Electrospun polyurethane nanofibrous composite impregnated with metallic copper for wound-healing application

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Abstract

In this study, a wound dressing based on polyurethane (PU) blended with copper sulphate nanofibers was developed using an electrospinning technique. The prepared PU and PU nanocomposites showed smooth fibers without any bead defects. The prepared nanocomposites showed smaller fiber (663 ± 156.30 nm) and pore (888 ± 70.93 nm) diameter compared to the pristine PU (fiber diameter 1159 ± 147.48 nm and pore diameter 1087 ± 62.51 nm). The interaction of PU with copper sulphate was evident in the infrared spectrum through hydrogen-bond formation. Thermal analysis displayed enhanced weight residue at higher temperature suggesting interaction of PU with copper sulphate. The contact angle measurements revealed the hydrophilic nature of the prepared nanocomposites ($71^\circ \pm 2.309^\circ$) compared with pure PU ($100^\circ \pm 0.5774^\circ$). The addition of copper sulphate into the PU matrix increased the surface roughness, as revealed in the atomic force microscopy (AFM) analysis. Mechanical testing demonstrated the enhanced tensile strength behavior of the fabricated nanocomposites (18.58 MPa) compared with the pristine PU (7.12 MPa). The coagulation assays indicated the enhanced blood compatibility of the developed nanocomposites [activated partial thromboplastin time (APTT)— 179 ± 3.606 s and partial thromboplastin time (PT)— 105 ± 2.646 s] by showing a prolonged blood clotting time compared with the pristine PU (APTT— 147.7 ± 3.512 s and PT— 84.67 ± 2.517 s). Furthermore, the hemolysis and cytotoxicity studies suggested a less toxicity nature of prepared nanocomposites by displaying low hemolytic index and enhanced cell viability rates compared with the PU membrane. It was observed that the fabricated novel wound dressing possesses better physicochemical and enhanced blood compatibility properties, and may be utilized for wound-healing applications.

Keywords Polyurethane · Copper sulphate · Electrospun nanofibers · Wound-healing · Physicochemical characteristics · Bio compatibility

Introduction

Wound dressing plays an important role in managing certain types of open wounds, including traumatic, thermal, or chronic wounds. In wound-healing, microbial growth is a major factor which affect the rate of the healing and is influenced by the environment of wound beds (Unnithan et al. 2012a, b; Wright et al. 2002). Furthermore, bacterial colonization caused by various substance like toxins, proteases, and pro-inflammatory molecules might cause a prolonged inflammatory response in the host tissue affecting the wound-healing process (Jones et al 2004; Leaper 2006). Hence, if the skin is damaged, the main importance is immediate care to prevent the pathogen invading and trans-epidermal water loss for facilitating wound remodeling (Unnithan et al. 2012a, b). The small pore sizes were able to reduce the bacterial growth by restricting bacterial cell division

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(Premuzic and Woodhead 1993). Microbial growth was also reduced by loading any agent that had antimicrobial activity. Hence, the wound dressing should be fabricated with small pore size and an agent with antimicrobial activity. The ideal wound dressing must perform certain functions such as absorbing of the lost fluids and liquids from the wound zone, maintenance of a moist environment, and mimicry of the native tissue structure (Kamoun et al. 2017). Furthermore, the ideal wound dressing must be non-allergenic, non-toxic, non-adherent, and non-stress inducing (Manikandan et al. 2017).

Growth in the field of nanotechnology enables the fabrication of wound dressings in the form of nanofibers which possess desirable characteristics like architectural features mimicking the original structure of the natural extracellular matrix (ECM) (Unnithan et al. 2012a, b; Jayakumar et al. 2010). Recently, nanofibers based on an electrospinning technique have gained wide attention in biomedical applications. Electrospinning is a versatile and cost-effective system which utilizes high applied voltage to generate fine nanofibers from the various polymers available on the market (Manikandan et al. 2017). A wound dressing fabricated using nanofibers provides many advantages compared with a conventional wound dressing (Supaphol et al. 2012). Rather et al. (2018) fabricated PCL–gelatin electrospun fibers loaded with cerium oxide for wound-healing applications. It was reported that the PCL–gelatin-loaded cerium oxide nanofibrous scaffold showed enhanced proliferation of 3T3-L1 cells and suggested it as a suitable candidate for the wound-healing applications. In another study, Chen et al. (2010) fabricated bioactive polyurethane wound dressing scaffold loaded with silver nanoparticles. *In vivo* studies reported that the nanofibrous membranes showed a better wound-healing rate than the commercial gauzes and collagen sponge wound dressing. Similarly, Augustine et al. (2014) fabricated PCL scaffold loaded with zinc oxide nanoparticles. It was that the polyurethane loaded with zinc oxide nanoparticles showed enhanced fibroblast adhesion and concluded it as a potential candidate for wound dressing applications. Moreover, the ultimate aim in wound management is to provide a structure which should mimic the ECM to grow the host cells on the ECM. Nanofibers produced by the electrospinning technique have a huge surface area and microporous structure which could quickly allow the pathway for fibroblast adhesion and proliferation for new tissue regeneration (Bolgen et al. 2007; Hong 2007; Prabakaran et al. 2012).

