Nanomed Res J 3(3):133-139, Summer 2018

RESEARCH ARTICLE

Silk suture reinforced with Cefixime nanoparticles using polymer hydrogel (CFX@PVA); Preparation, Bacterial resistance and Mechanical properties

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ARTICLE INFO

Article History:
Received 19 March 2018
Accepted 08 June 2018
Published 01 July 2018

Keywords:
Antibacterial Activity
Cefixime Trihydrate
Mechanical Properties
Nanoparticle
Polyvinyl Alcohol
Silk Suture

ABSTRACT

Objective(s): The objective of the current study was to prevent surgical site infection (SSI) by creating a new antibacterial silk suture.

Methods: Cefixime trihydrate (CFX) was prepared as nanoparticles via mixing with polyvinyl alcohol (PVA) hydrogel by covalent cross-linkage. The mixture was stirred vigorously to obtain a homogenous gel. Under this condition the polymer chains separate CFX as nanoparticles and trap them (CFX@PVA). The enrichment of silk suture was performed by immersing it in the CFX@PVA solution. The trapped CFX nanoparticles in PVA hydrogel on the surface of sutures were confirmed by SEM. The effect of CFX@PVA silk sutures on tensile strength was analyzed, using a Santam machine controller. The antibacterial activity of the reinforced silk suture was tested on *E. coli* (ATCC25922) and *S. aureus* (ATCC25924).

Results: All antibacterial studies clearly showed that the use of novel CFX@PVA silk sutures could represent clinical advantages, in terms of prevention of resistant bacteria, such as *Staphylococcus aureus (S. aureus)*, the same as the sensitive bacteria, for 15 days. The maximum elongation of composite before rupture, modulus and extension, showed statistically significant difference between reinforced silk sutures and untreated silk suture. No statistically significant difference was found between the Failure load, Stress, Bending and Energy.

Conclusions: Our data indicate that CFX@ Silk sutures are capable of reducing the risk of SSIs, and has a good mechanical strength to keep the wound sides closed, during early healing recovery.

How to cite this article
Alirezaie Alavijeh A, Dadpay M, Barati M, Molamirzaie A, . Silk suture reinforced with Cefixime nanoparticles using polymer hydrogel (CFX@PVA); Preparation, Bacterial resistance and Mechanical properties. Nanomed Res J, 2018; 3(3): 133-139.
DOI: 10.22034/nmrj.2018.03.003

INTRODUCTION

Surgical site infections (SSIs), usually are acquired from *Staphylococcus aureus (S. aureus)* and *Escherichia coli* (*E. coli*) and impede wound healing with separation of the wound edges; Therefore, mortality risk and healthcare have increased. mortality risk and healthcare costs [1-3]. Due to the effects of surgical sutures that hold wounds borders closed, they can be a major cause of complete healing, also they allow bacterial infection providing SSIs. Therefore, sutures have been developed with antibacterial agents to reduce bacterial adherence to suture materials [4]. Silk has a non-absorbable braided structure, made of material that is widely used for different surgeries like ocular, neural and cardiovascular [5, 6].

Polymeric agent with antibacterial properties is applied in silk sutures and wound healing, evaluated

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in recent studies [7]. Sutures with triclosan coating were applied in healing, pediatric general surgery [8], pediatric neurosurgery [9], thoracic [10], and abdominal surgery [11, 12] and were shown to perform quite well. Cefixime is a broad spectrum third-generation cephalosporin antibiotic and has little activity against *S. aureus* [13].

Nanostructure and microstructure of hydrogels are used to apply for processing new composites [14-16]. Hydrogel structure, such as a polymer network is embedded with special reservoirs of nanodispersions, microparticles and other polymers [17, 18]. Hydrogels' structure is similar to the body components and their chemical and physical cross-linked characteristic has made hydrogel suitable for metabolite exchange and mechanical behaviors, in clinical and fundamental application [19, 20, 21]. The water or other aqueous solutions were absorbed into the three-dimensional cross-linked polymeric hydrogels (containing up to 3000 times by weight of water, in its network) [22, 23], and were dispersed based on the linkage power and general properties of hydrogels [24-26]. The covalent linkage, and physical crosslinking are two different mechanisms in gelation process of PVA [27, 28]. Polyvinyl alcohol (PVA) is a hydrophilic polymer that has biocompatibility properties, easily formed, without toxicity and with high swelling characteristic [29, 30]. Biomedical applications and attention to re-engineering of PVA physical hydrogels make this material, suitable in bio and engineering science, including textile, paper, adhesives, food, biomedical, and pharmaceutical in particular [31-33].

