Design and fabrication of non-woven PCL Electrospun Nanofiber (ESNF) loaded with streptomycin.

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ABSTRACT *The aim of the study was to fabricate a non-woven electrospun PCL nanofiber mat (PCL-ESNF mat) which was spiked with the streptomycin to treat microbes in contaminated water. The process parameters were optimized to fabricate PCL-ESNF mat. The diameter of fiber, rate of release of drug and the concentration of streptomycin spiked on nanofibers were optimized.*

The streptomycin loaded fibers retarded the growth of all bacterial strains studied, which include human and plant bacteria's. The control fibers (Blank PCL-ESNF mat) showed no antimicrobial activity. The time taken for fibers to release the loaded drug was found to be 300 seconds. Calculation for the zone of inhibition of bacterial growth with known strain of bacteria was found to be 3.5 cm on average. This indicates that the release profile of the drug from the fibers is timely, to inhibit the growth of bacteria. In case of agar plates well method, the concentration required to exhibit bacteria kill was half that of one with agar plates without well method. This shows that with careful design, lower concentration of drugs might be more efficient to kill the microbes in the waste water. Hence, it can be concluded that the streptomycin loaded PCL-ESNF mat was a effective method to kill microbial contamination in contaminated water.

Keywords*: Electrospun Nanofibers, Polycaprolactone, Streptomycin, Drug release.*

Introduction

The long-term development of the global water situation is closely connected to the growth of the world population and global climate change. Constant growth of the world's population, which is forecasted to be nearly doubled from 3.4 billion in 2009 to 6.3 billion people in 2050. Thus, the demand for fresh water is growing dramatically, in particular for food production, since 70% of the world's freshwater withdrawals are already accounted for by agricultural irrigation. Currently, 64 billion cubic meters of fresh water are progressively consumed each year[1-3].

A common problem in developing countries is drinking water that is contaminated with bacteria and viruses, which are the main cause of water-borne diseases. Due to change in climatic conditions, growing pollution, water will become even scarer, especially in developing countries. Moreover, in these countries, available water is often unsafe to drink[4].

Wastewater treatment processes are designed to achieve improvements in the quality of the wastewater. The various treatment processes may reduce: (i) Suspended solids (ii) Biodegradable organics (iii) Pathogenic bacteria (iv) Nitrates and phosphates. Wastewater treatment is classified into three types: (a) Primary (b) Secondary and (c) Tertiary treatments. Based on the type of treatment and stage of purification, nanomaterials are selected for the effective removal of contaminants from the water systems. Nanotechnology can also be adopted for the removal of microbial contamination in contaminated water[5-9].

Nanotechnology based solution for a biomedical problem is a new area of research. In this fabrication of non-woven fiber matrix for controlled release of drug through the process of electrospinning has gain lot of attention**.** Electrospinning is a simple and versatile process by which polymeric nanofibers can be produced using an electrostatically driven jet of polymer solution[10-15]. These nanofibers can be loaded with both hydrophobic and hydrophilic drugs and used for controlled release of drugs for various biomedical application. These nanofibers can be prepared from a natural, biodegradable and biocompatible polymer or synthetic biodegradable polymer to release the drugs[16-17]. The aim of this work is to develop a nanotechnology-based controlled release drug delivery system to treat microbial contamination in contaminated water. Using electrospun nanofiber, drug (streptomycin) will be released such that drug prevents the buildup of microbial load in the contaminated water. There are several experimental variables that can be altered to tailor the release of the drug from within these electrospun nanofibers to achieve the

desired outcome to treat contaminated water. Therefore, fabrication of such drug loaded ESNF mat is a novel approach to treat microbial contamination in contaminated water.

2.0 Material and method

2.1 Material

Streptomycin was a gift sample from Natco Pharma Ltd. (Hyderabad), Ninhydrin was obtained from Sigma-Aldrich (Bangalore). Sodium hydroxide, Chloroform, Methanol, Nutrient agar – (beef extract, peptone, NaCl), were purchased from Merck Specialties Pvt. Ltd. (Mumbai). Bacteria cultures (strain) was provided by the Department of microbiology, Faculty of life science, JSS University Mysore.

3.0 Method

3.1 Standard calibration curve of streptomycin

Streptomycin can be determined by colorimetric method by complexation with Ninhydrin in basic medium which results in the formation of a pale violet colored complex with an absorption maximum at 420 nm.

3.1.1 Preparation of stock solution (1mg/ml) of streptomycin

25mg of streptomycin was dissolved into 25ml of distilled water in a 25ml of volumetric flask and vortex to dissolve the drug in the distilled water.

