Nanofiber-based sutures induce endogenous antimicrobial peptide

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Aim: The aim of this study was to develop nanofiber-based sutures capable of inducing endogenous antimicrobial peptide production. **Methods:** We used co-axial electrospinning deposition and rolling to fabricate sutures containing pam3CSK4 peptide and 25-hydroxyvitamin D₃ (25D₃). **Results:** The diameters and mechanical properties of the sutures were adjustable to meet the criteria of United States Pharmacopeia designation. 25D₃ exhibited a sustained release from nanofiber sutures over 4 weeks. Pam3CSK4 peptide also showed an initial burst followed by a sustained release over 4 weeks. The co-delivery of 25D₃ and pam3CSK4 peptide enhanced cathelicidin antimicrobial peptide production from U937 cells and keratinocytes compared with 25D₃ delivery alone. In addition, the 25D₃/pam3CSK4 peptide co-loaded nanofiber sutures did not significantly influence proliferation of keratinocytes, fibroblasts, or the monocytic cell lines U937 and HL-60. **Conclusion:** The use of 25D₃/pam3CSK4 peptide co-loaded nanofiber sutures could potentially induce endogenous antimicrobial peptide production and reduce surgical site infections.

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Infections that occur in wounds created by an invasive surgical procedure are classified as surgical site infections (SSIs) [1]. SSIs are one of the most important causes of healthcare-associated infections, where 300,000–500,000 SSIs occur each year in the United States affecting 2–5% of surgical patients [2]. These postsurgical infections increase the length of postoperative hospital stays between 5 and 20 days, and account for nearly \$3.5–10 billion annually in additional healthcare costs [2,3]. Even worse, SSIs are associated with considerable morbidity and over a third of postoperative deaths [4]. Interventions that can reduce the rate of SSIs could potentially save thousands of lives and dramatically decrease healthcare costs. The use of sutures that activate innate antimicrobial protection during surgical wound closure may provide a novel tool to protect surrounding tissues against infection [5,6] (Figure 1).

Different therapeutic agents have been incorporated into sutures to elicit an antimicrobial effect [7]. These agents (triclosan, chlorhexidine and silver) require direct contact with microorganisms to elicit an antimicrobial effect. Commercially available triclosan-coated sutures can reduce SSIs in different surgical scenarios. Leaper *et al.* demonstrated in a meta-analysis that triclosan-coated polyglactin 910 sutures effectively prevented SSIs across various surgical wound types and resulted in significant cost savings [8]. Schilling *et al.* demonstrated that a 2–0 triclosan-impregnated polydioxanone closing suture can decrease wound infections in patients having a laparotomy for general and abdominal vascular procedures [9]. Watanabe *et al.* showed that triclosan-coated polydioxanone sutures could help prevent the development of SSIs after gastroenterologic surgery [10]. However, Tianotti *et al.*

Figure 1. Schematic illustrating the mechanism of 25-hydroxyvitamin D₃ and pam3Cys-Ser-(Lys)4 peptide co-loaded poly(ε-caprolactone) nanofiber sutures which promote antimicrobial peptide LL-37 production.

reported that triclosan-coated sutures failed to prevent the occurrence of SSI after elective colorectal resections [11]. Due to the emergence of triclosan resistance, chlorhexidine, an oral antiseptic, has been used as a coating on sutures.

Obermeier *et al.* used chlorhexidine coatings at three different antiseptic concentrations based on palmitic and lauric acid carriers to obtain 96 h of slow drug release [12]. Also, antibiotics can be incorporated into sutures. Hans *et al.* manufactured monofilament sutures composed of poly(L-lactide), polyethylene glycol and levofloxacin using wet electrospinning for local, postoperative delivery of antibiotics following ophthalmic procedures [13]. For silver-based strategies, reports of *in situ* photochemical deposition of silver clusters on absorbable and silk sutures show promise in the fight against SSIs. [14,15]. In addition, Xie *et al.* recently created a twisting method for fabrication of antimicrobial nanofiber sutures with incorporation of both gentamycin and silver nanoparticles, and demonstrated a superior antibacterial efficacy compared with nanofiber sutures loaded with gentamycin or silver alone [16]. Although these novel sutures show promising antibacterial effects, they can be associated with many side effects (i.e., toxicity issues) and possibly development of antibiotic resistant strains [17,18]. To address these problems, enhancing human innate immunity to induce endogenous antimicrobial peptide production has become a novel strategy for preventing and treating SSIs [19].

