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Twisting Electrospun Nanofiber Fine Strips into Functional Sutures for Sustained Co-delivery of Gentamicin and Silver

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Abstract

Surgical site infections (SSIs) represent the most common nosocomial infection among surgical patients. In order to prevent SSIs in a sustained manner and lessen side effects, we developed a twisting method for generation of nanofiber-based sutures capable of simultaneous delivery of silver and gentamicin. The prepared sutures are composed of core-sheath nanofibers with gentamicin/pluronic F127 in the core and silver/PCL in the sheath produced by co-axial electrospinning. The diameters of obtained sutures range from ~ 80 μm to ~ 1.2 mm. The *in vitro* release profiles of silver and gentamicin exhibit an initial burst followed by a sustained release over 5 weeks. The co-encapsulated sutures were able to kill bacteria much more effectively than gentamicin or silver alone loaded nanofiber sutures, without showing obvious impact on proliferation and migration of dermal fibroblasts and keratinocytes. The gentamicin and silver co-loaded PCL nanofiber sutures may hold great potential for prevention of SSIs.

Graphical Abstract

We report a twisting method for the fabrication of nanofiber-based sutures capable of simultaneous delivery of both silver and gentamicin in a sustained manner for prevention of surgical site

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infections. We also demonstrated that the gentamicin and silver co-encapsulated nanofiber sutures were able to kill bacteria much more effectively than gentamicin or silver alone loaded PCL nanofiber sutures.

Keywords

Electrospun nanofibers; Co-delivery; Suture; Anti-bacterial; Surgical site infections

Background

Surgical site infections (SSIs) comprise ~22% of all healthcare-associated infections (HAIs) and represent the most common HAI among surgical patients.¹ In the United States, approximately 300,000–500,000 SSI occur within 30 days of an operation and kill more than 13,000 people each year.² These infections account for nearly \$3.5–10 billion annually in additional healthcare costs.² These postsurgical infections can increase the length of postoperative hospital stays from anywhere between 5 to 20 days. A patient with an SSI is 2 to 11 times more likely to die compared to those patients without an SSI and 75% of SSI-associated deaths are directly attributed to the SSI.³ Interventions that can reduce the rate of SSIs could save thousands of lives and dramatically decrease healthcare costs. Suture presence in a wound strengthens surrounding tissues susceptible to infection.^{4,5}

The FDA has approved clinical use of Polyglactin 910 suture coated with triclosan, Vicryl Plus, since 2002.⁶ Although triclosan-coated sutures show certain efficacy in reducing SSIs for abdominal surgeries,⁷ triclosan as an antimicrobial agent has raised many questions concerning environmental pollution, food allergy, and developing bacterial resistance and cross-resistance.⁸ In addition, triclosan has shown a wide range of health risks.⁹ Hence, there is an imperative need to develop novel antibacterial sutures for prevention of SSIs.

Silver has been recognized as a broad-spectrum and highly effective antimicrobial agent.^{10,11} Importantly, silver was demonstrated to be effective at killing the antibiotic-resistant strains.¹² A higher concentration of silver ions leads to more effective antimicrobial activity. However, the toxicity is also increased.¹³ Previous studies suggested that silver could accumulate in many tissues¹⁴ and high concentrations of silver can activate the

apoptotic pathway resulting in cell death.¹⁵ Similarly, gentamicin is an antibiotic widely used to treat many types of bacterial infections seen in the clinic.¹⁶ Nevertheless, high doses of gentamicin can cause many side effects, including low red blood cell counts, allergic responses, neuromuscular problems, and drug resistance.^{17,18} Intriguingly, recent studies showed that silver can make antibiotics thousands of times more effective when co-administered together in terms of the antibacterial capability.¹⁹

Poly(ϵ -caprolactone) (PCL) is an FDA approved, biocompatible, and biodegradable polymer, which has been used in certain clinical applications.²⁰ Co-axial electrospinning has attracted great attention in the generation of nanofiber-based biomaterials with its biocompatibility and suitable mechanical properties for use in the biomedical fields (e.g., tissue engineering and drug delivery).^{21,22} Thus in this study, we aim to use co-axial electrospinning to develop nanofiber-based sutures with co-encapsulation of gentamicin and silver and test their antimicrobial efficacy and biocompatibility *in vitro*.

