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Biomimetic electrospun polyurethane matrix composites with tailor made properties for bone tissue engineering scaffolds

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ABSTRACT

Bone tissue scaffolds require appropriate properties conducive for new tissue growth. In this study, we prepared a novel electrospun nanofiber scaffold using polyurethane (PU), rosemary (RM) oil and copper sulphate ($CuSO_4$) respectively. The properties of the developed membranes were established through scanning electron microscopy (FESEM), atomic force microscopy (AFM), attenuated total reflectance Fourier-transform infrared spectroscopy (ATR-FTIR), thermal gravimetric analysis (TGA), contact angle and mechanical testing. Further, blood compatibility and cytocompatibility assay were carried out to evaluate their biological responses. The developed composites rendered appropriate surface morphology with tailor made wettability and roughness. Composites with engineered physicochemical properties improved the blood and cytocompatible properties which can be potentially exploited for bone tissue engineering applications.

1. Introduction

Bone fraction as a result of trauma and bone diseases leads to a large bone defect which is quite a common problem and needs treatment to remodel the damaged tissue [1]. The conventional method used to remodel the damaged bone tissue was autogenous and allogenous bone grafting. They are considered as a golden standard for repairing the bone tissue and it was limited in clinical applications owing to some problems like limited donor supply, low level of immunity and infections [2]. The demand for orthopaedics devices was increasing day by day. The current global market value of orthopaedics technologies was reported to be $41.9 billion and it will reach $56.2 billion at the end of the year 2023 with CAGR of 4.7% [3]. With the advent of the technology, tissue engineering holds promising alternate for because it was reported to overcome the limitations of the conventional method. Tissue engineering (TE) comprises of three basic components (scaffolds, cells and scaffold and growth factors) in order to regenerate the new tissue growth. Among three components, scaffold is an important component as a substrate for cell adhesion, migration and growth [4,5]. Further, the scaffold should resemble native extra cellular matrix (ECM) of the bone in order to support the new tissue formation. In addition, the rapid absorption of plasma proteins will occur initially when the material contacts with the blood. In progress, it would facilitate the platelet surface interaction which might cause the formation of the thrombus. Finally, it results in the failure of the fabricated material [6]. Hence, blood compatibility assessments play a key role in deciding the usage of the fabricated material in clinical applications.

It has been reported that the scaffold based on synthetic and natural polymers was wide spread in tissue engineering application owing to their structural resemblance with the collagen fiber organization in the bone extracellular matrix [7]. There are many techniques utilized for scaffold fabrication such as drawing, self-assembly, phase separation, template synthesis, electrospinning etc., [8]. Among these techniques, the scaffolds based on electrospinning technique was wide spread in biomedical applications especially in tissue engineering application. Electrospinning technique having the ability to produce fine fibers with a diameter ranging from um to nm [9]. The nanofibers attained through electrospinning technique possess desirable characteristics like high

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surface area with interconnected pores [10,11]. Because of these characteristics, it could able to resemble the ECM matrix of the human tissue. Further, the nanofibers have the ability to support the cell adhesion and proliferation for new tissue generation [12]. The electrospinning has been widely used to electrospun different biodegradable polymers for scaffold fabrication such as poly-L-lactic Acid (PLLA), polycaprolactone (PCL), polyglycolic acid (PGA), polyurethane (PU), polylactic acid (PLA) and poly (lactic-*co*-glycolic) acid (PLGA). In this research, PU was used to fabricate the nanofibers [13]. PU was selected because it possesses biocompatibility, biodegradability, good barrier properties and better oxidation stability [14,15].

The scaffold used for bone tissue engineering should be bioactive to influence the cellular response and better mechanical strength to support the new tissue growth. Jaganathan et al. electrospun PU scaffold added with different oils namely corn, sunflower, grape seed oil for bone tissue engineering [16–18]. It was observed that the addition of oil enhanced the biocompatibility behaviour of the PU. From these studies, it was evident that the essential oil plays a critical role in enhancing the cellular response. Further, Silva et al. electrospun nanofibrous scaffold based on alginate loaded with magnesium oxide (MgO). It was reported that the addition of magnesium oxide resulted in the enhancement of mechanical strength [19]. Tobías et al. fabricated PLA scaffold incorporated with zinc oxide (ZnO) and showed that the addition of zinc oxide exhibited improvement of the tensile properties. Hence, the addition of metallic particles influenced the mechanical strength [20].

In this research rosemary (RM) oil and copper sulphate ($CuSO_4$) was selected as key constituents to fabricate the scaffold. Rosemary having botanical name *Rosmarinus officinalis L.* belongs to the Lamiaceae family which is a perennial shrub that found in several regions of the world. This oil reported having twenty bioactive compounds identified. The main constituents are 44.02% of p-cymene, 20.5% of linalool, 16.62% of g-terpinene, 1.81% of thymol, 3.61% of b-pinene, 2.83% of a-pinene and 2.64% of eucalyptol. The usage of rosemary oil is widely documented in traditional medicine for treating choleretic, colagoguic and also as a pulmonary antiseptic. It has also antidiarrhoic and antirheumatic properties [21]. Few studies had reported the antifungal and antioxidant activity of the rosemary [22]. To improve the mechanical strength of the scaffold, $CuSO_4$ was added into the electrospun scaffold. Scaffold containing copper particles showed increased antibacterial activity and also non-toxicity to the osteoprogenitor cells. Beyond their excellent antibacterial properties, the copper is reported to influencing the osteoblast activity, bone mineralization and enhancing the osteoblast cell adhesion and proliferation [23]. This study aims to electrospun and decipher the properties of the developed bone scaffold based on polyurethane added with RM and $CuSO_4$.

2. Experimental

2.1. Materials

PU with a grade name of Tecoflex EG 80A (Molecular weight (M_w) 1000 g/mol) was purchased from Lubrizol, Wickliffe, OH, USA. Dimethylformamide (DMF) was supplied from Merck, Burlington, NJ, USA. RM oil was procured from the local market. Copper sulphate ($CuSO_4{\cdot}5H_2O$) was supplied by Sigma-Aldrich, Sigma-Aldrich, Gillingham, UK. All coagulant reagents used in activated partial thromboplastin time (APTT) and prothrombin time (PT) assay were received from Diagnostic Enterprise, India.

2.2. Preparation of the PU and blend solutions

PU was dissolved in pure DMF at a weight fraction of 9 wt%. PU and RM complex (v/v = 8:1) solution was obtained by mixing homogenous RM solution (4 v/v%) in the PU homogeneous solution (9 wt%) respectively. Similarly PU, RM and $CuSO_4$ complex (v/v = 8:0.5:0.5) solution was obtained by mixing homogenous RM (4 v/v%) and $CuSO_4$ solution (4 wt%) in the PU homogeneous solution (9 wt%) respectively.