Humans synthesize previtamin D₃ or calcidiol from 7-dehydrocholesterol in the skin upon exposure to UVB irradiation from the sun or artificial light. Hydroxylation of calcidiol by vitamin D 25-hydroxylase (CYP27A1) in the liver generates 25D₃ and the kidney produces active 1,25-hydroxyvitamin D₃ (1,25D₃) via hydroxylation of 25D₃ by 25D₃ 1-α-hydroxylase (CYP27B1). Synthesis of active 1,25D₃ occurs in cells at numerous extra-renal sites (e.g., macrophages, keratinocytes and epithelia cells) that express both CYP27B1 and the vitamin D receptor (VDR), a ligand-activated transcription factor [20]. The synthesized 1,25D₃ binds to the VDR that induces expression of the cathelicidin antimicrobial peptide (*CAMP*) gene and increases levels of the protein hCAP18. The pro-protein hCAP18 is generally considered inactive. Upon cleavage by serine proteases, a small cationic peptide termed LL-37 is generated. LL-37 kills microbes by disrupting their membranes [21]. The current model describing the role of vitamin D in the human innate immune response in skin follows: upon epidermal wounding, Toll-like receptors (TLRs; e.g., TLR2) are activated by the presence of microbes; TLR2 upregulates CYP27B1 levels, thus increasing the conversion of 25D₃ to active 1,25D₃; and this induces the expression of hCAP18/LL-37 through binding of VDR to a vitamin D response element in the *CAMP* promoter (Figure 1). These studies showed

Figure 2. Schematic illustrating the method of fabricating nanofiber sutures.

the scientific feasibility of utilizing the vitamin D₃ signaling pathway for endogenous induction of antimicrobial peptides; however, a delivery system for practical applications is lacking.

In a recently published study, we demonstrated for the first time that incorporation of 25D₃ into poly(ϵ -caprolactone) (PCL) and poly(L-lactic acid) (PLA) fibers via electrospinning allowed sustained release over a period of 4 weeks, and the coculture of 25D₃ loaded PCL fibers with keratinocytes and monocytes greatly boosted the endogenous LL-37 production compared with the free drug [22]. Based on the model in Figure 1, co-delivery of a TLR agonist (e.g., pam3CSK4 peptide) and 25D₃ could enhance CYP27B1 expression and promote the conversion from 25D₃ to 1,25D₃, resulting in production of more LL-37 peptide. In this study, we coencapsulated 25D₃ and pam3CSK4 peptide in PCL/F-127 nanofiber-based sutures via co-axial electrospinning and rolling. We hypothesized that co-delivery of 25D₃ and pam3CSK4 peptide from nanofiber-based sutures could promote endogenous induction of the antimicrobial peptide LL-37 from keratinocytes and monocytes. Our results suggest a promising approach to manufacture novel antibacterial sutures for preventing SSIs.

Materials & methods

Chemicals & materials

PCL (Mw: 70–90 kDa) and pluronic[®] F-127 were purchased from Sigma-Aldrich (MO, USA). Dimethylsulfoxide (DMSO), dichloromethane and dimethylformamide were acquired from Thermo-Fisher Scientific (MA, USA). 25-hydroxyvitamin D₃ was purchased from Santa Cruz Biotechnology, Inc. (TX, USA). Pam3CSK4 and trihydrochloride was obtained from Enzo Life Sciences (NY, USA). Dulbecco's modified eagle medium, fetal bovine serum, and penicillin-streptomycin were obtained from Invitrogen (CA, USA). 25-hydroxyvitamin D₃ and LL-37 ELISA kits were purchased from Diagnostika GmbH (Hamburg, Germany) and Hycult Biotech (PA, USA), respectively. M-PER mammalian protein extraction reagent was obtained from Bio-Rad (GA, USA). FITC-dextran (Mw: 3000 Da) was purchased from Thermo-Fisher Scientific.