Firstly, we prepared PCL nanofiber membranes, gentamicin/pluronic F127-loaded PCL nanofiber membranes, silver-loaded PCL nanofiber membrane, and gentamicin/pluronic F127-silver/PCL core-sheath nanofiber membranes by electrospinning.²³ Then, we cut the membranes into nanofiber strips with different widths.²⁴ Finally, we twisted the strips to form antibacterial sutures.²⁵ We hypothesized that the antibacterial activity of co-encapsulated nanofiber sutures would be significantly enhanced relative to gentamicin or silver alone loaded sutures, while the toxicity of co-encapsulated sutures would be greatly reduced because of lower drug concentrations applied.

Methods

Poly(ϵ -caprolactone) (PCL, Mw=70–90kDa), pluronic® F-127, silver nanopowders, gentamicin, and 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (MTT) powders were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dimethylsulfoxide (DMSO), dichloromethane (DCM), and dimethylformamide (DMF) were acquired from Thermo Fisher Scientific (Waltham, MA, USA). Dulbecco's modified eagle medium (DMEM), fetal bovine serum (FBS), and penicillin-streptomycin were obtained from Invitrogen (Carlsbad, CA, USA).

Preparation of PCL Nanofiber-based Sutures

One gram PCL beads were dissolved in 8 ml DCM, 2 ml DMF and 300 μ l DMSO mixed solution. After the PCL solution was clear, one milliliter PCL solution was pumped at a flow rate of 0.6 ml/h using a syringe pump while a potential of 12 kV was applied between the spinneret (22 Gauge needle) and a grounded collector. A rotating drum was used to collect membranes composed of aligned PCL nanofibers. Then, the membranes were cut into fiber strips with different widths (e.g., 3, 6, 9, and 12 mm). Subsequently, the PCL nanofiber strips were twisted to form sutures with different diameters.

Preparation of Nanofiber-based Antibacterial Sutures

Ten mg silver nanopowders were dispersed in 10 ml DMSO to form 1 mg/ml solution. Then, 500 μ l silver suspension was added to 20 ml 10% PCL solution and mixed uniformly using

ultrasound to form 50 µg/ml silver containing PCL solution. Five mg gentamicin was dissolved in 20 ml 10% pluronic F-127 solution. The gentamicin containing pluronic F-127 solution and the silver containing PCL solution were fed to the inner and outer nozzles of the co-axial spinneret during electrospinning. Similarly, a rotating drum was used to collect membranes composed of aligned gentamicin/pluronic F127-silver/PCL core-sheath nanofibers. Then, the membranes were cut into fiber strips with different widths (e.g., 3, 6, 9, 12 mm). Subsequently, the core-sheath nanofiber strips were twisted to form antibacterial sutures with different diameters.

Characterization of Morphology

The morphology of PCL nanofiber strips, gentamicin/silver co-loaded PCL nanofiber strips, and sutures made of PCL nanofiber strips and gentamicin/silver co-loaded PCL nanofiber strips were recorded by a digital camera. The surface and inner structures were characterized by scanning electron microscopy (SEM, FEI, Quanta 200, Oregon, USA).

Mechanical Test

The diameters of sutures were measured by SEM. Sutures were fixed within a hollow cardboard and then samples were mounted on the INSTRON Tensile Tester. The cardboard partitions were cut along the discontinuous lines before stretching the fiber. Six samples were stretched to failure at a constant rate of 1 mm/min at room temperature.

In Vitro Release

Ten mg gentamicin-loaded sutures, silver-loaded sutures, gentamicin/silver-co-loaded sutures were immersed in 10 ml PBS solution at 37 °C. At indicated time points, the supernatant was collected and subsequently 10 ml fresh PBS solution was added. The concentration of gentamicin at each time point was measured by ultraviolet (UV) spectrum at 232 nm. The concentration of silver at each time point was measured by inductively coupled plasma-mass spectrometry (ICP-MS).

