and PCL 60 blends showed no phase separation, however, small pores appeared.

Figure 9 shows the SEM images of the enzyme degraded samples. PCL did not show any change in the morphology before and after degradation and is in agreement with the mass loss results. Blends, PCL 80 and PCL 60 showed patches of degraded sections, which increased with increasing JM content in the blends indicating JM, had degraded.

The porosity of JM was calculated to be 92% while PCL was 64%, close to the reported values in the range of

TABLE 2 Mechanical properties of PCL and PCL:JM blends

Sample	Elastic modulus (MPa)	Tensile strength (N)	Strain at break (%)
PCL	4.70	33.80	998
PCL 80	2.79	26.83	602
PCL 60	0.04	12.47	397

Abbreviation: PCL, poly(ε-caprolactone).

64 to 74%.[40] The high porosity in JM is due to the hydrophilic nature of the compounds present. In the blends, PCL80 and PCL60, the porosity was calculated to be 30 and 34%, respectively, indicating the increase in hydrophilicity.

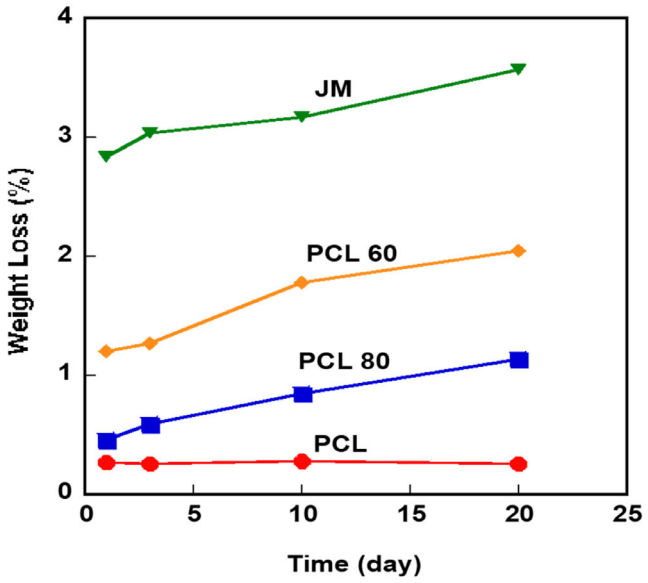

FIGURE 7 Weight loss profiles of neat poly(ε-caprolactone) (PCL), jackfruit mucilage (JM), and the blends in buffer solution containing pancreatic porcine lipase at 37°C at various times [Color figure can be viewed at wileyonlinelibrary.com]

3.8 | CGI assay

Figure 10 shows the percentage of L929 cell growth inhibition and cell viability with PCL, JM, the two blends, and the controls. It was observed that the cell growth inhibition for neat PCL and neat JM was very similar to silicone, which was the positive control. The cell viability of the neat samples showed a higher percentage indicating a higher number of cells survived. PCL has been reported to have zero toxicity toward L929 fibroblast cell[41] and our results are in good agreement. The L929 cells showed evidence of good adhesion, proliferation rate, and mitochondrial activity on the PCL films.[42, 43] Similarly, a low cell growth inhibition was also observed for JM indicating the potential it possess to support cell growth. Although there are no previous reports on L929 cells and JM, some work has been reported[18] on JM and NIH3T3 cell lines. It was found the cell inhibitory results were similar to our work and JM was reported to be nontoxic. JM has been reported to contain polysaccharides, lignins, and isoflavones in different amounts, which possess properties, such as anti-cancer, anti-oxidant, and anti-ulcer.[14-15] Polysaccharides provide a positive