Preparation of poly(ϵ -caprolactone) nanofiber-based sutures

PCL beads (2 g) were dissolved in mixed solvent containing dichloromethane (16 ml), dimethylformamide (4 ml) and DMSO (600 μ l). Then PCL solution (5 ml) 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 PCL nanofibers. The degree of fiber alignment from random-oriented to uniaxially-aligned was controlled by the rotating speed of the drum. After 5 min of collection, the deposited nanofiber membrane on the surface of the drum was rolled up, and the suture was formed and cut by a blade (Figure 2).

Preparation of 25D₃ & pam3CSK4 peptide co-loaded nanofiber sutures

25D₃ (1 mg) was dissolved in DMSO (500 μ l), then added to a 10% PCL solution (10 ml) and mixed uniformly. The pam3CSK4 peptide was dissolved in distilled water at a concentration of 200 μ g/ml and stored at -20°C. The

peptide solution (25 μ l) was added to a 10% pluronic F-127 solution (1 ml) forming a low-concentration peptide (4.88 μ g/ml) containing pluronic F-127 solution. The peptide solution (100 μ l) was added to a 10% pluronic F-127 solution (1 ml) forming a high-concentration peptide (18.18 μ g/ml) containing pluronic F-127 solution. Finally, the pam3CSK4 peptide containing pluronic F-127 solution and the 25D₃ containing PCL solution were fed to the inner (0.06 ml/h) and outer nozzles (0.6 ml/h) of co-axial spinnerets during co-axial electrospinning [23]. Similarly, a rotating drum was used to collect the core-sheath nanofibers. After collection for 5 min, the nanofiber membrane on the surface of drum was rolled up and the suture was formed and cut by a blade.

Characterization of morphology

The morphology of sutures made of random or aligned PCL nanofibers, and 25D₃ and pam3CSK4 peptide co-loaded PCL/F-127 nanofibers were recorded with a digital camera. The surface and cross-section structures of nanofiber-based sutures were characterized by scanning electron microscopy (FEI, Quanta 200, OR, USA).

Mechanical test

The nanofiber-based sutures were fixed within hollow cardboard and then mounted on the INSTRON Tensile Tester. The cardboard partitions were cut along the discontinuous lines before stretching the suture. Six samples were stretched to failure at a constant rate of 1 mm/min at room temperature. The diameters of nanofiber-based sutures were measured through scanning electron microscope images.

In vitro release

Ten milligrams 25D₃ loaded nanofiber sutures and 25D₃/pam3CSK4 peptide co-loaded nanofiber sutures were immersed in 10 ml phosphate-buffered saline (PBS) solution at 37°C. At each indicated time-point, the supernatant was collected and subsequently 10 ml fresh PBS solution was added. The concentration of 25-hydroxyvitamin D₃ at each time-point was measured using an ELISA kit. FITC-labeled dextran was used to simulate the release profiles of pam3CSK4 peptide. Similarly, 10 mg FITC-dextran loaded PCL/pluronic F-127 nanofiber sutures were immersed in 10 ml PBS solution at 37°C. The amount of FITC-dextran released from sutures was measured using a fluorescence spectrometer at an excitation wavelength of 488 nm and an emission wavelength of 530 nm, respectively.

