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Polyurethane foam is currently used as an exudate absorptive wound dressing material. In this study silver (Ag) nanoparticles were incorporated into electrospun polyurethane (PU) nanofiber to enhance the antibacterial as well as wound healing properties. The electrospinning parameters were optimized for PU with and without silver nanoparticles. Silver nanoparticles were synthesized by aqueous and organic methods. The water absorption, antibacterial and cytocompatibility of the PU-Ag nanofibers were studied and compared to that of conventional PU foam. The results indicated that the PU-Ag nanofibers could be used for wound dressing applications.

Keywords: Polyurethane nanofibers, silver nanoparticles, antibacterial, wound healing

1 Introduction

Silver has good antimicrobial activity with a broad-spectrum activity. As an antiseptic, it has a far lower propensity to induce bacterial resistance than antibiotics. The medicinal use of silver has been exploited for over 2000 years and it has been in common use as an antimicrobial since the 19th century. Bacterial resistance to silver is extremely low compared to other antimicrobial agents that help in accentuating wound healing. In wounds, silver decreases surface inflammation, surface metalloprotease activity, exudates matrix metalloprotease (MMP) activity and promote zinc for utilization in wound healing. Silver increases wound surface calcium thereby stimulating epithelialization. Nelson Durán has studied the antibacterial effect of silver nanoparticles (1) and demonstrated the application of biological synthesis to silver nanoparticles production and its incorporation in cloths, providing them sterile properties.

In order to make use of these properties of silver (Ag) in wound healing, it was incorporated in polyurethane (PU), a polymer formed by the reaction of a monomer with at least two isocyanate functional groups with another monomer containing at least two alcohol groups in the presence of a catalyst. They represent a major class of synthetic elastomers applied for long term medical implants (2). PU has tunable chemical and excellent mechanical properties, good biocompatibility and can be designed to degrade in a biological environment. L-lysine derived diisocyanates (LDI) or 1,4-diisocyanatobutane is an example of a medical grade PU that does not release toxic diamines like conventional isocyanates during their degradation (3).

PU foams have properties such as mechanical strength, swelling ratio and cell adhesion that aid in wound healing application. It also showed that PU foams containing alginate and hyaluronic acid with AgSD showed good wound healing (4). Recent studies showed that PU Ag nanocomposite have a free radical scavenging effect compared to plain PU (5).

Electrospinning is now an established technology which helps produce fibers in the micro and nanometer range, a size range otherwise difficult to access by conventional non-woven fiber fabrication techniques (6). Electrospinning has been used for applications such as filtration of subatomic particles, tissue engineering, wound dressings, drug delivery, artificial organs and vascular grafts (7–10). Electrospun nanofiber scaffolds possess an extremely high

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surface-to-volume ratio, tunable porosity and malleability to conform over a wide variety of sizes and shapes. The pore size within an electrospun nanofiber mesh allows it to be an excellent candidate for wound healing, haemostatic devices, and burn treatments. Similarity between electrospun nanofibers and the natural extracellular matrix allows it to support new healthy tissue growth in an injured area, which can reduce the formation of scar tissue and decrease the healing time required. Nano-pore sizes also help to protect injured tissue from bacteria that could otherwise infect a vulnerable wounded tissue. High porosity and surface area encourage fluid absorption and wound healing (11–15).

In our study, PU was electrospun and the electrospinning parameters were optimized. Silver nanoparticles were synthesized through two routes; aqueous and organic was incorporated into PU solution and PU-Ag nanofibers were produced by electrospinning with the standardized protocol. Material characterization was done by UV-spectrophotometer. The fiber size of the silver incorporated PU fiber was compared to PU nanofiber. A comparative study on water retention was done between sponge, PU nanofiber mat and Ag incorporated PU mat. Antibacterial and cytotoxicity studies were performed to determine the effectiveness of PU-Ag nanofiber mat for future use in wound dressings and prosthetic materials.

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2 Experimental

2.1 Materials

Polyurethane beads of medical grade were obtained from Dupont (UK). Silver nitrate and Sodium borohydrate was purchased from Sigma Aldrich, India. DMF (Dimethyl Formamide) and THF (Tetrahydrofuran) were purchased from Qualigens, India and all products were of medical grade. All reagents were used as received without further purification.

