Self Synthesize of Silver Nanoparticles in/on Polyurethane Nanofibers: Nano-Biotechnological Approach

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ABSTRACT: In this study, we are introducing a new class of Polyurethane (PU) nanofibers containing silver nanoparticles (NPs) by electrospinning. A simple method not depending on the addition of foreign chemicals has been used to self-synthesize of silver NPs in/on PU nanofibers. Typically, a sol−gel consisting of $AgNO_3$/PU/*N*,*N*-dimethylformamide (DMF) has been electrospun and aged for a week, so silver NPs have been created in/on PU nanofibers. Syntheses of silver NPs were carried out by exploiting the reduction ability of the DMF solvent which is the main constituent to obtain PU electrospun nanofibers in decomposition of silver nitrate precursor into silver NPs. Physiochemical characterizations confirmed well oriented nanofibers and good dispersing of pure silver NPs. Various parameters affecting utilizing of the prepared nanofibers on various nano-biotechnological fields have been studied. For instance, the obtained nanofiber mats were checked for mechanical properties which showed the improvement of the tensile strength upon increase in silver NPs content. Moreover, the nanofibers were subjected to 10 times successive washing experiments with using solid to liquid ratio of 3 : 5000 for 25 h, UV spectroscopy analysis reveals no losses of silver NPs from the PU nanofibers. 3T3-L1 fibroblasts were cultured in presence of the designed nanofibers. The morphological features of the cells attached on nanofibers were examined by BIO-SEM, which showed well attachment of cells to fibrous mats. The cytotoxicity results indicated absence of toxic effect on the 3T3-L1 cells after cell culturing.
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Key words: electrospinning; nanofibers; silver nanoparticles; polyurethane; cell culture; cytotoxicity

INTRODUCTION

Generally, formation of super-bugs is the main problem upon the frequent use of the antibiotics. Because of genetic transformations, the pathogenic microorganisms are becoming more resistant against present antibiotics.[1] To have complete removal of pathogenic strains during wound healing process, there is a need to have an alternate strategy. Silver has been used since ancient times for purpose of wound healing materials. When silver comes in contact with microorganisms, it leads to sudden distortion of cell wall which later causes death of these organisms, and therefore, progressive steps are made for future use of these silver based materials.[2] Silver is considered to have broad-spectrum antimicrobial activities against many gram-negative and gram-positive bacterial strains. The mechanism for development of minimal resistance against silver has been well documented.[3] This ability to create minimal or no resistance in microorganisms can lead them to replace nonpotent antibiotics.[4] Besides its antimicrobial activity, it has been found that the use of silver based dressing materials enhances epithelialization in clean wounds of animal model, which indicates a synergistic effect of silver ions for antimicrobial and wound dressing agents.[5] Modification of silver and its compounds is important to be applicable in various medical and paramedical fields, which is quit challenging for scientists till now. Recently, studies had been carried out to modify the silver so that it can be used in various applications such as an antimicrobial filters,[6] wound dressing materials,[7] water disinfectants,[8] chemical sensors,[9] and protective cloths.[10]

An interesting strategy has been exploited to formulate silver in an applicable form is that incorporating of silver nanoparticles (NPs) on polymeric

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nanofiber matrix. In this regard, electrospinning technique has been paid considerable attention due to producing fibers having diameter in the range of few microns to nanometer level by applying high electric fields.[11–13] Nanofibers received from this technique have been drawn attention due to their small diameter web like nature which mimic the topology of extracellular matrix present in human body, and therefore, are used as scaffolds in tissue engineering.[14] Not only the silver NPs do have solitary importance but also the polymer matrix plays a distinct role, so, many polymers have been exploited. Generally, for the purpose of using a polymer as antimicrobial and wound healer material, it should have the following characters; good mechanical properties, water insolubility, appropriate pore size, non invasive to human cells so as to support the epithelialization. Recently, many polymers have been utilized; like cellulose acetate,[15] poly(acrylonitrile),[16] poly(caprolactone),[17] poly(methyl methacrylate),[18] poly(vinyl alcohol),[19] and polyimide fibers.[20] Unfortunately, all the reports in such a field have focused only on the synthesizing procedure and ignored some important parameters affecting utilizing the final products in nano-biotechnological applications, for instance:

1. Effect of the incorporated silver nanoparticles on the mechanical properties of the final matrix.
2. Fixedness of the obtained silver nanoparticles.
3. Interactions of these nanofibers with bioentities.

Therefore, preparing of silver NPs/polymer nanofiber matrix with taken in consideration all the aforementioned parameters was the aim of the present study. Poly(urethane) (PU) is thermoplastic and has excellent mechanical properties[21] and water insoluble polymer,[22] moreover, it can be used as biomaterial. As aforementioned, nano-fibrous shape strongly modifies the characteristics of any material. Therefore, these polymer nanofibers do have tremendous applications in various fields; biosensors,[23] protective cloths,[24] and epithelial enhancing material.[25] There are various articles regarding electrospinning in which PU has been modified as such which it can be used as antimicrobial fibers.[26,27] However, according to our best knowledge, there is no report dealing with producing PU nanofibers/silver NPs to exploit their prompt features to be used in epithelialization process. In this work, silver nitrate/PU sol−gel was electrospun. It is noteworthy mentioning that, the silver NPs/PU nanofiber matrices have been prepared without using any reducing agent to produce pure silver NPs. *N*,*N*-Dimethylformamide (DMF), which is a main constituent for production of smooth PU nanofibers[28] has been exploited for this task. Moreover, evaluation of the obtained nanofiber matrices for morphological properties crystalline structure and mechanical strength were investigated. Also, stagnancy of the obtained silver NPs and cell cytotoxicity of fibroblasts were checked. According to the obtained results, one can say that the prepared silver NPs/PU nanofibers matrix could be properly used as recommended candidate for many biological applications such as prolonged wound dressing materials, filter membranes, etc.

