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The Effect of Silk-nanocefixime Suture on Healing and Antibacterial Properties

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ABSTRACT

Background: Surgical sutures are sterile filaments to support the healing process. Also Suture as a major part of surgical site management can challenge surgical wound healing. The objective of the current study was to prevent surgical site infection by creating a new antibacterial silk-nano cefixime suture.

Methods: The silk suture became silk-nano cefixime suture by immersing in the 1, 2, and 3 wt % cefixime solutions. The antibacterial activity of silk-nano cefixime suture was tested on *E. coli (ATCC25922).* The healing effect of silk-nano cefixime suture was evaluated by H&E staining sections and compared between the control and treated groups on days 3, 8 and 14. The obtained quantitative data were presented as mean ± standard deviation. In order to compare the mean of quantitative variables between the studied groups, general linear model of statistical analysis test and 3-D SPSS charts were used ($P \leq 0.05$).

Results: The inhibition zone diameter of *E. coli (ATCC25922)* was 25 mm and no statistically significant difference was found between the healing properties ($P \geq .05$). There was a significant difference in the mean of Polymorphonuclear leukocytes (PMN) and macrophage infiltration between the control and treated groups on day 8 ($p \leq 0.05$).

Conclusion: Our data indicate that silk-nano cefixime suture has the potential to reduce the risk of surgical site infections. Finally, it is strongly recommended to introduce silk-nano cefixime suture as a reliable substitute for current surgical sutures.

Keyword: Silk suture, Nano-Cefixime trihydrate, Antibacterial activity, Healing properties

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Introduction

Infection and dehiscence of wounds are considered as post-surgery complications. Delayed process of wound healing, increased post-treatment costs, and deformity of surgical sites are the outcomes of weak control on surgical wounds (1). As foreign agents, surgical sutures and external pathogens cause infection in surgical sites (2, 3). Bacteria such as staphylococcus aureus and *E. coli* invade the wound site and cause infection in the process of wound healing (4, 5). Surgical sutures connect the edges of the wound and complement the recovery process. But, the contact of suture thread with various bacteria causes secondary infection in the wound site. Suture threads with anti-bacterial bases decrease the contact of invading bacteria with the thread (6).

Different types of suture threads include absorbable suture threads, non-absorbable suture threads, single-thread and multi-thread ones (7). Silk suture threads have multi-structural and non-absorbable structure used in different surgeries such as cardiovascular, neural and oral surgeries (8, 9). Using anti-bacterial surgical sutures for the purpose of controlling and curing the surgical wounds have been studied over the past years. For example, suture threads coated with Triclosan antibiotic has been successfully used in general surgeries, abdominal surgeries and chest surgeries (10). Also, polymer-based materials such as carboxylic, chitosan, and alginate have been used for making the threads anti-bacterial (11).

Wound recovery occurs through four phases of hemostasis, inflammation, proliferation and maturation. This process starts with the presence of blood platelets and continues with the invasion of inflammatory cells then, proliferation and recovery of granulated tissue and epithelium, and finally, it ends with the proliferation, change of collagen and contraction of the wound (12). Wound healing usually takes 5-10 days, while some surgical processes take 14-28 days (13). Evaluation of tissue reactions and checking the recovery process by reviewing the histopathologic features are useful methods which have been employed in various studies (14, 15). The type of the suture thread can decrease or increase the healing speed through changing the inflammation phase. Therefore, tissue reactions with external materials are considered as one of the main factors for choosing appropriate materials for controlling the healing process (16).

This study aimed at processing silk-nano cefixime suture threat and investigating its anti-bacterial effects on *E. coli (ATCC25922)* in countering wound infection. Also, the healing process resulted from stitching the skin of mouse by this suture thread was compared with the ordinary threads. This study also investigated using or not using the antibiotics in wound management.

Methods

Materials, tools, and the process of preparing silk- nano cefixime suture thread

In the present study, 2-0 non-absorbable silk suture produced by SOPA Co., cefixime trihydrate (400) tab produced by Kosar Co. (Tehran-Iran), acetic acid produced by Merck Co., and *E. coli (ATCC25922)* were provided from the local laboratories. The coverage of cefixime 400 tabs was removed then 1, 2, and 3% cefixime solutions were prepared by deionized water and after that the adequate amount of acetic acid was mixed. Silk suture thread was immersed in the prepared solutions. Silk-nano cefixime anti-bacterial threads were used for the tissue and bacterial study after the laboratory environment was dried.