Nanoparticles are synthesized with various techniques, such as vapor deposition, lithographic, reverse micro-emulsion and solid-state processes[34]. The reverse micro-emulsion methods create some micro and nano-spherical structures with the possibility of controlling the final size [35].

In the current study, CFX@PVA silk sutures characterization was compared with the simple silk suture. The effect of mechanical properties of yarn and the physicochemical properties of Cefixime antibiotics, in CFX@PVA silk sutures were also evaluated. The silk suture was enriched by Cefixime that dispersed in PVA hydrogel as nanoparticles, and surface topography of new suture was evaluated by scanning electronic microscopy (SEM). The antibacterial activity of CFX@PVA silk suture was tested on *E. coli* (ATCC25922) and *S. aureus* (ATCC25924), as gram-negative and gram-positive bacteria for 15 days. Cefixime is particularly active against many enterobacteriaceae and little activity against *S. aureus*. Mechanical properties, such as the force (N), elongation break (percent), extension (mm), stress (MPa), modulus (MPa), energy (J) and bending (MPa)) were measured, using a Santam machine controller.

MATERIAL AND METHODS

Materials and Instruments

The non-absorbable silk sutures were supplied by SOPA Medical Device (Iran). The suture size was 2.0 USP, according to United States Pharmacopeia (USP 2) and Cefixime (as trihydrate) tablets (400 mg) were purchased from COSAR Pharmaceutical Co, Tehran, Iran. Polyvinyl alcohol (85% hydrolysis) was prepared from JAPAN VAM & POVAL CO., LTD. (JVP) and acetic acid was purchased from Merck. *S. aureus* (ATCC25923) and *E. coli* (ATCC25922) were obtained from a local clinical laboratory, and used as test strains.

CFX@PVA silk suture preparation

A solution with 7 weight percentage of PVA was prepared in distilled water and heated for 3 hours at 90°C, until a clear jelly like solution was obtained (solution 1) [12]. The certain amount of Cefixime trihydrate powder was added to distilled water. The aqueous acetic acid was added to this solution for pH adjustment that is necessary for Cefexime dissolving (solution 2). The solutions 1 and 2 were mixed and stirred vigorously with a magnetic stirrer at 60°C to prepare 1-3 wt % of Cefixime in PVA hydrogel, as a drug-loaded polymeric nano-composite (CFX@PVA). Silk suture samples were immersed in the final mixture of polymer and antibiotics. Subsequently, sutures were dried at room temperature.

CFX@PVA silk suture characterization

The surface morphology of new suture was characterized, using scanning electron microscopy (SEM). The SEM micrographs were taken with different magnification and resolutions, in order to confirm the CFX grafting on the PVA hydrogel matrix and coating of silk sutures. The technique also was carried out for an approximate evaluation of CFX particle sizes.

The effect of CFX@PVA on tensile strength of silk sutures were analyzed, using a Santam machine controller. Behavior of CFX@PVA effect on silk

suture was investigated by measuring the force (N), elongation break (percent), extension (mm), stress (MPa), modulus (MPa), energy (J) and bending (MPa).

Antibacterial effects in 15 days

The antibacterial performance of CFX@PVA silk sutures examined via the agar diffusion test for 15 days. The suspensions of *S. aureus* (ATCC25923) and *E. coli* (ATCC25922) were obtained to an optical density of 0.5 McFarland standards. Then, 1 mL of this suspension was plated uniformly on Mueller Hinton II Agar plates and Pieces of sutures were placed at each plate. The plates were incubated at 37°C for 15 days to determine the persistent antibacterial activity, until no detectable inhibition zone remained.

Statistics

Data were described as mean ± standard deviation, the experiments were run in triplicate (n=3). Statistical analysis was performed using t-test, ANOV, and dancan with significant level $p < 0.05$.