EXPERIMENTAL WORK

Materials

Polyurethane (PU, MW = 110,000, medical grade) and silver nitrate ($AgNO_3$) were purchased from Cardio Tech. Intern. Japan, and Junsei Chemical, Japan, respectively. Tetrahydrofuran (THF) and *N*,*N*-dimethylformamide (DMF) (analytical grade, Showa Chemicals, Japan,) were used as solvents without further purification. For cell cytotoxicity test, murine 3T3-L1 fibroblasts were purchased from ATCC. The cells were cultured in DMEM (ATCC) supplemented with 10% fetal bovine serum (Gibco, USA), with 2 mL glutamine, 100 μg/mL penicillin, and 100 μg/mL streptomycin (Sigma).

Characterization

The morphology of the nanofiber mats had been analyzed by JEOL JSM-5900 scanning electron microscope, JEOL, Japan. The average diameter and diameter distribution were obtained by using image analyzer (Image-Proplus, Media Cybrernetics). Additionally, morphologies were assayed by field-emission scanning electron microscope (FE-SEM, Hitachi S-7400, Japan). Transmission electron microscopy (TEM) was done by JEOL JEM 2010 (TEM) operating at 200 kV, JEOL, Japan. The electrical conductivity of the polymer/precursor mixture was measured by EC meter CM 40 G Ver 1.09 (DKK TOA, Japan). The mechanical properties of the nanofiber mats were investigated by universal testing machine (UTM, AG-5000G, Shimadzu, Japan) under a crosshead speed of 5 mm/min. Samples were prepared in the form of standard dumbbell shaped according to guidelines of ASTM standard via die cutting from nonwoven mats and tested in the machine direction at least five specimens were tested for each sample. Information about the phase and crystallinity was obtained by using Rigaku X-ray diffractometer (XRD, Rigaku, Japan) with Cu Kα ($\lambda = 1.540$ Å) radiation over Bragg angle ranging from 30 to 80°. UV-Visible measurements were performed by using HP

8453 UV-Visible spectroscopy system (Germany), the absorbance was measured from 400 nm to 1100 nm; wavelength. The spectra obtained were analyzed by HP ChemiStation software 5890 series.

To investigate fibroblast proliferation on the nanofiber mats, the cell morphologies were examined by Bio-SEM (Hitachi, Japan). Cell cultured samples were fixed in 2.5% glutaraldehyde in a phosphate buffer (pH 7.4) for 1 h at room temperature, dehydrated through a series of graded ethanol, dried under vacuum, mounted onto aluminum stubs, and sputter coated with osmium.

Procedure

Fabrication of nanofibers by electrospinning

Pure PU 10 wt % was prepared by stepwise dissolving in THF and DMF. Initially, PU pellets were overnight dissolved in THF after that DMF was added to produce final sol−gel containing 10 wt % of PU in THF/DMF (1 : 1, w/w). $AgNO_3$/DMF solutions were prepared and added to the PU sol−gel to have final mixtures containing silver nitrate of 2, 5, 7, and 10 wt %; with respect to the polymer. A care was taken to protect the samples from light. A high voltage power supply (CPS-60 K02V1, Chungpa EMT, Republic of Korea), capable of generating voltages up to 60 kV, was used as a source of electric field. Polymer solution to be electrospun was supplied through a glass syringe attached to a capillary tip. The copper wire originating from positive electrode (anode) connected with graphite pin was inserted into the polymer solution and a negative electrode (cathode) was attached to a metallic collector. Briefly, the solutions were electrospun at 20 kV voltage and 15 cm working distance (the distance between the needle tip and the collector). The as-spun fibers were stored for 1 week then vacuously dried for 24 h to remove the residual solvents.

Cell culture studies

To find the capabilities of the designed nanofibers for use in wound healing and to use as safe water filter membranes, cell culture studies were performed. Briefly, round dick shaped nanofiber mats measuring diameter of 1 × 1 cm having thickness of around 1 μm were cut by cork borer. The nanofiber mats were sterilized by ethylene oxide gas (EO gas) method for 8 h prior to cell seeding. Samples were loaded in 6-well cell culture plate (Costar) and were seeded with cells under aseptic conditions. The cell-seeded disks were maintained at humidified atmosphere of 95% air and 5% CO_2 at 37°C. After every 1 day, exhausted culture media was replaced with fresh media to grow cells in a continuous phase. During cell culturing, cell viability and morphological changes occurring were visibly monitored by inverted light microscope (Olympus Optical) as precautionary measures. Briefly, all the nanofiber compositions were checked for cell interactions for 4 days. Further on samples were analyzed for further investigations.

Cytotoxicity

Evaluation of the cytotoxicity was performed by the MTT assay. Briefly, 3T3-L1 cells were inoculated on culture media supplemented with 10% fetal bovine serum and antibiotics in 6 well culture plates and incubated in a humidified atmosphere containing 5% CO_2 at 37°C for 24 h.[29] After the cell culture, media was collected and utilized for colorimetric analyses. About 50 μL of MTT solution (5 mg/mL in PBS) were added to each well. The plates were incubated for an additional 4 h at 37°C. Next, MTT containing medium was aspirated off and 450 μL of DMSO were added to dissolve the crystals formed by living cells. Absorbance was measured at 570 nm, using a spectrophotometer (SpectraMax flourometer with SoftMax Program Molecular Probes). The cell viability (%) was calculated according to the following equation as Cell viability (%) = [intensity (OD) measured at 570 (sample)/intensity (OD) 570 (control)] × 100. The cytotoxicity PU containing silver NPs was evaluated in comparison with pristine nanofiber containing 0% of silver NPs.

