FABRICATION AND CHARACTERIZATION OF FIBROUS POLYCAPROLACTONE BLENDED WITH NATURAL GREEN TEA EXTRACTS USING DUAL SOLVENT SYSTEMS

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1. Introduction

The need for wound care is increasing sharply, and the search for novel wound dressing is explored by scientists globally. Millions of people suffer from acute wounds, chronic wounds, or burns every year. Problems such as infection, increase inflammation, and scarring further complicated wound care. It is estimated that 1–2% of the population in developed countries experience a chronic wound in their lifetime [1].

Wound healing process is the physiological response of the human body to restore the integrity and function of the tissue upon injury. The goal of wound care is to shorten the time of wound healing and reduce the risks of undesired complications. Wound healing is a complex pathophysiological process involving cellular and chemical processes, such as inflammation, angiogenesis, and collagen deposition. Usually, prolong inflammation and insufficient vessel formation cause delay in wound healing. Wound fibrosis or abnormal accumulation of collagen in the wound could lead to the formation of an unpleasant scar [2].

Cells in the skin require an appropriate matrix for adhesion and growth. Extracellular matrix (ECM) serves this purpose by providing suitable mechanical and biochemical clues conducive for the cells to regenerate. Scientists try to mimic the structure through several intuitive manufacturing processes that can produce the structural features of ECM. Nanofibrous manufacturing techniques, such as drawing, template synthesis, phase separation, and self-assembly, are widely experimented in producing structures closer to ECM. However, electrospinning is one of the widely used methods for the fabrication of nanofibers for medical applications. Electrospinning renders cost-effectiveness, scalability, versatility, and simplicity in manufacturing nanofibers from the polymers [3]. Polymeric nanofibers are of great interest in biomedical applications, specifically in the production of tissue engineering’s scaffold, drug delivery systems, and as wound healing material.

Recently, several natural products were blended with the polymer to enhance its physicochemical and biological properties. Recent work has shown that polyurethane blended with honey and papaya significantly enhances the specific protein adsorption and improved properties for burn wound healing [4]. In line with this research, electrospun fibers blended with...
with grapes [5] and garlic [6] were manufactured, and they were found to possess conductive properties necessary for wound healing application. Similarly, a recent work fabricated polycaprolactone (PCL) blended with aloe vera for guided tissue regeneration. It was deciphered that electrospun PCL/ aloe vera is promoting cell adhesion and proliferation than the pure PCL nanofibers [7].

Synthetic polymer, poly(ε-caprolactone) (PCL), has been widely used for biomedical engineering applications, largely due to its biocompatibility and biodegradability nature [8]. Green tea, a nonfermented tea derived from the leaves of *Camellia sinensis*, is not only consumed as a beverage but also incorporated into a product such as skincare. Green tea has been associated with various medicinal properties including the wound healing property. Some of the favorable health effects of green tea are antioxidant, anti-inflammatory, and antimicrobial qualities [9]. This work would like to utilize the beneficial properties of green tea by blending with PCL for wound healing application. Further, PCL/green tea composite was electrospun using dual solvent systems, namely, chloroform/dimethylformamide (DMF), and acetone/DMF. The fabricated PCL/green tea extract along with pure PCL will be characterized for its morphology, wettability, and water uptake to evaluate its feasibility for wound healing.

2. Materials and methods

PCL pellets, chloroform, acetone, and DMF were purchased from Sigma-Aldrich (M) Sdn Bhd, Malaysia. The green tea powder was purchased from Green Pot Tea Pte. Ltd., Singapore.