2.3. Fabrication of the scaffolds

The scaffolds form the homogeneous solution was prepared using electrospinning apparatus (Progene Link Sdn Bhd, Selangor, Malaysia). All homogenous solutions were electrospun at constant electrospinning parameters such as an applied voltage of 11 kV, collection distance of 20 cm and a flow rate of 0.3 ml/h respectively. The deposited fibers on the aluminium foil were dried under vacuum at room temperature to remove any residual DMF.

2.4. Characterizations

2.4.1. Field emission scanning electron microscopy

The images of the as-spun nanofibrous membrane were obtained by FESEM unit (Hitachi SU8020, Tokyo, Japan). The obtain images were transferred to the imaging software to determine the average fiber diameter by selecting 30 individual fibers randomly.

2.4.2. Infrared (IR) analysis

IR analysis was done to examine the functional groups present in the electrospun membranes and was examined in Nicolet iS 5, Thermo Fischer Scientific, Waltham, MA, USA. A small piece of the sample was recorded in wavelength between 600 and 4000 cm^{-1} at 32 scans per minute with a resolution of 4 cm^{-1}. Then, the obtained spectra were normalized using Spekwin 32 software to examine the chemical groups present in it.

2.4.3. Contact angle measurements

Surface wettability of the as-spun nanofibrous membrane was determined by static water contact angle measurement unit (AST Products, Inc., Billerica, MA, USA). The liquid used was distilled water and a droplet of 0.5 µl was dropped on the electrospun scaffolds. The static image of the water droplet was visualized and captured using a video camera. The manual angles between the surface and water droplet was determined computer integrated software and the experiments were repeated for 3 trials.

2.4.4. Mechanical properties test

The tensile properties of the as spun nanofibrous membrane were tested using universal machine tester (Gotech Testing Machines, AI-3000). All samples with a size of 40 mm × 15 mm was cut and clamped at the grip end of the tester machine. The gauge length utilized was 20 mm and the specimen was pulled at a strain rate of 10 mm/min until failure. The machine recorded data displays the tensile curve from which average strength was determined.

2.4.5. AFM analysis

Further, the surface analysis was performed through atomic force microscopy unit (NanoWizard®, JPK Instruments, Berlin, Germany). The scanning of the fibres was performed under a normal atmosphere in tapping mode. The scanned size was 20 * 20 µm area with 256-sample resolution.

2.4.6. Thermal characterisation

TGA analysis was carried out in PerkinElmer, Waltham, MA, USA to determine the thermal degradation of the fabricated membranes. In this study, the degradation of samples was evaluated by heating the sample at a rate of 10 °C/min in the temperature between 30 °C and 1000 °C under normal atmosphere.

2.5. Coagulation assays

APTT, PT and hemolysis assay were performed to investigate the

blood compatibility of the electrospun membranes. The assays were done according to the protocol as reported previously [17].

2.6. Cell viability and proliferation

Fibroblast cells were grown in Dulbecco's Modified Eagle Media (DMEM) supplied with 10% fetal bovine serum (FBS). Cells were incubated at 37°C and 5% carbon dioxide (CO_2) and the medium was replaced every 5 days. Electrospun scaffolds with small size were cut and placed in 96-well plates. After cells were grown, it was seeded onto the electrospun mats at 10 * 10^3 cells/cm^2 density. Cell viability was monitored on day 5 after cell seeding. At the stated time point, cell cultured on the electrospun mats were taken out and incubated with 20% MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3- carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H tetrazolium, inner salt) for 4 h. Finally, the spectrophotometric plate reader used to record the absorbance at 490 nm which determines cell viability rates.

2.7. Statistical analysis

All experiments were done thrice independently. One way ANOVA followed by dunnett *post hoc* test which was performed in GraphPad Prism 6.0 to calculate the statistical significance ($p < 0.05$). The attained results are expressed as mean ± SD. In case for qualitative experiments, an illustrative of three images is indicated.

3. Result and discussion

3.1. FESEM investigation

Fig. 1 depicts the FESEM micrographs and fiber diameter distributions of as spun PU, PU/RM, and PU/RM/$CuSO_4$ prepared by electrospinning technique. The images depicted that the scaffolds are non-woven having smooth, bead les fibres with randomly oriented structure. The electrospun pure PU nanofibers had an average diameter of 875 ± 154 nm, while as spun PU/RM and PU/RM/$CuSO_4$ nanofibers exhibited diameter of 745 ± 133 nm and 414 ± 156 nm respectively. Further, the average size of the copper particles in the electrospun PU/RM/$CuSO_4$ was reported to be 276 ± 184 nm. The results depicted that the incorporation of RM and $CuSO_4$ resulted in the reduction of the size of the fiber. The added constituents play a major role in influencing the properties of the electrospun solution. Further, the decrease in the fiber diameter of the pristine PU was due to the change in the viscosity or conductivity while adding RM oil and $CuSO_4$ [24,25]. Further, few studies have been reported that the decrease in the polymer concentration will result in the smaller fiber diameter [26,27]. The decrease in the fiber diameter was might also due to the decreasing in the polymer concentration while adding and respectively. Prabhakaran et al. reported that the electrospun scaffold with smaller fiber diameter would exhibit improved proliferation of osteoblast cell [28]. Since, our smaller fiber diameter of the developed nanocomposites might favour the new bone tissue growth. Further Energy-dispersive X-ray spectroscopy (EDX) study was carried to confirm the presence of copper in the polyurethane matrix. As shown in Fig. 2, it was evident that the polyurethane matrix contains copper content (1.9%) in the PU/RM/$CuSO_4$.

3.2. FTIR analysis

The characteristics peaks associated with PU, PU/RM and PU/RM/$CuSO_4$ were indicated in Fig. 3a. The PU scan showed a broad peak fixed at 3323 cm^{-1} indicates the NH stretch and its vibrations are at 1531 cm^{-1} and 1597 cm^{-1} respectively. The bands were seen at 2940 cm^{-1} and 2854 cm-1 denotes the CH stretch and the peaks seen at 1414 cm^{-1} attributes to the CH vibrations. The peaks located in 1702 and 1730 cm^{-1} is attributed to the carboxylic CO group. Further, the characteristic peak was depicted between 500 and 1200 cm^{-1} region. The peaks 1220 cm^{-1}, 1105 cm^{-1}, 1078 cm^{-1} and 770 cm^{-1} represents the characteristics peaks of PU and it might be ascribed to the C–O–C and C–OH stretch respectively [29–31]. The spectra of PU/RM and PU/RM/$CuSO_4$ showed the same peaks like that of pristine PU and there was no new peak formation with the addition of RM and $CuSO_4$. However, there was alteration in peak intensity of PU while the incorporation of RM and $CuSO_4$. It was observed that the CH peak intensity of the PU was increased with the addition of RM and $CuSO_4$ which might due to strong hydrogen formation as shown in Fig. 3b. It has been reported that the formation of inter hydrogen bonding between two different macromolecules was stronger than the bonding between the molecules of the same polymer [32]. The formation of hydrogen bond was because of linking of CH and NH molecules present in the PU with the components of PU and $CuSO_4$. The strong hydrogen bond formation confirms the presence of RM and $CuSO_4$ in the polyurethane matrix.

