



## The Effect of Silver Coated External Fixation Pin Usage on Biofilm Formation and Bacteria Adhesion

### KEYWORDS

Cancer: citric acid; complementary treatment.

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### ABSTRACT

**Introduction.** Pin tract infection is a common complication in fracture cases treated with stainless steel external fixation pin.

Silver has an antimicrobial effect which effectively address bacteria that contaminate and infect wound. By the same principles, researcher used a external fixator pin that had been overlaid with silver ion and assess the effectiveness of using silver (silver coated pin) on pin tract infection cases

**Material and methods.** This study is an experimental laboratory research with complete randomized design with repeated observation. The research subjects were adult male rabbits that met the inclusion criteria. Consecutive sampling method was performed on 2 group, each of them consist of 10 rabbits. Group A (control) : we perform installation of stainless steel external fixation in the left tibia of each research subject, Group B (Experiment) : we perform installation of silver coated external fixation in the left tibia of each research subject. We performed inoculation of *Staphylococcus aureus* bacterial colonies as much as 0,5 MacFarland at the puncture site of external fixation pin. We evaluated the number of the bacterial colonies in puncture site by histopathology, in all research subject at day 7 and 14

**Results.** The results of the study showed that at day 14, the number of colonies of both group was decreasing, group A (Mean±Std 110.87) and group B (Mean±Std 88.16) with p value of 0.02 (p< 0.05) which means that these result was statistically significant

**Conclusion.** The results of this study indicate that the usage of silver coated external fixation pin is better to reduce the bacterial colonization and adhesion.

### INTRODUCTION

Pin tract infection is a common complication in fracture cases treated with external fixation. The incidence of pin tract infection in Indonesia was high, between 30-85%. Stainless steel, the most common material for external fixation is a hydrophobic substrate, which cause larger adhesion of bacteria on the surface of the implant.<sup>1,2</sup>

Silver has an antimicrobial effect which effectively address bacteria that contaminate and infect wound. By the same principles, researcher used a external fixator pin that had been overlaid with silver ion and assess the effectiveness of using silver (silver coated pin) on pin tract infection cases.<sup>1,2,3</sup>

### MATERIAL AND METHODS

This study is an experimental laboratory research with complete randomized design with repeated observation, using clinical trial method.

The research subjects were adult male rabbits that met the inclusion criteria, namely: male rabbit, 3 months old, weight 3-4 kg. Number of research subjects was determined based on the Federer formula. From the results of calculation of sample size, we obtained the number of minimal research subject was 16, then to anticipate drop out, the number of research subjects was set at 20 adult male rabbits. Consecutive sampling method was performed on 2 group, each of them consist of 10 rabbits. Group A (Control) : we perform installation of stainless steel external fixation in the left tibia of each research subject, Group B (Experiment) : we perform installation of silver coated external fixation in the left tibia of each research subject.

We performed inoculation of *Staphylococcus aureus* bacterial colonies as much as 0,5 MacFarland at the puncture site of external fixation pin, covered with moist gauze (NaCl 0,9%) which was fixed with plaster. The dressing was changed every 24 hours and we performed observation for 5 days (the incubation period of

*Staphylococcus aureus*).

We evaluated the number of the bacterial colonies in puncture site by histopathology, in all research subject at day 7 and 14.

The research was conducted at the Laboratory of Medical Research Unit of the Faculty of Medicine Padjadjaran University, Bandung, Indonesia from April - May 2016.

### Research Procedures

The initial step of this study was to determine the number of samples. The number of samples was determined by the Federer formula. Samples in this study were 20 male rabbits. After determining the number of samples, male rabbits were grouped into 2 groups: group A and group B. During the experiments carried out, the amount and type of food intake in each group was the same.

The research procedure was as follows:

1. On the first day, each research subject was anesthetized with ketamine 50 mg / kg intramuscularly and diazepam 5 mg / kg.
2. After given anesthesia, the hair on the leg of research subjects of about 20 x 60 mm<sup>2</sup> were shaved.
3. The research subjects were grouped into 2 groups, namely Group A and Group B administered with the following treatments:  
Group A: The control group consisted of 10 male rabbits, we perform installation of stainless steel external fixation in the left tibia of each research subject,  
Group B: The experimental group consisted of 10 male rabbits, we perform installation of silver coated external fixation in the left tibia of each research subject.
4. The puncture site of external fixation pin in each research subject was inoculated by *Staphylococcus aureus* bacterial colonies as much as 1 MacFarland, closed with moisted screen (NaCl 0,9%) covered

with moist gauze (NaCl 0.9%) which was fixed with plaster. The dressing was changed every 24 hours and we performed observation for 5 days (the incubation period of *Staphylococcus aureus*)

5. We retrieve a sample at day 7 and 14 to evaluate the number of the bacterial colonies in puncture site by histopathology, in all research subject.

## RESULTS

The results of the study showed that at day 7, the number of colonies in group A (214.12) was greater than group B (205.65). Statistically, there was no significant differences between both groups, with p value of 0.155 ( $p > 0.05$ ).

On day 14, the number of colonies of both group was decreasing, group A (110.87) and group B (88.16) with p value of 0.02 ( $p < 0.05$ ) which means that these result was statistically significant (shown in table).

The results of this study indicate that the usage of silver coated external fixation pin is better to reduce the bacterial colonization and adhesion.