2.1. Preparation of sample

About 5 g of green tea leaf powder sample was mixed with 10 mL of water and then the mixture was boiled for 15 min. After this, the mixture was filtered and the green tea extract was stored in an Eppendorf tube at 4°C for further use. Table 1 shows six samples manufactured through electrospinning. The electrospinning parameters were fixed at applied voltage 12 kV, flow rate 0.5 mL/h, and 20 cm distance between the tip of the needle and the collector. Finally, the fabricated fibers obtained were left to dry at a vacuum for a day to ensure the complete removal of the solvent from the sample.

| Solvent system       | Sample solutions                           |
|----------------------|--------------------------------------------|
| Chloroform-DMF       | 12 mL 12% PCL                             |
|                      | 11 mL 12% PCL + 1 mL 12% green tea extract |
|                      | 10 mL 12% PCL + 2 mL 12% green tea extract |
| Acetone-DMF          | 12 mL 12% PCL                             |
|                      | 11 mL 12% PCL + 1 mL 12% green tea extract |
|                      | 10 mL 12% PCL + 2 mL 12% green tea extract |

2.2. Scanning electron microscopy

Scanning electron microscopy (SEM) was used to analyze the morphology of fiber produced [5, 6]. SEM unit (Hitachi Tabletop TM3000, Tokyo, Japan) was used to obtain the SEM image of the fabricated fibrous membrane. To begin with, a small square was cut from the sample and then placed on the coin. To obtain clear SEM images, the prepared samples were gold-coated using a low vacuum coater (Leica EM ACE200, Leica Microsystems, Wetzlar, Germany). To measure the diameter of fibre by using ImageJ software, SEM images were imported in the software (Java version, National Institutes of Health, USA). Fifty fibers were randomly picked and measured.

2.3. Water contact angle measurements

To analyze the wettability of the fabricated fibrous membrane [5, 6], the water contact angle was measured. The VCA Optima contact angle measurement unit (AST Products, Inc., Billerica, MA, USA) was used in this study. A rectangle of 1 cm × 5 cm was cut from the samples. Then, it was placed on top of the stage of VCA Optima. A water-filled syringe was used to drop a 2 μL of water onto the sample. The image of the static liquid deposition was captured using a high-resolution camera. For each sample, the water contact angle was measured at three different trials. The contact angle was measured in degree (°), and the results were expressed in mean ± SD.

2.4. Water uptake analysis

Water uptake analysis was performed to analyze the permeability of the fabricated fibrous membrane [6]. A rectangle of size 2 cm × 8 cm was cut from the samples. Then, it was weighed on the analytical scale. The weight measured was designated as the dry weight (Wd) at 0 min. The sample was immersed in the water. At 2, 5, 10, 15, 20, 25, 30, 45, and 60 min, the sample was taken out of the water. The sample was dried on tissue paper to remove excess water. Then, the weight of the sample was immediately recorded before it was immersed in water again.

The percentages of water uptake were calculated using the following equation:

\[
\text{Water uptake(%) } = \left( \frac{W_w - W_d}{W_d} \right) \times 100\%
\]

Table 1. Details of the samples manufactured via electrospinning

| Solvent system       | Sample solutions                           |
|----------------------|--------------------------------------------|
| Chloroform-DMF       | 12 mL 12% PCL                             |
|                      | 11 mL 12% PCL + 1 mL 12% green tea extract |
|                      | 10 mL 12% PCL + 2 mL 12% green tea extract |
| Acetone-DMF          | 12 mL 12% PCL                             |
|                      | 11 mL 12% PCL + 1 mL 12% green tea extract |
|                      | 10 mL 12% PCL + 2 mL 12% green tea extract |

DMF, dimethylformamide; PCL, polycaprolactone.
where Wd refers to dry weight (before the sample was immersed in water at 0 min) and Ww refers wet weights (after the sample was immersed in water).

2.5. Attenuated total reflectance Fourier transform infrared spectroscopy analysis

Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) analysis was performed to study the chemical composition of the fabricated fibrous membranes [5]. The ATR-FTIR unit (Nicolet iS5, Thermo Fischer Scientific, Waltham, MA, USA) was used to measure the spectra of each sample. A small square sample was cut and placed on top of the sensor surface. The spectra of each sample were measured over the range of 4,000–400 cm⁻¹ at 32 scans/min and averaged at resolution 4 cm⁻¹.