RESULTS AND DISCUSSION

Morphological analysis of CFX@PVA silk sutures

The distribution and size evaluation of CFX particle on silk suture were investigated, using SEM. Fig.1 shows CFX@PVA silk sutures with different magnifying scales. Moreover, the presence of particles was also visible, among the filaments of the suture. The nanoparticles were clearly trapped into the PVA hydrogel, and filled the pore between fibers of the yarn. The SEM analysis showed that CFX particle distribution in the PVA hydrogel was relatively good. The micrographs also showed CFX particles gained proximate sizes between 100 and 300 nm. CFX powder consists of large, irregular, rod shaped particles Whereas, the morphology of nanoparticles have a specified shape [36]. The size of CFX particles affected solubility and improved dissolution performance [37]. It was the main idea of this study that particles attached and trapped in the surface or penetrated in bulk of silk-PVA, were releasing slowly around the wound and absorbed the yarn. It is a fact that smaller particles have higher surface area and better performance of

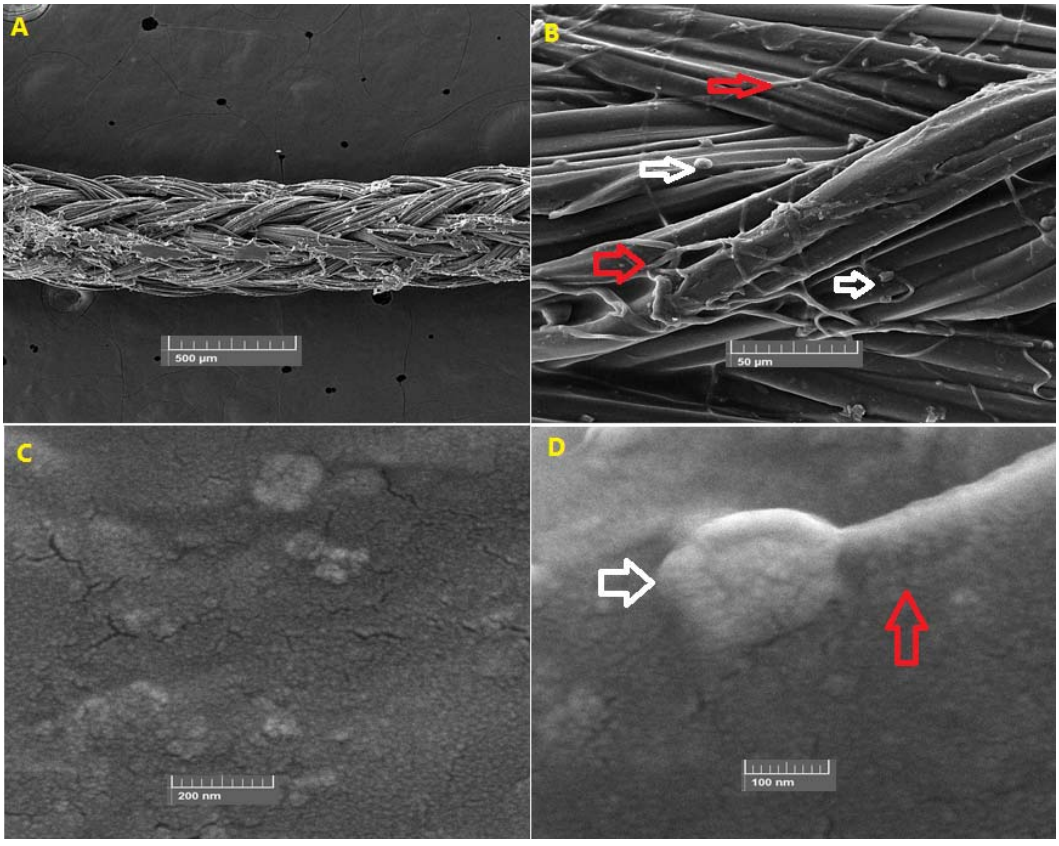

Fig. 1: Scanning electron microscopy (SEM) of CFX@PVA silk sutures with different magnifying scales. (Red arrows: PVA and white arrows: CFX)

activation and polymer coated sutures with the antibacterial agent showed a soft surface [38].

Antibacterial activity

The microbiological tests were performed on CFX@PVA silk sutures, and simple silk suture as the control group.