**Table. Comparison of the of colony of bacteria in group A and B**

Variable	Group		
	A N=10	B N=10	P value
Colony of Bacteria Day 7			
Mean±Std	214.12± 9.291	205.65± 8.981	0.155*
Median	216.54	207.22	
Range (min-max)	185.35-236.16	172.65-234.75	
Colony of Bacteria Day 14			
Mean±Std	110.87± 15.725	88.16± 14.219	0.002**
Median	114.75	90.65	
Range (min-max)	70.14-137.88	55.10-108.50	

**Note :** The mark \* shows statistically not significant or not meaningful on day 7.

The mark \*\* shows statistically significant or meaningful on day 14.

## DISCUSSION

External fixation is a fast and atraumatic technique for the stabilization of bone fragments. The most significant complication with external fixators is pin tract infection, which has been reported in up to 63% of patients. At our own facility, pin tract complications have been reported in various studies at rates ranging from 30% to 75% of patients.<sup>4,5</sup>

This high infection rate has been attributed to the conduit that the pins provide between the skin and underlying soft tissue and bone. Complications related to pin tract infection include need for pin change or removal, failure of fracture healing, septic arthritis, and osteomyelitis. A method to decrease the rate of pin infection, therefore, has tremendous clinical appeal.<sup>4,5</sup>

Silver (Ag) is a topical antiseptic that effectively address organisms that contaminate and infect wounds and rarely cause resistance. These agents also effectively penetrate the biofilm where silver itself will be bound to the proteoglycan in the cell walls of microbes, while the walls of mammalian cells lack proteoglycans so they are not affected by silver.<sup>3,4</sup>

This is important, because every wound therapy products should be able to provide space for the components of wound healing, such as epithelium. Silver works by causing denaturation of microbial proteins, interfere with the process of food digestion, reduce metabolism, and inhibit growth. In addition, silver also known to influence the microbial DNA.<sup>3,5,6</sup>

Microbial resistance against silver is uncommon compared to resistance to antibiotics. To work effectively, silver should reach tissue in the form of ions in sufficient concentration. Silver has two forms of anti-microbial, namely in the form of active methods and passive methods.<sup>3,4</sup>

Active method, by activating the proteolytic enzymes in the body of microbes that will cause necrosis or damage the cell membranes of microbes. The process of elimination of microbes in this way is quick but could not last long.<sup>3,7</sup>

Passive methods, by the nucleation mechanism to form a silver nanoparticle solution. The method is slow but has several advantages, such as: the ratio between surface area and volume is large, it will optimizing dissipation capacity of silver, more stable to heat and is not toxic.<sup>3,7</sup>

Silver, with its potent, broad-spectrum antibacterial activity, has many clinical uses. Silver-based creams for wound care and silver coatings for catheters have decreased infection rates with minimal systemic effects. Silver coating has been advocated for use on external fixation pins to decrease infection rate, and a small, animal study has demonstrated decreased infection and motion at the pin-bone interface. Although silver coated pins are now commercially available, no clinical study has been performed to confirm their efficacy.<sup>7,8,9</sup>

Silver-coating external fixation pins has been proposed as one means to decrease the pin infection rate and subsequent pin tract complications. Bacteria colonize the surface of the pin and form a resistant biofilm of polysaccharides that serves as a barrier to antibiotics and the body's immune system. This film therefore serves as a conduit for bacteria to migrate from the surface of the skin via the pin to the bone. The silver coating provides an antimicrobial layer on the pin that prevents bacterial colonization and pin tract infections.<sup>4,5,10</sup>

The parameters used in this study is the number of bacterial colonies. These parameters are calculated in the proliferative phase of wound healing.<sup>11</sup>

The process of counting of bacterial cells was done by calculation method in the petri dish, performed by diluting samples of bacterial suspension in nutrient broth agar. Dilution is done so that after incubation, colonies formed on the plate in an amount which can be calculated.<sup>11,12</sup>

The principle of dilution is to lower the amount so that more number of dilutions performed, the fewer the number of microbes. Principle of cup count method is to grow microbial cells are still alive, so that the microbial cells will multiply and form colonies that can be viewed directly by the eye without a microscope.<sup>11,13</sup>

Cup count method can be divided into two ways: method of casting (pour plate) and methods of surface / scatter (surface / spread plate). To meet the statistical requirements, the cup was chosen to be calculated to contain 30-300 colonies. The number of microbes in the sample is determined by multiplying the number of colonies by the dilution factor at the plate in question. The unit used to express the number of bacterial colonies or is cfu / mL (cfu = colony forming units).<sup>14,15</sup>

Based on the results of research, the usage of silver coated external fixator pin show a decrease in bacteria colonies better than the usage of conventional stainless steel external fixator pin.

Silver application suppress the number of bacteria colonies because silver effectively penetrate the biofilm formed by bacteria and bind to proteoglycans in the cell walls of bacteria, it also causes protein denaturation bacterial cells, disrupt metabolism and inhibiting the

growth because of bacterial DNA damage occurs.<sup>11,13</sup>

Silver also activate proteolytic enzymes in bacteria, causing necrosis or damage to the bacterial cell membrane. In addition, through the mechanism of nucleation silver will form an argentic solution that has nanoparticles characteristic.<sup>16</sup>

## CONCLUSION

It can be concluded that there is a decrease in the number of bacterial colonies on the proliferative phase of wound healing to the use of external fixation pins that have been coated with silver.

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