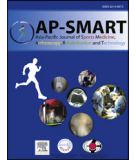Original article

Cefazolin-containing poly(ε-caprolactone) sponge pad to reduce pin tract infection rate in rabbits

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Abstract

In our previous study, a fibroblast growth factor-2 (FGF-2)−apatite composite layer coated on titanium screws effectively prevented pin tract infection in rabbits because of enhanced wound healing; however, the FGF-2−apatite composite layers did not completely prevent pin tract infection. Thus, we recently developed a poly(ε-caprolactone) (PCL) sponge pad embedded with cefazolin sodium (+CEZ), which has a fast-acting bactericidal effect. The pad is placed on the skin around the screws. The purpose of this study was to determine the anti-infective efficacy of the +CEZ pad on the pin−skin interface of the FGF-2−apatite-coated titanium screws. The +CEZ pads were prepared by mixing PCL and CEZ in 1,4-dioxane, followed by freeze-drying and compaction. They were analyzed regarding their surface structure, *in vitro* CEZ release profile, and bactericidal activity. The FGF-2−apatite-coated screws were implanted percutaneously in bilateral rabbit proximal tibial metaphyses—with and without the +CEZ pad—for 4 weeks ($n = 20$). The + CEZ pads consisted of a porous matrix of PCL in which CEZ was embedded. The CEZ-release profile showed an initial burst on Day 1 and a sustained release lasting for 30 days. The +CEZ pad retained its bactericidal activity against *Staphylococcus aureus* after preincubation on an agar plate for 7 days. Based on visual inspection, the pin tract infection rate was successfully reduced from 72.2% to 15.0% with the +CEZ pad ($p < 0.05$), which reduced the bacterial count, especially *S. aureus* ($p < 0.05$). The histological inflammation rate of the soft tissues was also significantly lower with the +CEZ pad than without it ($p < 0.05$). The pin tract infection rate was reduced to one-fifth with the +CEZ pad. Using it as described improves infection resistance during percutaneous implantation.

Introduction

Titanium screws coated with fibroblast growth factor-2 (FGF-2)−apatite composite layers have greatly improved resistance against pin tract infection because of enhanced wound healing during percutaneous implantation.[1−3] Pin tract infection is the most common complication associated with external fixation.[4−6] When FGF-2−apatite is used, the FGF-2 enhances wound healing by accelerating fibroblast proliferation and vascularisation,[7−9] and the apatite facilitates integration between the screw and the surrounding tissues. In a rabbit percutaneous screw implantation model, the titanium screws coated with FGF-2−apatite reduced by one-half (from 93.8% to 43.8%) the rate of macroscopic pin tract infection, compared to titanium screws without the FGF-2−apatite.[2] In addition, the interfascial soft tissue that is attached to the FGF-2−apatite composite layer contains a Sharpey's fibre-like tissue rich in blood vessels.[3] This suggests that the FGF-2−apatite enhances the formation of Sharpey's fibre-like

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tissue in the process of wound healing, thereby reducing the pin tract infection rate.

Despite this success, pin tract infection is not completely prevented. One possible reason is bacterial invasion via the pin tract immediately after implantation of the percutaneous screw. Because wound healing requires time, even in the presence of FGF-2−apatite, completely preventing bacterial invasion especially at the early postoperative stage is important.

Therefore, we developed a sponge-like pad containing cefazolin sodium as the antibiotic (+CEZ pad) that is placed on the skin around the screws coated with the FGF-2−apatite. The +CEZ pad consists of a porous poly(ε-caprolactone) (PCL) matrix with CEZ embedded in the matrix. Poly(ε-caprolactone) is a biocompatible and resorbable material that has been explored as a promising drug carrier.[10−12] An advantage of PCL over other resorbable polymers is that it avoids acidification of the surrounding tissue during resorption. Acidification of tissue may have a negative impact on FGF-2-mediated skin tissue regeneration. Cefazolin sodium is a first-generation cephalosporin antibiotic that is clinically effective against infections caused by *Staphylococcus aureus*, which causes most pin tract infections.[1−3] Therefore, we used CEZ embedded in PCL. The +CEZ pad may have a fast-acting effect on bacterial elimination around the screws by releasing CEZ on contact with blood and/or fluid discharge from the wound. Therefore, the combination of the +CEZ pad and titanium screws coated with FGF-2−apatite can greatly reduce the pin tract infection rate through their complementary effects, compared to the results without the +CEZ pad. The purpose of this study was to determine the anti-infective efficacy of the +CEZ pad on the pin−skin interface of the FGF-2−apatite-coated titanium screws.