Proliferation assay

U937 (human monocyte histiocytic lymphoma cell-line), HL-60 (human promyelocytic leukemia cell line), HaCaT cells (human keratinocyte cell line) and human primary dermal fibroblasts were first seeded in 12-well plates at a density of 2×10^4 cells/well. PCL nanofiber sutures, 25D₃ loaded PCL nanofiber sutures, 25D₃/pam3CSK4 peptide (4.88 μ g/ml) co-loaded PCL/pluronic F-127 nanofiber sutures, 25D₃/pam3CSK4 peptide (18.18 μ g/ml) co-loaded PCL/pluronic F-127 nanofiber sutures were then added at a concentration of 1 mg/ml and incubated in 5% CO₂ at 37°C for 1, 3 and 5 days, respectively. At each indicated time-point, 10 μ l of CCK-8 solution was added and the cells were cultured for another 3 h. Absorbance was detected at 450 nm.

Endogenous LL-37 induction

U937, HL60 and HaCaT cells were first seeded in 10-cm culture dishes. PCL nanofiber sutures, 25-hydroxyvitamin D₃ loaded PCL nanofiber sutures, 25D₃/pam3CSK4 peptide (4.88 μ g/ml) co-loaded PCL/pluronic F-127 nanofiber sutures, 25D₃/pam3CSK4 peptide (18.18 μ g/ml) co-loaded PCL/pluronic F-127 nanofiber sutures were then added at a concentration of 1 mg/ml and incubated in 5% CO₂ at 37°C for 1, 3 and 5 days, respectively. At indicated time-points, keratinocytes were washed with PBS twice, and then 1 ml M-PER mammalian protein extraction reagent (Bio-Rad, CA, USA) was added and cells were lysed for 5 min. U937 and HL60 cells were centrifuged and resuspended with PBS twice, and then 300 μ l M-PER mammalian protein extraction reagent was added to lyse the U937 and HL60 cell pellets for 10 min. All procedures were performed on ice. The lysates of HaCaT, HL60 and U937 cells were collected and transferred to 1.5-ml microcentrifuge tubes. The tubes were then centrifuged at $13,000 \times g$ for 30 min at 4°C and the supernatant transferred to a new tube for analysis. LL-37 in cell lysates was quantified by an ELISA assay kit according to the manufacturer's instructions.

Figure 3. Characterization of sutures made of random and aligned poly(ϵ -caprolactone) nanofibers. (A) Photographs and scanning electron microscope images showing the morphology, surface structure and cross-section of sutures. **(B)** The strain–stress curves of sutures. **(C)** The break forces of sutures. These tests ($n = 3$) were repeated three times.

** $p < 0.01$.

Statistical analysis

The data were presented as the mean $\pm$ standard deviation, and the statistical analysis was performed using SPSS 13.0 software. Differences between two groups were assessed using a paired-student T-test. Differences among four groups were assessed using one-way ANOVA followed by Fisher's Least Significance Difference test. The values of $p < 0.05$ were considered statistically significant. The values of $p < 0.01$ were considered statistically very significant. The significance levels were set at * $p < 0.05$ and ** $p < 0.01$.

Results

Effect of the degree of nanofiber alignment on mechanical properties of sutures

By controlling the voltage applied to the motor of the rotating drum, we prepared sutures made of PCL nanofibers with different degrees of alignment (i.e., 3 v: random-oriented and 12 v: uniaxially aligned) by rolling up the nanofiber membrane deposited on the drum as shown in Figure 2. We then examined the morphologies, surface structures and cross-section structures of sutures (Figure 3A), indicating the sutures were made of random and

Figure 4. Characterization of sutures made of poly(ϵ -caprolactone) aligned nanofibers and poly(ϵ -caprolactone)/F-127 co-axial aligned nanofibers. (A) Photographs and scanning electron microscope images illustrating morphology, surface structure and cross-section of sutures made of PCL/F-127 co-axial nanofibers. (B) The strain–stress curves of sutures made of PCL nanofibers and PCL/F-127 co-axial nanofibers. (C) The break forces of sutures made of PCL nanofibers and PCL/F-127 co-axial nanofibers. These tests ($n = 3$) were repeated three times. PCL: Poly(ϵ -caprolactone).