2.2 Synthesis of Electrospun PU Fibers

The PU nanofibers were prepared by an electrospinning technique. The conditions required for efficient electrospinning of PU fibers for various concentrations (4, 6, 8, 10, 12 and 16%) of PU in THF was optimized. The parameters optimized include distance, voltage, and flow rate at room temperature. The viscosity and conductivity of the solution was also measured (Table 1).

Table 1. The viscosity and conductivity values of PU solutions

PU (wt%)	16	12	10	8	6	4
Viscosity (cP)	658	343	147	90	36	28
Distance (cm)	10	10	10	10	10	10
Voltage (kW)	13	13	13	13	13	13
Flow rate (mL/hr)	1	1	1	1	1	1

2.3 Methods of Silver Incorporation in Nanofibers

2.3.1. *Preparation of Silver Nanoparticles Incorporated PU Nanofibers by Organic Method*

Silver nanoparticles were synthesized by organic method using DMF as a reducing agent using the following reaction:

$$CONMe_2+2Ag^+ + H_2O \longrightarrow 2Ag^0 + Me_2NCOOH + 2H^+ \quad (1)$$

The reduction of Ag^+ ions by *N*, *N*-dimethylformamide (DMF), in the presence of PU yields silver nanoparticles. To prepare silver nanoparticles, 500 ml of 0.01 wt% $AgNO_3$ in DMF was mixed with 4 μl of 0.25 wt% sodium borohydride in DMF under constant stirring. To this solution, PU was added to attain a final concentration of 12%. The PU solution was stirred for 4 h to make it homogenous. This PU solution resulted in the formation of a brown colored solution. This was characterized using UV spectroscopy. The prepared 0.015, 0.010, 0.005 wt% silver nanoparticles containing PU solution were electrospun at suitable conditions. The parameters for electrospinning were optimized again due to the higher conductivity of PU solution containing silver. The flow rate of the solution was reduced to 0.1 ml/hr.

2.3.2. *Preparation of Silver Nanoparticles Incorporated PU Nanofibers by the Aqueous Method*

To synthesize colloidal Ag by the aqueous route, $AgNO_3$ can be reduced and capped using PEI to produce Ag nanoparticles. Equal amounts of 0.1 M Ag NO_3 and 1 wt% PEI were mixed and added as drops of 25 μl to NaOH every 10 min till a total of 600 ul was added .The samples were analyzed by a spectrophotometer. Silver nanoparticles synthesized using the aqueous method was lyophilized and the powder was mixed into 12% PU in THF solution to get final concentrations of 0.015, 0.010, 0.005 wt% and electrospun.

2.4 Characterization

Characterization was done using SEM, FTIR and XRD. An electrospun sample in aluminum foil was pasted on the stub and coated with platinum prior to SEM evaluation using a JEOL analytical Scanning Electron Microscope (SEM) [JSM-6490 LA]. In FTIR, both PU nanofiber mat and PU-Ag nanofiber mat mixed separately with KBr and FTIR was carried out separately by a Perkin-Elmer Spectrum RX1 in the range between 4000 and 400 cm^{-1}, with a resolution of 2 cm^{-1}. In XRD, a PU-Ag nanofiber mat was spun on to the cover slip and analyzed using a PANalytical X'PERT PRO X ray diffractometer.

2.5 Water Absorption Studies

Water absorption studies were done on the silver incorporated PU nanofiber mats. The mats were weighed and 5 mg of the mat was soaked in PBS buffer at 37°C and the weight difference was measured to calculate the rate of water absorption. The same experiment was carried out for various time periods, which are 0.5, 0.10, 0.3, 1, 2, 4, 8, 12, 24 and 48 h.

2.6 Antibacterial Study of the PU Nanofiber Mats

The Kirby disc diffusion method was used and this test was performed on *Klebsiella.* The appropriate disks were placed evenly (no closer than 24 mm from center to center) on the surface of the agar plate by using a sterile forceps. The disk should not to be moved once it has come in contact with the agar surface since some of the compound diffuses almost instantaneously. The plate was then immediately inverted and placed in an incubator at 3°C. The plates were then incubated aerobically (without CO_2) and after 16–18 h of incubation, the plates were examined and the diameters of the zones measured for complete inhibition, including the diameter of the disk. The zones were measured to the nearest millimeter using a ruler.