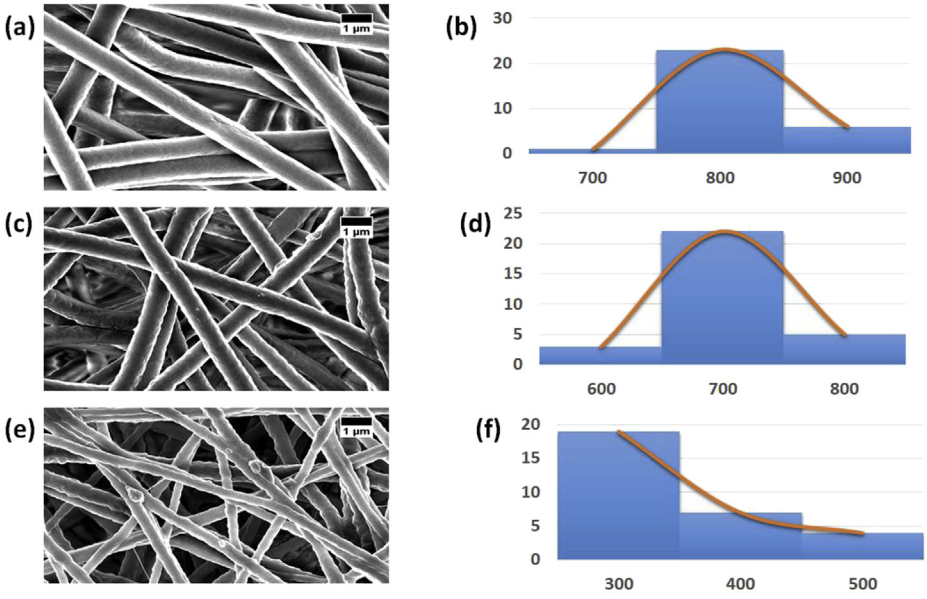


Fig. 1. FESEM images of a) PU, c) PU/RM and e) PU/RM/$CuSO_4$ and fiber diameter distribution of b) PU, d) PU/RM and f) PU/RM/$CuSO_4$.

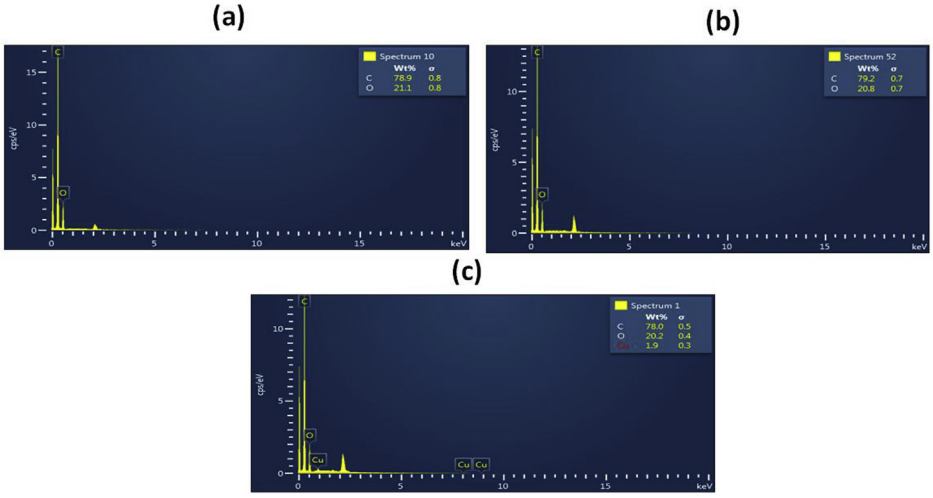


Fig. 2. EDX of a) PU, b) PU/RM and c) PU/RM/$CuSO_4$.