aligned nanofibers, respectively. The average diameters of sutures made of random and aligned nanofibers were $332.33 \pm 11.34 \mu\text{m}$ and $292.43 \pm 6.84 \mu\text{m}$, respectively. Based on the United States Pharmacopeia (USP) designation, the sizes of sutures made of random and aligned nanofibers prepared in this study ranged from 5–0 to 10, which matches the sizes of most commercial sutures in Supplementary Table 1. Initial tensile strength is a measure of the amount of tension necessary to break the suturing material. We also examined the strain–stress curves of sutures made of random and aligned nanofibers (Figure 3B), showing that sutures made of aligned nanofibers had much higher Young's modulus and ultimate strength than those made of random nanofibers. Similarly, the break force of sutures made of aligned nanofibers was significantly higher than that of sutures made of random nanofibers ($p < 0.01$) (Figure 3C). Interestingly, we could tailor the mechanical properties of nanofiber-based sutures by simply varying the degree of fiber alignment via changing the voltages applied to motor of the rotating drum (Supplementary Figure 1).

Preparation & characterization of sutures composed of poly(ϵ -caprolactone)/pluronic F-127 co-axial nanofibers

Pam3CSK4 peptides are water soluble, therefore, to prolong the release, we encapsulated the peptides in a core of PCL/pluronic F-127 core-shell nanofibers by co-axial electrospinning following our previous protocol [23]. The surface and cross-section structures of sutures made of PCL/pluronic F-127 co-axial nanofibers were similar to the aforementioned PCL nanofiber sutures (Figure 4A). Aligned PCL/pluronic F-127 co-axial nanofibers were seen on the surface of sutures made of PCL/pluronic F-127 co-axial nanofibers. The average diameter of this suture was about $296 \pm 7.38 \mu\text{m}$. Figure 4B shows the strain–stress curve of sutures made of PCL/pluronic F-127 co-axial

Figure 5. *In vitro* release profiles of 25-hydroxyvitamin D₃ and FITC-dextran from sutures. (A) The release profile of 25-hydroxyvitamin D₃ alone loaded sutures (VD suture). (B) The release profiles of 25-hydroxyvitamin D₃ and FITC-dextran (4.88 µg/ml) co-loaded sutures (VD + LC-dextran suture). (C) The release profiles of 25-hydroxyvitamin D₃ and FITC-dextran (18.18 µg/ml) co-loaded sutures (VD + HC-dextran suture). These tests (n = 3) were repeated three times.

nanofibers. A slight reduction in Young's modulus and ultimate strength of sutures composed of PCL/pluronic F-127 co-axial nanofibers was observed compared with sutures made of PCL nanofibers. However, there was no statistical difference in break force between sutures made of PCL/pluronic F-127 co-axial nanofibers and PCL nanofibers (Figure 4C).

In vitro release of 25-hydroxyvitamin D₃ & pam3Cys-Ser-(Lys)₄ peptide

We further examined the release profiles of 25D₃ and pam3CSK4 peptide from sutures made of pam3CSK4 peptide/pluronic F127 - 25D₃/PCL core-sheath nanofibers. In this study, we chose FITC-labeled dextran with a molecular weight of 3000 Da to simulate the *in vitro* release kinetics of pam3CSK4 peptides. Figure 5A shows the *in vitro* release profile of 25D₃ from sutures made of 25D₃-loaded PCL nanofibers, indicating an initial burst followed by a sustained release over 4 weeks. The *in vitro* release of 25D₃ from co-loaded sutures showed a similar profile as that for 25D₃-loaded sutures. The percentages of 25D₃ released after incubation for 4 weeks were $9.67 \pm 0.35\%$, $10 \pm 0.38\%$ and $10.52 \pm 0.53\%$ for the 25-hydroxyvitamin D₃-loaded sutures (VD suture), 25-hydroxyvitamin D₃/FITC-dextran (4.88 µg/ml) co-loaded sutures (VD + LC-dextran suture) and 25-hydroxyvitamin D₃/FITC-dextran (18.18 µg/ml) co-loaded sutures (VD + HC-dextran suture), respectively. The *in vitro* release of FITC-dextran from the co-loaded sutures also showed an initial burst followed by a sustained