2.7 Cytotoxicity Assay

MTT assay was performed to study the cytotoxicity of PU nanofiber mats. The cells were seeded in the 96 well plates at a predetermined density. The plate was then incubated for 24 h to allow the cells to attach to the well surface. Nanofiber mats, which were sterilized previously and leached in MEM, were added to each well containing the cells at varying concentrations. Each concentration was in triplicate. Triplicates of a positive control (Triton) and a negative control (Complete media alone) were also included. The cells treated with the leached out sample was incubated for the required time period in the CO_2 incubator. The media containing leached out sample was removed after 24 h incubation. 100 μl fresh media and 10 μl MTT were added to the wells,. The plate was incubated for 4 h and the solubilization buffer was added After 1 h incubation, the solution in the wells was mixed thoroughly. The absorbance in each well was then measured using the ELISA plate reader at 575 nm.

3 Results and Discussion

3.1 Effect of PU Concentration on the Morphology of the Electrospun Fiber

The different concentrations of PU were used to prepare the nanofiber mat. 12 wt% of PU in THF was optimized which had a viscosity of 343.4 at 20 rpm at 24.6°C for getting good nanofibers with a diameter of 1.2 to 1.5 μm.

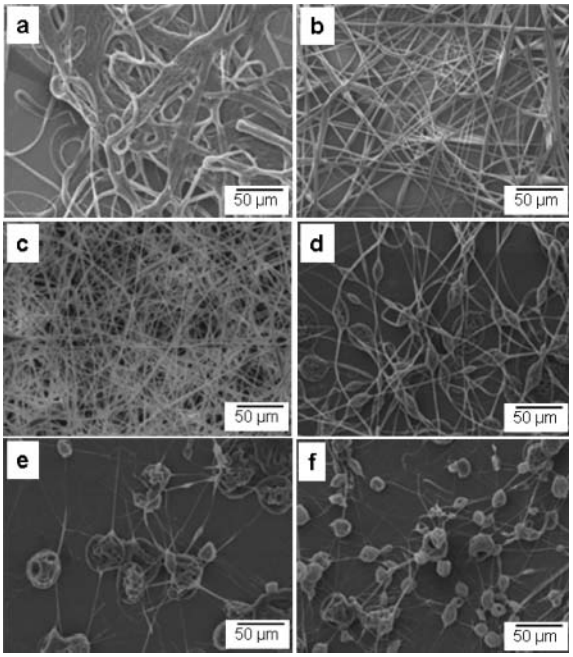

Fig. 1. SEM images of PU fibers at different concentrations (a) 16; (b) 14; (c) 12; (d) 10; (e) 8; and (f) 4%.

Whereas only beads were obtained with 4, 6 and 8 wt% of PU; at 10 wt% fibers along with beads were obtained and at 14 and 16 wt% pancake-like structures were obtained. The parameters optimized were the tip to target distance of 10 cm at a voltage of 13KV and a flow rate of 5ml/hour. The morphology and size of the nanofibers were assessed using SEM images (Fig. 1).

The Ag nanoparticles of different concentrations such as 0.005, 0.010 and 0.015 wt% were incorporated into the PU solution. The Ag NP was synthesized in a range of 5 to 20 nm that was measured through the UV spectrophotometer. The conductivity of the solution was increasing from 1.75 μs for plain PU solution to 96 μs for 0.015 wt% solution. The parameters for electrospinning were kept unchanged except the flow rate that was reduced to 0.1ml/hr because of the possibility that increased conductivity could cause increased attraction towards the target. The fiber diameter was found in the range of 150–250 nm with the highest silver concentration. Thus with the increase in Ag concentration, there was a ten time reduction in fiber diameter. This may be due to increased stretching of fiber as a result of increased conductivity (16). The SEM images of different fiber morphologies are shown in Figure 2.