The inhibition zone diameter of *S. aureus* (ATCC25923) started with 25 mm, which moderately plunged until the 7th day, followed by a considerate decrease by the 10th day, then, it remains steady for 5 days in less than 5 mm. The inhibition zone of *E. coli* (ATCC25922) was about 23 mm at first. There was a slight increase in first week of about 27 mm, and it reduced insignificantly from second weeks to about 20 mm. Antibacterial effect was approximately the same for two different strains of bacteria in the first 7 days, after that *E. coli* (ATCC25922) is affected by CFX@PVA silk sutures more than *S. aureus* (ATCC25923 (Fig. 2). The treated sutures were confirmed effective, against *S. aureus* (ATCC25923) and *E. coli* (ATCC25922). There was a significant difference between the mean of inhibition zone in the *E. coli* (ATCC25922) and *aureus* (ATCC25923) on days 10 and 15 ($p< 0.05$). No significant difference was observed between on days 1, 3, 5, and 7 (Table 1). By contrast, no zone of inhibition was exhibited in untreated sutures, against both *S. aureus* and *E. coli* (Fig. 3).

Table 1: Antimicrobial activity of CFX@PVA silk sutures

Time	S. aureus	E. coli
1	25±1.32	23±1
3	24±.866	24±1.73
5	24±1.8	25±1.32
7	23±1	27±1
10	3±.5	23±.5*
15	2±.86	21±.5*

Values are (means ± SD).
*: Significant difference between groups ($P < 0.05$).

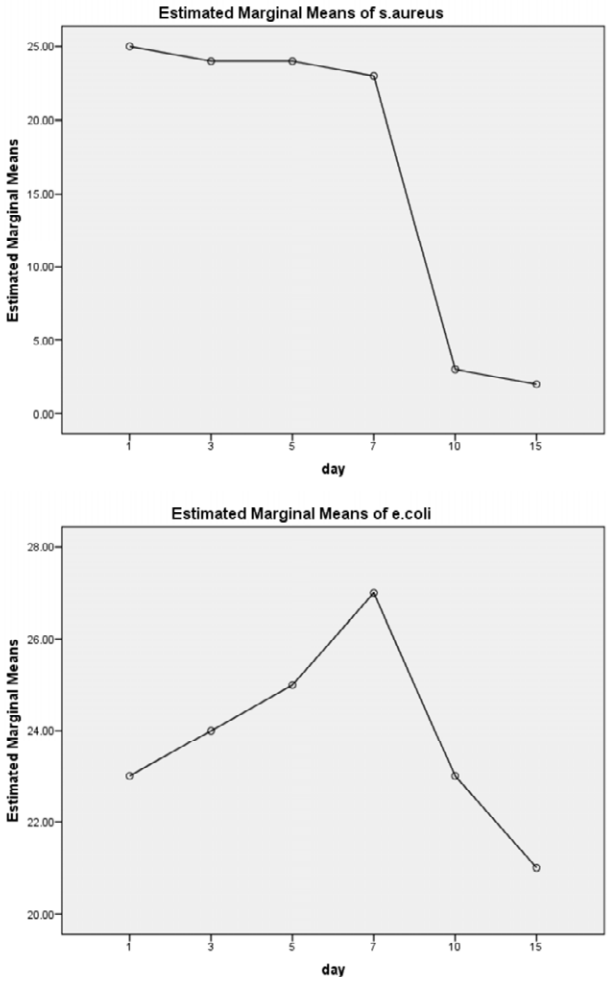

Fig. 2: Variations in inhibition zone diameter for *S. aureus* (ATCC25923) and *E. coli* (ATCC25922) as a function of CFX@PVA silk sutures in 15 days

Table2: The values are presented as mean ± standard deviation

	Force (N)	Extension (mm)	Stress (MPa)	Elongation (%)	Module (MPa)	Energy (j)	Bending (MPa)
Control	20.10±0.46	12.07±2.14	6.39±0.14	18.57±3.29	35.15±6.07	125.453±16.16	516±18.76
Treated	21.97±1.3	18.35±1.94*	6.67±0.7	28.7±2.79*	24.63±1.01*	196.24±45.34	442.46±36.78

*: Meaningful difference between groups ($P < 0.05$).