3.1.2 Preparation of 1% (w/v) Ninhydrin solution

0.25g of Ninhydrin was dissolved in 25ml of distilled water in 25 ml of volumetric flask and vortex to dissolve the ninhydrin in distilled water.

3.1.3 Preparation of 0.1N NaOH solution

400mg of NaOH was dissolved into 100ml of distilled water and the magnetic stirrer was used to dissolve the pellets of NaOH.

3.1.4 Preparation of calibration curve using streptomycin-Ninhydrin complex (43)

Various concentration of streptomycin (0.8 mg– 2.2mg) from the stock solution was taken out in 10 ml volumetric flask using micropipette and mixed with 1ml of Ninhydrin solution and 1ml of NaOH. The solution was diluted with distilled water to make up to 10ml solution. The sample/aliquots bottles were placed in water bath shaker for 45 minutes at 80 - 100°C. All samples/aliquots were sealed with parafilm tape to avoid spilling before or after removal from water bath shaker. The vials were placed into beakers with water for uniform distribution of heat. All sample/aliquots were pale yellow at the time of mixing with Ninhydrin but changed the color to pale greenish color after around 10minutes of mixing. The final solution after boiling resulted in the pale violet solution. The different concentration of streptomycin was measured at 560nm. Table 1 and figure 1 shows the concentration used for plotting calibration curve.

Table 1: Different concentration used to make 1mg/ml of streptomycin for calibration curve.

Streptomycin µl (mg)	**NaOH**	**Ninhydrin**	**Distilled water**	**Absorbance**
800µl (0.8 mg)	1ml	1ml	7.29µl/ml	0.498
1000µl (1.0 mg)	1ml	1ml	7.09µl/ml	0.6
1200µl (1.2 mg)	1ml	1ml	6.89µl/ml	0.83
1400µl (1.4 mg)	1ml	1ml	6.69µl/ml	0.928
1600µl (1.6 mg)	1ml	1ml	6.49µl/ml	1.124
1800µl (1.8 mg)	1ml	1ml	6.29µl/ml	1.254
2000µl (2.0 mg)	1ml	1ml	6.09µl/ml	1.434
2200µl (2.2 mg)	1ml	1ml	5.89µl/ml	1.643

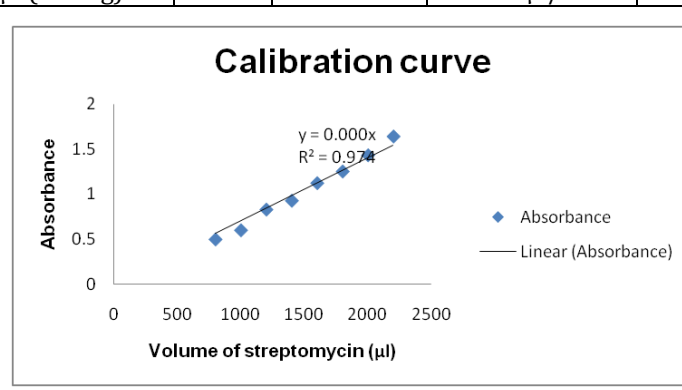


Figure 1:- Calibration curve of streptomycin using streptomycin-Ninhydrin complex.

3.2. Optimization of electrospinning process parameter for the fabrication of PCL-ESNF.

Different process parameters were optimized to fabricate the PCL ESNF using electrospinning technique. The parameter which was affecting the nanofiber morphology was selected and optimized i.e. polymer concentration, solvent system, distance between the needle tip and the collector drum, rpm, type of the syringe and the applied voltage.

3.3 Fabrication of optimized electrospun PCL nanofibers using electrospinning technique (42)

500mg of PCL was weighed and transferred to 15ml sample bottle. Five (5ml) mixtures of methanol and chloroform in ratio of 1:3 that is (1.25ml of methanol and 3.75ml of chloroform) was transferred to the above said sample bottles. Optimized parameters used for electrospinning process were as follow: rpm 1000, voltage 20 kV, flow rate 2ml/h, distance between needle and collection drum/plate 12cm, total spinning time of 15 minutes and volume of syringe used was 5ml.The above mixture (solution) was electrospun using electrospinning machine. Prepared electrospun nanofiber was collected on aluminum foil sheet (12cm x 15 cm) dimension of sheets.

3.4 Characterization of the prepared PCL ESNF

The prepared optimized PCL ESNF was characterized for its surface morphology using SEM.