3.2 Effect of Concentration of PU on the Viscosity of the Solution

The effect of silver nanoparticles concentration on conductivity of the PU solution was studied and it was observed (as seen from Table 2) that as the concentration of silver

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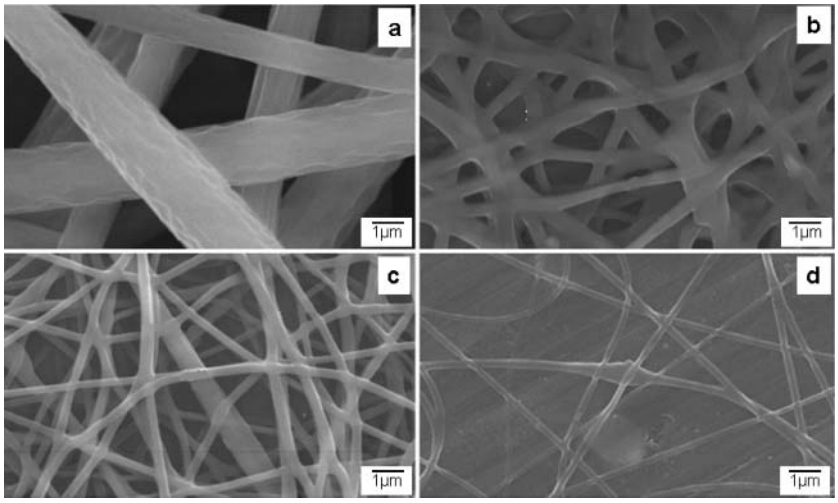

Fig. 2. SEM images of PU fiber and silver nanoparticles incorporated PU fibers (a) PU fiber; (b) PU fiber with (0.005% Ag); (c) PU fiber (with 0.01% Ag) and; (d) PU fiber (with 0.015% Ag).

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nanoparticles increases the conductivity also increases. The UV spectrum of Ag nanoparticles is shown in Figure 3.

3.3 Characterization

3.3.1. *FTIR studies*

The reduction of shouldering at peak 1737 cm^{-1} in PU with silver compared to PU alone (Fig. 4) is due to the formation of the NH-Ag bond. The peak at 3446 $cm^{-1}$is due to the amine group in PU and when silver was incorporated, its intensity also increased. This also indicated the increase in crystallinity of PU due to silver incorporation.

3.3.2. *XRD studies*

Figure 5 shows the XRD data of the silver containing nanofibers. A sharp peak at $2\theta = 38^{\circ}$ of silver indicating the incorporation of silver nanoparticle into polyurethane. So the XRD studies confirmed the incorporation of silver in nano fibrous scaffold (17, 18).

3.4 Water Absorption Studies

Water absorption studies of PU and PU-Ag nanofibers mats were carried out and are shown in Figure 6. The PU-Ag nanofibrous mat showed higher water absorption than the PU mat. PU-Ag nanofibrous mat have higher porous and surface areas than the PU mat (19, 20). So, PU-Ag nanofibers showed higher water adsorption than the PU mat. The results indicated that the PU-Ag nanofibers mat could be used for wound dressing applications.

Table 2. Variation in conductivity for PU solution with Ag nanoparticle concentration

Solution	*(%)*	*Conductivity (us)*
PU	12	1.75
Ag	0.005	26
Ag	0.010	54
Ag	0.015	96

3.5 Antibacterial Activity

Antibacterial activity of the PU-Ag nanofibers was examined using *Klebsiella* bacteria. With *Klebsiella*, Ag incorporated PU showed 1.1 cm zone of inhibition compared to 3cm zone of inhibition for GM disc and no zone of inhibition for plain PU and PU sponge (Fig. 7). Ag

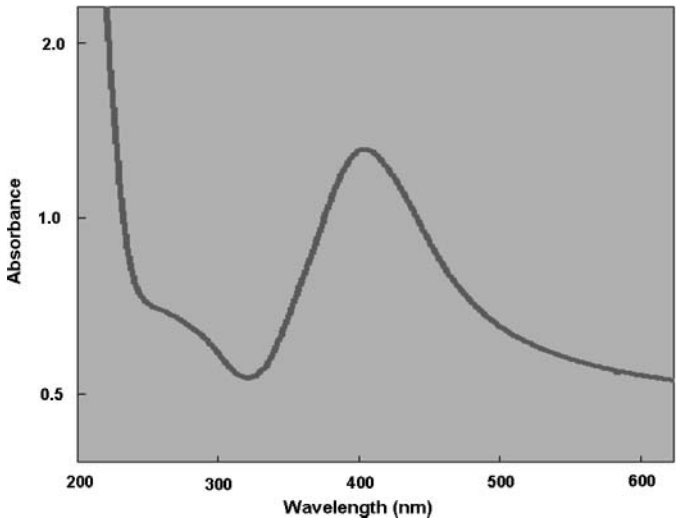

Fig. 3. UV spectrum of Ag nanoparticles.