RESULTS AND DISCUSSION

Previous works have indicated that DMF has the ability to reduce some metallic precursors to the corresponding metal NPs,[30,31] particularly, this organic solvent has been exploited to synthesize silver NPs from silver nitrate at room temperature without using any catalyst.[31,32] Moreover, as aforementioned, DMF is a main solvent to obtain PU nanofibers.[28] Therefore, we have exploited these observations to self-synthesize silver NPs within PU/(THF/DMF) electrospun nanofiber mats. To get full decomposition of silver nitrate into silver NPs, after electrospinning; the nanofiber mats were stored for 1 week, during this storing time; visible changes in color was observed in the electrospun mats. These mats exhibited light pinkish to light brown color due to reduction of silver salts into silver NPs.

Figures 1 and 2 show SEM and FE-SEM images for all nanofiber formulations. As sown in Figure 1, PU/$AgNO_3$ produces smooth and bead-free nanofibers. Observable NPs can be noticed with high silver nitrate contents (Figs. 1 and 2, panels D and E). It is noteworthy mentioning that the average nanofibers diameter was decreasing with increasing the silver

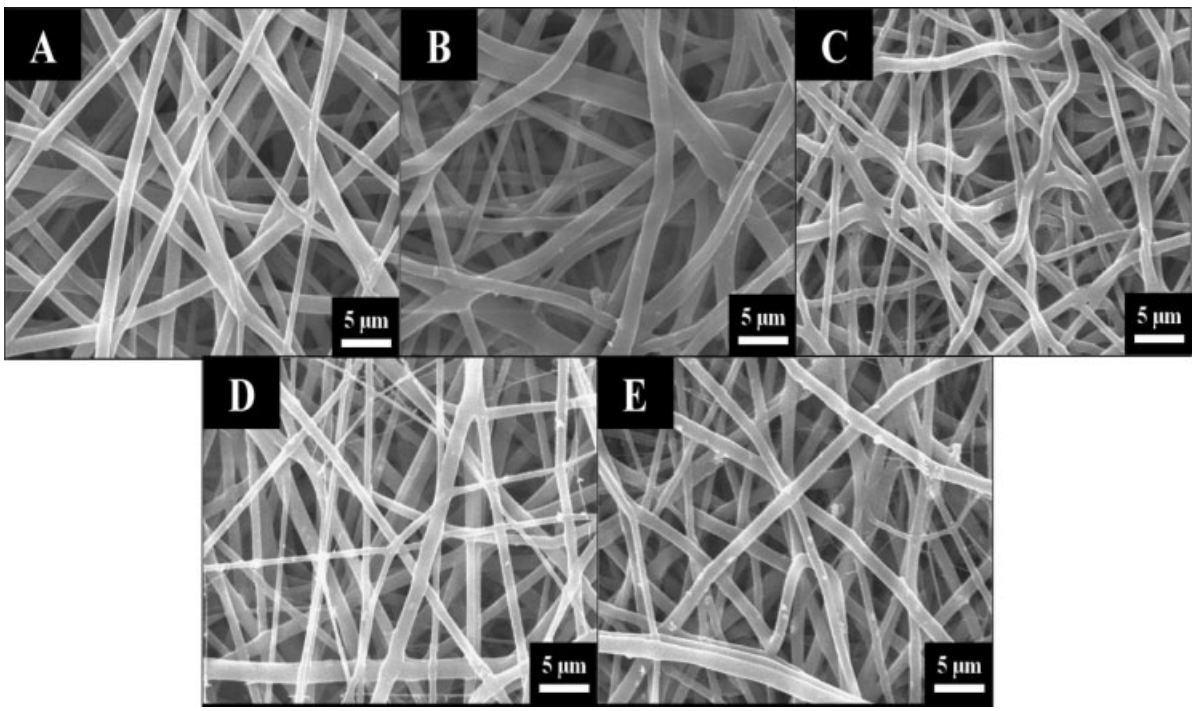


Figure 1 Scanning electron microscopy images of the PU nanofibers containing different amounts of silver nitrate: 0% (A), 2% (B), 5% (C), 7% (D), and 10% (E) silver nitrate with respect to PU.

nitrate content in the sol–gel. Figure 3(A) shows the frequency diameter distribution for the prepared nanofiber mats. An acceptable explanation for this phenomenon was provided by many previous works concluded that salts play an important role in decreasing of the fiber diameter during electrospinning.[33,34] To prove these findings; we analyzed the conductivities of PU/silver nitrate solutions and the