Proliferation Assay

For the proliferation assay, the sutures' extracted medium in different concentrations was first prepared. Briefly, 20 g silver and gentamicin co-loaded sutures were immersed in 10 ml DMEM and incubated at 37°C for 1 week. Then, 2 mg/ml antibacterial suture extracted medium was obtained and filtered with a needle filter. The 0.5 and 1 mg/ml antibacterial suture extracted mediums were prepared by diluting the 2 mg/ml antibacterial suture extracted medium with DMEM. Following HaCaT cells (human keratinocyte cell line) and human dermal fibroblasts (a gift from Dr. Mark A. Carlson) were seeded in 96-well plates at a density of 5000 cells/well and incubated in 5% CO₂ at 37 °C for 12 h. After the cells attached, the medium was replaced by the extracted medium in which they were incubated with 0.5, 1, and 2 mg/ml extracted medium and cultured for 1, 3, and 5 days. Cells cultured with DMEM, DMEM + free drug (5 µg/ml silver and 2.5 µg/ml gentamicin) served as a control. At indicated time points, 10 µl of 5 mg/ml MTT solution was added and cultured for another 3 h. Then, the cultured medium was removed and 100 µl DMSO was added. Finally,

the absorbance was detected at 450 nm.²⁶ The relative growth rate (RGR) was defined as $RGR (\%) = (\text{Absorbance of sample} / \text{Absorbance of negative control}) \times 100\%$.

Migration Assay

HaCaT cells (human keratinocyte cell line) were seeded in 3 cm culture dishes at a density of 1×10^6 cells/well and incubated in 5% CO₂ at 37 °C for 12 h. After the cells grew to 90% confluence, a scratched wound was created with 20 µl-micropipette tips. The medium was replaced by the 0.5, 1, and 2 mg/ml extracted medium and cultured for 24, 48, and 72 h. Cells cultured with DMEM, DMEM + free drug (5 µg/ml silver and 2.5 µg/ml gentamicin) served as a control. At each time point, the scratched wounds were recorded by a phase contrast microscope. Similarly, human dermal fibroblasts were seeded in 3 cm culture dishes at a density of 1×10^6 cells/well and incubated in 5% CO₂ at 37 °C for 12 h. After the cells grew to 90% confluence, they were treated with 50 µg/ml mitomycin for 30 min. Then, a scratched wound was created with 20 µl-micropipette tips. The medium was replaced by the extracted medium and cultured for 12 and 24 h. Cells cultured with DMEM, DMEM + free drug (5 µg/ml silver and 2.5 µg/ml gentamicin) served as a control. At each time point, the scratched wounds were also recorded by a phase contrast microscope.

Colony Forming Unit Test

Pseudomonas aeruginosa were cultured in liquid Luria-Bertani (LB) medium overnight and then diluted to 10^4 CFUs/ml in PBS. Ten mg PCL nanofiber-based sutures, gentamicin-loaded PCL nanofiber-based sutures, silver-loaded PCL nanofiber-based sutures, gentamicin/silver co-loaded PCL nanofiber-based sutures were added to the 10 ml bacterial PBS solution, and co-cultured at 37 °C for 1 and 2 h. Then, 20 µl bacterial solution was added to LB agar plate and spread mildly. After 12 h incubation, the number of colonies was counted.

Statistical analysis

In this study, the data were expressed as the mean $\pm$ S.D, and the statistical analysis was performed using SPSS 13.0 software. Differences among four groups were assessed using one-way ANOVA. P values less than 0.05 were considered statistically significant, and the significance levels were set at * $p < 0.05$ and ** $p < 0.01$.