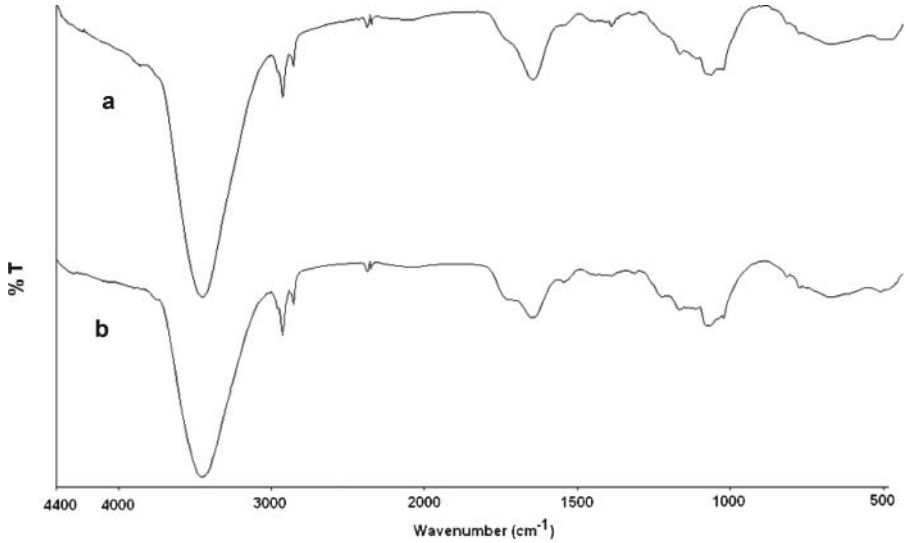

Fig. 4. FTIR of (a) PU nanofibers with Ag nanoparticles and, (b) PU nanofibers.

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nanoparticles interact with the thiol group of the respiratory enzyme present in the bacterial cell wall. Apart from that, nanoparticles interact with the DNA and RNA of bacteria and hence, stop the replication (17, 18, 21).

3.6 Cytotoxicity Studies of Silver Nanoparticles Incorporated Nanofiber Mat

Figure 8 shows the cytotoxicity results of PU-Ag nanofibers. The cytotoxicity of PU-Ag fiber by aqueous and organic route was compared with plain sponge and plain PU. The organic route was shown to be toxic which may be due to the fact that DMF is toxic to the cell. The boiling point of DMF is 160°C and hence, the traces of DMF were not vaporized even after heating at 60°C for one hour. The melting point of PU is around 200°C and hence, heating at a high temperature of 160°C could alter the fiber morphology. In the other case, the boiling point of THF is 60°C and therefore, all the experiments were carried out after heating the mats at 60°C for one hour. The cytoxicity study was repeated in L929 and hMSC and both were shown to be non-toxic ((Fig. 8 (a and b)).

The PU-Ag nanofibers can be used for wounds with less exudations and also as occlusive dressings, prosthetic material such as mesh and sling operation to prevent mesh infection and to give mechanical support during the surgeries such as uterine prolapse, sacrocolpopexy, sacrospinous fixation, infra coxccygeal sacropexy with or

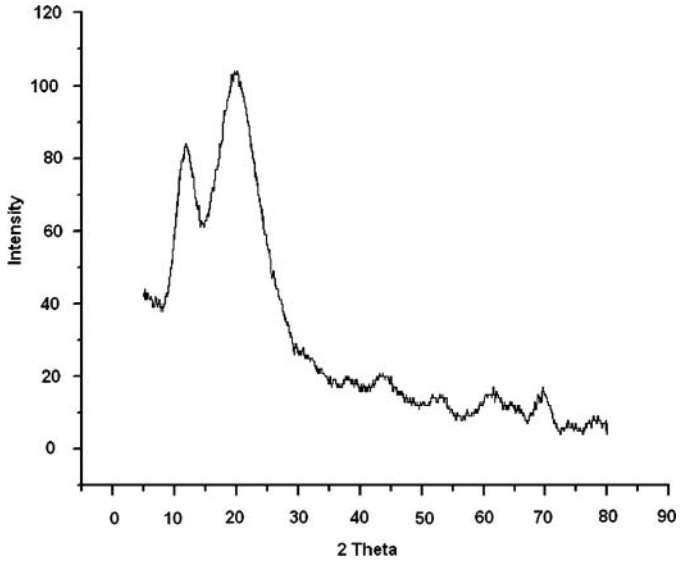

Fig. 5. XRD spectrum of PU-Ag fibers.

