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Sustained release of drug loaded nanofibers for wound dressing applications

Manvi Singh[1,2,3#], Deepika Chauhan[1#], Renu Gill[3], Zeenat Iqbal[2]* & Partima Solanki[1]*

[1]Special Centre for Nanoscience, Jawaharlal Nehru University, New Delhi-110 067, Delhi, India

[2]Department of Pharmaceutics, School of Pharmaceutical Education and Research, Jamia Hamdard, New Delhi-110 062, Delhi, India

[3]Department of Pharmaceutics, SGT College of Pharmacy, SGT University, Gurugram-122 505, Haryana, India

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Global burden of chronic wounds has increased drasticallyas they are vulnerable to bacterial infections that causes inflammation, thereby leads to a delay in the healing process. Furthermore, wound care and dressing industry is subjected to a global market of $30.4 billion by 2024. Our work entails fabrication of polymeric electrospun nanofibers loaded with different concentration of the amoxicillin (AMX) antibiotic. Biodegradable and biocompatible poly (vinyl) alcohol (PVA)/poly(meth)(methacrylate)(PMMA) polymerswere blended with different AMX concentration (100, 150, 200 and 250 mg) and fabricated by electrospinning technique. Morphology, structural properties and drug release from electrospun nanofibers depend on the different concentrations of drug incorporated in PVA:PMMA blend of polymer. Furthermore, these studies revealed drug-excipient compatibility and drug encapsulation within the nanofiber. In-vitro release study showed the AMX release time from PVA: PMMA: AMX was extended up to 7 days for AMX-250 with an initial burst release of 70% and further sustained drug release. Electrospun nanofibers of PVA:PMMA:AMX showed greater zone of inhibition of S. *aureus* as 2.1±0.4 cm for 100-AMX, 2.3±0.5 cm for 150-AMX, 2.4±0.1 for 200-AMX and 3.4±0.3 cm for 250-AMX. These results demonstrate that AMX retains the anti-bacterial activity and hence can be used as a potential wound dressing candidate.

Keywords: Amoxicillin, Electrospun nanofibers, Sustained release, Wound healing

Fabrication of nano and microfibers made up of natural and synthetic polymers takes place by a versatile technique called as "electrospinning". Electrospinning helps in the accumulation of aligned or non-woven fibers called as fibrous fabrics, fibrous membranes or fibrous mats. The polymeric composite fibrous mats produced through this process have high surface to volume ratio. Various processing properties such as type of collector, voltage, flow rate of the solution, needle to collector distance, needle diameter and the solution properties, like concentration, viscosity, electrical conductivity and surface tension can impact the morphology and other properties of the electrospun fibrous mats. The processing and solution parameters can be optimized accordingly and the final product with required characteristics can be obtained[1-5].

Electrospun nanofibers can be utilized in several fields, for instance, drug delivery, wound healing, filtration, protective clothing and in biomedical areas as scaffolds for tissue engineering. The main area for research in drug delivery is prevention of infection which is carried out by wound dressing[6]. An ideal candidate for wound dressing should have the following properties:

- Site specific drug delivery
- High amount of drug at the wound site
- Should function structurally and biologically like the natural extracellular matrix protein
- Regulation of the cellular activities and offers support
- Maintenance of normal state of differentiation inside the cellular section
- Biodegradable and biocompatible
- No adverse effects[7-10].

The above characteristics can be fulfilled by biocompatible polymer electrospun fibrous mats, loaded with antibiotics for specific applications.

Amoxicillin (AMX) is penicillin like antibiotic, has bacteriolytic action used to treat bacterial infections in skin and commonly used antibiotic for children. It can also be used before surgery to prevent the risk of infections[11]. Chitin (polysaccharides from arthropods) nanofibers were electrospun and prepared, with size ranging from 40-600 nM indiameter[12]. A composite