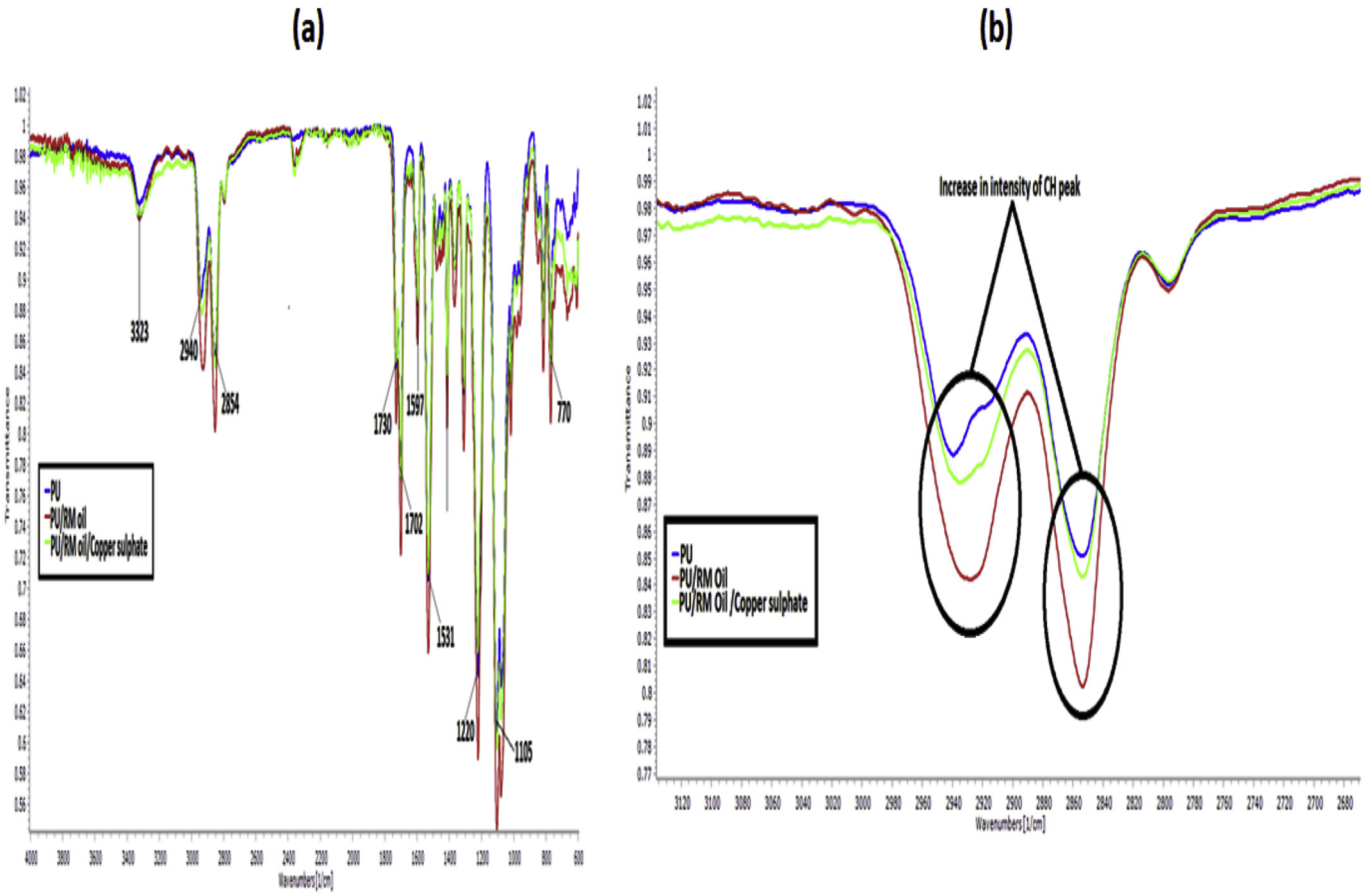


Fig. 3. a) FTIR spectrum of PU, PU/RM and PU/RM/$CuSO_4$ and b) Change in CH peak intensity of the composite membranes.

3.3. Contact angle measurements

The wettability measurements of the electrospun membranes were presented and their images were shown in Fig. 4. As observed the contact angle of PU scaffold was about 106 ± 3° which suggests that the scaffold is hydrophobic. On adding RM, the angle was increased to 113 ± 1° which was highly hydrophobic than the pure PU. While adding $CuSO_4$, the contact angle was decreased to 79 ± 6° suggesting hydrophilic behaviour. Hence, the addition of $CuSO_4$ improved the wettability of the scaffold. It was reported that the wettability in the range of is optimum for an improved cellular response for new tissue growth. While adding RM to the polyurethane matrix, the contact angle was beyond the reported range (106°) which might reduce the cellular response [33]. On another hand, while reinforcing $CuSO_4$ to the PU/RM resulted in improved wettability having angle with in the reported optimal range (less than 106°) and might be suitable for the improved cellular response for the new tissue growth. Hassan et al. electrospun a bone scaffold utilizing poly (ϵ-caprolactone) and hydroxyapatite nanofibers. It was found that the PCL/hydroxyapatite matrix showed improved wettability than the pristine PCL and concluded that the

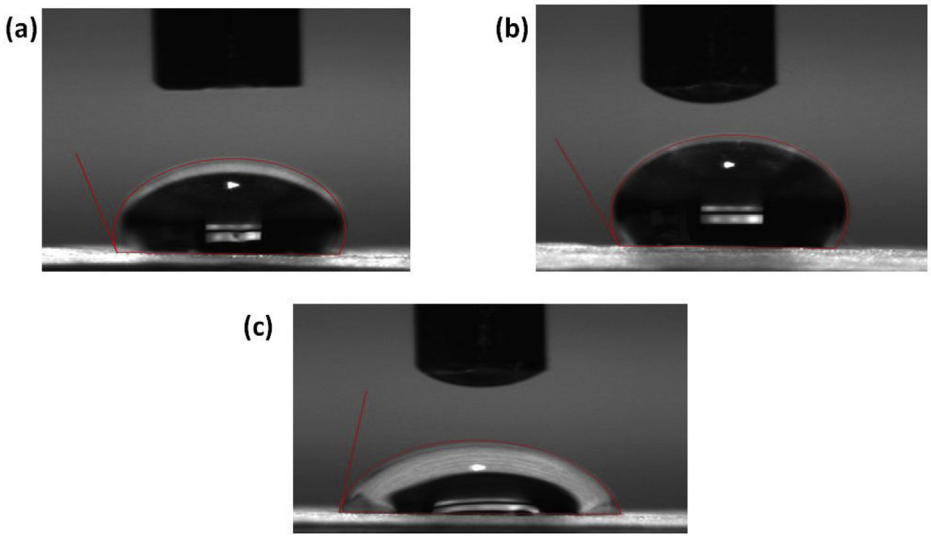


Fig. 4. Contact angle images of a) PU, b) PU/RM and c) PU/RM/$CuSO_4$.

improved wettability might improve osteoblast growth [34]. Hence, our hydrophilic behaviour of the PU/RM/$CuSO_4$ might be suitable for the osteoblast cell growth.

3.4. Thermal behaviour

The thermal degradation behaviour of PU, PU/RM and PU/RM/$CuSO_4$ nanofibrous scaffolds are shown in Fig. 5. TGA curve patterns of the electrospun polyurethane and its composites were not similar indicating the presence of added constituents. Further, it was clearly observed that the degradation temperature of PU/RM was higher than pristine PU and but for PU/RM/$CuSO_4$ it was lower than PU samples. The initial decomposition temperature of the PU was found to 266 °C and for electrospun PU/RM and PU/RM/$CuSO_4$, it was reported to 277 °C and 230 °C respectively. The results showed the incorporation of RM enhanced the thermal stability of the pristine PU while adding $CuSO_4$ it decreases the thermal stability. The decrease in the thermal stability was might due to the evaporation of water molecules present in the copper sulphate pentahydrate which is similar to the recently reported works [35,36]. Further, the pristine PU exhibited remaining residual weight percentage of 16.46%, while the electrospun PU/RM and PU/RM/$CuSO_4$ showed remaining weight residual percentage of

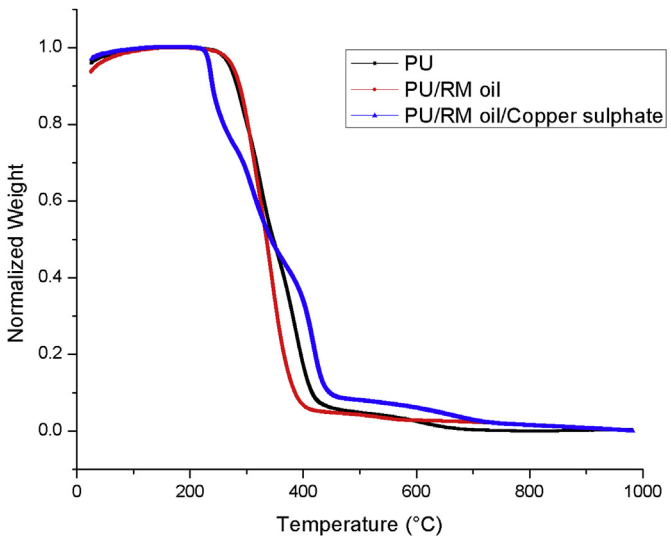


Fig. 5. TGA of PU, PU/RM and PU/RM/$CuSO_4$.