Figure 6. Cytotoxicity test of nanofiber sutures. The proliferation of U937 (A), HL60 (B), keratinocytes (C) and dermal fibroblasts (D) after treatment with 1 mg/ml PCL sutures (control), 25-hydroxyvitamin D₃ loaded PCL nanofiber sutures containing 100 µg/ml 25-hydroxyvitamin D₃ (VD suture), 25-hydroxyvitamin D₃ and peptide co-loaded PCL/pluronic F127 co-axial nanofiber sutures containing 100 µg/ml 25-hydroxyvitamin D₃ and 4.88 µg/ml peptide (VD + LC-peptide suture), 25-hydroxyvitamin D₃ and peptide co-loaded PCL/pluronic F127 co-axial nanofiber sutures containing 100 µg/ml 25-hydroxyvitamin D₃ and 18.18 µg/ml peptide (VD + HC-peptide suture) for 1, 3 and 5 days. These tests (n = 3) were repeated three times. PCL: Poly(ε-caprolactone).

release over 4 weeks. The percentages of FITC-dextran release after incubation for 4 weeks were $74.00 \pm 5.93\%$ and $49.01 \pm 3.59\%$ for the VD + LC-dextran suture and VD + HC-dextran suture, respectively.

Cell proliferation after incubation with 25D₃ & pam3CSK4 peptide co-loaded sutures

To determine the potential negative effects of fabricated 25D₃ and pam3CSK4 peptide co-loaded sutures, we first examined their influence on the proliferation of skin cells (i.e., keratinocytes and dermal fibroblasts) and immune cells (i.e., monocytes and neutrophils), which play important roles in the wound healing process. We cultured U937 cells, HL60 cells, HaCaT cells and dermal fibroblasts in the presences of 1 mg/ml sutures. At each indicated time-point, no significant differences in U937 cell proliferation were observed among the control, VD suture, VD + LC-peptide suture and VD + HC-peptide suture groups (Figure 6A). For HL60 cells, there was no significant difference in their proliferation among the four groups on day 1 and 3, while the proliferation of HL60 cells was obviously lower in VD + HC-peptide suture group than other groups on day 5 ($p < 0.05$). For keratinocytes, the proliferation of keratinocytes in control group was higher than other groups on day 3 ($p < 0.05$), while no significant difference was showed among the four tested groups on day 1 and 5. For fibroblasts, there was no significant difference in their proliferation among the four groups at each indicated time-point. These results suggest that 25D₃ and pam3CSK4 peptide co-loaded sutures marginally affected the proliferation of skin cells and immune cells.

Figure 7. Quantification of antimicrobial peptide LL-37 expression. The LL-37 expression of U937 cells (A) and keratinocytes (B) after treatment with 1 mg/ml PCL sutures (control), 25-hydroxyvitamin D₃ loaded PCL nanofiber sutures containing 100 µg/ml 25-hydroxyvitamin D₃ (VD suture), 25-hydroxyvitamin D₃ and peptide co-loaded PCL/pluronic F127 co-axial nanofiber sutures containing 100 µg/ml 25-hydroxyvitamin D₃ and 4.88 µg/ml peptide (VD + LC-peptide suture), 25-hydroxyvitamin D₃ and peptide co-loaded PCL/pluronic F127 co-axial nanofiber sutures containing 100 µg/ml 25-hydroxyvitamin D₃ and 18.18 µg/ml peptide (VD + HC-peptide suture) for 1, 3 and 5 days. These tests (n = 3) were repeated three times. PCL: Poly(ε-caprolactone).

Endogenous LL-37 induction

To endogenously induce the antimicrobial peptide production, we cultured U937 cells and keratinocytes in the presence of 1 mg/ml sutures. We then quantified the induction of LL-37 using an ELISA kit. Although there was no statistical difference in induction of LL-37 from U937 cells among the four groups on day 1, the induction of LL-37 was higher in the VD + HC-peptide suture treatment group than control, VD suture and VD + LC-peptide suture groups on day 3 and 5 ($p < 0.05$) (Figure 7A). The VD + LC-peptide suture group showed higher induction of LL-37 from keratinocytes than control, VD suture and VD + HC-peptide suture groups ($p < 0.05$) on day 5. There was no statistical difference in LL-37 induction among the four groups on day 1 and 3 (Figure 7B).

