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Potential Use of Antibiotic-Loaded Polycaprolactone Electrospun Nanofibers to Prevent Implant Related Infections

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Abstract - Covering the surface of a bone implant with electrospun micro/nanofibers can substantially improve its biocompatibility. Since these fibers are usually made of a biodegradable polymer, if they are loaded with a drug, the release rate can be easily controlled. Therefore, loading the fibers with an antimicrobial drug will allow the slow release of the drug during the healing period and can prevent implant-related infections. In this paper, the effectiveness of polycaprolactone (PCL) fibers loaded with ampicillin and silver nanospheres against Escherichia coli growth is described. The results revealed that ampicillin-loaded fibers displayed significant antibacterial activity whereas fibers containing silver nanospheres did not show any activity, possibly due to the low concentration of silver used in this study. Additionally, the release rate of ampicillin was examined and the fibers were able to maintain the ampicillin for a significantly longer time compared to when the sample was simply soaked in an ampicillin solution.

Keywords**:** Electrospinning, Polycaprolactone (PCL), Silver nano particles, Antibiotics, Ampicillin.

1. Introduction

Implant related infections are one of the most important issues that may arise after implanting an artificial object inside the body. They can cause severe pain and implant rejection and the only effective treatment is removing the implant and redoing the surgery, which can be very problematic especially for people of advanced age [1]. The main cause of implant infection is bacterial contamination, which can be substantially reduced by covering the surface of the implant with a material containing antibiotics or other antibacterial agents [2]. Ideally, antimicrobial drugs should remain on the surface for a relatively long period, until the fusion between the implant and bone is achieved. If the surface is simply covered with antibiotics, they will be released into the body in just a few hours and infection may still occur. To overcome this issue, antimicrobial drugs are usually incorporated into a biodegradable material to reduce the release rate. Adding vancomycin to polylactide-co-glycolide polymer [3] and various antibiotics such as cephalothin, carbenicillin, amoxicillin, cefamandol, tobramycin, gentamicin and vancomycin to hydroxyapatite [2,4] are some examples that have been previously studied.

Another strategy is to add the antimicrobial agents to electrospun polymeric fibers. In addition to controlling drug release rate, these fibers can be beneficial to the osseointegration process, since they are highly biocompatible and provide a porous structure for the bone cells to grow in [5]. Some previous studies have examined the effects of adding antibiotics to fibers for implant fabrication purposes. Li et al. added gentamicin to PLGA/PEO fibers and the fibers showed significant antibacterial activity [1]. Zhang et al. studied the effect of adding vancomycin to PLGA fibers. They showed that vancomycin has a very slow release rate and the fibers were able to kill the bacteria even after 28 days [6]. Gilchrist et al. showed the high antibacterial activity of fusidic acid and rifampicin loaded poly(D,L-lactic acid-co-glycolic acid) (PLGA) fibers using both in-vitro and in-vivo tests [7]. Another study on PLGA fibers was conducted by Kim et al. where they incorporated Mefoxin® and sodium cefoxitin to the fibers and these remained effective against bacteria for up to one week [8].

In this study, the effect of adding the water-soluble beta-lactam antibiotic ampicillin to polycaprolactone (PCL) fibers was examined. In addition, as an alternative to antibiotics, the incorporation of silver nanospheres to PCL fibers was also studied. Silver is a very strong bacteriostatic material, which means it can stop the bacteria from multiplying without killing them. The main advantage of silver over antibiotics is that bacteria cannot develop resistance to this agent and accordingly silver nanoparticles have recently been considered for clinical applications. One issue associated with silver is the toxicity of the element. However, the high surface to volume ratio of silver nanoparticles allows them to effectively stop the bacteria from reproducing even at very small doses that are not toxic to humans [9, 10].