Results

Morphology Characterization

Figure 1 shows the preparation process of nanofiber-based sutures. Briefly, nanofiber membranes (e.g., PCL nanofiber membranes, gentamicin/pluronic F127-loaded PCL nanofiber membranes, silver-loaded PCL nanofiber membranes, and gentamicin/pluronic F127-silver PCL core-sheath nanofiber membranes) were first prepared by electrospinning or co-axial electrospinning. To achieve aligned nanofiber membranes, the nanofibers were collected using a high-speed rotating mandrel during electrospinning or co-axial electrospinning. Then, the membranes were cut into strips with different widths. Lastly, the strips were twisted to form nanofiber-based sutures. The EDX result shows that silver has been successfully incorporated to fibers (Figure S1). Figure 2A shows gentamicin/pluronic

F127-silver PCL core-sheath nanofiber strips with 3, 6, 9, and 12 mm wide. Figure 2B shows the photographs of functional sutures formed by twisting the corresponding nanofiber strips in Figure 2A, indicating the uniformity in diameter. The SEM images of fabricated nanofiber sutures were shown in Figure 2, C-E. The diameters of nanofiber-based sutures were 189 μm and 476 μm when made by 3 mm and 12 mm wide fiber strips (Figure 2C). Based on the United States Pharmacopeia (USP) designation, the sizes of nanofiber-based sutures prepared in this study ranged from 6-0 to 10 (metric size/gauge no.: 0.7 to 12), which can match the sizes of most commercial sutures in Table S1. The sutures were composed of twisted and aligned nanofibers. The minimum diameter we were able to achieve was $\sim 82 \mu\text{m}$ by making use of $\sim 34 \mu\text{m}$ thick and 1 mm wide strips (data not shown). The similar morphology for PCL nanofiber-based sutures was shown in Figure S2.

Mechanical Test

Initial tensile strength is a measure of the amount of tension applied in a horizontal plane necessary to break the suturing material. To test the tensile strength, we measured the strain-stress curves of nanofiber-based sutures made of 3, 6, 9, and 12 mm wide fiber strips (Figure 3, A and B). The sutures made of gentamicin/pluronic F127-silver/PCL core-sheath nanofibers had lower ultimate tensile strength than the ones composed of PCL nanofibers. Figure 3C shows that the breaking force of PCL nanofiber-based sutures increased with increasing width of fiber strips. The breaking forces of PCL nanofiber-based sutures made of 9 and 12 mm wide fiber strips were significantly higher than those composed of 3 and 6 mm wide fiber strips. Figure 3D suggests that the breaking forces of gentamicin/pluronic F127-silver/PCL core-sheath nanofiber-based sutures made of 6, 9, and 12 mm strips were greatly higher than the one made of 3 mm strips. No significant differences were observed among breaking forces for 6, 9, and 12 mm-strip made antibacterial sutures. The sutures made of gentamicin/pluronic F127-silver/PCL core-sheath nanofibers exhibited lower breaking forces than the ones composed of PCL nanofibers. Figure 3, E and F, shows the Young's modulus of sutures made of PCL nanofibers and gentamicin/pluronic F127-silver/PCL core-sheath nanofibers. The values of Young's modulus usually ranged from 64.61 to 168.24 MPa, which seemed to be independent on the widths of nanofiber strips.

Gentamicin and Silver Release Profiles

We further examined the release profiles of silver and gentamicin from sutures made of gentamicin/pluronic F127-silver/PCL core-sheath nanofiber strips in four different widths (Figure 4). The sutures in four different diameters shared the similar characteristics in their silver and gentamicin release kinetics - an initial burst followed by a sustained release over 5 weeks. The amount of gentamicin released after incubation for 5 weeks were 2.35 ± 0.13 , 2.06 ± 0.12 , 2.08 ± 0.22 , and $2.21 \pm 0.16 \mu\text{g/mg}$ for sutures made of 3, 6, 9, 12 mm wide core-sheath nanofiber strips. By comparison, the amounts of silver released within the same incubation time were 30.99 ± 2.49 , 31.25 ± 1.91 , 28.91 ± 3.27 , $25.49 \pm 1.63 \text{ ppb}$ for the corresponding sutures.