In this research, the wound dressing scaffold was fabricated using polyurethane polymer. PU is commonly used in wound dressing applications owing to its good barrier properties and oxygen permeability (Unnithan et al. 2012a, b; Lakshmi et al. 2010). It is important that the wound dressing should possess antimicrobial agents. For that purpose,

copper sulphate nanofibers were loaded into the PU matrix. Copper is well known for its antimicrobial activity and is also reported to be chemically stable and have low toxicity (Amna et al. 2014). Moreover, copper is involved in several physiological and metabolic processes by facilitating the growth of endothelial cells, angiogenesis, and maintenance of extracellular skin proteins (Sen et al. 2002). The objective of this study is to develop a novel wound dressing based on PU incorporated with copper sulphate nanofibers. The various physicochemical, blood compatible, and cytotoxic properties of the developed scaffold are determined.

Materials and methodology

Materials

Medical grade Tecoflex EG-80A polyurethane was purchased from Lubrizol and dimethylformamide (DMF) was obtained from Sigma-Aldrich, UK. Copper sulphate was supplied by Sigma-Aldrich, UK. The chemicals such as phosphate-buffered saline (PBS, Biotech Grade) and sodium chloride physiological saline (0.9% w/v) were obtained from Sigma-Aldrich, Malaysia. The APTT and PT reagents were obtained from Diagnostic Enterprises, India.

Solution preparation and the fabrication of the electrospun membranes

The homogeneous solution of PU with concentration of 9 wt% was made by dissolving a calculated amount of PU pellets in DMF and stirring overnight. Similarly, the solution of copper sulphate was prepared at a concentration of 9 wt% by adding a calculated amount of copper sulphate in the DMF and stirring for 2 h. Finally, the PU/copper sulphate solution was prepared at a volume ratio of 8:1 (v/v%). Then, the prepared homogeneous was loaded in a 10 ml glass syringe fitted into electrospinning apparatus. A voltage of 10 kV was used to fabricate the nanofibers at a flow rate of 0.2 ml/hr. The nanofibers were deposited on the collector drum which was placed 15 cm away from the needle. To remove the residual DMF present in the electrospun membranes, it was dried under vacuum for 24 h at room temperature.

Scaffold morphology

Scanning electron microscopy (SEM) was utilized to determine the scaffold morphology of the electrospun PU and PU/copper sulphate membranes. The electrospun PU and PU/copper sulphate membranes were sputtered with gold before attaining the SEM images. From the attained SEM

image, the mean fiber and pore diameters were calculated using Image J.

Fourier transform infrared (FTIR) analysis

IR spectra of the PU and PU/copper sulphate membranes were obtained through FTIR. A small-size sample was placed on the recording surface, and the IR spectra of the electrospun membranes were recorded at a 600 and 4000 cm^{-1} wavelength at a resolution of 4 cm^{-1} with 32 scans per minute.

Contact angle measurement

The VCA Optima contact angle system mounted with a video cam was used to determine the water contact angles of the electrospun PU and PU/copper sulphate membranes. A small piece of a nanofibrous scaffold was placed on the surface and a droplet of distilled water was made to fall on the electrospun surface. Then, the water droplet image was captured using video cam and the mean contact angle was calculated using computer integrated software.

Thermogravimetric (TGA) analysis

To begin, a small piece of sample weighing 3 mg was placed on the aluminum pan of the TGA equipment at a nitrogen atmosphere. Then, the data were recorded by applying heat to the samples with temperature range from 30 to 1000 °C at rate of 10°C/min respectively.

Atomic force microscopy (AFM) analysis

AFM was used to determine the surface topography of the electrospun PU and PU/copper sulphate membranes. A small piece of sample was placed on the scanning surface and was scanned in 20 $\mu\text{m} \times 20 \mu\text{m}$ area. A 3D image with pixels of around 256 $\times$ 256 pixels was obtained using JPKSPM data processing software.