3.4.1 SEM of the blank PCL ESNF

Scanning electron microscopy of blank PCL ENSF mat was obtained using FE-SEM. The fabricated blank PCL ESNF mat were mounted on the carbon tape and placed inside the SEM chamber. The SEM images were taken at 5KV(22).

3.5 Post loading treatment of blank PCL nanofibers using NaOH

1 x 1 cm^2 PCL nanofibers was cut from the 10x12 cm of fiber sheets. These nanofiber mats were immersed in 0.5M NaOH solution for 12hours.After 2 minutes of reaction with NaOH, bubbles were observed and gas was produced by reaction between PCL and NaOH.

3.6 Preparation of drug loaded nanofiber mats (drug loading)

200mg of Streptomycin was dissolved in 1ml of distilled water for this study. 50µl of drug solution was pipette and transferred to 1 x 1cm^2 of nanofibers, which were previously treated with NaOH solution. The drug loaded nanofibers were dried under ambient conditions for 24hours. The NaOH treated PCL ESNF surface was get charged with ions and leading to the physical adsorption of the streptomycin.

3.7 Determination of drug release from electrospun nanofibers

To quantify and study the release of streptomycin from the PCL ESNF mats, drug loaded mat was immersed in 10 mL distilled water. Aliquot of 100 µl was taken at different time intervals (30, 60, 120, 180, 300, 420 and 600 sec) and fresh distilled water was added to maintain sink condition. The aliquot was mixed with the other reagents to form the complex for Spectrophotometric quantification. From the absorbance value and using the standard curve, the quantity of streptomycin released at various time points was measured.

3.8 Bacterial inhibitor test on various bacterial strains

3.8.1 Preparation of the nutrient agar

The Beef extract 3g, Peptone 5g, NaCl 5g, Agar 15g were weighted and dissolved in 1000ml of distilled water and the pH was maintained at 5 – 7. The flask was heated with agitation to dissolve the ingredients. The flask was sealed with cotton plug and wrap with a craft paper to avoid access water from entering the flask. The flask was autoclaved at 121°C for 15 minutes. Remove the flask from the autoclave and while in molten state the solution was poured onto the Petri dishes/plates either ½ to ¾ and allowed to solidify for 15minutes.

3.8.2 Preparation of Agar plate for the Bacterial inhibitor test

In molten state the solution was poured onto the Petri dishes/plates either ½ to ¾ and allowed to solidify for 15minutes. Spread plate technique was used to spread microorganisms on to the solid medium. With the spread plate method, a volume of an appropriately diluted culture of not greater than 0.1 ml or a small aliquot was transferred to an agar plate and spread over the surface of an agar plate using a sterile L shaped glass rod or strong L-spreader or loop as sometimes called. The bacteria were then distributed evenly over the surface. Bacterial strains were spread with the L-shaped loop. Then six types of bacteria were selected for this study which were *Staphylococci* (staphla) strain, *Salmonella typhae* strain, *Escherichia coli* strain (gram-negative), *Klebsiella* strain, *Pseudomonas aeruginosa* strain and *Ralstonia solanaceam* strain a plant bacteria (gram-negative in nature) as model microorganism. The spread plate were inoculated and incubated for 24hours at 37° C ambient temperature. . After incubation, an even growth of bacteria, or lawn, should have covered the plate.

3.8.3 Preparation of Agar well method for the Bacterial inhibitor test

Sterilized Nutrient agar which was prepared according to the standard protocol for preparation of Nutrient agar was poured into 12 disposable Petri plates under aseptic condition and was left to solidify in aseptic

conditions of Laminar air flow. The 12 petri plates containing solidified nutrient agar were then inoculated with *Staphylococci* (staphla) strain, *Salmonella typhae* strain, *Escherichia coli* strain (gram-negative), *Klebsiella* strain, *Pseudomonas aeruginosa* strain and *Ralstonia solanaceam* strain in triplicates respectively using spread plate method. Now using sterile croak and borer 2 wells were made in the agar and the streptomycin loaded PCL ESNF were added to the wells so that each plate contained an antibiotic loaded PCL ESNF. And to each plate a standard PCL ESNF (blank) was added. The plates were kept for 18-24hours incubation in the incubator.

3.8.4 Loading of control and the drug loaded PCL ENSF mat

Antibacterial activities of streptomycin loaded electrospun nanofibers were investigated by zone of inhibition method. Firstly, the 1 x 1cm^2 nanofibers were placed onto the agar plate. Secondly, plate labeling was done with the bacteria name, divided into 3 sectors.