#Equal first author
*Correspondence:
E-mail: zeenatiqbal@jamiahamdard.ac.in (ZI);
partima@mail.jnu.ac.in (PS)

consisting of water-soluble poly-vinyl alcohol and carboxyethyl chitosan were electrospun to produce a wound dressing. These nanofibers showed notoxicity when tested on mouse fibroblast L929 cellline, and helped in cell proliferation and attachment. Chitosan acetate bandages were prepared and found to be effective as anti-microbial dressing. Their efficacy was also tested on BALB/c mice having burn wounds which were likely to be infected with *Proteus mirabilis* and *Pseudomonas aeruginosa*[13]. Silver-loaded zirconium phosphate was electrospun with poly ε-caprolactone polymer to produce nanofibers. The strains of *S. aureus* and *E. coli* showed a growth inhibition of 99.27% and 98.44%, respectively, when they were cultured in the presence of these nanofibers. Proliferation of human dermal fibroblasts attached to the nanofibers was suggesting them to be used as material for wound dressings[14].

In the present paper, PVA:PMMA:AMXelectrospun nanofibers were produced by electrospinning technique. For this purpose, different weight ratios of the two polymers were used and different amounts of amoxicillin were loaded into PVA:PMMA nanofibers. The structure, morphology and drug release behaviour of the electrospunfibers were analysed. The release behaviour of the amoxicillin, at different concentrations, was studied in physiological solution and anti-bacterial activity of these nanofibers were assessed.

Materials and Methods

Materials

PVA (Molecular Weight= 85, 000 Da, hydrolysis 89%) and PMMA (Molecular Weight= 1, 20, 000 Da) were procured from Sigma Aldrich (Missouri, USA).Formic acid (85%) was purchased from Thomas Baker (Mumbai, India) and Acetic acid (99%) was purchased from, s d fine-chem limited (SDFCL) (Mumbai, India). The drug Amoxicillin was obtained from Sigma Aldrich (Missouri, USA). All the chemicals used during the research have been received and used without any modification or purification.

Fabrication of electrospun nanofibers

PVA and PMMA were dissolved in a solution of formic acid and acetic acid in the ratio of 1:3 *v/v* to form a homogeneous solution. In order to prepare 10% *w/v* PVA solution 2 g of the PVA powder was dissolved in 20 mL of the solvent solution mentioned above, whereas, for preparing 25% PMMA solution 5 g of PMMA powder was weighed and dissolved in 20 mL of the solvent solution and stirred at 25 rpm overnight at 50°C on the magnetic stirrer. Distinct drug loaded fibers of PVA and PMMA were fabricated using the process of electrospinning. PVA drug loaded nanofibers were obtained with a solution flow rate of 0.2 mL/h, 10 kV as the applied positive voltage and the distance from the tip to the collector was measured as 13.5 cm. Similarly, PMMA drug loaded nanofibers were prepared with a solution flow rate of 0.2 mL/h, 12.7 kV as the voltage, and a distance of 13.5 cm[33]. Thereafter, PVA and PMMA electrospun nanofibers were prepared and optimized from 1:9 to 9:1 ratio. Amoxicillin (AMX) in the concentration of 100 mg, 150 mg, 200 mg, 250 mg was added to each solution of PVA: PMMA (5:5) 2 h prior to electrospinning and termed as100-AMX, 150-AMX, 200-AMX and 250-AMX, respectively, (Table 1). The resulting solutions were transferred to 5 mL syringe and placed at right angle to the collector. The nanofibers formed were collected on the aluminium foil and the electrospinning process were carried out at 25°C[15].

Scanning electron microscopy (SEM) analysis

The morphology, alignment and structure of the electrospun nanofibers were analysed using Field Emission Scanning Electron Microscopy (FE-SEM) (Zeiss EVO40, Switzerland) having 15 kV as an accelerating voltage and 8.70 mM is the working distance (WD). For each sample the average diameter was determined from the SEM image. The SEM image was segmented randomly from 100 different sections and the average diameter was evaluated using Image J software[16].

X-ray diffraction (XRD) analysis

X-ray diffraction (XRD) studies were carried to analyze the structural characteristics of the electrospun nanofibers. To determine the degree of crystallinity of AMX and the drug loaded nanofibers, XRD patterns were obtained using a diffractometer

Table 1 — Electrospinning Parameters for different concentration of Amoxicillin

Concentration of Amoxicillin (mg)	Flow Rate (mL/h)	Voltage (kV)	Distance from the tip of the needle to the aluminium collector (cm)
100	0.2	7.9	14
150	0.2	9.5	14
200	0.2	9.3	14
250	0.2	10.2	14

(Rigaku Miniflex 600, Japan). The scanning range for XRD analysis was taken from 2θ = 10°-60°[17].