Antibacterial test

Antibacterial activity of silk- nano cefixime suture thread was evaluated by measuring the diameter of the corona created around the *E. coli (ATCC25922)* compared with the control sample (17). An amount of 1.2×10^{8} $CFU.mL^{-1}$ of the bacteria was cultivated in the plate. Silk-nano cefixime suture thread and ordinary silk thread were put at the two sides of the thread and the plate was placed in the incubator for 24 hours in order to measure the antibacterial property.

Tissue evaluation in mouse model

Eighteen male mature mice with an average weight of 25-30 g were bought from the animal laboratory of College of Pharmacy, Isfahan University of Medical Sciences. After being kept in standard conditions for one week, the mice were divided into two groups of tests treated and control. In the treated group, the lumbar skin of the mice was cut and then, stitched with silk-nano cefixime suture thread. In the control group, the skin of the mice was cut and then, stitched with the control silk suture. Biopsy was done on the skin of the wound site in the mice on the 3rd, 8th, and 14th days and the samples were put in a container of formalin 10%. The skin samples were transferred to the laboratory and then, they were crossed with a thickness of 5 mm and colored with Hematoxylin and Eosin. The tissue crosses were studied by optical microscope using the semi-quantitative method. The structure and changes of the wounds, epithelium, Polymorphonuclear leukocytes (PMN), tissue macrophages, angiogenesis and fibrosis were recorded based on the grade of the observed cells as the following. The scores included inexistence (-), mild (+1), mild to moderate (02), moderate (+3), and severe (+4) (18).

Statistical analysis

The obtained quantitative data were presented as mean ± standard deviation. In order to compare the mean of quantitative variables between the studied groups, general linear model of statistical analysis tests and 3-D SPSS charts were used ($P \leq 0.05$).

Results

The morphology of silk-Cefixime nanoparticles (CFX NPs)

The distribution of CFX particle on silk suture was evaluated by SEM. Moreover, the presence of particles among the filaments of the suture was visible. The nanoparticles clearly covered the suture surface by filling the pore between fibers of the yarn (Figure 1).

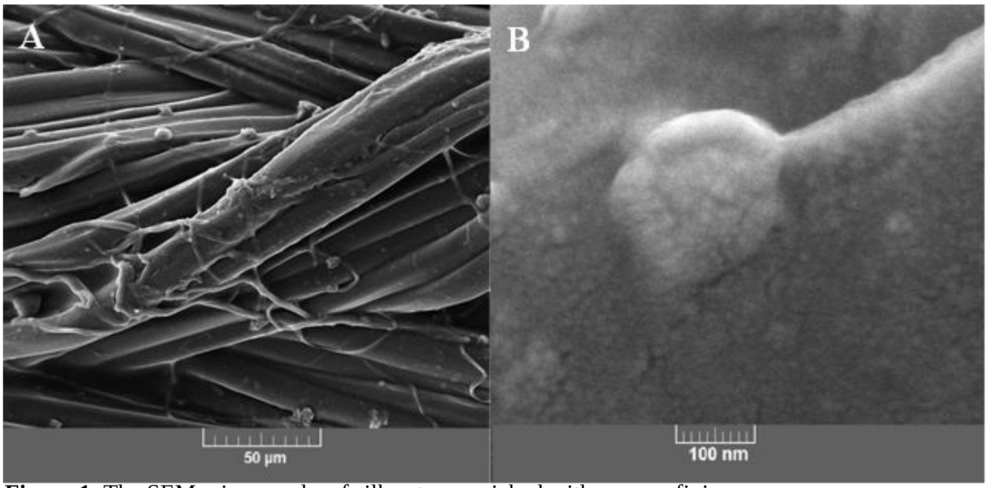

Figure 1. The SEM micrographs of silk suture enriched with nano-cefixime
A) 1000 times magnification, B) 330000 times magnification.

CFX NPs anti-bacterial activity

The microbiological characterizations were performed on Silk-nano cefixime. The zone of *E. coli* inhibition was compared with control model. The zone of *E. coli* around the silk-nano cefixime thread was measured after 24 hours and recorded as 25 mm (Figure 2).