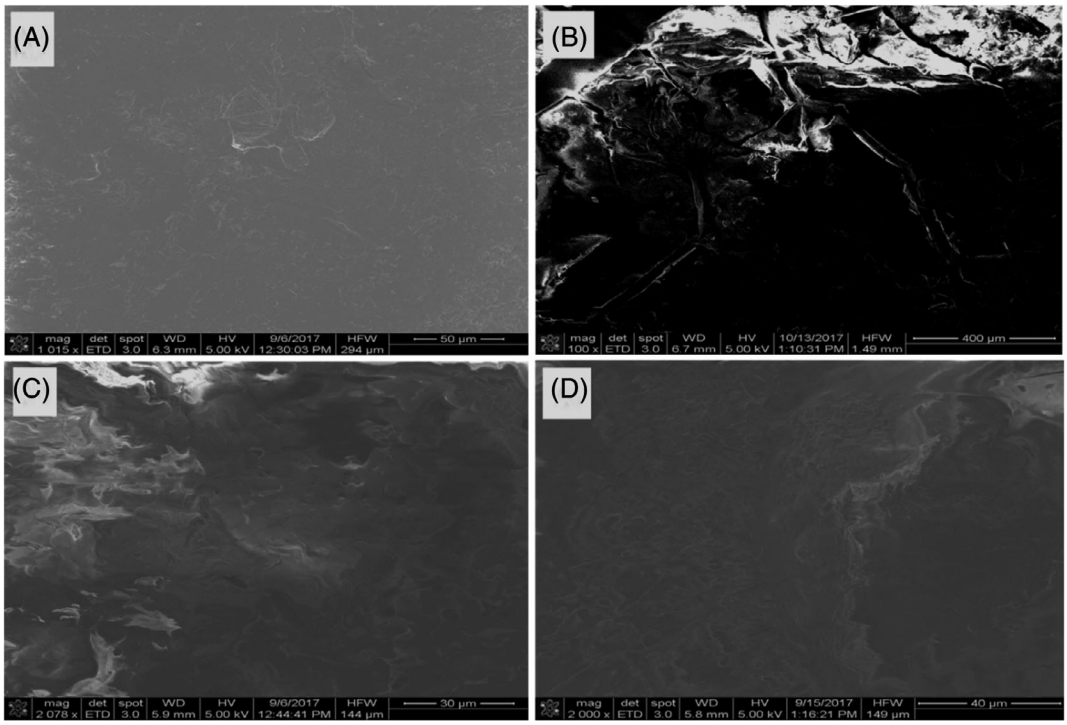

FIGURE 8 Scanning electron microscopy (SEM) images showing external surface before degradation. Neat poly(ε-caprolactone) (PCL) (A), jackfruit mucilage (JM) (100×) (B), PCL 80 (C), and PCL 60 (D), at ×2000 magnification

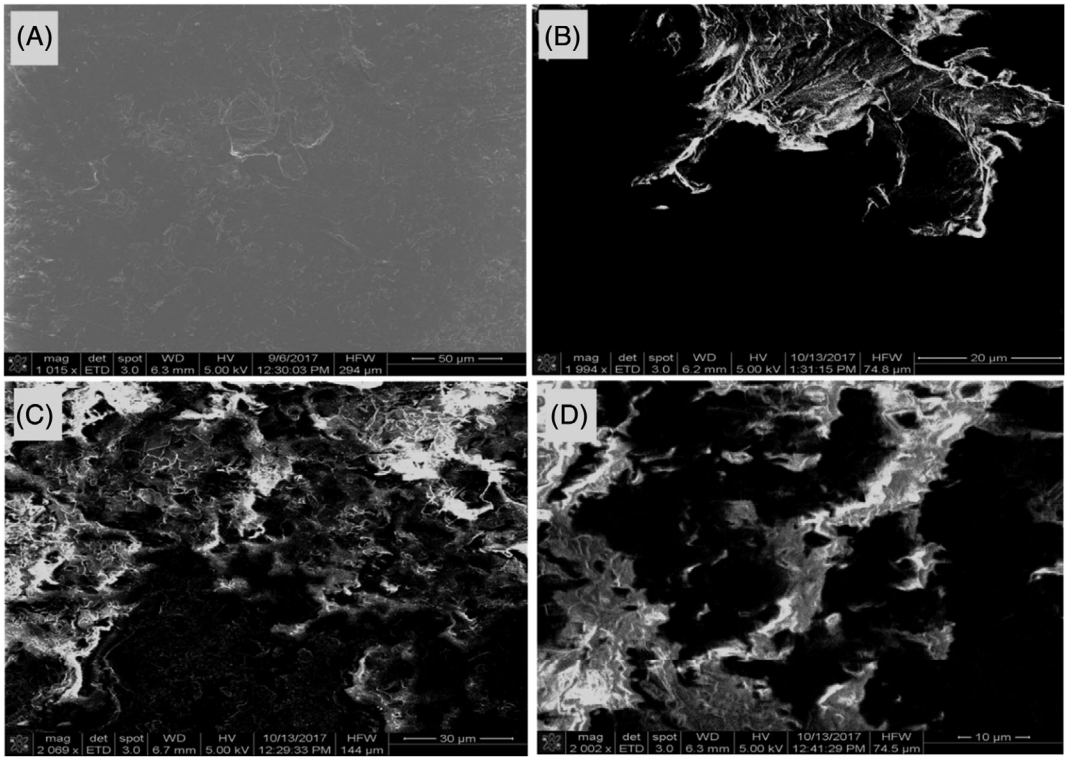

FIGURE 9 Scanning electron microscopy (SEM) images after 10 days of enzymatic degradation in buffer solution at 37°C. Neat poly(ε-caprolactone) (PCL) (A), jackfruit mucilage (JM) (B), PCL 80 (C), and PCL 60 (D), at ×2000 magnification