To help with the removal of fibers from the foil about 100µl of water was added to the each eppendorf tube and both drug loaded & control nanofibers were peeled off from the foil. Antibiotic's loaded PCL ESNF were transferred to the plates using forceps by firmly pressing them on onto the agar in three (3) of the sectors on the plate. Sterilization of the forceps was done each time by dipping it in ethanol and then flaming it for sometimes. Zones of inhibition were determined by calculating the diameters in centimeters of the clear area formed around each of the nanofibers.

4.0 Result and Discussion

4.1 Optimization of electrospinning process parameter for the fabrication of PCL-ESNF.

Different concentration of the solution have been explored at different time ranges and it came out that, the solution with a concentration of 10% PCL was best compared to concentration of 20% PCL due to its viscous flow properties, whereas 8% concentration solution was found to be too watery. Chloroform and methanol was utilized as solvents because of their capability to dry-up fast and form fibers that are robust. The distance between the needle tip and the collector was maintained at 12cm, as the distance of 10 cm produced bead formation in the nanofibers while distance of 15cm barely formed a fiber. Furthermore, the flow rate of 2.0 ml/h was utilized as other flow rate were either too fast or too slow to form the desired nanofibers. Meanwhile, 5ml syringe were used in the process for solution transferring during electrospinning. The applied voltage to obtain good uniform fibers was found to be 20kV for/ with 1000 Rotation per Minute (RMP).

Table:1 Optimization of electrospinning parameter utilized

Parameter	Value
Ambient temperature	0-36 degrees Celsius
Needle to collector distance	12 cm
Voltage	20 kV
Collector rotational speed	1000 RPM
Solution feed rate	2.0 ml/hour
Solution concentration	10%
Methanol and chloroform	1.25ml and 3.75ml

4.2 Fabrication of optimized electrospun PCL nanofibers using electrospinning technique

The PCL ESNF was fabricated using electrospinng technique. Here 500 mg of PCL was dissloved in methanol (0.5ml) and chlorofrom (4.5ml) mixture. The slurry was electrospun at 1000 rpm, 20 kV, 2 ml/hr flow rate and 12cm distance between needle and collector plate.

When the concentration of PCL was less than 10 % w/v the fabrication of ESNF did happen since there was no formation of Taylor cone which is required for the fabrication of nanofibers.

When the voltage applied was less than 10 kV, ESNF mats were not formed as this was lower than the minumium potential that is required between the needle and the collector plate for spontanous sprying of polymer slurry.

Therefore the effect of voltage on fiber diameter, was examined on conditions 1, 2, 3 where each represents a series of fibers formed at voltages of 15, 20 and 25kV respectively. Higher voltage led to bigger diameter for each condition. At low voltages, the electrostatic force was not enough to pull all the fibers to the collector plate as many fibers fell between the gap of the collector plate and the needle. The thicker fibers at the lowest voltage indicate that there was not enough electrostatic force to completely overcome and break the Taylor cone on the tip of the needle.

After many trail runs, the optimum parameter required for making nanofiber with required diameter and thickness was found. A representative photograph of the ESNF mat is shown in Figure 2.

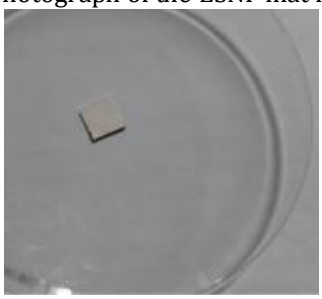


Figure 2: Photograph of PCL ESNF.

Meanwhile concentration was another parameter with noticeable influence as concentration of 20% PCL formed thicker diameter, whereas 8% concentration solution was found to be too watery as noted above and barely formed any desirable fibers, led to beads rather. This is the same as noted by Audrey *et al* (32) and Z, Li & C, Wang (30).

Solubility of streptomycin was not an embedment in this study, as the drug is having moderate solubility and solubilized at the beginning of the experiment.

4.3 Characetization of the fabricated PCL ESNF mat

4.3.1 SEM

The fabricated blank PCL ESNF mat was charaterized using SEM to study the surface morphology of the prepared nanofiber. From the SEM images it can be clearly obsrerved that thte favricated PCL ESNF mat is free from beading ashsown in figure 3.