Fourier transform-infrared spectroscopy (FT-IR) analysis

FT-IR technique was used to determine the characteristic peaks of the functional groups present in AMX and drug loaded electrospun nanofibers. The scanning range was taken from 1000-4000 cm^{-1} using Perkin-Elmer Spectrumspectrometer[18].

Atomic force microscopy

AFM was carried out (Park XE-70, Korea) to evaluate the roughness of the surface for the electrospun nanofibers. For this the electrospun nanofibers were deposited on the aluminium sheets having a size of 1×1 cm^2 and placed on the microscopic slide. The imaging of these electrospun nanofibers was carried out using cantilever from a distance of 5 mM, 10 mM and 20 mM[19].

Drug Release Study

Drug release from the electrospun nanofibers was calculated by immersion of known mass of the nanofibers (approx. 5 mg) in 5 mL of acetate buffer with pH 5.5 at 100 rpm and 37°C in shaker bath incubator. At regular time intervals, a known amount (3 mL) of sample was withdrawn from the buffer solution and substituted with an equal amount of fresh buffer. Drug release was quantified at λ_{max} = 280 nM and estimated using UV spectrophotometer. The experiments for each sample taken were performed in triplicates, and the average values were evaluated[20].

Anti-bacterial activity

Electrospun nanofiber samples 100-AMX, 150-AMX, 200-AMX and 250-AMX were examined for the *Staphylococcus aureus* (ATCC 9542) according to the CLSI protocol[21]. Mueller Hinton Agar plates having microbial suspensions (1×10^6 cells m/L) was kept for overnight growth. Nanofiber samples were prepared and cut into small disk shape (diameter 1.5 cm) and placed on the agar plates. These agar plates were then subjected to the incubator for 24 h at 37°C. The inhibition zone diameter was measured using Vernier caliper[21].

Results and Discussion

Scanning electron microscopy

Various ratio for preparation of the electrospun nanofibers (PVA: PMMA) from 1:9 to 9:1 was taken for optimization of the nanofibers. Placebo nanofibers were prepared and their average diameters were calculated and the ratio having the smallest size was selected. 5:5 ratio was selected as the optimized ratio for drug loaded nanofiber preparation as these nanofibers were smallest in size and the morphology is found to be smooth and uniform. Furthermore, AMXwas loaded at different concentrations of 100 mg, 150 mg, 200 mg, 250 mg to the PVA: PMMA (5:5) composition. As shown in Figure 1 the average diameter of various nanofibers loaded with different AMX concentrations have been found to be 231.3 nM for 100-AMX, 439.45 nM for 150-AMX, 505.75 nM for 200-AMX and 571.9 nM for 250-AMX. On increasing the concentration of AMX in the electrospun nanofibers the average diameter of the nanofibers increases[22]. Figure 1 depicts the structure of all the fabricated electrospun nanofibers having uniform and randomly oriented fibers**.**

X-ray diffraction (XRD)

In Figure 2, XRD pattern for different electrospun nanofibers have been shown. Curve A depicts characteristics peaks at 2θ = 38.16° and 44.34°, as it shows regular crystallization[23]. Curve B, C, D, E shows nanofibers with the blend of PVA: PMMA:AMX with different AMX concentrations portraying the peak disappearance of AMX concluding that the drug is in the amorphous phase and is completely dispersed in the nanofiber matrix. These electrospun nanofibers are suitable for the drug delivery application. The blend of PVA: PMMA shows an amorphous structure, on which the incorporation of AMX regardless of their concentration, does not induce relevant changes.