Discussion

SSIs are one of the most important causes of healthcare-associated infections, which increase patients' suffering, length of hospital stays and cost of care. Current antimicrobial sutures are made by incorporating antimicrobial agents through coating and encapsulation. Due to resistance and toxicity concerns, alternative approaches have been explored including co-delivery of antimicrobial agents and alterations in surface nanotopography [24]. In this study, we took a different approach that uses the host's innate immune system to enhance immunity by inducing endogenous antimicrobial peptide LL-37 expression using vitamin D. To achieve this goal, we developed a new method to fabricate nanofiber-based sutures composed of aligned co-axial nanofibers with pam3CSK4 peptide/pluronic in the core and 25D₃/PCL in the shell via electrospinning and rolling.

In this proof-of-concept study, we chose PCL/pluronic F-127 as raw materials to fabricate nanofiber-based functional sutures capable of codelivering 25D₃ and pam3CSK4 peptide in a sustained manner for endogenous antimicrobial peptide induction. To meet different clinical requirements, we can tailor the properties of nanofiber-based sutures developed in this study in terms of degradation/resorption profiles and break forces. We can easily switch to other absorbable materials (e.g., silk, polyglyconate, polyglactin 910 and collagen) and nonbiodegradable materials (e.g., nylon, polyester and polypropylene) to satisfy different degradation profiles [25–27]. We can regulate the speed of rotating drum to obtain different degrees of nanofiber alignment, resulting in different mechanical properties (e.g., Young's modulus, ultimate strength and break force) of sutures (Supplementary Figure 1). We can also fabricate sutures from different materials to attain desired mechanical properties [28,29]. In our recent study, we showed a twisting method of fabricating multifunctional nanofiber sutures [16]. The smallest diameter of sutures produced by the twisting method was 0.070–0.099 mm (USP size 6–0), while the lowest diameter of sutures produced by the rolling method was 0.050–0.059 mm (USP size 7–0).

Our recent work demonstrated that the incorporation of 25D₃ in electrospun nanofiber membranes could induce endogenous expression of the antimicrobial peptide, LL-37 [22]. To further analyze the induction of antimicrobial peptides, this study developed a method to coencapsulate 25D₃ and pam3CSK4 peptide into nanofiber sutures. Vitamin D₃ plays an important role in regulating the innate human immune system against infectious pathogens containing gram negative and positive bacteria, fungi and mycobacteria [30,31]. Of note, 25-hydroxyvitamin D₃ can induce LL-37 expression in monocytes, macrophages, neutrophils and epithelial cells. LL-37 is expressed at integumentary barrier sites such as the respiratory epithelium to resist infection and at sites of tissue injury including surgical incisions [22,32]. Dickerson *et al.* reported that only 4% of adult patients admitted to the trauma intensive care unit were vitamin D sufficient [33]. Since the production of antimicrobial peptides, like LL-37, is critically dependent on the locally available 25D₃, making a suture that delivers this to the injury site in vitamin D-deficient patients could provide a novel way to minimize the risk of SSIs [22]. The induction of LL-37 is vitamin D-dependent as shown in Figure 1 and involves the TLR. Liu *et al.* reported that the activation of TLRs on human macrophages upregulated the expression of the VDR and the vitamin D-1 α -hydroxylase gene, leading to synthesis of 1,25D₃ from 25D₃ and the induction of the LL-37 peptide. This subsequently enhanced the killing of intracellular *Mycobacterium tuberculosis* by monocyte/macrophages [34]. The activation of TLR2/1 results in a direct antimicrobial response in monocytes and macrophages *in vitro* [35].