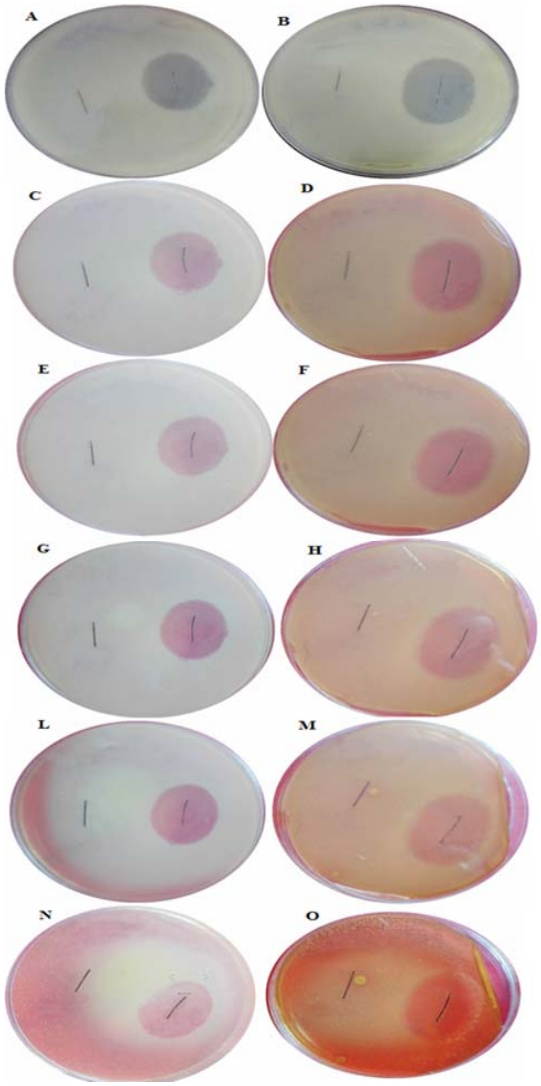

Fig. 3: Antimicrobial activity of CFX@PVA silk sutures. A,C,E,G,L, N show sustained efficacy assay against *S. aureus* and B,D,F,H,M and O show sustained efficacy assay against *E. coli*, on the day 1, 3, 5, 7,10, and 15, respectively.

CFX is affected on a broad spectrum of bacteria, such as *Enterobacteriaceae*, *Haemophilus* influenzae, *Streptococcus pyogenes*, Streptococcus *pneumoniae* and *Branhamella catarrhalis*, and has little activity against *S. aureus* [39]. The current study revealed that CFX@PVA has similar effect on resistant bacteria, including *S. aureus* and *Enterobacteriaceae*

Most recent studies have manufactured the antimicrobial silk sutures with biodegradable polymers, combined with antibacterial drugs [40], such as coating a braided silk suture with the synergistic drug levofloxacin-tinidazole and the biodegradable polymer Chitosan. Results showed an acceptable antibacterial activity and persistence against both gram-positive and gram- negative test organisms [41].

Mechanical testing

Mean and standard deviation values extension diameter (mm), failure load (N), percent elongation to failure (%), failure stress (MPa or $N/m^2 \times 106$), modulus (MPa) and bending (MPa) are presented for CFX@PVA silk suture and control in the Table 1. Table 2 also shows the results of statistical analysis.

The sutures with improved tensile properties show better performance in keeping the wound sides closed, and provide stronger effect on initial wound healing recovery [42, 43]. Tensile testing is characterized by different factors: Elongation, as the increase of length until it is broken, and it is defined as capacity to handle without failure and modulus shows the binding energy of atoms, meaning that higher forces are needed to separate the atoms [27, 29, 44].

The maximum elongation of composite before rupture (**P**=0.015), modulus (**P**=0.042) and extension (**P**=0.020) showed statistically significant difference between CFX@PVA silk sutures and the control sample ($P < 0.05$), and Statistically difference between Failure load (**P**=0.093), Stress (**P**=0.519), Bending (**P**=0.532) and Energy (**P**=0.063) was not significant.

CONCLUSION

Cefixime nanoparticles were successfully prepared. PVA hydrogels were used as carrier to load nanoparticles on silk sutures. Reinforced silk suture was applied against resistant and non-resistant bacteria. Fine grafting of nanoparticles was confirmed with SEM micrographs, mechanical properties of treated sutures were improved and antibacterial activity was confirmed on 15 days. The new suture was compared with untreated silk

suture, and proposed for wound management and preventing SSIs. Further studies should focus on the drug-release with antibacterial effect and biodegradation of the silk *in vivo*.

ACKNOWLEDGEMENT

The authors are grateful to the University of AJA for financial support.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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