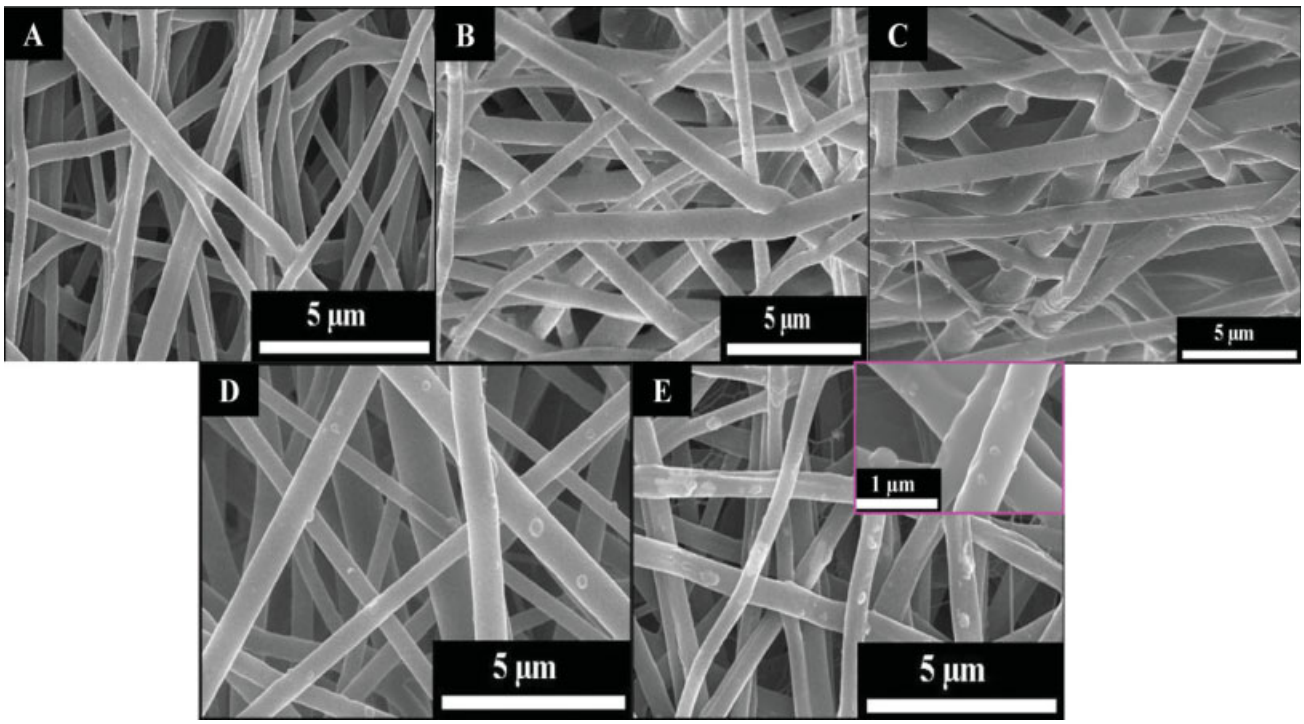


Figure 2 Field-emission scanning electron microscopy images of the PU nanofibers containing different amounts of silver nitrate: 0% (A), 2% (B), 5% (C), 7% (D), and 10% (E) silver nitrate with respect to PU. Inset in the panel (E) represents high magnification image of 10% silver nitrate content. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

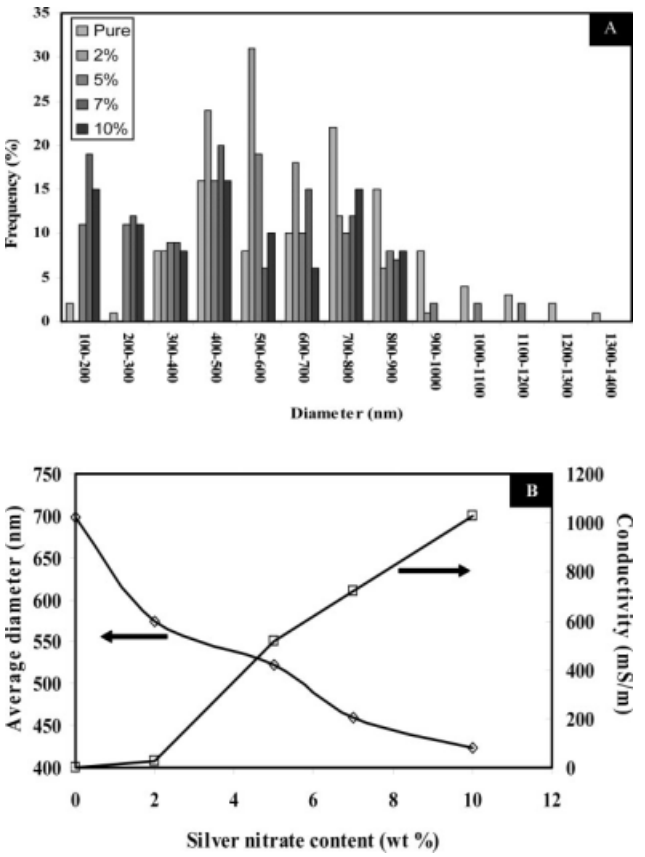


Figure 3 (A) Average frequency size distribution of nanofibers for all compositions. (B) Effect of silver nitrate content in silver nitrate/PU mixture on the conductivity of the sol−gel and the average diameter of the electrospun nanofiber mats.

corresponding average diameter of the obtained nanofibers. At this stage, Figure 3(B) represents the effect of silver nitrate content on conductivity and consequently on the average diameter of nanofibers. It indicates that the conductivity of the polymeric solution increases with increasing the silver nitrate content in the sol–gel. However, the fiber diameter decreases gradually upon the addition of silver nitrate. Actually, increase in the salt concentration results in increase in the conductivity of the polymeric solution, which leads to high charge values during electrospinning process and possibly forming thinner fiber diameter.[34]

The typical XRD patterns for pristine and PU composite nanofiber mats are presented in Figure 4. The diffraction peaks at 2θ values of 38.11, 44.27, 64.42, and 77.47° corresponding to (111), (200), (220), and (311) crystal planes implies the persistence of crystalline silver NPs (JCDPS, card no 04–0783). As shown in Figure 4, all the standard peaks of pure silver are observed in the case of nanofibers obtained from PU/silver nitrate sol–gels. While as in case of pristine ones no such peaks were observed as compared with other modified counterparts. This indicates that the obtained nanofiber mats from PU/silver nitrates sol–gels do have silver particles and simultaneously confirming ability of DMF as reducing agent. Moreover, it can also be observed from this figure that, the intensity at (111) main plane in the standard silver crystal lattice (at 2θ values of 38.11°) increases with increasing the silver nitrate content in the original sol−gel, which leads to more silver NPs in the electrospun nanofiber mats.