In this study, we discovered that the released 25D₃ from nanofiber sutures can induce LL-37 production from U937 cells and keratinocytes, which is consistent with our previous study [22]. Moreover, co-delivery of 25D₃ and pam3CSK4 peptide from nanofiber sutures could significantly enhance production of LL-37 from U937 cells. The maximal concentration of induced LL-37 in U937 cells using co-loaded nanofiber sutures was 59.38 $\pm$ 0.68 ng/ml (Figure 7). However, the effective concentration of LL-37 needed for killing the most common pathogenic bacteria causing SSIs is in the μ M range (*Staphylococcus aureus* 32 μ M [144 μ g/ml], *Escherichia coli* [32 μ M], *Pseudomonas aeruginosa* [16 μ g/ml] and *Proteus vulgaris* [2.5 μ g/ml]) [36–38]. Thus, the concentration of induced LL-37 in this study may be insufficient to treat developed infections. However, using a single cell infection model, Liu *et al.* used *M. tuberculosis* to infect macrophages (multiplicity of infection = 2), which were then treated with TLR2/1 peptide [34]. The expression of VDR and Cyp27B1 were upregulated and the viability of intracellular *M. tuberculosis* was reduced. Moreover, when treating macrophages with 25D₃ and a TLR2/1 agonist, the expression of LL-37 was dramatically increased above levels from either 25-hydroxyvitamin D₃ or TLR2/1 peptide alone [34]. Therefore, the sustained co-delivery of 25D₃ and a TLR2/1 agonist from nanofiber-based sutures could enhance innate immunity mediated induction of LL-37. This type of suture could be suitable for preventing SSIs rather than treating established SSIs.

Owing to the difficulty in quantifying pam3CSK4 peptide, we chose a FITC-conjugated dextran molecule with a molecular weight close to the peptide to simulate its release kinetics. This may not be very accurate due to the differences in molecular weight, molecular structure, solubility and diffusivity between these two molecules.

The induction of LL-37 could be further enhanced using the active form of vitamin D₃ (1,25D₃) in combination with other immune modulating compounds (e.g., resveratrol or phenylbutyrate) [39,40]. Based on the same principle, we can incorporate other therapeutic molecules into nanofiber-based sutures for imparting multiple functions to the sutures. For example, immune modulating agents, growth factors, anti-inflammatory drugs or anesthetic drugs could be incorporated through direct coencapsulation/immobilization or deposition of therapeutic agent-loaded particles on the nanofibers before rolling for control of immune response, modulation of inflammatory response, promotion of wound healing or pain relief at surgical sites [41–43].

Conclusion

In this study, we demonstrated a simple and robust method of fabricating nanofiber-based sutures via electrospinning deposition and rolling. We also demonstrated the control of mechanical properties of nanofiber-based sutures by varying the degree of fiber alignment. We further demonstrated that such sutures were capable of co-delivering 25D₃ and pam3CSK4 peptide in a sustained manner over 4 weeks, which promoted endogenous induction of antimicrobial peptide LL-37 in monocytes and keratinocytes. In addition, the 25D₃/pam3CSK4 peptide co-loaded nanofiber sutures had no significant negative influence on the proliferation of U937 cells, HL60 cells, keratinocytes and dermal fibroblasts *in vitro*. In future work, we will test the *in vivo* biocompatibility and efficacy of prevention of SSIs. The nanofiber sutures developed in this study may be used as general sutures, cosmetic surgery sutures and surgical sutures in many elective surgeries.

Financial & competing interests disclosure

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Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: www.futuremedicine.com/doi/full/10.2217/nnm-2017-0161

Summary points

- We demonstrated a simple and robust method of fabricating nanofiber-based sutures.
- The mechanical properties of nanofiber-based sutures can be tailored by varying the degree of alignment of fibers.
- The nanofiber-based sutures are capable of sustained co-delivery of pam3CSK4 peptide and 25D₃ over 4 weeks.
- The pam3CSK4 peptide, a TLR2/1 receptor agonist, is codelivered with 25D₃ to enhance endogenous induction of the antimicrobial peptide LL-37 through the vitamin D₃ signaling pathway.

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