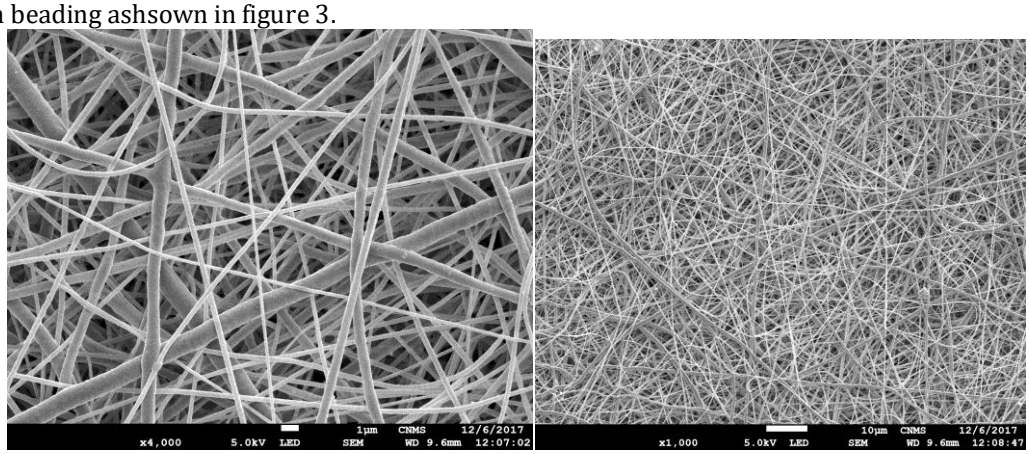


Figure 3:- SEM image of thr fabricated PCL ESNF mat.

4.4 NaOH treatment of PCL ESNF

When the PCL ESNF mat was fabricated using electrospinnig technique the surface of fiber was hydrophobic. Inorder to make the surface of PCL ESNF mat hydrophilic, it was treated with 0.5M NaOH solution for 12 hr. This results in the creation of negative charge on the surface of PCL ESNF mat. The antibiotic, Streptomycin is positively charged chemical compund which can electrostatically adsorp on the surface of treated PCL ESNF mat.

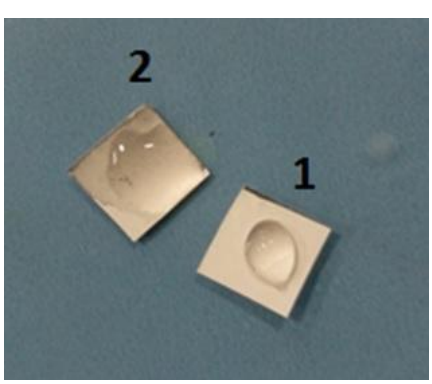


Figure 4:- ESNF mat (1) PCL ESNF not treated with NaOH (2) PCL ESNF treated with NaOH

This is demonstrated through Figure 4 which shows the hydrophobic PCL ESNF mat (1) with a droplet intact on the surface. After treatment, the surface of PCL ESNF mat (2) is rendered hydrophilic and as a result the droplet of water spreads immediately across the whole surface.

4.5 Loading of streptomycin on to PCL ESNF mat.

The amount of streptomycin to be loaded on to the surface of PCL ESNF mat was calculated to be 10 mg. This concentration was chosen from pervious experiments carried out in our laboratory to study the minimum concentration needed for effective kill of bacteria strain on agar plates. This 10 mg of drug was dispersed in 50µl of distilled water and introduced on to the surface of PCL ESNF mat. The rationale for choosing 50µl as the volume to be introduced on the PCL ESNF mat was that 50µl of the drug loaded solution was the optimum volume that could stay on the surface without spilling out of the 1cm x 1cm fiber mat.

4.6 Release behaviour of streptomycin from the PCL ESNF

Drug release of streptomycin from PCL ESNF mat was carried out to evaluate the amount and rate of drug release over a period of 24 hours. As shown in figure 6, there was an initial burst release of streptomycin within the first 120 sec as shown in figure 5. This was due to the physical adsorption of the drug on to the fiber making is ease for its release from the fiber upon contacting with the release media. Subsequently, after the initial burst release, there was sustained release of the drug molecules that was encapsulated and entrapped between the pores of the ESNF fiber mat leading to the sustained release 100 % of the drug was released in 10 min. Therefore, 120 sec play a crucial role in the streptomycin release from fiber of 0.001µm.