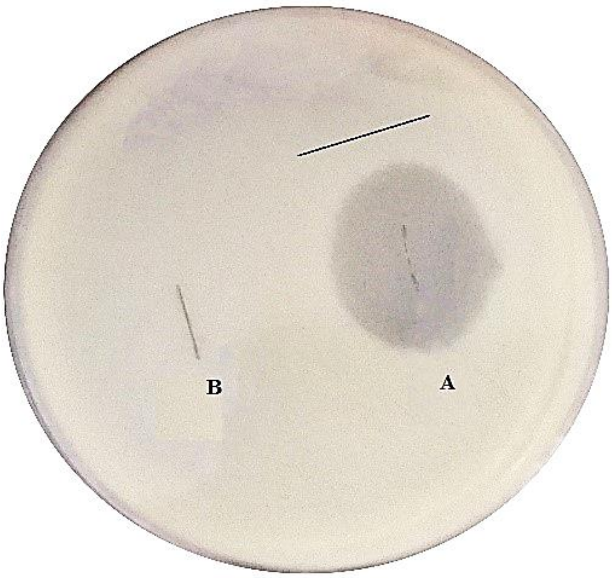

Figure 2. The antibacterial activity of silk-nano cefixime suture threads (A), control suture thread (B)

Tissue healing and changes

The section of sample was stained with Hematoxylin and Eosin (H&E). H&E staining section was compared between the control and treated group on the 3rd, 8th, and 14th days. Figure 3 shows the semi-quantitative analysis of tissue structure and changes for the 3rd, 8th and 14th days after the surgery.