Fourier- Transform Infrared Spectroscopy (FT-IR) analysis

As per the literature, amoxicillin characteristics peaks were obtained at 1586, 1686 and 1775 cm^{-1} displaying the presence of C=O carbonyl stretching and quinolones, respectively. For the pure PVA polymer, a small characteristics peak was observed at 3300 cm^{-1} depicting the presence of -OH stretching and symmetric and asymmetric stretching was observed at a peak of 2900 cm^{-1} [24]. For pure PMMA polymer, characteristics peaks were observed at 3000-2900 cm^{-1} showing stretching vibrations and the peak at 1750 cm^{-1} depicted the presence of carbonyl group[25]. Addition of AMX at different concentrations in the PVA:PMMA blend displayed the disappearance ofthe AMX peaks showing that the drug is entirely encapsulated into the polymer matrix. Moreover, the peaks did not deviate or disappear on increasing the AMX concentration (Fig. 3).

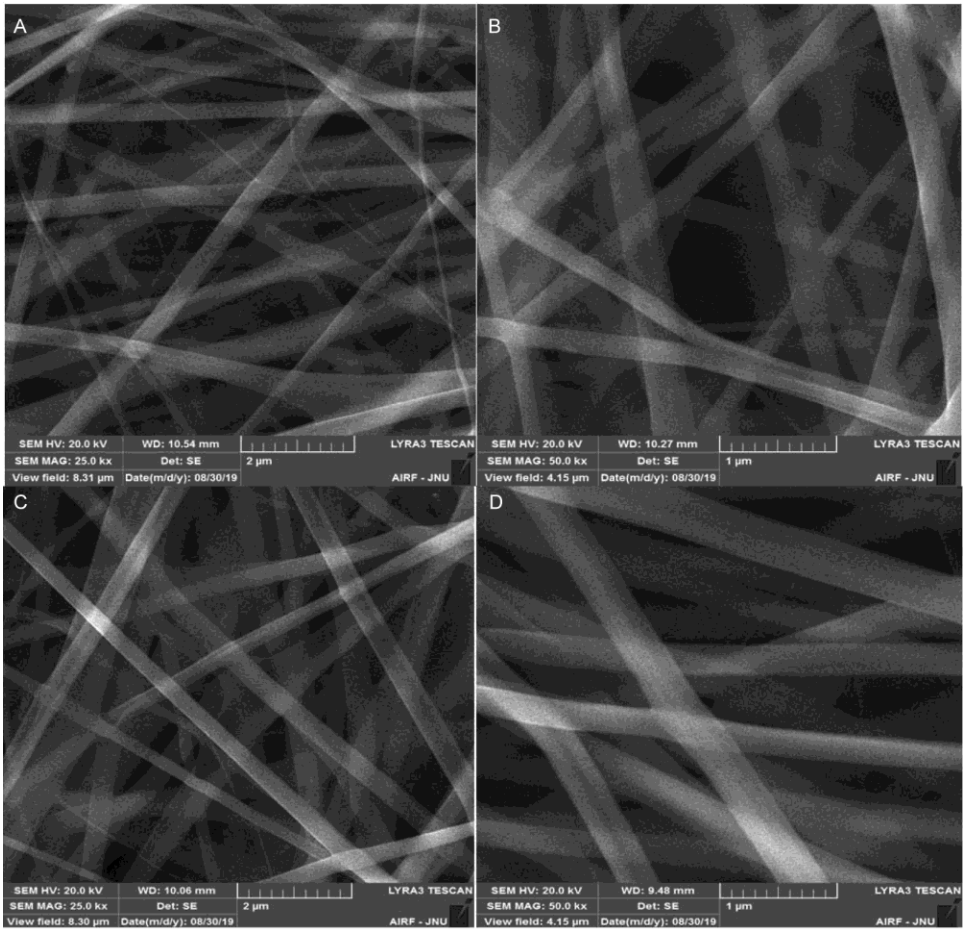

Fig. 1 — SEM images with their average diameters (A) AMX-100; (B) AMX-150; (C) AMX-200; and (D) AMX-250