2.6. Statistical analysis

All works were performed thrice and the quantitative results were expressed as mean ± SD, whereas for qualitative imaging experiments one of the three images is shown.

3. Results

3.1. SEM analysis

Before the SEM analysis, the thickness of the sample was measured using a digital micrometer. The PCL membrane showed an average thickness of 137 μm, whereas the PCL/green tea showed 128 μm and 152 μm for 11:1 and 10:2 chloroform/DMF solvent system. In the case of acetone/DMF, PCL showed a thickness of 190 μm and its composites exhibited 187 μm and 201 μm. The morphology of fabricated nanofibrous composites was analyzed based on fiber diameter measurement from the SEM images. Table 2 summarizes the results obtained from the measurement of fiber diameter. Figures 1 and 2 show the SEM images at ×2.5k magnification and the graph of diameter distribution of the electrospun fibers. The difference between the dual solvent-systems (chloroform-DMF and acetone-DMF) can be clearly observed from the fiber diameter measurements. In the chloroform/DMF solvent system, the mean average fiber diameter of the pure PCL was found to be 673 nm and the PCL/green tea extract exhibited reduced fiber diameter of 601 nm (11:1 sample) and 663 nm (10:2 sample). A similar trend of fiber diameter reduction was observed in the acetone-DMF solvent system. The mean fiber diameter of pure PCL was 1,104 nm, and it was reduced when blending with green tea extract as 896 nm and 901 nm in 11:1 and 10:2 ratios. This reduction in fiber diameter is mainly attributed to the interaction of PCL with green tea extract components, which would have altered the fiber diameter. This was in agreement with recently published work, which integrated honey and papaya in the polymer matrix [4].

In the chloroform/DMF solvent system, polymer displayed smaller average fiber diameter, whereas acetone-DMF-diluted polymer produced larger average fiber diameter. This could be elucidated by the wide array of solvent properties that play a crucial part in morphology and attributes of fibers produced [10]. Chloroform has a higher boiling point compared to acetone and hence during the process acetone evaporates rapidly and thus allowing less time for the jet to solidify, resulting in larger fiber diameter. However, in both solvent systems, composite fibers (PCL/green tea) diameter was reduced compared with PCL indicating the influence of green tea on the fibers.

In a recent report [11], the reduced diameter of the composite system found to be encouraging in wound healing. The morphology of the produced fibers was compared between the dual solvent systems. Both solvent systems produce fibers that were almost even along with minor beads in the chloroform-DMF system, whereas in the acetone-DMF, fibers were smooth and uniform. This may be attributed to the solvent viscous properties as reported in the recent research [12].

3.2. Wettability analysis

PCL is known to be a hydrophobic polymer. As can be seen in Table 3, both chloroform/DMF and acetone/DMF-diluted pure PCL fibers have shown a water contact angle measurement of 104° indicating that electrospun pure PCL fibers were hydrophobic. PCL/green tea prepared using chloroform/DMF rendered a contact angle of 100° on average for both ratios (11:1 and 10:2), whereas the acetone/DMF showed an average of 96° and 94° for 11:1 and 10:2 ratios, respectively. Water contact angle measurement of the PCL/green tea membrane is reduced indicating the wettability of the composite membrane is enhanced because of the incorporation of green tea extract into the PCL solution. It has been reported that smaller the fiber diameter wettability of the membrane will be enhanced [5, 6]. Based on the SEM analysis, PCL/green tea composite had reduced fiber diameter, which has a putative role in the contact angle reduction. Hence, the enhanced wettability can

| Fiber diameter of electrospun fibers of PCL and PCL/green tea composites | Range of diameter (nm) | Average diameter (nm) |
|-------------------------------------------------|-------------------------|-----------------------|
| | Chloroform-DMF | Acetone-DMF | Chloroform-DMF | Acetone-DMF |
| Pure PCL | 310–1,719 | 432–3,644 | 673 ± 288 | 1,104 ± 646 |
| 11:1 PCL: green tea extract | 430–1,169 | 430–1,805 | 601 ± 170 | 896 ± 269 |
| 10:2 PCL: green tea extract | 430–1,121 | 440–1,640 | 663 ± 159 | 901 ± 215 |