Mechanical testing

The uniaxial testing machine was used to determine the tensile strength of the electrospun PU and PU/copper sulphate membranes. The samples were cut into 4 cm $\times$ 1.5 cm pieces and fitted vertically on the tensile machine. Then, the test was carried out and the load deformation data were obtained at a strain rate of 5 mm/min with load unit of 500 N. Finally, from the machine constructed stress strain curves, the tensile strength of the electrospun scaffolds was calculated.

Blood compatibility assays

Activated partial thromboplastin test (APTT)

The measurement of APTT was performed to determine the interdiction of the intrinsic pathway for the developed membranes. To start the assay, the test samples were added to 50 μL of platelet poor plasma (PPP) and incubated with 50 μL of rabbit brain encephalin for 3 min at 37 °C followed by adding 50 μL of calcium chloride (CaCl_2). Finally, the initiation of the blood clot was detected using a steel hook and stopwatch (Balaji et al. 2016).

Prothrombin test (PT)

The measurement of prothrombin time was performed for the electrospun membranes to determine the interdiction of extrinsic pathway of the fabricated membranes. To start the assay, the test samples were incubated with 50 μL of PPP followed by adding 50 μL of sodium chloride (NaCl)–thromboplastin (Factor III). Finally, the initiation of the blood clot was calculated using a steel hook and stopwatch (Balaji et al. 2016).

Hemolysis assay

The hemolysis assay was used to investigate the toxicity of red blood cells with the electrospun membranes. To start the assay, the test samples were soaked in 0.9% w/v of physiologic saline for 30 min at 37 °C. Then, the soaked samples were added to the mixture of citrated blood and diluted saline prepared at a ratio of 4:5 (v/v%) for 1 h at 37 °C. The samples were taken out and centrifuged at 3000 rpm for 15 min. Finally, the supernatant was pipetted and the OD was measured at 542 nm which denotes the release the hemoglobin. The hemolysis percentage was calculated as described in the previous work (Balaji et al. 2016).

Cytocompatibility studies

HDF cells (Human Skin Fibroblast Cells 1184, ECACC, UK, primary cells obtained from the dermis of the skin) were cultured in Dulbecco's Modified Eagle's Medium (DMEM), supplemented with 10% Bovine serum and maintained at standard conditions (37 °C and 5% CO_2). The cultured medium was refreshed every 3 days. To begin the assay, the electrospun scaffolds were cut into round pieces and placed in 24-well plates. Prior to cell seeding, the electrospun membranes were sterilized with 75% alcohol for 3 h and then cleaned with PBS. Afterwards, HDF cells were seeded on an electrospun scaffold with a density of 10×10^3 cells/ cm^2 and cultured for 3 days. The cell viability rates of the electrospun membranes were determined through

3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2 H tetrazolium, inner salt (MTS) assay. For the MTS assay, the electrospun membranes with cells were extracted after 3 day culturing and further incubated with 20% MTS reagent for 4 h. Finally, the medium was extracted and the absorbance at 490 nm was recorded to determine the proliferation of the seeded HDF cells in the electrospun membranes.

Statistical analysis

All experiments were performed three times and an unpaired *t* test was used to find the statistical significance. The obtained experimental results are expressed as mean $\pm$ SD. In the case of qualitative experiments, an illustration of three images is shown.

Results and discussion

Figure 1a and b shows SEM micrographs of electrospun PU and PU/copper sulphate nanofiber mats. The morphology of the electrospun membranes showed the smooth fibers without any bead defects. It was observed that the PU/copper sulphate nanofibers had a decreased diameter compared to the pristine PU. The decrease in the fiber diameter might be due to the increase in the conductivity of the PU solution with the addition of copper sulphate. The fiber diameter of

pristine PU was found to be 1159 ± 147.48 nm, while the PU/copper sulphate nanofibers showed a fiber diameter of 663 ± 156.30 nm [mean differences were significant compared with pure PU ($p < 0.05$)]. The corresponding fiber distribution curve is shown in Fig. 2a and b. Unnithan et al. (2012a, b) developed a wound dressing scaffold based on PU blended with dextran nanofibers. It was observed that the addition of dextran into the PU matrix reduced the fiber diameter, and exhibited enhanced fibroblast adhesion and proliferation compared to the pure PU. In our study, the developed nanocomposites showed reduced fiber size which can serve as a valid justification for enhanced fibroblast proliferation for the new tissue growth. In another study, Augustine et al. (2014) fabricated PCL scaffold loaded with zinc oxide nanoparticles for wound-healing applications. They reported a maximum reduced diameter of 1340 nm in case of PU/ZnO fibers which exhibited showed higher fibroblast adhesion than the pristine. Since, our fibers displayed significantly reduced mean fiber diameter of 663 ± 156.30 nm which was below than the reported values and it might be suitable for the wound-healing applications.