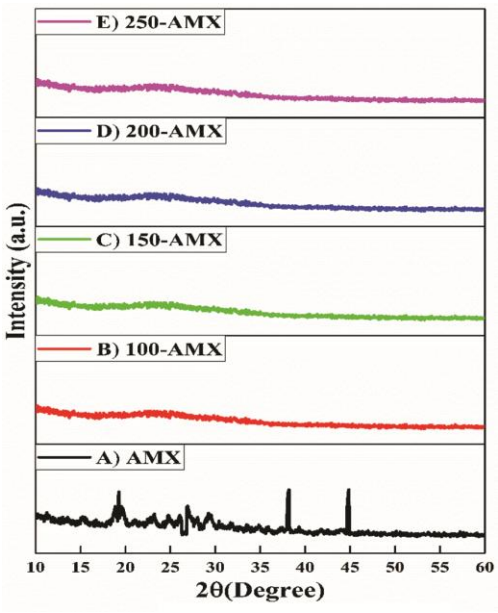

Fig. 2 — XRD pattern for various nanofibers and drug (A) Drug Amoxicillin; (B) 100-AMX; (C) 150-AMX; (D) 200-AMX; and (E) 250-AMX

Atomic force microscopy

Figure 4 displays the AFM topographic images of the electrospun nanofibers that demonstrates changes due to addition of different AMX concentrations into the polymer matrix. Figure 4A-D shows nanofibers with a smooth surface but the diameter of 250-AMX nanofibers is on the larger side when compared with the other nanofibers. On increasing the concentration of the drug AMX, the average diameter of the electrospun nanofibers increases as seen in the above images of AFM[26].

Drug release study

Figure 5 shows the AMX release profiles from the electrospun nanofibers containing 100 mg, 150 mg, 200 mg, 250 mg of drug. These electrospun nanofibers showed an initial burst and rapid drug release within the first 6 h and further a sustained release behaviour was observed. The initial drug release may be attributed to several mechanisms such as pore diffusion, surface desorption, or the lack of a diffusion front barrier to regulate the diffusive process. This initial drug release period is called as "burst" as there

could be quick release of AMX molecules that are deposited on surface area of nanofibers. For the antibiotic delivery, an initial burst is ideal to eliminate the interfering bacteria before they begin their proliferation[27]. However, a few micro-organisms continue to survive the initial burst, a controlled and sustained release of antibiotic is necessary to prevent their growth. The second stage of drug release has a slow phenomenon which is attributed primarily to permeation or diffusion of the drug molecules through polymer matrix into the release medium[28]. *In vitro* release studies were carried out in acetate buffer having a pH of 5.5, as bacterial biofilms can only grow at acidic pH when compared with the healthy micro-environment of the living system[29]. Cumulative drug release from the various nanofibers can be seen in the above figure. The graph shows that AMX released from blend of PVA: PMMA at different concentrations. For 100-AMX (Fig 5A), the initial burst release was about 66% for 6 h and a cumulative release of 82.99% for 7 days, for 150-AMX (Fig. 5B) the first 6 h release was almost similar (66.6%) to the 100-AMX nanofibers, for 200-AMX (Fig. 5C) the initial burst release was 71.4% and 94.9 % for a period of 7 days and for 250-AMX, (Fig. 5D) almost all of the drug was released in the first few hours of the release study.