Proliferation Assay

To determine any negative effects of the fabricated antibacterial sutures, we first examined their influence on the proliferation of skin cells including keratinocytes and dermal

fibroblasts, which play an important role in the wound healing process (Figure 5). The OD values for both keratinocytes and dermal fibroblasts increased from days 1 to 5 after treatment with 0.5, 1, and 2 mg/ml extracted medium and silver-gentamicin free drug (Figure 5, A and C). The OD values showed no significant dissimilarities between different treatment groups at the same time point. It seems that the treatment with antibacterial suture extracts and silver-gentamicin free drug had no significant impact on the cell proliferation compared to the control. Furthermore, as shown in Figure 5B and 5D, the relative growth rates of keratinocytes and fibroblasts after treatment with silver-gentamicin free drug and 0.5, 1, 2 mg/ml antibacterial suture extracts were 88.66%, 90.40%, 86.25%, and 87.81%, respectively at each indicated time point, while the relative growth rates of fibroblast after treatment with silver-gentamicin free drug and antibacterial suture extracts were 91.11%, 87.45%, 87.21%, and 88.26%, respectively at each indicated time point. Both the relative growth rates of keratinocytes and fibroblast were higher than 75%, indicating no significant *in vitro* cytotoxicity according to the cytotoxicity grading criteria (Table S2).²⁶

Migration Assay

To further determine if there are any negative effects of the fabricated antibacterial sutures on skin cells, a scratch wound healing assay was used to investigate their impact on skin cell migration which is another critical factor affecting wound healing. The *in vitro* wound-healing assays were made by preparing and culturing keratinocytes or dermal fibroblasts, with cell-scratched regions in the center (Figure 6). The artificial wounds were treated with the 0.5, 1, and 2 mg/ml extracted medium and silver-gentamicin free drug to test their effect on cell migration. The artificial wound gaps decreased with increasing incubation time (Figure 6, A and B). Based on the statistical analysis of the keratinocyte migration data, p values between control and treatment groups with free drug and 1mg/ml antibacterial suture extract for 72 h were 0.019 and 0.003. Similarly, based on the statistical analysis of the fibroblast migration data, p value between control and treatment group with free drug for 12 h was 0.06. This indicates there are significant differences between these treatment groups. However, the treatment with 1 mg/ml antibacterial suture extract for 72 h showed a faster wound-healing rate, suggesting a positive influence on the keratinocyte migration. Other p values were larger than or equal to 0.5. Therefore, the treatment with 0.5, 1, and 2 mg/ml antibacterial suture extracted medium showed no negative effect on the skin cell migration compared to the control. Additionally, keratinocytes seemed to take ~ 72 h to close 80% of the artificial wound surface, while dermal fibroblasts only needed 24 h to completely close the same wound surface (Figure 6, C and D).

Antibacterial Test

To characterize the antibacterial effect, we examined sutures made of gentamicin/pluronic F127-silver/PCL core-sheath nanofiber strips against *Pseudomonas Aeruginosa* (Figure 7). Figure 7A shows the CFU counting after treatment of sutures made of PCL nanofibers, gentamicin-loaded nanofibers, silver-loaded nanofibers, and silver/gentamicin co-loaded nanofibers for 1 h. PCL nanofiber-based sutures showed no obvious antibacterial effect when compared with the control (without any treatment). However, gentamicin-loaded, silver-loaded, and silver/gentamicin co-loaded, nanofiber-based sutures showed significant bacterial inhibition compared with PCL nanofiber-based sutures. By comparison, the

numbers of CFUs after treatment with silver/gentamicin co-loaded sutures were much lower than those after treatment with either gentamicin-loaded sutures ($p < 0.01$) or silver-loaded sutures ($p < 0.05$). Intriguingly, after treatment/co-incubation for 2 h, silver/gentamicin co-loaded sutures were able to completely eliminate bacteria while treatment with gentamicin-loaded or silver-loaded sutures only suppressed the bacterial growth to a certain degree (Figure 7B).

Discussion

In this proof-of-concept study, we chose PCL as raw materials to fabricate nanofiber-based functional sutures capable of co-delivering gentamicin and silver in a sustained manner for prevention of SSIs. In order to meet different clinical scenarios, we can easily switch to other materials with appropriate tensile strength and degradation/resorption profiles including absorbable materials (e.g., silk,²⁷ polyglyconate,²⁸ polyglactin 910, and collagen²⁹) and non-biodegradable materials (e.g., nylon,³⁰ polyester and polypropylene³¹).