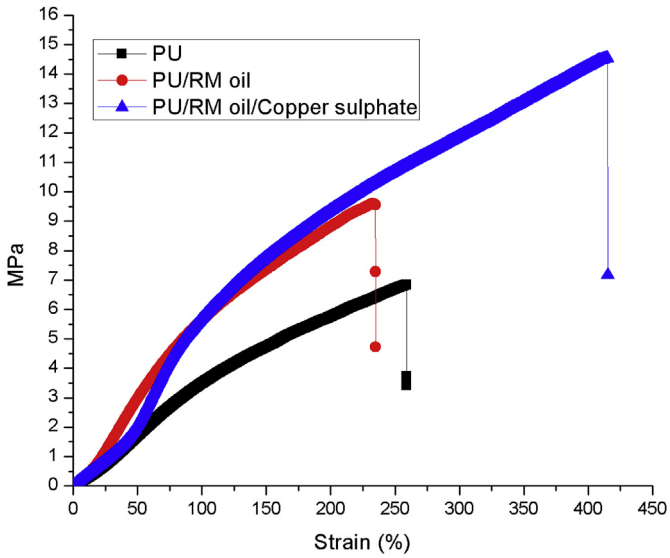


Fig. 6. Tensile strength of PU, PU/RM and PU/RM/$CuSO_4$.

8.09% and 15.14% respectively.

3.5. Mechanical testing

The typical stress-strain curves of PU and PU reinforced with RM and $CuSO_4$ nanofibers are presented in Fig. 6. The results of the mechanical tests depicted that the tensile strength of reinforced composites was increased compared to the pristine PU. The pure PU showed an average tensile strength of 6.83 MPa, while reinforcing RM and $CuSO_4$, the strength was increased to 9.60 MPa and 14.54 MPa respectively. The enhancement of the tensile strength was due to the adhesion of fibers resulting in bonding between the polyurethane and RM oil molecules. This is reflected in FTIR study as identified by the formation of strong hydrogen bond in the fabricated composites. Similar observations were reported in a recent work by Unnithan et al. who had ascribed the enhanced tensile strength of PU/emu oil composite to the adhesive property of emu oil [32]. To further add, Jaganathan et al. developed polyurethane scaffold incorporated with zinc nitrate. They attributed the enhanced tensile strength to their smaller fiber diameter of the fabricated composites [36]. Concurrently, Prabhakaran et al. reported that the scaffold with smaller fiber diameter

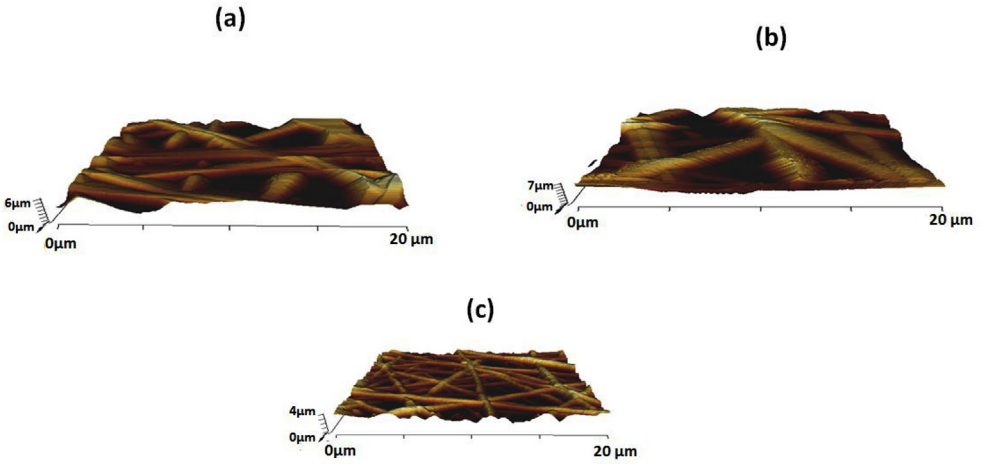


Fig. 7. AFM images of a) PU, b) PU/RM and c) PU/RM/$CuSO_4$.

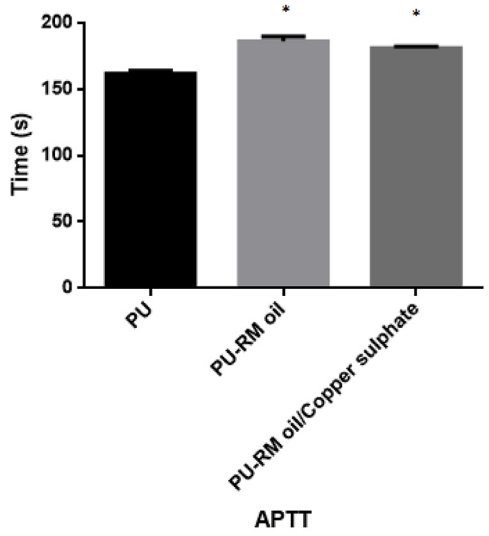
results in reduced pore size [37]. Hence, the fabricated composites with smaller fiber diameter might possess low pore size which had played a putative role in the improved tensile strength. Salifu et al. reported tensile strength in the range of 4–10 MPa suitable for bone tissue growth [38]. Our developed nanocomposites showed better tensile values than the reported representing its suitability for bone tissue engineering.

3.6. AFM analysis

AFM analysis for as-spun membranes was done to examine their surface properties and 3D images of the electrospun membranes were indicated in Fig. 7. Surface measurements depicted that the as-spun PU membranes exhibited average surface roughness of 776 nm, while the PU/RM and PU/RM/$CuSO_4$ showed the roughness of 1052 nm and 310 nm respectively. The addition of RM showed rougher surfaces and while adding $CuSO_4$ the surface becomes smoother. Addition of oil resulted in the enhanced surface roughness of the pristine PU. This might be attributed to the coarse active constituents present in the RM oil. However, with the addition of $CuSO_4$, it resulted in the smoother surfaces which might be due to the interaction of RM oil constituents with the $CuSO_4$ particles. Ribeiro et al. developed poly (L-lactide) membranes and investigated the influence of surface roughness on osteoblast cell response. It was reported that the fabricated membranes showed enhanced osteoblast cell response with lower surface roughness compared to the higher roughness surfaces [39]. Hence, our electrospun nanocomposites displayed lower surface roughness which might facilitate the improved osteoblast cell adhesion and proliferation.