The pore size of electrospun PU and PU/copper sulphate nanofibers mats was measured through Image J. It was observed that the pore size was reduced with addition of the copper sulphate into the PU. The electrospun PU showed a pore size of 1087 ± 62.51 nm, while the pristine PU/copper sulphate nanofibers showed a pore size of about 888 ± 70.93 nm [mean differences are significant compared

Fig. 1 SEM images of **a** PU membrane and **b** PU/copper sulphate nanocomposites

Fig. 2 Fiber diameter distribution of **a** PU membrane and **b** PU/copper sulphate nanocomposites

with pure PU ($p < 0.05$) and their graphical representation is indicated in Fig. 3a, b. It was suggested that the small pore sizes were able to reduce the bacterial growth by restricting

bacterial cell division (Premuzic and Woodhead 1993), and in our fabricated nanocomposites, the pore size was observed to be low which might also restrict the bacterial cell growth.

Fig. 3 Pore-size distribution of **a** PU membrane and **b** PU/copper sulphate nanocomposites

The functional groups of prepared PU and PU/copper sulphate scaffolds are shown in Fig. 4. In the spectra PU, a wide peak seen at 3328 cm^{-1} was attributed to N-H stretching, while the peaks observed at 1596 and 1530 cm^{-1} indicated the vibrations of NH group. The sharp peaks observed at 2938 and 2853 cm^{-1} denoted the CH_2 stretching and their vibrations were observed at a peak of 1413 cm^{-1} . The C=O stretching corresponded to the carboxylic groups seen at 1730 and 1702 cm^{-1} , and the vibrations attributed to the alcohol group were seen at 1220 and 1105 cm^{-1} (Kim et al. 2009; Li et al. 2014). In the spectra of PU/copper sulphate nanocomposites, it was observed that no new bands formed, but their intensity was increased owing to the formation of hydrogen bonds (Zhou et al. 2011; Pant et al. 2010). The interaction of PU with copper sulphate was revealed by peak shifting of NH in pure PU from 3328 to 3314 cm^{-1} in PU/copper sulphate nanofibrous mats; this demonstrated the existence of copper sulphate content in polyurethane matrix (Tijing et al. 2012).

The surface wettability of the fibrous mats was measured through water contact angle measurements. The pristine PU

nanofibrous mats showed a contact angle of $100^\circ \pm 0.5774$ indicating their hydrophobic nature. The reason might be the presence of CH_2 groups in the PU matrix that leads to the hydrophobic properties. Meanwhile, the contact angle of the PU/copper sulphate nanofibrous mats was decreased to $71^\circ \pm 2.309$ [mean differences were significant compared with pure PU ($p < 0.05$)] indicating hydrophilic behavior. The contact angle images of the electrospun membranes are indicated in Fig. 5. The reason might be due to the presence of hydroxyl and carboxyl groups, which correlates with the FTIR studies. Unnithan et al. (2014) studied an electrospun scaffold based on PU incorporated with cellulose acetate and zein nanofibers for wound dressing. It was observed that the addition of cellulose acetate and zein into the PU matrix improved the hydrophilic nature of the electrospun scaffold, and showed enhanced fibroblast adhesion and proliferation. Hence, in our study, the addition of copper sulphate improved the wettability of the PU membrane, and this might lead to the enhanced fibroblast adhesion and proliferations for new tissue growth.

The typical tensile stress-strain curves of PU and PU/copper sulphate nanofibrous mats are indicated in Fig. 6a, b.