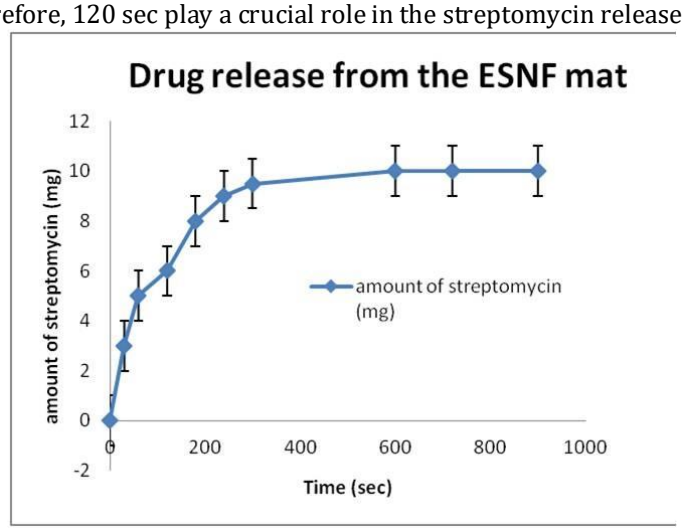
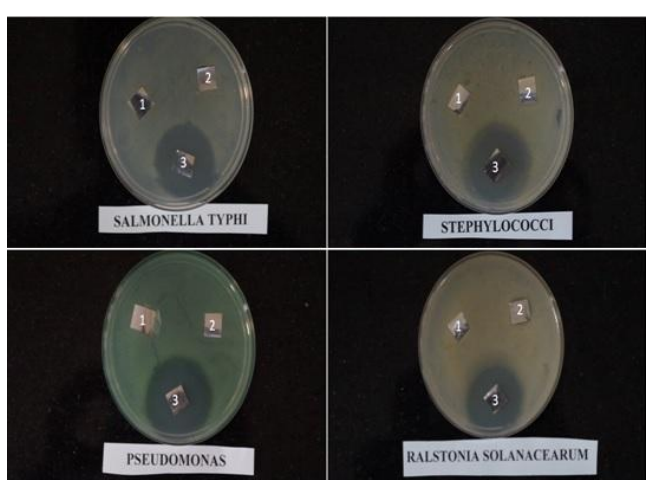


Figure 5 Drug release from PCL ESNF mat.

4.7 Anti bacterial acitivity of PCL ESNF on various bacterial strain using agar plate method

The fabricated PCL ESNF was treated with NaOH to make the surface hydrophilic. Streptomycin (50µl, 200mg/mL) was then introduced on the surface of treated PCL ESNF mat. These samples, along with controls were placed on agar plates with different bacterial strains and growth of bacterial colonies was studied.



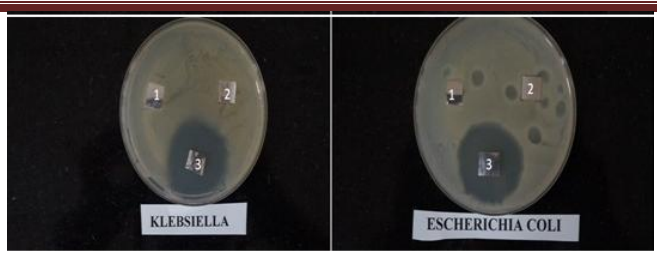


Figure 6 Zone of inhibition for various bacterial strain as seen on agar plates. (1) blank aluminum foil (2) aluminum foil with PCL ESNF (3) aluminum foil with PCL ESNF loaded with streptomycin

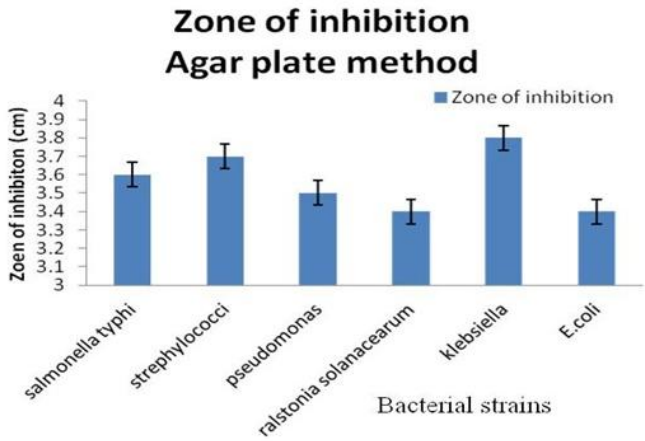


Figure 7: Zone of inhibition in agar plate method on different bacterial strain

As seen in figure 6, blank aluminun foil (1) and aluminium foil with PCL ESNF (2) did not show any inhibitor activity when compared to aluminium foil with PCL ESNF loaded with streptomycin. This clearly indicates that the drug released from the PCL ESNF mat was able to induce inhibitory effect on the growth of bacterial stain.