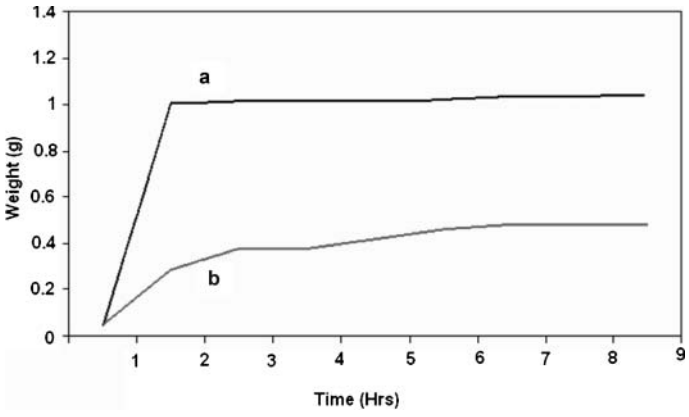

Fig. 6. Water absorption study of PU nanofiber mat with and without Ag nanoparticles. (a) PU nanofiber with 0.015% Ag, (b) Plane PU nanofiber mat.

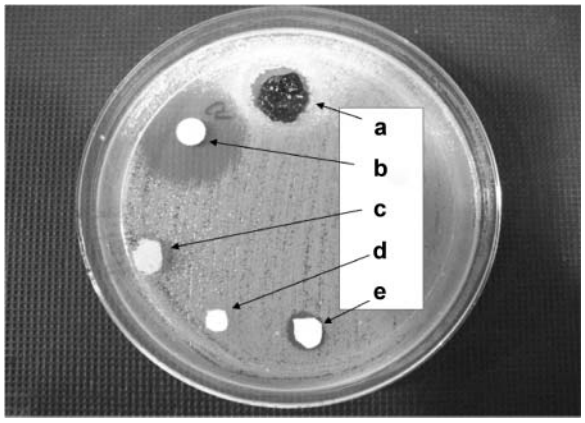

Fig. 7. Photograph of antibacterial activity of different PU materials with and without Ag nanoparticles by Kirby disc diffusion method using Klebsiella (a) Silver nanoparticles; (b) Gentamycin disc; (c) conventional PU sponge; (d) PU nanofiber and; (e) PU-Ag nanofiber (0.015% Ag).

without biofuntionalisation. Ag+PU systems also can be used for non-medical applications, such as water filters, ICU or operation theater instruments, dialysis membranes etc.

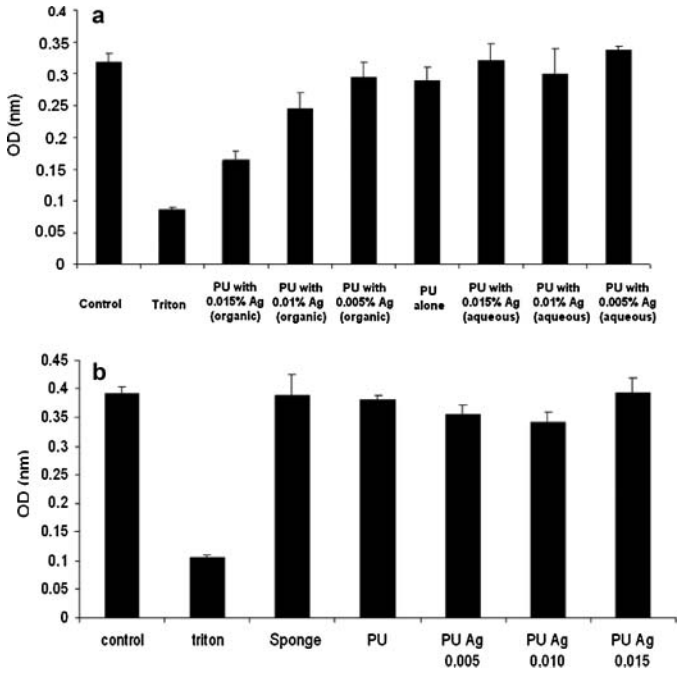

Fig. 8. (a) Cytotoxicity studies of PU nanofibers with and without Ag nanoparticles prepared by aqueous and organic methods using L929 and, (b) Cytotoxicity studies of conventional PU sponge, PU nanofibers and PU nanofibers with Ag nanoparticles prepared by aqueous method using hMSC.