Materials and methods

Preparation of the pad

Poly(ε-caprolactone) foam with or without CEZ was prepared by a freeze-drying process (Fig. 1).[13] To prepare the PCL foam for the +CEA pad, 2 g of PCL (MW = 40,000) and 0.2 g of CEZ were added to 60 g of 1,4-dioxane in a glass bottle and stirred for 90 minutes for complete dissolution of the PCL. The solution was cooled to 0°C with vigorous stirring to make a sherbet-like mixture. The mixture was vigorously stirred for 16 minutes at 0°C, and then stirred for 3 minutes at room temperature. The sherbet-like mixture was frozen at −70°C for 1 hour, and then freeze-dried for 48 hours to remove the 1,4-dioxane from the PCL framework. For the non-CEZ (−CEZ) pad, the PCL foam was similarly prepared, but without CEZ. All reagents used for preparing the foam were obtained from the Wako Pure Chemical Industries (Tokyo, Japan).

The PCL foam was sliced with a razor blade into 2-mm sections and pressed at 10 MPa to a 1-mm thickness. Circular or doughnut-shaped +CEZ and −CEZ pads were punched out of the 1-mm PCL foam sections. Circular pads with a 6.35-
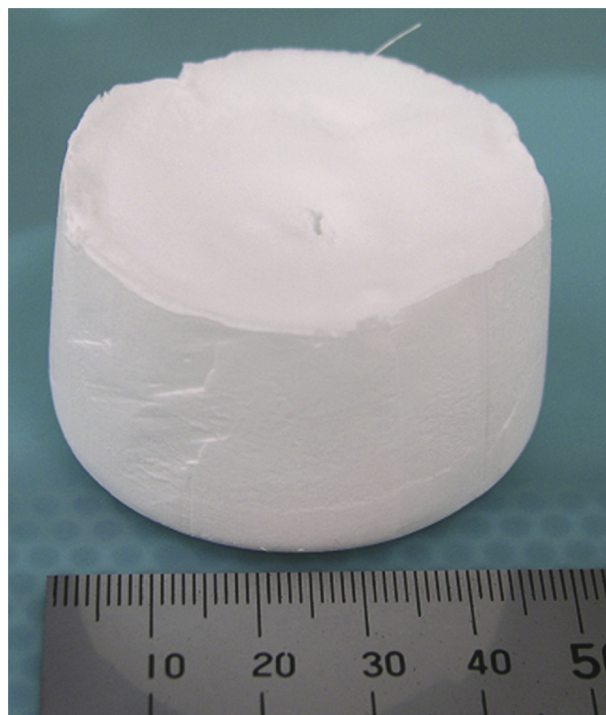

Fig. 1. Macroscopic view of poly(ε-caprolactone) (PCL) sponge foam embedded with cefazolin sodium (+CEZ).

mm diameter were used for material characterization and for the *in vitro* test. The doughnut-shaped pads with outer and inner diameters of 13.0 mm and 3.0 mm, respectively, were used for the *in vivo* test. The amount of CEZ in the 6.35-mm pads and 13.0-mm pads were 0.9 mg and 3.5 mg, respectively.

Characterization of pads

After coating the pads with a thin gold film, the surfaces of the +CEZ and −CEZ pads were analyzed by a scanning electron microscope (SEM; XL30; FEI, Tokyo, Japan) and by an energy dispersive electron probe X-ray (EDX) analyzer (Genesis 2000; EDAX Japan, Tokyo, Japan).