2. Materials and methods

2.1. Materials

Polycaprolactone (PCL) was the polymer used in this study for the electrospinning process and was purchased from Sigma-Aldrich (Polycaprolactone, average Mn 80,000). Acetone (Acetone, ACS reagent >99.5%, Sigma-Aldrich) served as solvent to prepare the electrospinning solution. Silver nanospheres suspended in water at 0.02mg/ml with the average sizes of 20, 40 and 80 nm were purchased from Sigma-Aldrich and Ampicillin (Na salt) was purchased from Fisher Scientific. All the materials in this study were directly used as purchased.

2.2. Electrospinning process

The solution was prepared by dissolving PCL in acetone at 10 wt% using an ultrasonic cleaner and a thermocline for about 2 hrs. The silver suspension or ampicillin powder was directly added to the electrospinning solution at different concentrations (5 wt% for silver and 16 wt% for ampicillin). The electrospinning machine used in this study was YFlow® StartUp Electrospinning Lab Device with a vertical setup. The voltage was set at 10 kV and the solution was pumped to the needle at the flow rate of 1 ml/hr. Additionally, the distance between the needle and the collector was 20 cm. The fibers were deposited on the surface of a thin titanium sheet.

2.3. Antibacterial test

The antibacterial activity of silver nanospheres and ampicillin was assessed using the agar diffusion test. In these tests, wild type gram-negative E. coli strain DH5α and an ampicillin-resistant derivative carrying plasmid pET16b was cultured overnight at 37 °C with shaking in LB (Lysogeny Broth). 5 µl of the overnight culture was added to 4 ml top agar (LB supplemented with 0.8% agar), mixed by inversion, and overlaid upon a LB agar plate. After the top agar solidified, the PCL samples were placed polymer side down on the surface of the top agar and were incubated for 24 hours at 37 °C. If a circular clear zone (zone of inhibition) was formed around a sample, it means that the sample had antibacterial activity and killed all the bacteria inside the zone.

2.4. Materials characterization

Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray (EDX) analysis was performed using a JEOL 6400 Digital SEM.

3. Results and discussion

Table 1: Fiber sizes obtained through image processing of the SEM images.

	PCL Without ampicillin	Ampicillin-loaded fibers
Average diameter (µm)	1.36	1.31
Maximum diameter (µm)	2.4	2.5
Minimum diameter (µm)	0.5	0.5
Standard deviation (µm)	0.43	0.49

SEM images were produced of electrospun fibers generated with or without ampicillin (Figure 1). A beadles web of fibers was generated in both cases which shows adding ampicillin did not affect the quality of fibers. The diameter of the fibers was measured through image analysis using 50 random fibers from each picture and the results are tabulated at Table 1, which indicates that the presence of ampicillin did not have any noticeable effect on the size of fibers.

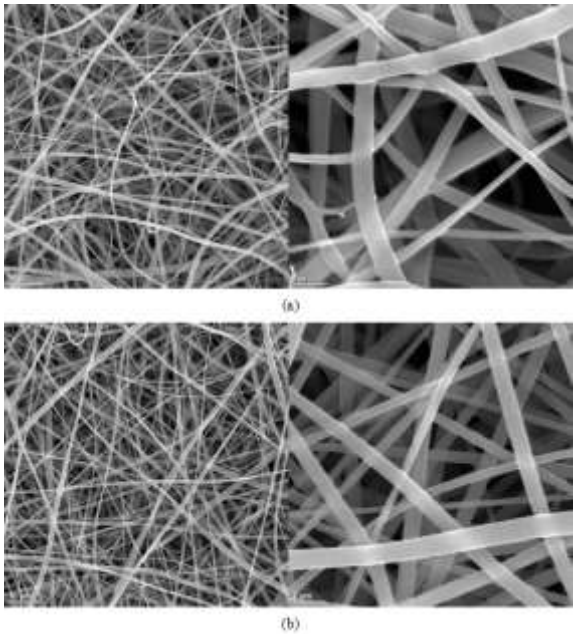

Fig. 1: SEM images of the electrospun fibers at two different magnifications: 1000X (left) and 5000X (right) for: (a) simple PCL fibers, (b) ampicillin-loaded fibers.