Fig. 4 IR spectrum of PU membrane and PU/copper sulphate nanocomposites

Fig. 5 Contact angle images of **a** PU membrane and **b** PU/copper sulphate nanocomposites

It was observed that the tensile strength of the PU mat was increased with the addition of the copper sulphate. The tensile strength for the pristine PU was found to be 7.12 MPa, while the electrospun PU/copper sulphate nanofibrous membrane exhibited tensile strength of about 18.58 MPa [mean differences were significant compared with pure PU ($p < 0.05$)]. It was observed there was a significant increase

in the ultimate strength of the PU with blending the copper sulphate. Wong et al. (2008) reported that fibers with a small diameter might result in enhanced tensile strength. In our study, our developed nanocomposites had a smaller diameter compared to the pristine PU which might have favored the enhanced tensile strength. In another study, Unnithan et al. (2012a, b) fabricated polyurethane wound dressing

Fig. 6 Mechanical testing of **a** PU membrane and **b** PU/copper sulphate nanocomposites

scaffold added with emu oil nanofibers. The tensile strength of the composite scaffold was found to be increased with the addition of emu oil nanofibers. Furthermore, the observed strength was found to be around in the range of 7 MPa. Our tensile strength of the fabricated composites showed a better tensile strength than the reported values suggesting its superiority for the wound-healing applications.

The thermal properties of the electrospun PU and PU/copper sulphate nanofibers are shown in Fig. 7a and b. In the case of electrospun PU/copper sulphate nanofibers, the initial onset degradation temperature was found at 202 °C, and in the case of the PU membrane, the initial onset degradation temperature was observed at 276 °C which indicating the reduced thermal stability of the fabricated nanocomposites.

The reason for low onset degradation temperature was due to moisture vaporization. Furthermore, at 1000 °C, the remaining weight percentage for pure PU membrane was observed to be 0.47%, while the electrospun PU/copper sulphate nanofibers showed a weight percentage of 11.26% which was higher compared to the PU, confirming its enhanced thermal stability. Moreover, the results of the derivative weight loss curve for the electrospun membranes are shown in Fig. 8a and b. There were two large weight loss peaks in the pure PU membrane in which the first major and second weight loss occurred at 223–348 and 348–446 °C, respectively. Furthermore, a third small peak occurred at 557–684 °C indicating a small level of weight loss. In the case of the electrospun PU/copper sulphate nanofibers, there were three weight loss

Fig. 7 TGA analysis of **a** PU membrane and **b** PU/copper sulphate nanocomposites

Fig. 8 Weight residue percentage of **a** PU membrane and **b** PU/copper sulphate nanocomposites

points, from 180 to 266°C, 266 to 334, and 334 to 468 °C, respectively. The first weight loss might be due to moisture vaporization. Furthermore, the intensity of the two large weight loss peaks was lower compared to pure PU indicating low weight loss in the PU/copper sulphate matrix. Balen et al. (2016) prepared polymethyl methacrylate (PMMA)/ZnO nanocomposites for tissue engineering applications, and investigated the structural, thermal and cytotoxic properties. It was observed that the incorporation of ZnO influenced the thermal stability of the PMMA membrane and their observations correlate with our findings.

AFM was utilized to determine the surface roughness of electrospun nanofibers. From the measurement of surface roughness, it was observed that PU/copper sulphate nanofibers had highest surface roughness compared to the pristine PU. The measured surface roughness for the pristine PU was 216 nm and the electrospun PU/copper sulphate nanocomposites exhibited surface roughness of 262 nm [mean differences were significant compared with pure PU ($p < 0.05$)], as indicated in Fig. 9a, b. The incorporation of copper sulphate improved the surface roughness of the PU membrane. Ghorbani et al. (2015) prepared an electrospun nanocomposite based on polycaprolactone (PCL)/chitosan incorporated with zinc-doped hydroxyapatite nanoparticles (nZnHA). It was observed that the addition of hydroxyapatite nanoparticles into the PCL and chitosan matrix improved their surface roughness. In our study, the addition of copper oxide into PU matrix might have improved the surface roughness and correlates with their findings. In another study, Sharifi et al. (2016) surface modified polycaprolactone nanofibrous membrane and analyzed their fibroblast cell proliferation rate. It was observed that the surface modified PCL exhibited increased surface roughness. The observed surface roughness of 10, 40, and 70 min plasma-modified PCL scaffolds were found to be 205, 225, and 243 nm. Furthermore, the scaffold with improved surface roughness favored the enhanced fibroblast cell proliferation rate. In our study, the surface roughness of our developed composites was observed to within these reported values and might be favorable for the enhanced fibroblast cell proliferation rate.