This was the zone of inhibition recorded for *Salmonella typhi, Strephylococci, Pseudomonas, Ralstonia solanacearum, Klebsiella and E.coli* was 3.6, 3.7 , 3.5, 3.4, 3.8 and 3.4 cm respectivily. This is represented in a graphical form in figure 7. As shown in figure 6 and 7, *E.coli* and *Ralstonia solanacearum*showed the minimum antimicrobial activity when compared to other strain. On the other hand *Klebsiella* showed the maximum sensitivity against the drug streptomycin.

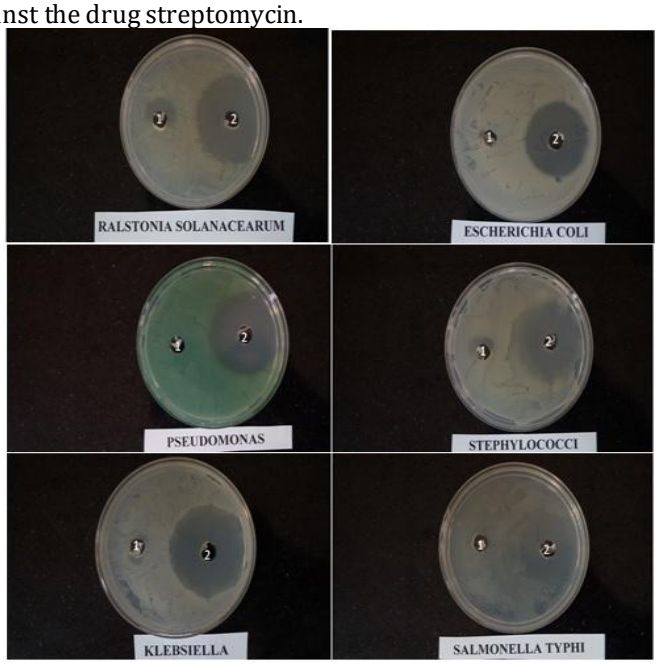


Figure 8 Zone of inhibition in agar well method on different bacterial strain. In all plates (L) is control – blank PCL ESNF and (R) sample – streptomycin-loaded PCL ESNF mat

The zone of inhibition was recorded for *Ralstonia solanacearum, E.coli, Pseudomonas, Strephylococci, Klebsiella and Salmonella typhi* was 4.7, 3.8, 4.5, 4.6, 4.6 and 3.7 cm respectivily.

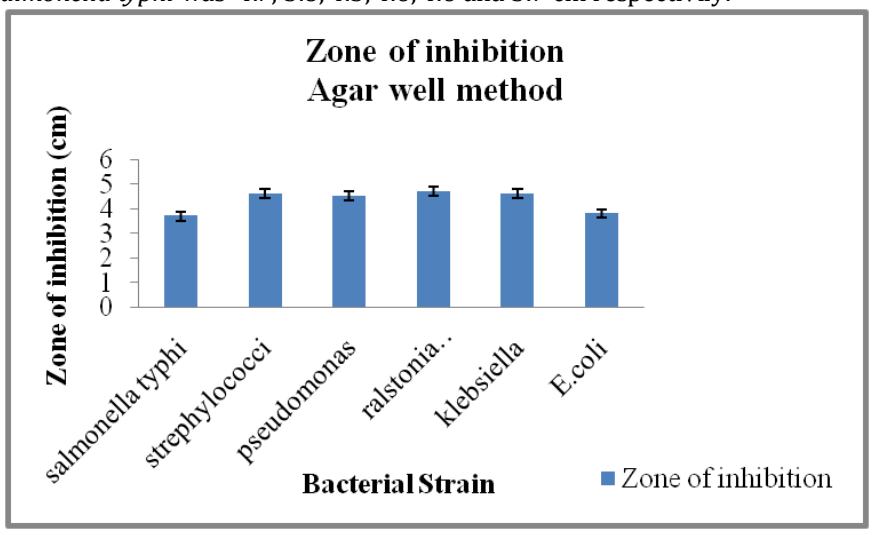


Figure 9 Zone of inhibition in agar well method on different bacterial strain

As shown on a graphical representation in figure 9, *E.coli* showed the maximum sensitivity towards streptomycin when compared to other strains. This was encountered because of the sensitivity of *E.coli* towards the streptomycin which is an aminoglycosides class of drug with act by inhibiting protein synthesis in the bacterial strain.