EDX analysis was conducted on simple PCL fibers and ampicillin-loaded fibers (Figure 2). A trace of ampicillin can be seen in the results where an obvious peak of sulfur, associated with the beta-lactam thiazolidine ring, can be seen at 2.3 keV. Moreover, a sodium peak is observed for the ampicillin-loaded fibers, which is expected since the sodium salt of ampicillin was used in this study.

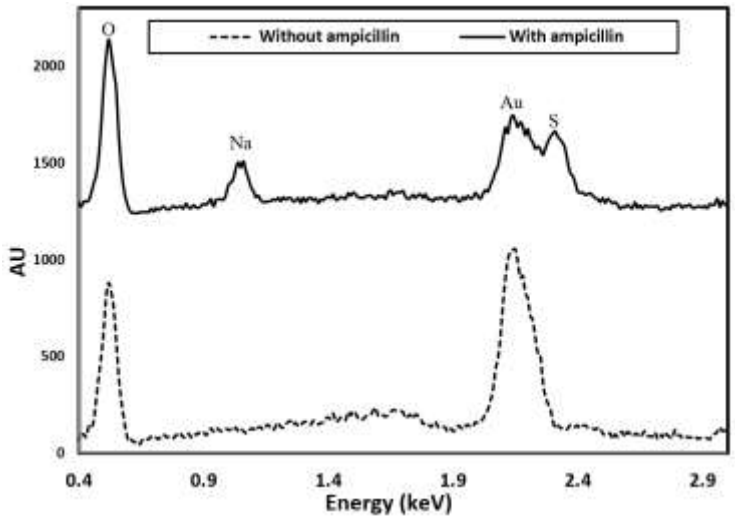

Fig. 2: EDX analysis of ampicillin-loaded and unloaded fibers.

To investigate silver as an antimicrobial agent, silver nanospheres with three different sizes (20, 40 and 80 nm) were suspended in a water-based solution at a concentration of 0.02 mg/ml. The silver suspension was added to the electrospinning solution at 5 wt%. It should be noted that, at higher concentrations, the solution was not electro-spinnable. No zone of inhibition was observed for either wild type (Figure 3) or ampicillin resistant E. coli (not shown). We conclude that the Ag nanoparticle concentrations still permitting effective fiber electrospinning were too low to inhibit bacterial growth. We are currently investigating alternative strategies toward incorporating silver particles into the fibers.

Fig. 3: Antibacterial testing of the fibers containing silver nanospheres against wild type E. coli. The samples are labelled as L (control sample), H (ampicillin-loaded fibers) and D (ampicillin soaked sample).

In testing ampicillin, we used the highest concentration (16%) that permitted effective electrospinning. In addition, samples without ampicillin and another sample that was only soaked in an ampicillin solution (100 mg/ml) post-spinning were used as controls. Ampicillin-resistant E. coli displayed no detectable zones of inhibition around any of the fiber samples (not shown). In contrast, wt E. coli was strongly inhibited by the fibers with incorporated ampicillin and where the fiber was soaked in an ampicillin solution post-spinning (Figure 4A).

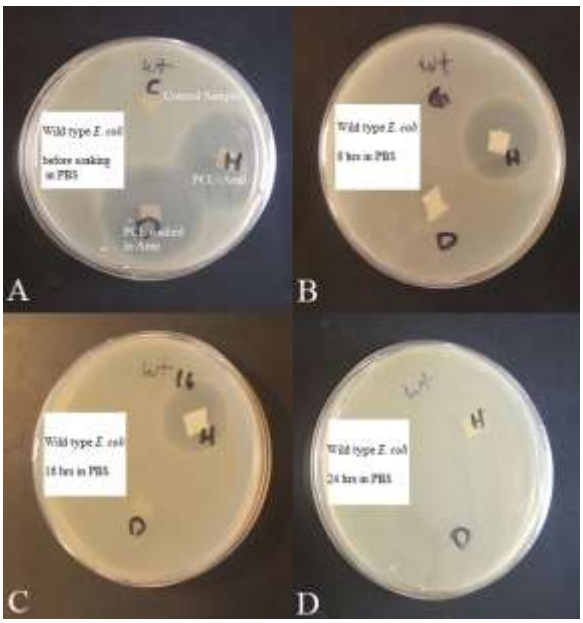

Fig. 4: Antibacterial testing of the ampicillin-loaded fibers (labeled as “H” in the images) and fibers soaked in ampicillin (labeled “D” in the images) against wild type E. coli.