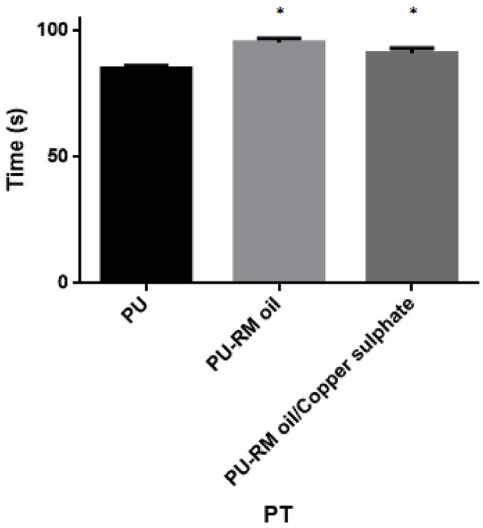
3.7. Blood compatibility measurements

Fig. 8 and Fig. 9 depicts the APTT and PT assay for the PU/RM and PU/$CuSO_4$. The results of APTT and PT assay depicted that the prepared nanocomposites displayed prolonged blood clotting time because of the addition of RM and $CuSO_4$ into the PU matrix. In APTT assay, the clotting time for the PU membrane was 162 ± 2 s, while for the electrospun PU/RM and PU/RM/$CuSO_4$ scaffold, clotting time was observed to be 186 ± 4 s and 181 ± 1 s and respectively. Similarly in PT assay, the clotting time for the PU membrane was 85 ± 1 s, while for the electrospun PU/RM and PU/RM/$CuSO_4$ scaffold, clotting time was observed to be 95 ± 2 s and 91 ± 2 s and respectively. Further, the hemolysis study was to investigate the osmotic stress of the electrospun membranes on the RBCs. The hemolytic percentage of pristine PU was 2.58% and for the PU/RM and PU/$CuSO_4$ scaffold, the measured hemolytic index was 1.63% and 1.70% respectively as shown in Fig. 10 suggesting less toxic behaviour of the fabricated nanocomposites with



*mean differences were significant compared with pure PU ($p < 0.05$)

Fig. 8. APTT assay of PU, PU/RM and PU/RM/$CuSO_4$.



*mean differences were significant compared with pure PU ($p < 0.05$)

Fig. 9. PT assay PU, PU/RM and PU/RM/$CuSO_4$.

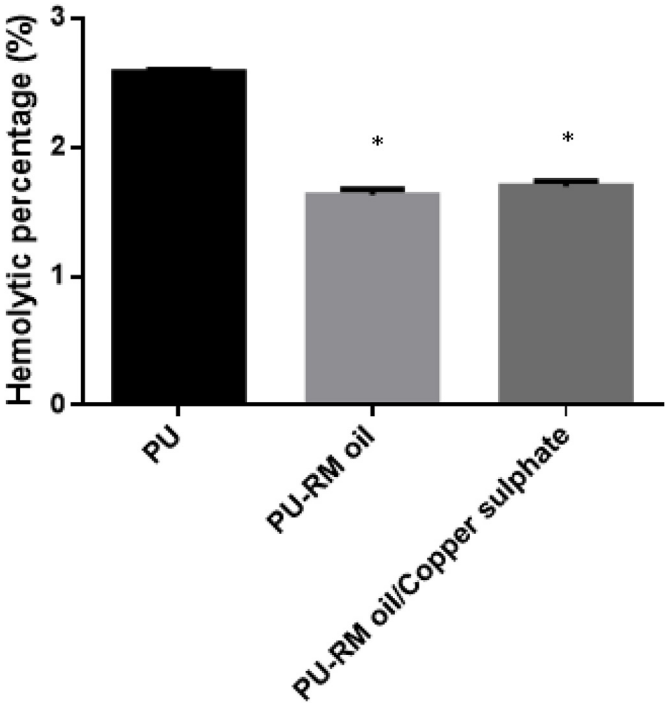
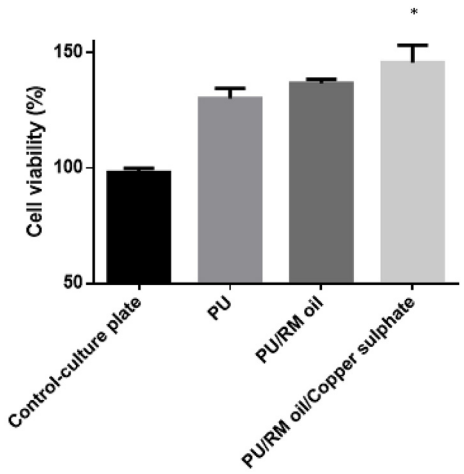


Fig. 10. Hemolytic assay PU, PU/RM and PU/RM/$CuSO_4$.

RBC. Moreover, the developed nanocomposites were assumed to be non-hemolytic material because its calculated index was less than 2% according to ASTMF756-00 (2000) [16–18]. Hydrophobic surfaces seem to adhere protein irreversibly thereby promoting the blood compatibility of the PU/RM composites significantly. Due to the addition of $CuSO_4$, the surface of composite shifted towards hydrophilicity. Hydrophilic surfaces tend to bind more proteins unspecifically which may because for the reduced blood compatibility of the PU/RM/$CuSO_4$ [40]. It has been reported that the blood compatibility of fabricated material is influenced by multiple physico-chemical properties (surface roughness, surface energy, surface tension, surface wettability, and fiber diameters) rather than a single factor. Jaganathan et al. investigated the physico-chemical and blood compatibility properties of electrospun polyurethane added with castor oil. It was reported that the polyurethane/castor oil membranes showed improved blood clotting time compared to pure polyurethane owing to an increase in surface roughness [41]. In another study, Jaganathan et al. electrospun polyurethane scaffold added with grape seed oil, honey and propolis nanofibers. It was reported that the grape seed oil incorporation improved the anticoagulant nature of the pristine PU which correlates with our findings. They attributed the enhanced blood compatibility to smaller fiber diameter and hydrophobic behaviour of the fabricated composites [18]. From these literature, it was evident that the prediction of a single parameter which can influence the blood compatibility is a complicated task. Hence, the enhanced blood compatibility behaviour of the fabricated composites might be due to reduced fiber diameter (PU/RM and PU/RM/$CuSO_4$), rough surfaces (PU/RM) and hydrophobic nature (PU/RM) of electrospun composites.