Recent studies showed that when boosted with a small amount of silver, antibiotics could kill between 10 and 10,000 times as many bacteria.¹⁹ The antibacterial efficacy test in the present study demonstrated that silver/gentamicin co-loaded sutures were more effective in killing bacteria than silver or gentamicin alone loaded sutures. Our results agreed well with previous findings.¹⁹ The difference is that antibacterial sutures can release gentamicin and silver simultaneously in a sustained manner. It is expected that this boosted bacterial-killing effect can last more than 5 weeks. Several steps could occur during the release of gentamicin from nanofiber sutures including 1) wetting suture (water penetration), 2) dissolution of encapsulated gentamicin, 3) diffusion of gentamicin through the nanofibers, and 4) diffusion of gentamicin through the sutures. In the step one, the nanofiber sutures became wet rapidly due to the incorporation of pluronic F127 and their hydrophilicity. In the step two, gentamicin can dissolve in an aqueous solution rapidly because it is a hydrophilic molecule. In the step three, the diffusion of encapsulated gentamicin through the nanofibers could be relatively slow due to the small pores, low porosity, and high tortuosity within nanofibers. In the step four, the diffusion of gentamicin could be fast due to the large pores and high porosity between nanofibers. Therefore, the diffusion of gentamicin through nanofibers could be the control step for its release. The sutures were made of strips with different widths but the same nanofibers. This could explain why the release profiles from sutures were not dependent on the strip width. The release profiles of silver ions are similar to gentamicin. The difference could lie in the disparity between the dissolution rates for silver ions from silver nanopowders and gentamicin and their diffusion rates within nanofibers, which may cause some divergence for the release profiles.

Previous studies showed that gentamicin and silver were commonly used for the treatment of serious infections.^{32,33} However, gentamicin is nephrotoxic and ototoxic while silver can accumulate in many organs, inducing cytotoxicity.^{34–36} These toxic side effects arise from high concentrations of gentamicin or silver.^{37–39} According to the standards of ISO10993-5, *in vitro* cytotoxicity is usually examined for the biological evaluation of medical devices.⁴⁰ The relative growth rates of keratinocytes and dermal fibroblasts after treatment of 0.5, 1, and 2 mg/ml antibacterial sutures extracted medium, indicate that the antibacterial sutures

fabricated in this study have no significant detrimental effects on cell proliferation. Further, our results revealed that the present dose of gentamicin and silver exhibited no evident influence on skin cell migration, which is key for re-epithelialization and granulation tissue formation during wound healing.⁴¹ This could be due to the much lower concentrations of gentamicin and silver used in the present study. In this study, the total released gentamicin and silver from the antibacterial suture within 28 days were 2.356 µg and 31.25 ppb. By comparison, Abraham *et al.* reported the released gentamicin from an injectable polymeric implant was up to 450 µg after eight-day release.⁴² The concentration of silver for bacteriostasis experiments ranged from 1000 to 20000 ppb.⁴³ The silver released from a modified branched PEG hydrogel within 14 days was up to 1000 ppb.⁴⁴ In contrast, the silver released from our sutures was only 26 ppb within the same timeframe. Thus it is expected that the silver-gentamicin free drug had no significant influence on the proliferation and migration of keratinocytes and fibroblasts due to the much lower concentrations used in this study.^{42–44}

In this study, we demonstrated the antimicrobial function of nanofiber-based sutures. Based on the same principle, we can incorporate other therapeutic molecules to nanofiber-based sutures for imparting multiple functions. For example, immune modulating agents, growth factors, or anesthetic drugs could be incorporated to nanofiber-based sutures through direct co-encapsulation/immobilization or deposition of growth factors-loaded particles on the nanofiber strips before twisting/scrolling for control of immune response, promotion of wound healing, or pain relief at surgical sites.^{25,45–47} We will test these functions of nanofiber-based sutures *in vitro* and *in vivo* in our future studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Schematic illustrating the preparation processes of PCL nanofiber-based sutures (A) and gentamicin/pluronic F127 - silver/PCL core-sheath nanofiber-based sutures (B). (C) Schematic illustrating the cross section of PCL nanofiber-based sutures and gentamicin/pluronic F127 - silver/PCL core-sheath nanofiber-based sutures.