DMF, dimethylformamide; PCL, polycaprolactone.
Figure 1. SEM images at x2.5k magnification of chloroform-DMF-diluted. (A) pure PCL, (B) 11:1 PCL:green tea extract, (C) 10:2 PCL:green tea extract, and the graph of fiber diameter of chloroform-DMF-diluted (D) pure PCL, (E) 11:1 PCL:green tea extract, and (F) 10:2 PCL:green tea extract. DMF, dimethylformamide; PCL, polycaprolactone.

Table 3. Water contact angle measurement of the PCL and PCL/green tea composites

| Sample                          | Chloroform-DMF | Acetone-DMF |
|---------------------------------|----------------|-------------|
| Pure PCL                        | 104 ± 2.4      | 104 ± 1.3   |
| 11:1 PCL:green tea extract     | 100 ± 2.5      | 96 ± 1.3    |
| 10:2 PCL:green tea extract     | 100 ± 6.1      | 94 ± 6.1    |

DMF, dimethylformamide; PCL, polycaprolactone.

Table 4. Percentage of water uptake of PCL and PCL/green tea composites after 30 min

| Sample                          | Chloroform-DMF | Acetone-DMF |
|---------------------------------|----------------|-------------|
| Pure PCL                        | 51 ± 10        | 60 ± 19     |
| 11:1 PCL:green tea extract     | 74 ± 18        | 170 ± 34    |
| 10:2 PCL:green tea extract     | 65 ± 22        | 350 ± 33    |

DMF, dimethylformamide; PCL, polycaprolactone.
PCL/green tea composites will favor the absorption of excess exudate from the wounds simulating the function of the ECM [13]. Further, the electrospun membrane using acetone/DMF is seemingly a more attractive choice compared to chloroform/DMF due to its enhanced water uptake percentage.

3.4. ATR-FTIR results

Based on the PCL FTIR spectra (Figure 4), the specific functional groups identified are –CH₃ and C=O groups. The peaks, observed at 2,944 cm⁻¹ and 2,865 cm⁻¹, are associated with the asymmetric and symmetric –CH₂ groups. The peaks at 1,721 cm⁻¹ correspond to stretching vibration of C=O groups. On observing the spectra, there are no new peaks in the PCL/green tea (10:2) in both solvent systems due to similar chemical composition between PCL (C₆H₁₀O₂)n and green tea catechins C₁₅H₄O₆. However, when their spectra were overlaid, a change in the intensity of the peaks was observed, which is due to the hydrogen bond formation. This is similar to some recently published reports where they attributed the peak intensity increase to the hydrogen bond formation. This is similar to some recently published reports where they attributed the peak intensity increase to the hydrogen bond formation.

3.3. Water uptake assay

The permeability of the electrospun fibrous membrane was studied by performing water uptake analysis. Figure 3 shows the time kinetics graph of water uptake of PCL and PCL/green tea. Grossly by observing the graph, it is evident that acetone/DMF fibers uptake more water for different ratios of PCL/green tea extract. This is in accordance with the contact angle results, which depicted lower values for acetone/DMF than chloroform/DMF membrane. After 30 min, the average water uptake percentage of pure PCL was 51%, and it was increased to 65% (10:2) and 74% (11:1) in a chloroform-DMF solvent. Similarly, in the case of acetone/DMF water uptake, the percentage of pure PCL was 60% and it increased to 170% (11:1) and 350% (10:2). The increased wettability of PCL/green tea would have favored more water uptake ability for the composite system than the pure PCL. Increasing the water uptake capacity of the PCL/green tea composites will favor the absorption of excess exudate from the wounds simulating the function of the ECM [13]. Further, the electrospun membrane using acetone/DMF is seemingly a more attractive choice compared to chloroform/DMF due to its enhanced water uptake percentage.
change to the interaction between the added component and the polymer matrix [5, 6].