3.8. Cytocompatibility measurements

The toxicity of the electrospun scaffold with HDF cells was investigated using MTS assay as shown in Fig. 11. It was reported that the HDF cells viability in all electrospun membranes were increased compared to the control plates. After 5 days culture, the electrospun PU/RM and PU/RM/$CuSO_4$ scaffold exhibited cell viability of 137 ± 2% and 146 ± 8%, while the pristine PU membrane showed cell viability rate of 130 ± 4%. The adhesion and proliferation of cells on the developed



*mean differences were significant compared with pure PU ($p < 0.05$)

Fig. 11. MTS assay PU, PU/RM and PU/RM/$CuSO_4$.

scaffold is a multifactorial process and it was influenced by various physico-chemical properties such fiber diameter, wettability, surface roughness, surface energy and surface chemistry [32,42–45]. It has been reported scaffold with reduced fiber diameter [32], hydrophilic behaviour [42] and increase surface roughness [43] will favour the enhanced cellular response. Our fabricated composites showed smaller fiber diameter (PU/RM and PU/RM/$CuSO_4$) morphology, hydrophilic behaviour (PU/RM/$CuSO_4$) and increase surface roughness (PU/RM) which might have favoured the enhanced cellular response than the pristine PU.

4. Conclusion

In this study, for the first time electrospun nanofibers scaffold using polyurethane (PU), rosemary (RM) oil and copper sulphate ($CuSO_4$) was fabricated. The results obtained showed that the mean diameter of PU nanofibers was reduced with the addition of RM and $CuSO_4$. PU interactions with RM and $CuSO_4$ was confirmed through FTIR and TGA analysis. Wettability measurements showed that the contact angle increased for PU/RM indicating hydrophobic and decreased for PU/RM/$CuSO_4$ suggesting hydrophilic. TGA study depicted that the thermal behaviour of PU with the addition of RM and decreased while incorporating $CuSO_4$.. The electrospun PU/RM composites showed the increased surface roughness and for PU/RM/$CuSO_4$ composites, the roughness was decreased. In the mechanical tests, the electrospun nanofibrous composites showed higher tensile strength than the pristine PU. The blood clotting time of the pristine PU was prolonged with the addition of RM and $CuSO_4$ as revealed in the coagulation study. Moreover, the cell proliferation increased for the electrospun composites than the pristine PU. Therefore, the desirable properties of electrospun nanocomposites may play a conducive role in bone tissue engineering. In future, it would be interesting to investigate the cell viability using osteoblast cells to assess their potential for bone tissue engineering.

Conflicts of interest

The authors declare that they have no conflict of interest.