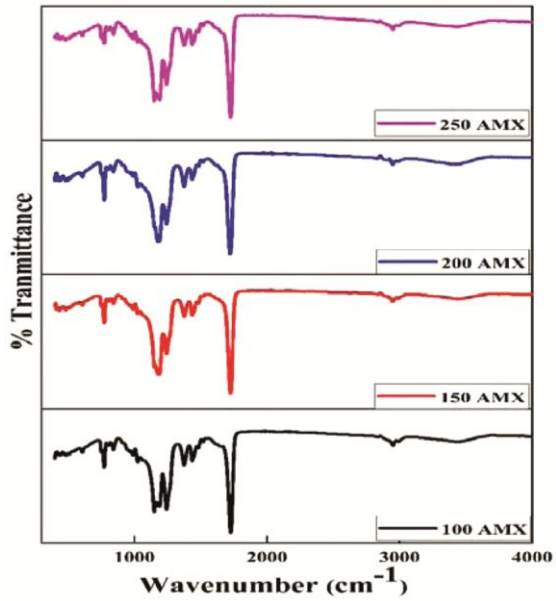

Fig. 3 — FT-IR peaks for 100-AMX, 150-AMX, 200-AMX and 250-AMX nanofibers

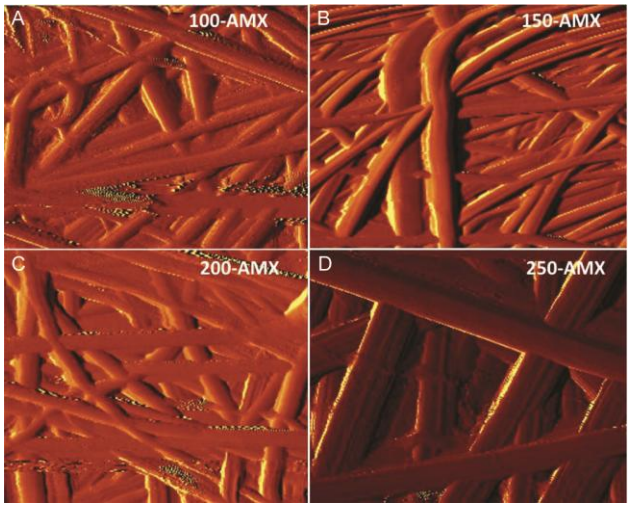

Fig. 4 — AFM Topographic image of (A) 100-AMX; (B) 150-AMX; (C) 200-AMX; (d) 250-AMX

Anti-bacterial activity

Amoxicillin is a broad-spectrum antibiotic for bacterial infections and is effective in treating wound infections[30]. AMX present at different concentrations in the electrospun nanofibers using polymer matrix of PVA: PMMA (5:5) were designed, developed and subjected to anti-microbial activity with *S. aureus* using agar diffusion test. All the electrospun nanofibers showed anti-bacterial activity. For control microbial plate, acetate buffer disks were used. Zone of inhibition was evaluated with different electrospunnanofibers as seen in (Table 2). It can be deduced that the electrospun nanofibers of PVA: PMMA: AMX showed greater zone of inhibition of *S. aureus* as 2.1±0.4 cm for 100-AMX, 2.3±0.5 cm for 150-AMX, 2.4±0.1 for 200-AMX and 3.4±0.3 cm for 250-AMX. These results demonstrate that AMX retains the anti-bacterial activity even after the electrospinning process[31-33], and hence can be used as

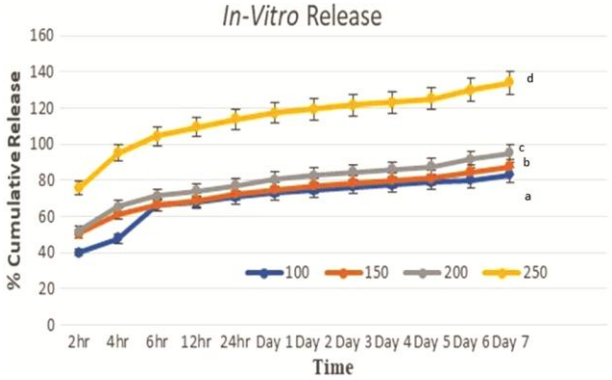

Fig. 5 — The % cumulative release pattern of CPX from various nanofibers

Table 2 — Zone of inhibition the electrospun nanofibers

	Zone of Inhibition (cm)			
Micro-organism	100-AMX	150-AMX	200-AMX	250-AMX
S.aureus	2.1±0.4	2.3±0.5	2.4±0.1	3.4±0.3

a potential wound dressing candidate due to its strategy to fabricate electrospun nanofibers containing antibiotics.

Conclusion

Biodegradable and biocompatible poly (vinyl) alcohol/poly(meth)(methacrylate) electrospun nanofibers loaded with amoxicillin were designed and developed by electrospinning technique. Morphology and structural properties of electrospun nanofibers depend on the drug incorporated at different concentrations in the PVA: PMMA blend of polymer. The time of AMX release from loaded fibrous membranes was extended up to 7 days. The release properties of these antibiotics loaded electrospun nanofibers and their capability for local delivery of active molecules make them suitable for biomedical applications such as wound and skin infections.

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Conflict of interest

All authors declare no conflict of interest.

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