Figure 5 shows the TEM images of the PU nanofibers containing silver NPs after electrospinning. As observed in all the combinations ranging from 2, 5, 7, to 10 wt %, nearly spherical silver NPs. Another observation can be also noticed when TEM images are compared with FE-SEM ones that the NPs density is higher in TEM images than FE-SEM for all silver nitrate contents. By taking in consideration that the electron beam in TEM analysis passes through the nanofibers, so, it can detect the silver NPs incorporated inside the nanofibers, however, in case of SEM and FE-SEM only the surface morphology is investigated, consequently, we can say that the number of silver NPs inside the obtained nanofibers are so many compared with the NPs synthesized on the surface. For instance, as shown in Figure 2(B), which reveals the nanofibers obtained from a sol–gels containing 2% silver nitrate, no NPs can be observed, however, in the corresponding TEM image [Fig. 5(A)] some crystalline NPs are clearly appeared. This observation might add good character to the prepared nanofiber matrix, since some authors have been proofed that PU is biodegradable[35] so with time passing more silver NPs will appeared which enhance the antibacterial activity and epithelization process for the prepared nanofiber mats. It is noteworthy mentioning that no stabilizers or dispersants

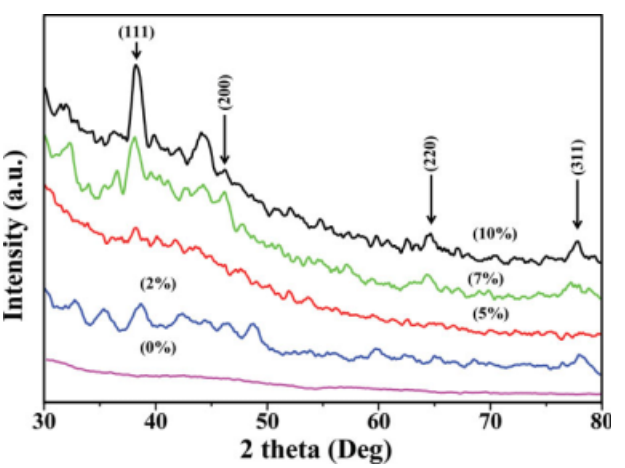


Figure 4 XRD results of obtained nanofiber mats, the percentage in the brackets above the curves represent the silver nitrate content in the original electrospun solutions. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

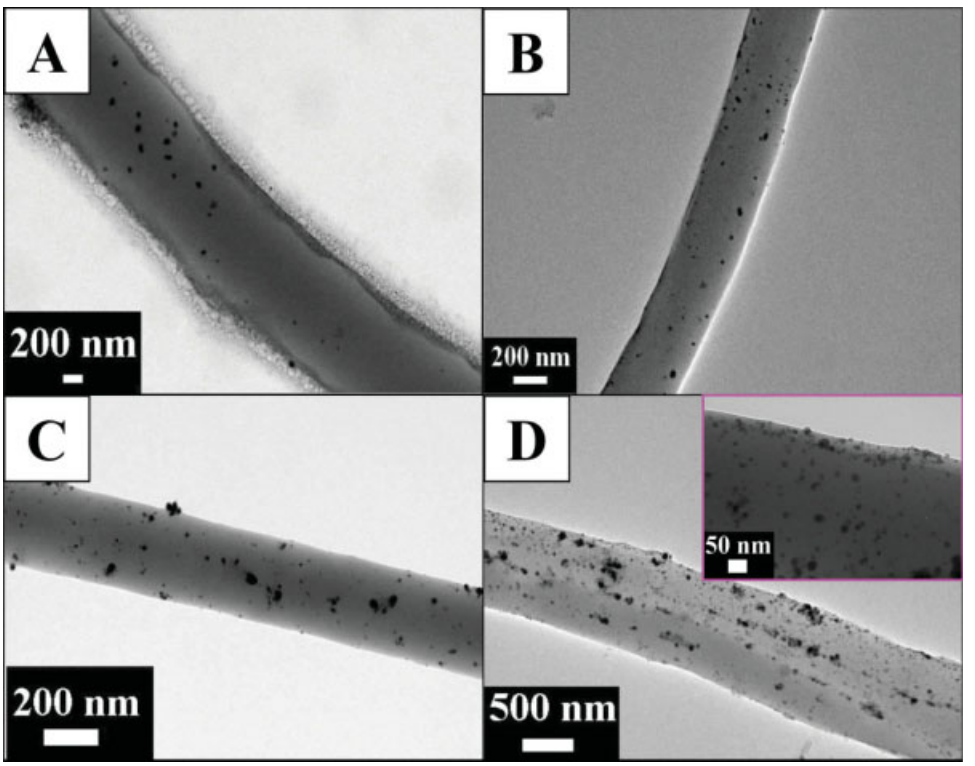


Figure 5 Transmission electron micrographs of the prepared nanofibers obtained from silver nitrate/PU sol–gels containing different amounts of silver nitrate: 0% (A), 2% (B), 5% (C), 7% (D), and 10% (E) silver nitrate with respect to PU. Inset in the panel (E) represents high magnification image of 10% silver nitrate content. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

agents have been invoked to get the well distributed and unagglomerated obtained NPs, we think using silver nitrate precursor as a solution, well mixing of PU/silver nitrate solution, and the stretching force during the electrospinning process are responsible for getting such well dispersed silver NPs within the PU nanofibers.