To test whether incorporating ampicillin during the electrospinning process increased retention time of the drug, ampicillin-incorporated and ampicillin-soaked fiber samples were soaked in phosphate-buffered saline (PBS) for different times (8, 16 and 24 hours) and were tested to assess their inhibitory effects. As predicted no zone of inhibition was observed when the dish was cultured with ampicillin resistant E. coli (not shown). Using wt E. coli however, a clear zone of inhibition was visible around the ampicillin-loaded fibers after 8 (Figure 4B) and 16 (Figure 4C) hrs of soaking in PBS. In contrast, no zone of inhibition was observed around the fiber samples that were merely soaked in an ampicillin solution. This indicates that incorporating the drug into the electrospun fibers improves drug retention rate and increases the effective time in which these fibers can mediate an antimicrobial effect. After soaking for 24 hrs in PBS (Figure 4D) even the ampicillin-incorporated samples had lost antimicrobial activity. Figure 5 quantifies the zones of inhibition formed around the samples prior soaking in PBS and after soaking in PBS for 8 and 16 hrs. The zones of inhibition gradually decreased in size as PBS soaking time was increased for the ampicillin-incorporated sample.

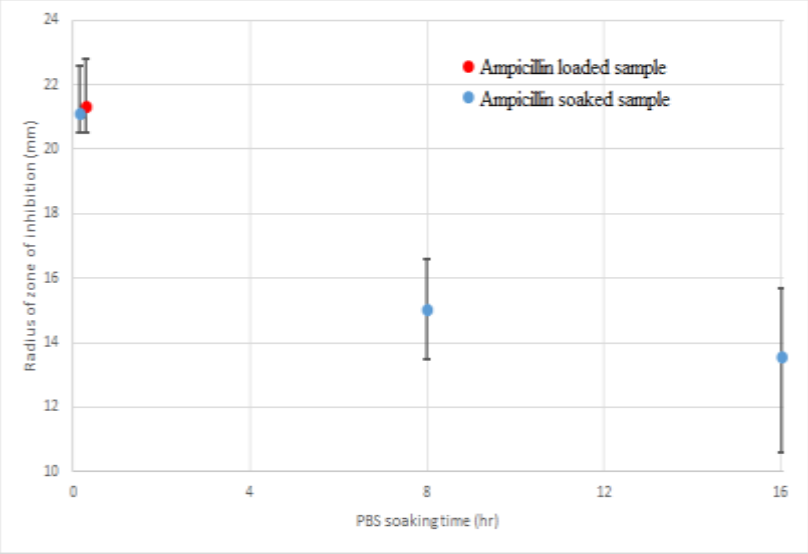

Fig. 5: Radius of zone of inhibition before and after 8 and 16 hours of soaking in PBS (Error bars: standard deviation; number of each tests: 3 times).

4. Conclusion

The effect of incorporating silver nanospheres and ampicillin into PCL fibers was studied. the concentration of silver in the suspension used in this study (0.02 mg/ml) was apparently too low to display antibacterial activity and higher concentrations of silver impaired the electrospinning process. We are investigating whether alternative methods of nanoparticle incorporation may allow for the use of higher concentrations in the future.

The results revealed that, even though PCL is hydrophobic and ampicillin is hydrophilic, it is possible to generate electrospun fibers containing these two materials. Incorporating ampicillin during the electrospinning increased the retention time of the drug relative to fibers merely soaked in an ampicillin solution post-spinning. Drug incorporation and increased retention time indicate that this procedure should be considered for surface modification of bone implants to increase their biocompatibility and reduce the chance of infection.

Acknowledgements

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