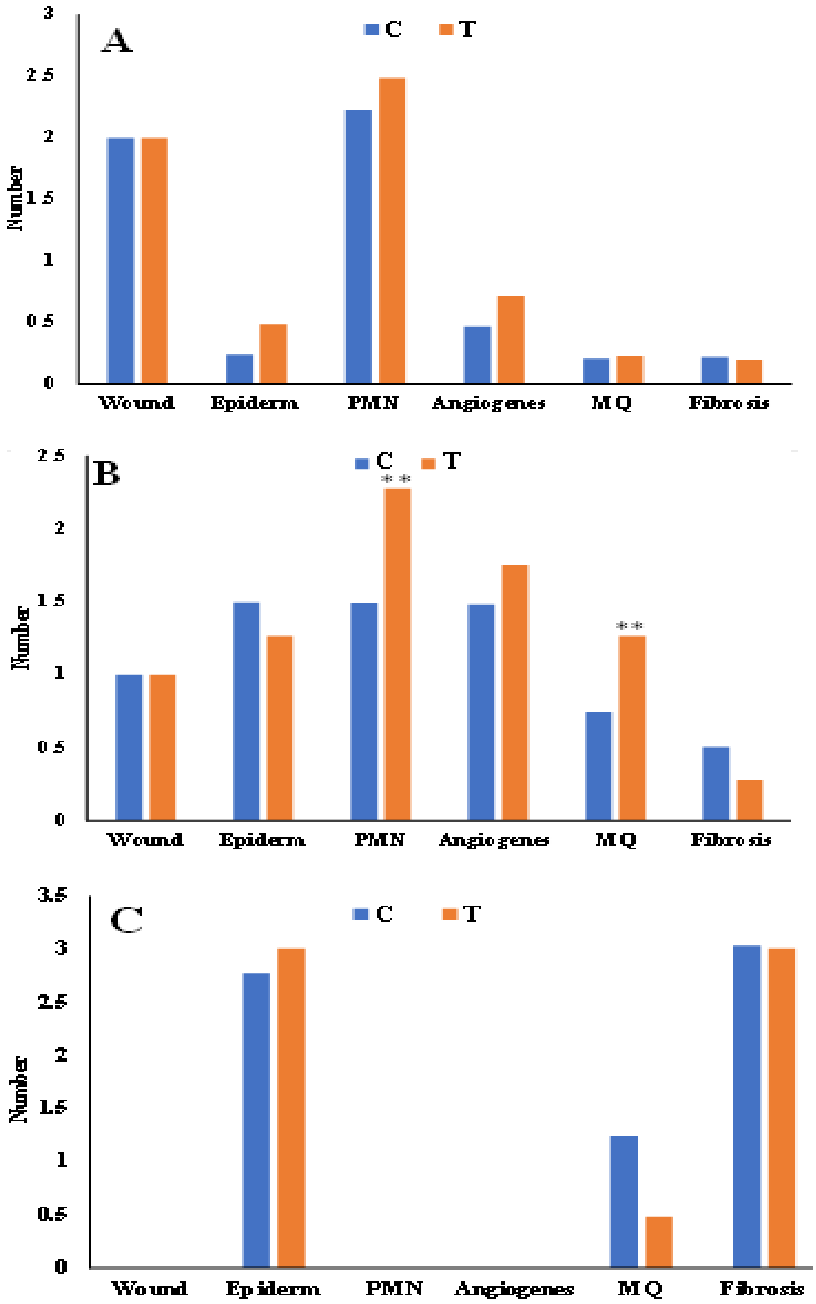

Figure 3. Semi-quantitative analysis of tissue structure and changes

A: the 3rd day after the surgery, B: the 8th day after the surgery, C: the 14th day after the surgery. The data are expressed based on mean ± standard deviation. *P ≤ 0.05

Analysis of the studied factors on the 3rd and 14th days was similar and without any significant difference. According to the statistical analysis, on the 8th day after the surgery, there was a significant difference ($P \leq 0.05$) between the control group and treated group in infiltration of PMN Polymorphonuclear leukocytes and existence of macrophage in the tissue. Figure 4 shows different characteristics of the samples (Figure 4A treated and figure 4B control on the 8th day. Figures 4C-F show that the infiltration of inflammatory cells at the site of healing could complete healing process with angiogenesis and fibrosis in the treated group).