5. CONCLUSION

Thus, using a nanotechnology-based platform, a novel drug loaded electrospun nanofiber mat was fabricated that showed excellent control on growth of bacteria. The versatility of this platform is that other drugs could be loaded instead of streptomycin or multiple drugs could be simultaneously loaded to inhibit the growth of bacteria. Furthermore, the rate of release of drug could be altered to have sustained release which could be then used to inhibit the growth of slow growing microbes. The streptomycin loaded PCL ESNF mats were successful in achieving inhibition in six different strains of bacteria using two different types of agar plates.

6. Acknowledgement

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7. Conflict of interest

The authors confirm that this article content has no conflict of interest.

8. References

1. Christopher Anand Scott, J. Antonio Zarazúa, Gilbert Levine (2000). Urban-wastewater Reuse for Crop Production in the Water-short Guanajuato river basin, Mexico. Research report 41. Colombia, Sri Lanka: International Water Management Institute pp. 1-31
2. G. G. Huebner (1915). Economic Aspects of the Panama Canal. The American Economic Review. Vol. 5, No. 4, pp. 816-829.
3. P. K. Behera (2014). Soil and solid waste analysis; a laboratory manual. Dominant publisher and distributor Pty. Ltd. New Delhi, India.
4. Wim van der Hoek, MehmoodUl Hassan, Jeroen H. J. Ensink, SabienaFeenstra, LiqaRaschid-Sally, SarfrazMunir, RizwanAslam, Nazim Ali, RaheelaHussain, Yutaka Matsuno, (2002).Urban Wastewater: A Valuable Resource for Agriculture: a Case Study from Haroonabad, Pakistan. Research report, 63. Colombia, Sri Lanka: International Water Management Institute pp. 54.67.
5. B.C. Punmia, Er. Ashok Kumar Jain and Arun K. Jain. (1988). Environmental Engineering – II, wastewater engineering (including air pollution). Laxmi Publications (P) LTD.
6. James Winpenny, Ingo Heinz, Sasha Koo-Oshima, Miguel Salgot, Jaime Collado, Francesc Hernandez, Roberta Torricelli (2010). The wealth of waste: The economics of wastewater use in agriculture. Food and agriculture organization of the United Nations. FAO, water reports 35.

7. Met Calf and Eddy (2003). Wastewater engineering treatment and re-use (4thed). Tata McGraw hill education private limited. New Delhi.
8. Naina M. Maier, Ian L. Pepper and Charles P. Gerba (2009). Environmental microbiology (second ed). Academic press.
9. Donglush Shi, "Nano science in Biomedicine," in Nano Science in Biomedicine., Beijing and Springer-Verlag GmbH, Heildelberg., Springer Science & Business Media (online ebook), 2009.
10. P. K. Supaphol (2010). "Preparation and Adsorption Behavior of Aminated Electrospun Polyacrylonitrile Nanofiber Mats for Heavy Metal Ion Removal.," The Petroleum and Petrochemical College and The Center for Petroleum, Petrochemicals and advanced materials, vol. Volume 2. No 12, pp 1232-1239.
11. S. H. Elbahri and a. Mady, "Nanocomposite Electrospun Nanofiber Membranes for Environmental Remediation.," pp. 1996-1944, 2012.
12. B. Nandana and C. K. and Subhas, "Research review paper Electrospinning: A fascinating fiber fabrication technique.," Current Opinion in Colloid and Interface Science, vol. 28, pp. 325-347, 2010.
13. Supaphol, K. Pimolpun and a. Pitt, "Preparation and Adsorption Behavior of Aminated Electrospun Polyacrylonitrile Nanofiber Mats for Heavy Metal Ion Removal.," The Petroleum and Petrochemical College and The Center for Petroleum, Petrochemicals and advanced materials, vol. Volume 2. No 12, 2010.
14. L. 1. Alessandro, V. C. 1. Eleonora, B. 1. Chiara and *. a. M. Caironi, "Electrospun Polymer Fibers for Electronic Applications.," Center for Nano Science and Technology, vol. Volume 7, pp. 906-947, 2014.
15. P. L. Ding and &. Bin, "Applications of Electrospun Fibers.," Recent patents on Nanotechnology, Vols. Vol. 2, No 3, 2008.
16. S. R. Seeram, F. 3. Kazutoshi, T. 1. Wee-Eong, Y. 3. Thomas, M. 1. Zuwei and R. 1. and Ramakrishna, "Electrospun nanofibers: solving global issues.," Nanoscience and Nanotechnology Initiative, 2006.
17. S. C. K. Nandana Bhardwaj (2010). "Research review paper Electrospinning: A fascinating fiber fabrication technique.," Biotechnology Advances , vol. Volume 28, pp. 325-347.