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References

[1] K.L. Ong, New biomaterials for orthopedic implants, Int. J. Nanomed. 7 (2015) 107–130.
[2] L. Zhao, J.L. Zhao, L. Wan, S.K. Wang, The study of the feasibility of segmental bone defect repair with tissue- engineered bone membrane: a qualitative observation, Strat. Trauma. Limb. Reconstr. 3 (2) (2008) 57–64.
[3] Available at: http://market-analysis.conferenceseries.com/bonetissue-engineering-market-reports.
[4] Z. Heydari, D. Mohebbi-Kalhori, M.S. Afarani, Engineered electrospun polycaprolactone (PCL)/octacalcium phosphate (OCP) scaffold for bone tissue engineering, Mater. Sci. Eng. C 81 (2017) 127–132.
[5] Q. Zhang, S. Lv, J. Lu, S. Jiang, L. Lin, Characterization of polycaprolactone/collagen fibrous scaffolds by electrospinning and their bioactivity, Int. J. Biol. Macromol. 76 (2015) 94–101.
[6] S.K. Jaganathan, M.M. Prasath, UV induced surface modification on improving the cytocompatibility of metallocene polyethylene, An Acad. Bras Ciências 90 (1) (2018) 195–204.
[7] B. Dhandayuthapani, Y. Yoshida, T. Maekawa, D.S. Kumar, Polymeric scaffolds in tissue engineering application: a review, Int. J. Polym. Sci. (2011) 2011.
[8] Z.M. Huang, Y.Z. Zhang, M. Kotaki, S. Ramakrishna, A review on polymer nanofibers by electrospinning and their applications in nanocomposites, Compos. Sci. Technol. 63 (15) (2003) 2223–2253.
[9] R. Bhattarai, R. Bachu, S. Boddu, S. Bhaduri, Biomedical applications of electrospun nanofibers: drug and nanoparticle delivery, Pharm. Times 11 (1) (2019) 5.
[10] T. Subbiah, G.S. Bhat, R.W. Tock, S. Parameswaran, S.S. Ramkumar, Electrospinning of nanofibers, J. Appl. Polym. Sci. 96 (2) (2005) 557–569.
[11] Z.M. Huang, Y.Z. Zhang, M. Kotaki, S. Ramakrishna, A review on polymer nanofibers by electrospinning and their applications in nanocomposites, Compos. Sci. Technol. 63 (15) (2003) 2223–2253.
[12] S.C. Wu, W.H. Chang, G.C. Dong, K.Y. Chen, Y.S. Chen, C.H. Yao, Cell adhesion and Proliferation enhancement by gelatin nanofiber scaffolds, J. Bioact. Compat Polym. 26 (6) (2011 Nov) 565–577 2011.
[13] J. Wang, L. Wang, Z. Zhou, L. Lai, P. Xu, L. Liao, J. Wei, Biodegradable polymer membranes applied in guided bone/tissue regeneration: a review, Polymer 29 8 (4) (2016) 115.
[14] Avaiblabe at: https://polymerdatabase.com/polymer%20classes/Polyurethane%20type.html.
[15] Z. Shen, D. Lu, Q. Li, Z. Zhang, Y. Zhu, Synthesis and characterization of biodegradable polyurethane for hypopharyngeal tissue engineering, BioMed Res. Int. (2015) 2015.
[16] S.K. Jaganathan, M.P. Mani, G. Nageswaran, N.P. Krishnasamy, M. Ayyar, Single stage electrospun multicomponent scaffold for bone tissue engineering application, Polym. Test. 70 (2018) 244–254.
[17] S.K. Jaganathan, M.P. Mani, S.K. Palaniappan, R. Rathanasamy, Fabrication and characterisation of nanofibrous polyurethane scaffold incorporated with corn and neem oil using single stage electrospinning technique for bone tissue engineering applications, J. Polym. Res. 25 (7) (2018) 146.
[18] C.Y. Chao, M.P. Mani, S.K. Jaganathan, Engineering electrospun multicomponent polyurethane scaffolding platform comprising grapeseed oil and honey/propolis for bone tissue regeneration, PLoS One 13 (10) (2018) e0205699.
[19] R.T. De Silva, M.M. Mantilaka, K.L. Goh, S.P. Ratnayake, G.A. Amaratunga, K.M. de Silva, Magnesium oxide nanoparticles reinforced electrospun alginate-based nanofibrous scaffolds with improved physical properties, Int. J. Biom. (2017) 2017.
[20] H. Rodríguez-Tobías, G. Morales, A. Ledezma, J. Romero, D. Grande, Novel antibacterial electrospun mats based on poly (d, l-lactide) nanofibers and zinc oxide nanoparticles, J. Mater. Sci. 49 (24) (2014) 8373–8385.
[21] M.M. Özcan, J.C. Chalchat, Chemical composition and antifungal activity of rosemary (Rosmarinus officinalis L.) oil from Turkey, Int. J. Food Sci. Nutr. 59 (7–8) (2008) 691–698.
[22] G. Nieto, G. Ros, J. Castillo, Antioxidant and antimicrobial properties of rosemary (Rosmarinus officinalis, L.), Rev. Med. (Paris) 5 (3) (2018) 98.
[23] J.C. Forero, E. Roa, J.G. Reyes, C. Acevedo, N. Osses, Development of useful biomaterial for bone tissue engineering by incorporating nano-copper-zinc alloy (nCuZn) in chitosan/gelatin/nano-hydroxyapatite (ch/G/nHAp) scaffold, Materials 10 (10) (2017) 1177.
[24] L.R. Lakshman, K.T. Shalumon, S.V. Nair, R. Jayakumar, S.V. Nair, Preparation of silver nanoparticles incorporated electrospun polyurethane nano-fibrous mat for wound dressing, J. Macromol. Sci. Pure Appl. Chem. 47 (10) (2010) 1012–1018.
[25] H. Maleki, A.A. Gharehaghaji, P.J. Dijkstra, A novel honey-based nanofibrous scaffold for wound dressing application, J. Appl. Polym. Sci. 127 (2013) 4086–4092 2013.
[26] G. Eda, Effects of Solution Rheology on Electrospinning of Polystyrene, MSc Thesis Worcester Polytechnic Institute., 2006.
[27] S.H. Tan, R. Inai, M. Kotaki, S. Ramakrishna, Systematic parameter study for ultrafine fiber fabrication via electrospinning Process, Polymer 46 (2005) 6128.
[28] M.P. Prabhakaran, J. Venugopal, S. Ramakrishna, Electrospun nanostructured scaffolds for bone tissue engineering, Acta Biomater. 5 (2009) 2884–2893.
[29] A. Mohammadi, M. Barikani, M. Barmar, Synthesis and investigation of thermal and mechanical properties of in situ prepared biocompatible Fe_3O_4/polyurethane elastomer nanocomposites, Polym. Bull. 72 (2) (2015) 219–234 2015.
[30] L. Jia, M.P. Prabhakaran, X. Qin, S. Ramakrishna, Guiding the orientation of smooth muscle cells on random and aligned polyurethane/collagen nanofibers, J. Biomater. Appl. 29 (2014) 364–377 2014.
[31] S.E. Kim, D.N. Heo, J.B. Lee, J.R. Kim, S.H. Park, S.H. Jeon, I.K. Kwon, Electrospun gelatin/polyurethane blended nanofibers for wound healing, Biomed. Mater. 4 (2009) 0441062009.
[32] A.R. Unnithan, P.T. Pichiah, G. Gnanasekaran, K. Seenivasan, N.A. Barakat, Y.S. Cha, C.H. Jung, A. Shanmugam, H.Y. Kim, Emu oil-based electrospun nanofibrous scaffolds for wound skin tissue engineering, Colloid. Surf. Physicochem. Eng. Asp. 415 (2012) 454–460.
[33] J. Wei, T. Igarashi, N. Okumori, T. Igarashi, T. Maetani, B. Liu, M. Yoshinari, Influence of surface wettability on competitive Protein adsorption and initial attachment of osteoblasts, Biomed. Mater. 4 (2009) 045002.
[34] M.I. Hassan, N. Sultana, S. Hamdan, Bioactivity assessment of poly (e-caprolactone)/hydroxyapatite electrospun fibers for bone tissue engineering application, J. Nanomater. 8 (2014) 1–6.
[35] S.K. Jaganathan, M.P. Mani, Electrospun polyurethane nanofibrous composite impregnated with metallic copper for wound-healing application, 3 Biotechol 8 (8) (2018 Aug 1) 327 2018.
[36] S.K. Jaganathan, M.P. Mani, Single-stage synthesis of electrospun polyurethane scaffold impregnated with zinc nitrate nanofibers for wound healing applications, J. Appl. Polym. Sci. 136 (3) (2019) 46942.
[37] M.P. Prabhakaran, D. Kai, L. Ghasemi-Mobarakeh, S. Ramakrishna, Electrospun biocomposite nanofibrous patch for cardiac tissue engineering, Biomed. Mater. 6 (2011) 0550012011.
[38] A.A. Salifu, C. Lekakou, F.H. Labeed, Electrospun oriented gelatin-hydroxyapatite fiber scaffolds for bone tissue engineering, J. Biomed. Mater. Res. A 105 (7) (2017) 1911–1926.
[39] C. Ribeiro, V. Sencadas, A.C. Areias, F.M. Gama, S. Lanceros-Méndez, Surface roughness dependent osteoblast and fibroblast response on poly (l-lactide) films and electrospun membranes, J. Biomed. Mater. Res. A 103 (7) (2015) 2260–2268.
[40] M. Szycher, High Performance Biomaterials: A Complete Guide to Medical and Pharmceutical Applications, CRC Press., 1991.
[41] S.K. Jaganathan, M.P. Mani, M. Ayyar, E. Supriyanto, Engineered electrospun polyurethane and castor oil nanocomposite scaffolds for cardiovascular applications, J. Mater. Sci. 52 (18) (2017 Sep 1) 10673–10685 2017.
[42] M.P. Mani, S.K. Jaganathan, A.F. Ismail, Appraisal of electrospun textile scaffold comprising polyurethane decorated with ginger nanofibers for wound healing applications, J. Ind. Text. (2018), https://doi.org/10.1177/1528083718795911.
[43] F. Sharifi, S. Irani, M. Zandi, M. Soleimani, S.M. Atyabi, Comparative of fibroblast and osteoblast cells adhesion on surface modified nanofibrous substrates based on polycaprolactone, Prog. Biomater. 5 (3–4) (2016) 213–222 2016.
[44] K. Anselme, L. Ploux, A. Ponche, Cell/material interfaces: influence of surface chemistry and surface topography on cell adhesion, J. Adhes. Sci. Technol. 24 (5) (2010) 831–852 2010.
[45] N.J. Hallab, K.J. Bundy, K. O'Connor, R.L. Moses, J.J. Jacobs, Evaluation of metallic and polymeric biomaterial surface energy and surface roughness characteristics for directed cell adhesion, Tissue Eng. 7 (1) (2001) 55–71 2001.