The obtained nanofibers were analyzed for the mechanical properties by following our early developed method.[36] Figure 6 reveals the obtained stress–strain curves. As shown in this figure, incorporating silver NPs in PU nanofibers do have noticeable positive effect on the mechanical properties since the fracture tensile strength increases with increasing the silver NPs content. A typical reason for this can be simply concluded due to decrease in the average diameter of nanofiber mats upon increasing silver nitrate content in the sol–gel [Fig. 3(B)]. Moreover, it has been suggested by many reporters that the silver NPs could act as nano-sized fillers for improving mechanical properties of polymer matrix.[37,38] Therefore, we can say that improvement in mechanical properties of the produced nanofibers is the results of parallel efforts from decreasing of the average diameter and incorporation of nano-sized fillers in the polymer matrix. Improving the mechanical properties of the obtained silver NPs/PU nanofibers mats strongly supports utilizing of prepared mats in many versatile applications such as filter membranes for water purification systems and wound dressing cloths.

Surface plasmon resonance (SPR) is an interesting feature for the metallic NPs colloids. SPR is a phenomenon which occurs when light is reflected off thin metal films or NPs. A fraction of the light energy incident at a sharply defined angle can interact with the delocalised electrons in the metal surface (plasmon) thus reducing the reflected light intensity.[39] In more details, when small metallic NPs are illuminated, the oscillating electric field causes the conduction electrons to oscillate coherently. In particular, silver and gold metals are the most popular materials used in this concern,[40] however, silver is most commonly utilized,[41] because its *d–s* band gap is in the UV region and does not damp out the plasmon mode as strongly as for gold.[42] This feature has been exploited by the researches to detect silver NPs in any solution using the UV-visible spectroscopy. Therefore, the polymeric nanofibers should be dissolved by using a proper solvent, then silver NPs can be spectroscopically detected. In a case of silver NPs/PU nanofibers matrix, THF has been used as solvent of the polymer leaving out silver NPs suspended in the solution. Figure 7(A) shows the obtained UV-Vis absorption

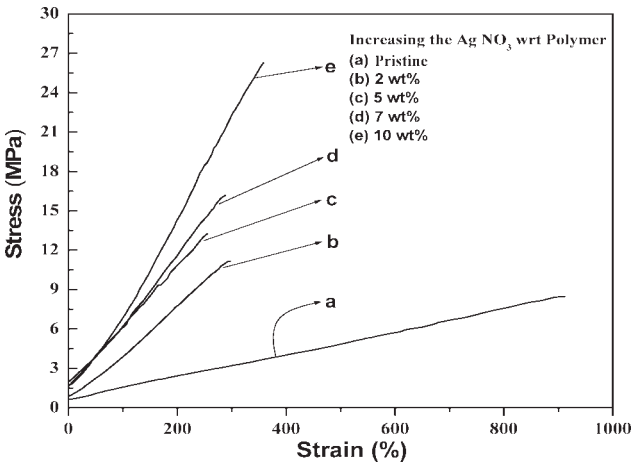


Figure 6 Stress over strain curves of the prepared silver NPs/PU nanofibers matrix obtained from silver nitrate/PU sol–gels containing different amounts of silver nitrate.

spectra of pristine PU and PU containing different amounts of silver NPs solutions. As shown in this figure, the pristine nanofibers exhibited nearly no absorption in the selected region, whereas nanofiber containing silver NPs showed absorbance band in the range of 450 nm and this absorbance is attributed to the characteristic surface plasmon resonance of silver NP.[43] Moreover, as can be observed from Figure 7(A) also, the absorbance intensity directly proportions with the silver content in the nanofibers that means more silver NPs being present in the corresponding formulations.

As shown in TEM and FE-SEM images, the synthesized silver NPs are well dispersed along with the nanofibers. Beside this, as was concluded from the washing experiments, the silver Nps have good stability in the polymeric matrix. Actually, we can explain these interesting findings as follow: polyurethane does have the ability to form hydrogen bond,[44] so it is expected that this character will enhance the distribution of the silver nitrate along the polymeric matrix during the preliminary preparation of the electrospun solution as nitrate group has oxygen atom. However, utilizing of DMF as a solvent leads to reduce the silver nitrate into silver metal as aforementioned, so zero oxidation state silver atoms will be produced and slightly agglomerate to form small NPs. The later will be imprisoned inside the polymer network chains. Considering we are using single polymer, so the similar chains will repel which results in formation of free spaces in the polymer matrix enclosing the synthesized silver NPs.[45] This hypothesis has been conducted with other polymers/silver NPs composites. For instance, well dispersion with very good stability of silver NPs in polyvinylpyrrolidone, polyurethane, and polyacrylonitrile polymers have been observed by other researchers.[46–48]