In this study, the coagulation assays, namely APTT and PT, were used to investigate the blood clotting time of the electrospun PU and PU/copper sulphate nanofibers. The PT determined the extrinsic pathway, while the APTT determined the intrinsic pathway. It was observed that the

prepared PU/copper sulphate nanocomposites showed prolonged blood clotting time compared to the pure PU membrane. The PU/copper sulphate scaffolds exhibited APTT of around 179 ± 3.606 s, while, for PU scaffold, the APTT was observed to be 147.7 ± 3.512 s, as depicted in Fig. 10. Similarly, the PT for the PU/copper sulphate scaffolds was 105 ± 2.646 s, while, for the PU scaffold, it was 84.67 ± 2.517 s as indicated in Fig. 11. The delay in APTT and PT times suggested that the prepared nanocomposite showed anticoagulant enhancement compared to the pristine PU. Next, the hemolytic assay was performed to determine the safety of the electrospun membranes with red blood cells. The hemolytic index of the electrospun PU/copper sulphate membrane was observed to be lower than the pristine PU indicating its lower toxicity to red blood cells (RBCs). The hemolytic index of PU was found to be 2.56%, while the electrospun PU/copper sulphate nanofibers showed a hemolytic index of 1.68%, as indicated in Fig. 12. It was reported that, if the hemolytic value was above 2%, the material was considered hemolytic and if the value was below 2%, then the material was non-hemolytic (Balaji et al. 2016). The measured value of hemolytic index for the

Fig. 10 APTT assay of PU membrane and PU/copper sulphate nanocomposites

Fig. 9 AFM images of **a** PU membrane and **b** PU/copper sulphate nanocomposites

Fig. 11 PT assay of PU membrane and PU/copper sulphate nanocomposites

Fig. 12 Hemolytic percentage of PU membrane and PU/copper sulphate nanocomposites

fabricated nanocomposites was found to be below 2% and it was considered to be a non-hemolytic material. It was reported that the surfaces with increased roughness might be less thrombogenic than the smooth surfaces (Baily et al. 1999). In another study, it was reported that the scaffolds with small diameters improved the blood compatibility by prolonging the blood clotting time (Vincent et al. 2012). In our study, the developed nanocomposites showed a smaller

fiber diameter and increased surface roughness which would have promoted enhanced blood compatibility.

At the wound site, fibroblast cells are present to support the wound-healing process. It has been reported that fibroblasts are actively participating in producing the ECM and collagen for new tissue growth (Bainbridge 2013). Hence, assessing the impact of the developed composite on fibroblast cell viability will promote this candidate for wound-healing application. To assess the toxicity of the electrospun scaffold to HDF cells, MTS assay was performed after 72 h cell culture. MTS is a commonly used method to analyze proliferation rates of the cultured cells. The tetrazolium salts present in the MTS reagent bind to the mitochondria of the cell, and reflect the cell viability with ease and in a more economic manner (Rai et al. 2018). After 3 days, the cell viability of the electrospun PU membrane was $132 \pm 3.606\%$ and the electrospun PU/copper sulphate membranes showed cell viability of around $174 \pm 3.464\%$, as depicted in Fig. 13. It was reported that the hydrophilicity nature favors the enhanced adhesion and spreading of fibroblast cells (Wei et al. 2007). Our electrospun nanocomposites showed a hydrophilic nature which might have favored the enhanced cell viability. Augustine et al. (2014) reported that the fibroblast proliferation of the PCL/ZnO scaffold was found to be $120 \pm 1.5\%$ and our fabricated PU-incorporated copper sulphate scaffold exhibited proliferation rate of $174 \pm 3.464\%$ which is higher indicating its better plausibility for wound-healing applications.

Fig. 13 MTS assay of PU membrane and PU/copper sulphate nanocomposites

Conclusion

In the present study a PU wound dressing blended with copper sulphate nanofibers was made using an electrospinning technique. The prepared PU and PU nanocomposites showed smooth fibers without any bead defects. The prepared nanocomposites showed smaller fiber and pore diameter compared to pristine PU. The interaction of PU with copper oxide was evident in FTIR and TGA analysis identified by hydrogen-bond formation and enhanced weight residue. The contact angle measurements revealed the hydrophilic nature of the prepared nanocomposites compared with pure PU. The addition of copper sulphate into the PU matrix increased the surface roughness and mechanical strength, and revealed by AFM and mechanical testing. The coagulation assays indicated the enhanced blood compatibility of the developed nanocomposites by showing prolonged blood clotting time compared with pristine PU. Furthermore, the hemolysis and cytotoxicity studies suggested a less toxic nature of the prepared nanocomposites by displaying a low hemolytic index and enhanced cell viability compared with the PU membrane. It was observed that the fabricated novel wound dressing might possess better physicochemical and enhanced blood compatibility properties which may be utilized for wound-healing applications.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