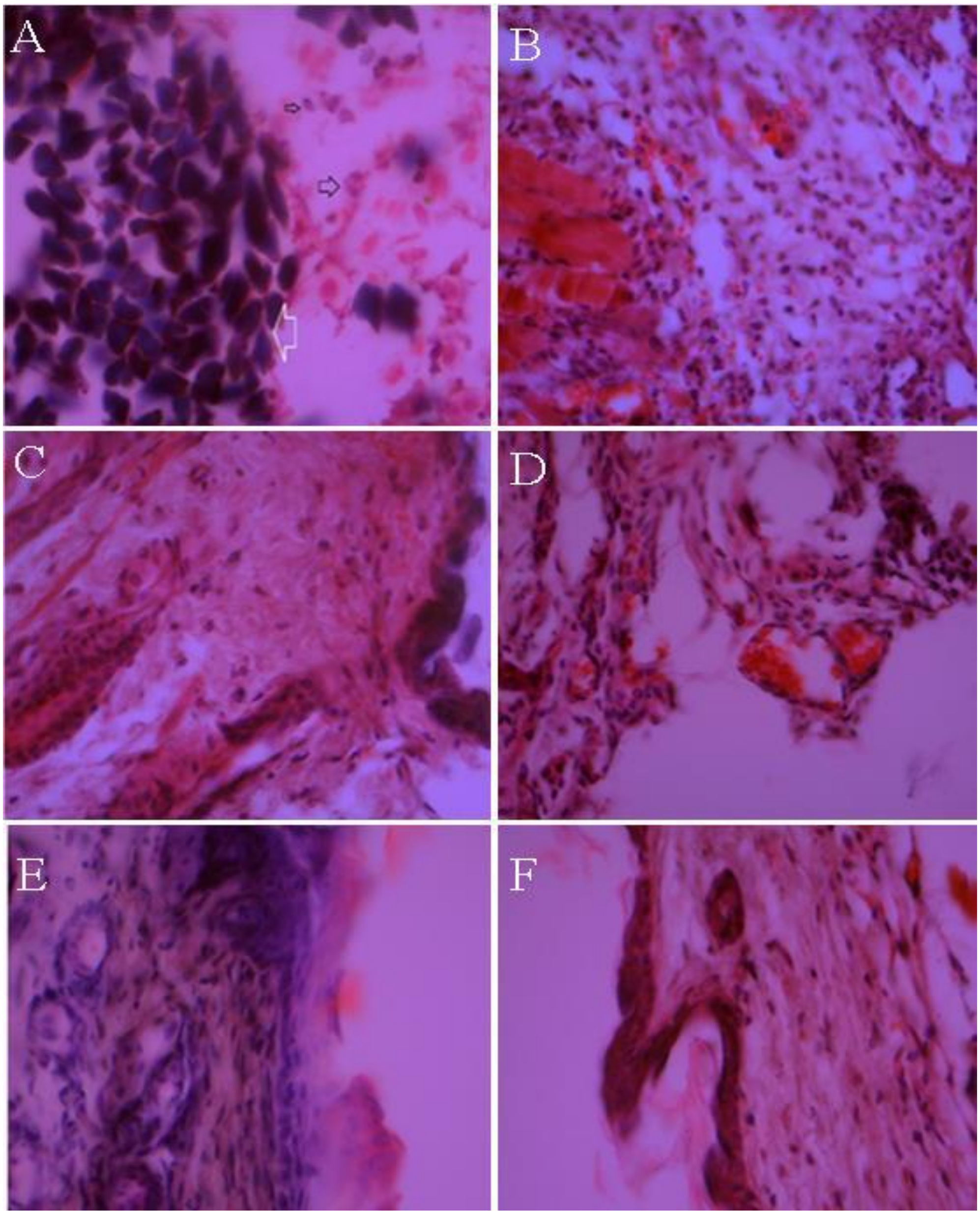

Figure 4. The images show different characteristics of the samples: A. PMN infiltration (treated group on 8th day), B. The presence of macrophages in the tissue (control group on the 8th day), C. angiogenesis, D. fibrosis, E. final epithelium formation, F. image magnification of final epithelium formation.

Discussion

Since 1960, wound contamination by suture thread and its effect on healing process have been investigated. The previous reports have investigated the number of bacterial bindings to the thread as well as, anti-bacterial effect on the threads coated with triclosan antibiotic (19-21). Bacteria adherence to the suture impact delayed wound healing and antibacterial activity and will be considered as a practical way to improve wound management. Surgical suture products and antibacterial properties have been improved by the polymer carriers such as polycaprolactone that is reported in the previous studies. Also, Vicryl Plus threads are used for clinically controlling secondary infections (22). Using nanoparticles such as silver for increasing the capacities of this thread has been studied over the past years (23). In the previous studies, capacities of silk suture thread coated with tetracycline antibiotic and natural fungal extract have been investigated (24).

Antibacterial study of silk- nano cefixime thread showed that this thread has a high capacity of encountering surgery site infections (SSIs). The antibacterial zone can be studied in aspect of polymer-antibiotic composite and nano-composite release in the surgery site (25). Coating the suture with poly lactic-glycolic acid and surgery implants do not affect the tissue inflammation. (26). Silk-levofloxacin designing test was effective in antibacterial zone inhibition (27). Designing silk suture threads with slow-release performance has also an important role in clinical wound management (28). Suture, as an external agent, reduces the dead space and make a moderate inflammation in the wounds (29). According to previous studies, 2-7 days after stitching, suture threads show different inflammatory responses due to their different structure and compounds (30, 31). In a tissue comparison made in jaw surgery, the occurrence of redness and pain on the days 1-7 and 14 after the surgery was reported with a higher level in the case of using silk suture threat (32). Antibacterial threads (triclosan-suture) do not have any negative effect on healing process in clinical study. Silk-nano cefixime sutures are capable of reducing the risk of SSIs, and are capable to keep the wound sides closed, during early healing recovery (33).

Conclusion

This study suggested that antibacterial suture threads do not have any effect on disinfection wound healing process. Although these threads caused increased inflammation over 8 days, they did not affect the healing process. During the experiment, the wounds started to become coagulated and closed, so that over 14 days, no sign of the wounds was observed in tissue sections. Over 14 days, epidermis increased in both treated and control groups and multi-core leukocytes decreased, so that with the recovery and healing of the wound site, the wounds were not observable in the site. Angiogenesis and healing were completed at the end of healing process and maximum presence of fibroblasts formation in the site was observed. All in all, using antibiotic in clean wounds is not suggested. Therefore, not only the unnecessary use of antibiotic is not useful for the patient, but also it raises the risk of infection. Design, surgical suture product or properties improvement of other surgical yarn affect tissue reaction and healthy and treatment cost directly. Every wound closure could be improved by upgrading one of these properties.

Conflict of interest

No conflict of interest is declared.

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