Figure 2.

The morphological characterization of nanofiber-based antibacterial sutures. A) The gentamicin/pluronic F127 - gentamicin/PCL core-sheath nanofiber membranes with different widths. B) The sutures made of core-sheath nanofiber membranes with 3, 6, 9, and 12 mm wide. C–E) SEM images showing the surface, inside (cutting parallel to the long axis of sutures), and cross-sectional area of the corresponding sutures in (B).

Figure 3.

The mechanical characterization of nanofiber-based sutures. A, B) The strain-stress curves of sutures made of PCL nanofiber membranes and gentamicin/pluronic F127 - gentamicin/PCL core-sheath nanofiber membranes with 3, 6, 9, and 12 mm wide. C, D) The breaking forces of the corresponding sutures. (E, F) The Young's modulus of the corresponding sutures. These tests (n=6) were repeated three times. *p<0.05, **p<0.01.

Data was presented as mean $\pm$ population standard deviation.

Figure 4.

The *in vitro* release profiles of silver and gentamicin from antibacterial sutures made of gentamicin/pluronic F127 - gentamicin/PCL core-sheath nanofiber membranes with (A) 3, (B) 6, (C) 9, and (D) 12 mm wide. These tests (n=3) were repeated three times. Data was presented as mean $\pm$ population standard deviation.

Figure 5.

Effect of nanofiber-based sutures on skin cell proliferation. A) The proliferation of keratinocytes treated with medium incubated with 0.5, 1, and 2 mg/ml antibacterial sutures extracted medium for 1, 3, and 5 days. The DMEM medium and DMEM + gentamicin-silver free drug were used as a control. B) The relative growth rate (%) of keratinocytes treated with medium incubated with 0.5, 1, and 2 mg/ml antibacterial sutures extracted medium for 1, 3, and 5 days, the DMEM + gentamicin-silver free drug was used as control. C) The proliferation of dermal fibroblasts treated with 0.5, 1, and 2 mg/ml antibacterial sutures extracted medium for 1, 3, and 5 days. The DMEM medium and DMEM + gentamicin-silver free drug were used as a control. D) The relative growth rate (%) of dermal fibroblasts treated with medium incubated with 0.5, 1, and 2 mg/ml antibacterial sutures extracted medium for 1, 3, and 5 days, the DMEM + gentamicin-silver free drug was used as control. These tests (n=6) were repeated three times. Data was presented as mean $\pm$ population standard deviation.

Figure 6.

Effect of antibacterial sutures on skin cell migration. A) Keratinocyte migration in the scratched keratinocyte free area after treatment with medium incubated with 0.5, 1, and 2 mg/ml antibacterial sutures for 0, 24, 48 and 72 h. The DMEM medium and DMEM + gentamicin-silver free drug were used as a control. B) Dermal fibroblast migration after treatment with medium incubated with 0.5, 1, and 2 mg/ml antibacterial sutures for 0, 12, and 24 h. The DMEM medium and DMEM + gentamicin-silver free drug were used as a control. C) Wound (scratched keratinocyte area) healing rate measured in percentage of wound surface. D) Wound (scratched dermal fibroblast area) healing rate measured in percentage of wound surface. These tests (n=3) were repeated three times. Data was presented as mean $\pm$ population standard deviation. (Keratinocyte:

$P(\text{between control and free drug at 72 h}) = 0.019$, $P(\text{between control and 1mg/ml at 72 h}) = 0.003$;
Fibroblast: $P(\text{between control and free drug at 12 h}) = 0.06$; others: $p = 0.05$

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Figure 7. Nanofiber-based sutures against *Pseudomonas aeruginosa in vitro*. The bacteria were quantified by counting CFU after treatment with 1 mg/ml PCL nanofiber sutures, gentamicin-loaded PCL nanofiber sutures, silver-loaded PCL nanofiber sutures, and gentamicin/pluronic F127-silver/PCL core-sheath nanofiber sutures for (A) 1 and (B) 2 h. These tests (n=3) were repeated three times. *p<0.05, **p<0.01. Data was presented as mean $\pm$ population standard deviation.