- Amna T, Hassan MS, Yang J, Khil MS, Song KD, Oh JD, Hwang I (2014) Virgin olive oil blended polyurethane micro/nanofibers ornamented with copper oxide nanocrystals for biomedical applications. *Int J Nanomed* 9:891
- Augustine R, Malik HN, Singhal DK, Mukherjee A, Malakar D, Kalarikkal N, Thomas S (2014) Electrospun polycaprolactone/ZnO nanocomposite membranes as biomaterials with antibacterial and cell adhesion properties. *J Polym Res* 21(3):347
- Bailly AL, Lautier A, Laurent A, Guiffant G, Dufaux J, Houdart E, Labarre D, Merland JJ (1999) Thrombosis of angiographic catheters in humans: experimental study. *Int J Artif Organs* 22(10):690–700
- Bainbridge P (2013) Wound healing and the role of fibroblasts. *J Wound Care* 22(8):407–408, 410–412
- Balaji A, Jaganathan SK, Ismail AF, 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. *Int J Nanomed* 11:4339–4355
- Balen R, da Costa WV, de Lara Andrade J, Piai JF, Muniz EC, Companhia MV, Nakamura TU, Lima SM, da Cunha Andrade LH, Bittencourt PR, Hechenleitner AA (2016) Structural, thermal, optical properties and cytotoxicity of PMMA/ZnO fibers and films: Potential application in tissue engineering. *Appl Surf Sci* 385:257–267
- Bolgen N, Vargel I, Korkusuz P, Menceloglu YZ, Piskin E (2007) In vivo performance of antibiotic embedded electrospun PCL membranes for prevention of abdominal adhesions. *J Biomed Mater Res Part B: Appl Biomater* 81:530–543
- Chen JP, Chiang Y (2010) Bioactive electrospun silver nanoparticles-containing polyurethane nanofibers as wound dressings. *J Nanosci Nanotechnol* 11(11):7560–7564
- Chong EJ, Phan TT, Lim IJ, Zhang YZ, Bay BH, Ramakrishna S, Lim CT (2007) Evaluation of electrospun PCL/gelatin nanofibrous scaffold for wound healing and layered dermal reconstitution. *Acta Biomater* 3(3):321–330
- Ghorbani FM, Kaffashi B, Shokrollahi P, Seyedjafari E, Ardeshtyrlajimi A (2015) PCL/chitosan/Zn-doped nHA electrospun nanocomposite scaffold promotes adipose derived stem cells adhesion and proliferation. *Carbohydr Polym* 118:133–142
- Hong KH (2007) Preparation and properties of electrospun poly (vinyl alcohol)/ silver fiber web as wound dressings. *Polym Eng Sci* 47:43–49
- Jayakumar R, Prabakaran M, Nair SV, Tamura H (2010) Novel chitin and chitosan nanofibers in biomedical applications. *Biotechnol Adv* 28:142–150
- Jia L, Prabhakaran MP, Qin X, Ramakrishna S (2014) Guiding the orientation of smooth muscle cells on random and aligned polyurethane/collagen nanofibers. *J Biomater Appl* 29:364–377
- Jones SA, Bowler PG, Walker M, Parsons D (2004) Controlling wound bioburden with a novel silver-containing hydro fiber dressing. *Wound Repair Regen* 12:288–294
- Kamoun EA, Kenawy ER, Chen X (2017) A review on polymeric hydrogel membranes for wound dressing applications: PVA-based hydrogel dressings. *J Adv Res* 8(3):217–233
- Kim SE, Heo DN, Lee JB, Kim JR, Park SH, Jeon SH, Kwon IK (2009) Electrospun gelatin/polyurethane blended nanofibers for wound healing. *Biomed Mater* 4:044106
- Lakshmi RL, Shalumon KT, Sreeja V, Jayakumar R, Nair SV (2010) Preparation of silver nanoparticles incorporated electrospun polyurethane nano-fibrous mat for wound dressing. *J Macromol Sci Pure Appl Chem* 47:1012–1018
- Leaper DJ (2006) Silver dressings: Their role in wound management. *Int Wound J* 3:282–294
- Manikandan A, Mani MP, Jaganathan SK, Rajasekar R, Jagannath M (2017) Formation of functional nanofibrous electrospun polyurethane and murivenna oil with improved haemocompatibility for wound healing. *Polym Test* 61:106–113
- Pant HR, Bajgai MP, Yi C, Nirmala R, Nam KT, Baek WI, Kim HY (2010) Effect of successive electrospinning and the strength of hydrogen bond on the morphology of electrospun nylon-6 nanofibers. *Colloids Surf A: Physicochem Eng Asp* 370(1):87–94
- Prabakaran M, Jayakumar R, Nair SV (2012) Electrospun nanofibrous scaffolds-current status and prospectus in drug delivery. *Adv Polym Sci* 246:241–262
- Premuzic ET, Woodhead A (1993) Microbial enhancement of oil recovery—recent advances. Elsevier, London
- Rai Y, Pathak R, Kumari N, Sah DK, Pandey S, Kalra N, Soni R, Dwarakanath BS, Bhatt AN (2018) Mitochondrial biogenesis and metabolic hyperactivation limits the application of MTT assay in the estimation of radiation induced growth inhibition. *Sci Rep* 8(1):1531
- Rather HA, Thakore R, Singh R, Jhala D, Singh S, Vasita R (2018) Antioxidative study of cerium oxide nanoparticle functionalised