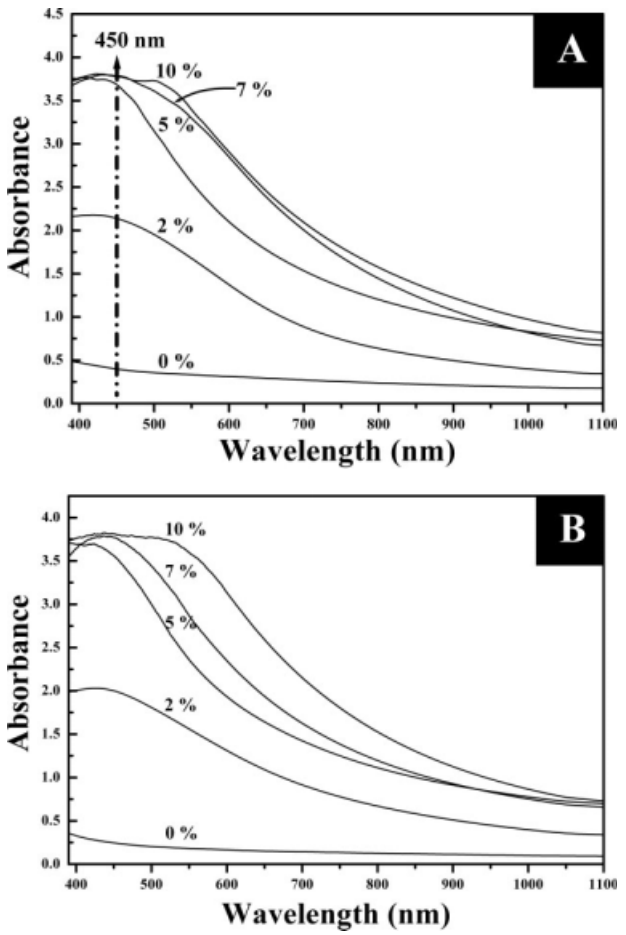
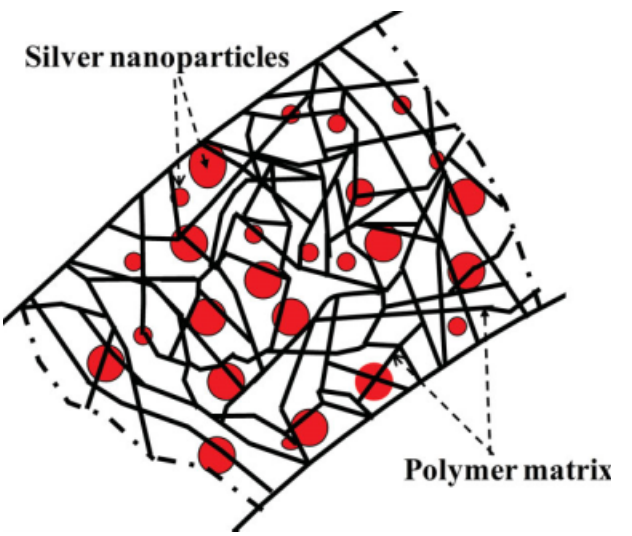


Figure 7 (A) UV-visible spectroscopy of silver NPs/PU nanofibers matrix containing different amounts of silver nanoparticles. (B) UV-visible spectroscopy for the same mats after washing process.



Scheme 1 Conceptual illustrations for the microstructure of the prepared silver NPs/PU nanofibers. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

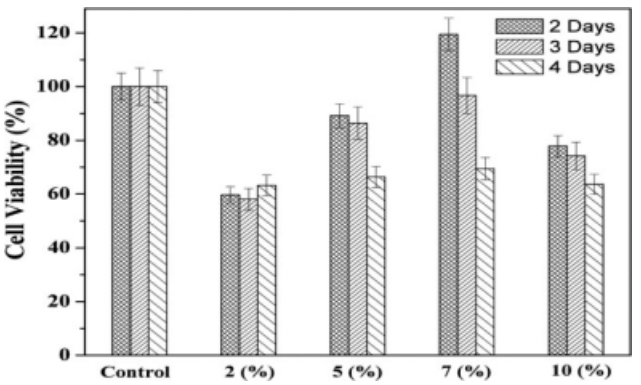


Figure 8 Cell viability results after cell culture for 2, 3, and 4 days.

As aforementioned, stagnancy of the silver NPs on the polymer matrix is an important parameter for utilizing the final product in the biotechnological applications. In a case of silver NPs/PU nanofibers matrix, to be sure that the nano silver present in nanofiber is firmly bound inside the nanofibers and will not be lost under harsh conditions like contact with water, we performed series of wash experiments; in that a pre-weighted amount of fiber was washed with distil water at solid to liquid ratio of 3 : 5000 under vigorously stirring for 25 h. The distilled water was replaced by fresh one every 2.5 h. Finally, the washed nanofiber mats were dried in vacuum and dissolved in equal amount of THF for spectroscopic examination. Figure 7(B) represents UV spectra for the washed nanofibers solutions. Nearly, no difference between the spectra obtained before and after washing with distilled water was observed. As can be indicated from this figure, silver NPs are still remaining in the washed nanofibers, almost no losses in the silver NPs had taken place. The absorbance intensities at 450 nm were not changed due to washing by this huge amount of distilled water during this long time. These results support TEM analyses (Fig. 5) indicating that most of the NPs are imprisoned inside the nanofibers. Moreover, these results also reveal that even the NPs present on the surface are well attached with the nanofibers since no change in the absorbance intensity even for the high silver NPs content nanofibers which contain many NPs on the outer surfaces (Fig. 1; panels D and E and Fig. 2; panels D and E). Therefore, one can recommend these nanofibers for long term contact with water operations; like antimicrobial water filters. As aforementioned, no stabilizing agents have been utilized to fix the produced silver NPs within the PU nanofibers, so, we are proposing a conceptual illustration shown in Scheme 1 to explain the microstructure of polyurethane nanofibers containing silver NPs. As shown in this