4. CONCLUSION

Electrospun PCL/green tea of different ratios using dual solvent systems was fabricated. The nanofibrous composite (PCL/green tea) rendered small fiber diameter, enhanced wettability, and water uptake capacity. The components present in the green tea are ascribed for enhancing the physicochemical properties of the composite. Further, membrane spun by acetone/DMF solvent system displayed enhanced water uptake capacity than the chloroform/DMF system. Hence, PCL/green tea composites will be attractive candidates for wound...
healing applications. However, further in vitro and in vivo trials are required to promote these candidates in clinical application.

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References

[1] Gottrup, F. A. (2004). Specialized wound-healing center concept: Importance of a multidisciplinary department structure and surgical treatment facilities in the treatment of chronic wounds. The American Journal of Surgery, 187, 38-43.

[2] Xue, M., Jackson, C. J. (2015). Extracellular matrix reorganization during wound healing and its impact on abnormal scarring. Advances in Wound Care, 4, 119-136.

[3] Kadavil, A., Zagho, M., Elzatahry, A., Altahtamouni, T. (2019). Sputtering of electrospun polymer-based nanofibres for biomedical applications: A perspective. Journal of Nanomaterials, 9, 77.

[4] Balaji, A., Jaganathan, S., Ismail, A. F., Rajasekar, R. (2016). Fabrication and hemocompatibility assessment of novel polyurethane-based bio-nanofibrous dressing loaded with honey and Carica papaya extract for the management of burn injuries. International Journal of Nanomedicine, 11, 4339-4355.

[5] Mani, M. P., Jaganathan, S. K. (2020). Fabrication and characterization of electrospun polyurethane blended with dietary grapes for skin tissue engineering. Journal of Industrial Textiles, 50, 655-674.

[6] Mani, M. P., Jaganathan, S. K. (2019). Physicochemical and blood compatibility characteristics of garlic incorporated polyurethane nanofibrous scaffold for wound dressing applications. The Journal of the Textile Institute, 110, 1615-1623.

[7] Princeton, C., Shekh, M. R., Narayan, B. (2016). Facile fabrication of aloe vera containing PCL nanofibers for barrier membrane application. Journal of Biomaterials Science, Polymer Edition, 27, 692-708.

[8] Malikmammadov, E., Tanir, T. E., Kiziltay, A., Hasirci, V., Hasirci, N. (2018). PCL and PCL-based materials in biomedical applications. Journal of Biomaterials Science, Polymer Edition 29, 863-893.

[9] Sabu, M. C., Priya, T. T., Ramadasan, K., Ikuo, N. (2010). Beneficial effects of green tea: A literature reviews. Chinese Medicine, 5, 13.

[10] Khajavi, R., Abbasipour, M. (2017). Controlling nanofibre morphology by the electrospinning process. Electrospun Nanofibres, Woodhead Publishing Series in Textiles (Duxford; Cambridge), pp. 109-123.

[11] Hollister, S. J. (2005). Porous scaffold design for tissue engineering. Nature Materials, 4, 518-524.

[12] Jinyou, L., Xianfeng, W., Jianyong, Y., Gang, S. U., Moran, W. (2012). Biomimicry via Electrospinning. Critical Reviews in Solid State and Materials Sciences, 37, 114.

[13] Mehmet, E. O., Ioannis, D., Karantas, Z. S., Neslihan, U. O., Panoraia, I. S. (2020). Recent trends on wound management: New therapeutic choices based on polymeric carriers. Asian Journal of Pharmaceutical Sciences. https://doi.org/10.1016/j.ajps.2019.11.008.

[14] Chong, L. H., Lim, M. M., Sultana, N. (2015). Fabrication and evaluation of polycaprolactone/gelatin-based electrospun nanofibres with antibacterial properties. Journal of Nanomaterials, 15, 1–8.