- PCL-Gelatin electrospun fibers for wound healing application. *Bioact Mater* 3(2):201–211
- Sen CK, Khanna S, Venojarvi M, Trikha P, Ellison EC, Hunt TK, Roy S (2002) Copper-induced vascular endothelial growth factor expression and wound healing. *Am J Physiol Heart Circ Physiol* 282(5):H1821–H1827
- Sharifi F, Irani S, Zandi M, Soleimani M, Atyabi SM (2016) Comparative of fibroblast and osteoblast cells adhesion on surface modified nanofibrous substrates based on polycaprolactone. *Prog Biomater* 5(3–4):213–222
- Supaphol P, Suwantong O, Sansanoh P, Sowmya S, Jayakumar R, Nair SV (2012) Electrospinning of biocompatible polymers and their potentials in biomedical applications. *Adv Polym Sci* 246:213–240
- Tijing LD, Ruelo MT, Amarjargal A, Pant HR, Park CH, Kim DW, Kim CS (2012) Antibacterial and superhydrophilic electrospun polyurethane nanocomposite fibers containing tourmaline nanoparticles. *Chem Eng J* 197:41–48
- Unnithan AR, Barakat NA, Pichiah PT, Gnanasekaran G, Nirmala R, Cha YS, Jung CH, El-Newehy M, Kim HY (2012a) Wound-dressing materials with antibacterial activity from electrospun polyurethane–dextran nanofiber mats containing ciprofloxacin HCl. *Carbohydr Polym* 90(4):1786–1793
- Unnithan AR, Pichiah PT, Gnanasekaran G, Seenivasan K, Barakat NA, Cha YS, Jung CH, Shanmugam A, Kim HY (2012b) Emu oil-based electrospun nanofibrous scaffolds for wound skin tissue engineering. *Colloids Surf A: Physicochem Eng Asp* 415:454–460
- Unnithan AR, Gnanasekaran G, Sathishkumar Y, Lee YS, Kim CS (2014) Electrospun antibacterial polyurethane–cellulose acetate–zein composite mats for wound dressing. *Carbohydr Polym* 102:884–892
- Vincent M, Thomas H, Heike H, Viola V, Daniel E (2012) Influence of fiber diameter and surface roughness of electrospun vascular grafts on blood activation. *Acta Biomater* 8(12):4349–4356
- Wei J, Yoshinari M, Takemoto S, Hattori M, Kawada E, Liu B, Oda Y (2007) Adhesion of mouse fibroblasts on hexamethyldisiloxane surfaces with wide range of wettability. *J Biomed Mater Res Part B: Appl Biomater* 81(1):66–75
- Wong SC, Baji A, Leng S (2008) Effect of fiber diameter on tensile properties of electrospun poly (ϵ -caprolactone). *Polymer* 49(21):4713–4722
- Wright JB, Lam K, Buret AG, Olson ME, Burrell RE (2002) Early healing events in a porcine model of contaminated wounds: effects of nanocrystalline silver on matrix metalloproteinases, cell apoptosis, and healing. *Wound Repair Regen* 10:141–151
- Zhou C, Chu R, Wu R, Wu Q (2011) Electrospun polyethylene oxide/cellulose nanocrystal composite nanofibrous mats with homogeneous and heterogeneous microstructures. *Biomacromolecules* 12(7):2617–2625