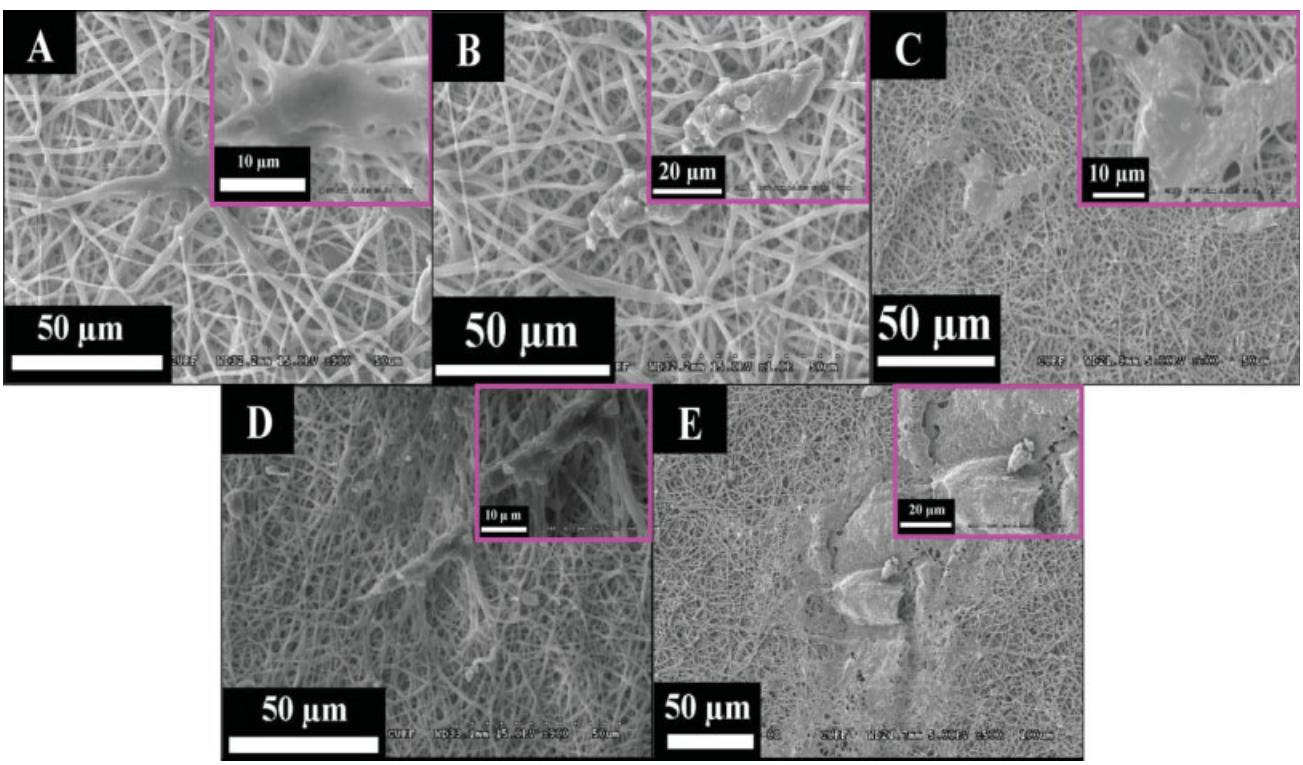


Figure 9 BIO-SEM images of the nanofiber mats showing cell attachments after 4 days of culturing cells for the prepared silver NPs/PU nanofiber matrices: 0% (A), 2% (B), 5% (C), 7% (D), and 10% (E). The inset in each figure shows high magnification image. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

scheme, the used branched PU polymer matrix imprisons the generated silver NPs inside the polymer web. Therefore, the silver NPs are well captured and did not escape during the washing process.

Silver is a well known antimicrobial agent.[5] It is also well established that PU enhances the epithelization.[25] Meanwhile, nitrates present in silver precursor is toxic to human body. To confirm that all nitrate content is completely eliminated and simultaneously affirm that the prepared nanofiber mats will not be toxic when utilized in biological or medical applications, we cultured murine fibroblasts in presence of the nanofiber mats. At this stage, 3T3-L1 fibroblasts cells were cultured in presence of sterilized nanofibers. Figure 8 reveals the cell viability quantitative analysis results. As can be indicated from Figure 8, all the combinations of silver are adequate for confluent growth of cells. Further on, to confirm the findings of cell viability we checked the morphological appearance of cells attachment on nanofiber mats after 4 days of culture as photographed in Figure 9. From this figure, one can clearly visualize the cell attachment and cell spreading in the nanofiber matrix. This phenomenon of cell growth on nanofiber membranes exactly matches with previously reported literatures.[49] Also, the observed morphologies in all the nanofiber compositions clearly support non toxic effect on cell proliferation. Therefore, beside the well known information that silver NPs are strongly recommended as antimicrobial agent,[50] the cell cytotoxicity test results provide open permission to use the prepared silver NPs/PU nanofibers in various medical and paramedical applications.

CONCLUSION

Silver nitrate/PU sol–gel can be easily electrospun by applying the same reported procedure for electrospinning of pure PU polymer. However, presence of DMF can result to reduce $AgNO_3$ to silver NPs in simple and biologically safe way. Therefore, silver NPs/PU nanofibers matrix can be prepared. Interestingly, presence of silver NPs in PU nanofibers distinctly improves the mechanical properties of the nanofiber mats. Moreover, the obtained silver NPs do have strong stagnancy since these NPs have not been released after many times washing of the nanofiber mats by big amount of fresh water for long time. Also, the cytotoxicity test has been conducted to ensure that the nitrous compounds byproducts have been completely removed from the prepared nanofiber mats; the obtained results were satisfactory. Avoiding using any foreign reducing chemicals, improving the mechanical properties of the final silver NPs/PU nanofibers matrix, and well stability of the NPs open a new avenue for the prepared nanofibers to be used in many medical, paramedical, and biotechnological applications.

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