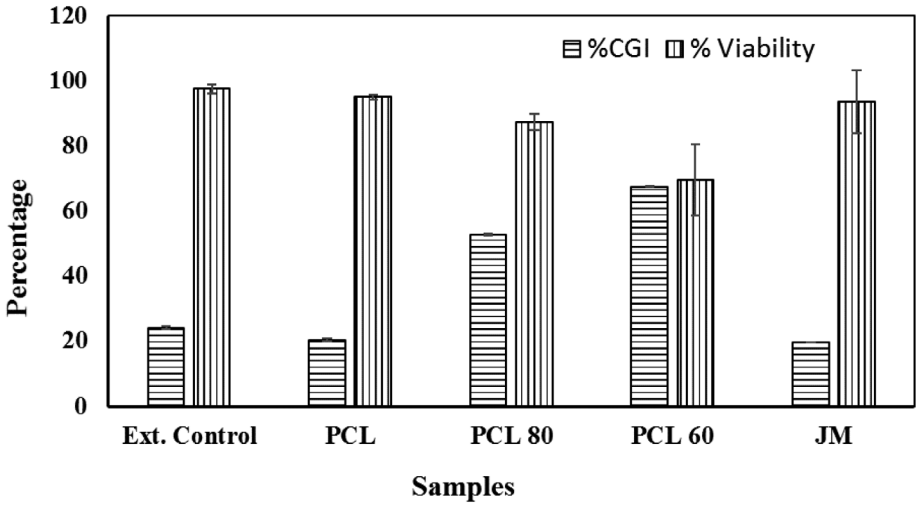

FIGURE 10 Percentage cell viability and inhibitory effect of neat poly(ε-caprolactone) (PCL), jackfruit mucilage (JM) and the blends on L929 cells. Error bars represent SD

environment for cell growth whereas lignins affect cell viability through a reduction in cell proliferation, rather than by inducing cytotoxic death, especially at high concentrations and long treatment times.[44] Isoflavones have been reported to strongly inhibit the cell growth of stomach cancer cell lines in vitro.[45, 46]

PCL/JM blends showed a higher cell inhibition behavior, which escalated with increasing PCL. PCL, JM, and the PCL/JM blends showed significant difference ($p \leq 0.05$) on the cell viability and inhibitory effect on L929 cells. This observation is contrary to the work reported on L929 cells with PCL/gelatin[47] and PCL/chitosan[48] blends where cell growth and proliferation increased on/within scaffolds and has been attributed to surface modification. The increased inhibition of the L929 cells observed in the blends in our study is probably due to the way polysaccharides, lignans, isoflavones, and saponins behaved that is present in JM when it was

blended with PCL. PCL/JM is a miscible crystalline/amorphous blend system (Figure 3) and the polysaccharide present in JM is suggested to be the component of JM present in the interlamellar regions of the blended PCL, rejecting the isoflavones, lignins and the saponins outside of the interlamellar region of the spherultic structure, which therefore, was easily extractable and induced cell growth inhibition. In blends with high JM content, more of these components were present in the extract because the polysaccharide had moved into the interlamellar region.

These results indicate this is an interesting blend system. For applications where cell growth is required, blends with low JM content can be used whereas in situation of cancer, blends with high JM content can be used. However, further studies such as contact angle measurement, evaluation of surface roughness, cell proliferation rate, and cell adhesion are required to extend PCL/JM blends for tissue engineering applications, which will be the basis of our future work.

4 | CONCLUSION

Melt-blended samples of PCL and JM were found to be miscible based on the depression in the equilibrium melting temperature of PCL in the blends and the presence of extinction rings in the spherulites of blended PCL. Infrared studies of the blends showed changes in the carbonyl and C—O—C bands confirming the involvement of these functional groups in the interactions. With increasing JM, the blends became soft and the porosity decreased. The viability of L929 fibroblast cell on PCL and JM extracts was around 90% but the viability decreased in the blends with increasing JM due to the increasing content of isoflavones in the mucilage. The results indicate this is an interesting blend system. For applications where cell growth is required, blends with low JM can be used whereas blends with high JM content can be used in cancer therapy. The biomaterial developed suggest to have significant potential in tissue engineering applications.

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