4 Conclusions

The PU-Ag nanofiber made by the aqueous route was found to be cytocompatable as it is nontoxic, while those synthesized by organic route was cytotoxic. FTIR studies indicated that silver incorporation increased the crystallinity of PU, thereby increasing the strength of the nanofiber mat. The Kirby disc diffusion study revealed antibacterial activity against *Klebsiella*. Water absorption studies showed 75% water absorption compared to the conventional PU sponge. As it prevents wound colonization with bacteria and also absorbs moderate amounts of wound exudates, it can be used as an occlusive wound dressing in acute and chronic wounds.

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References

1. Duran, N., Marcarto, P.D., H. De Souza, G.I., Alves, O.L. and Esposito, E. (2007) *J. Biomed. Nanotechnol.,* 3(2), 203–208.
2. Lamba, N., Woodhouse, K. and Cooper, S., CRC Press: New York, 205–241, 1998.
3. Guan, J., Fujimoto, K.L., Sacks, M.S. and Wagner, W.R. (2005) *Biomaterials*, 26(18), 3961–3971.
4. Lee, J.S., Cho, Y.S., Lee, J.W., Kim, H.J., Pyun, D.G., Park, M.H., Yoon, T.R., Lee, H.J. and Kuroyanagy, Y. (2001) *Artif. Organs*, 15(1), 4–6.
5. Sun, R.W., Chen, R., Chung, N.P., Ho, C.M., Lin, C.L. and Che, C.M. (2005) *Chem. Comun*., 40, 5059–5061.
6. Teo, W.E. and Ramakrishna, S. (2009) *Comp. Sci. Techn*., 69(11), 1804–1817.
7. Yoo, H.S., Kim, T.G. and Park, T.G. (2009) *Adv. Drug Deliv. Rev*., 61(12), 1033–1042.
8. Jayakumar, R., Prabaharan, M., Nair, S.V. and Tamura, H. (2010) *Biotech. Adv*., 28(1), 142–150.
9. Zhang, Y.Z., Su, B., Venugopal, J., Ramakrishna, S. and Lim, C.T. (2007) *Int. J. Nanomedicine*, 2(4), 623–638.
10. Vasita, R. and Katti, D.S. (2006) *Int. J. Nanomedicine*, 1(1), 15–30.
11. Lu, S., Gao, W. and Gu, H.Y. (2008) *Burns*, 34(5), 623–628.
12. Fong, J. and Wood, F. (2006) *Int. J. Nanomedicine*, 1(4), 441–449.
13. Slavin, J. (1996) *J. Pathol*, 178(1), 5–10.
14. Grinnell, F. (1994) *J. Cell Biol*., 124(4), 401–404.
15. Heggers, J.P. (1998) *J. Wound Care*, 7(8), 389–392.
16. Thompson, C.J., Chase, G.G., Yarin, A.L. and Reneker, D.H. (2007) *Polymer*, 48(20), 6913–6922.

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17. Sudheesh Kumar, P.T., Abhilash, S., Manzoor, K., Nair, S.V., Tamura, H. and Jayakumar, R. (2010) *Carbohydr. Polym*., 80(3), 761–767.
18. Madhumathi, K., Sudheesh Kumar, P.T., Abhilash, S., Sreeja, V., Tamura, H., Manzoor, K., Nair S.V. and Jayakumar, R. (2010) *J. Mater. Sci: Mater. Med.*, 21(2), 807–813.
19. Torres Vargas, E.A., do Vale Baracho, N.C., de Brito, J. and de Queiroz, A.A.A. (2010) *Acta Biomaterialia*, 6(3), 1069–1078.
20. Ana, H., Shin, C. and Chase, G.G. (2006) *J. Mem. Sci*., 283(1–2), 84–87.
21. Rai, M., Yadav, A. and Gade, A. (2008) *Biotech. Adv*., 27(1), 76–83.

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