In vitro release of CEZ

A +CEZ pad was immersed in 10 mL of Hanks' balanced salt solution (HBSS; Sigma−Aldrich, St. Louis, MO, USA), which was contained in a tightly capped 15-mL conical tube.[14,15] Ninety-six tubes were prepared and allowed to stand at 37°C. After various periods of time, ranging from 2 hours to 32 days, a 4-mL aliquot of the HBSS was collected from the tubes ($n = 6$) and analyzed for the amount of CEZ released from the +CEZ pad. To calculate the amount of released CEZ, standard solutions were prepared as follows: CEZ solution (100 μg/mL) in HBSS was incubated at 37°C for the same period as in the CEZ release test. Prior to each measurement, the incubated standard solution was diluted 2 times, 4 times, and 10 times with HBSS to use them as CEZ solutions of 50 μg/mL, 25 μg/mL, and 10 μg/mL, respectively. Because CEZ in aqueous solution has an absorption peak at 290 nm, the absorption of the collected HBSS was measured at 290 nm
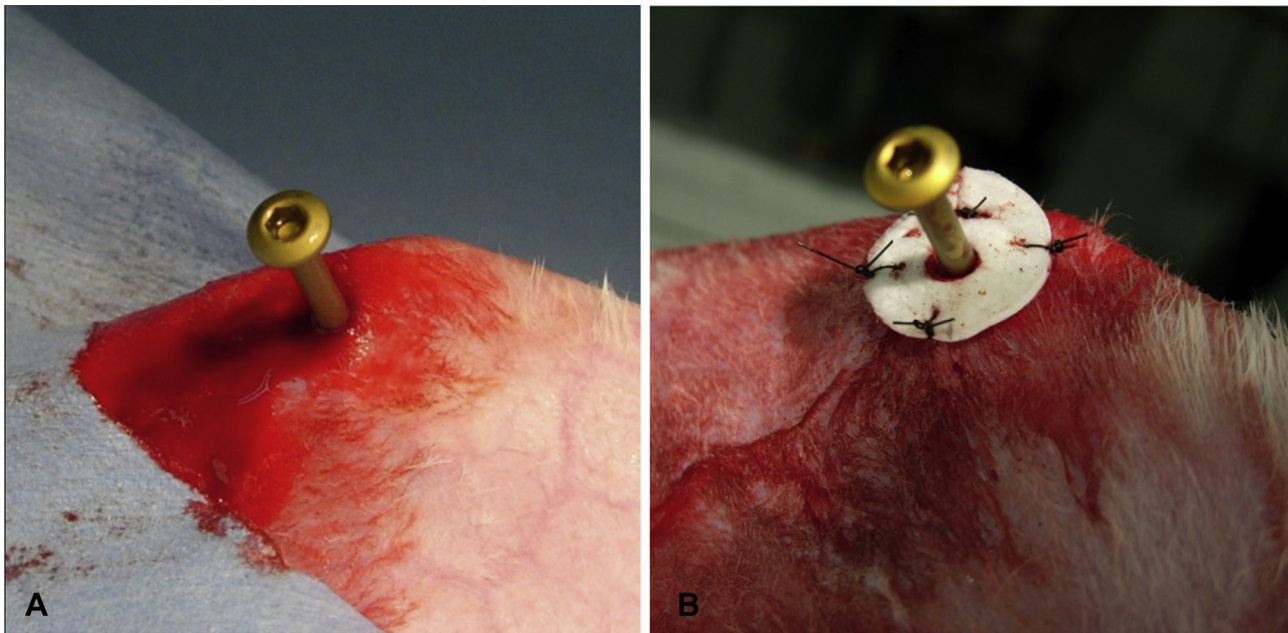
Fig. 2. Postoperative views of screws coated (A) with a fibroblast growth factor 2 (FGF-2)−apatite composite layer without CEZ (−CEZ pad) and (B) with CEZ (+CEZ pad) in the rabbit proximal tibial metaphysis. CEZ = cefazolin sodium.

with a UV visual spectrophotometer (U-3010; Hitachi High-Technologies, Tokyo, Japan).

In vitro bactericidal activity test

The *in vitro* bactericidal activity of the +CEZ pads was assayed by a method similar to the Kirby-Bauer (KB) antibiotic test. The −CEZ pads were the negative control; the KB disks (6.35 mm in diameter; CZ30; Becton, Dickinson, Tokyo, Japan) were the positive control; and +CEZ pads were placed on uniformly grown *Staphylococcus aureus* (ATCC: 29213) in agar plates (Mueller-Hinton) and incubated at 35°C for 24 hours ($n = 6$). The diameters of the inhibition zones were measured against *S. aureus* that formed around the pads or the KB disks. Prior to the bactericidal activity test, the +CEZ pads were preincubated on a freshly prepared aseptic agar plate at 35°C for 0 days, 3 days, 6 days, 7 days, 12 days, and 13 days to evaluate the sustained bactericidal activity under conditions similar to those during actual usage of the +CEZ pads on skin tissue. By following the manufacturer's instruction for the KB disk, the bactericidal activity of the +CEZ pad was estimated by the diameter of the inhibition zone. The bactericidal activity against *S. aureus* was judged clinically effective when the inhibition zone had a diameter greater than 18 mm.

Formation of the FGF-2−apatite composite layer on titanium screws

Titanium cancellous screws with a 142-nm thick anodic oxide layer on the surface (#407-030; SYNTHES, West Chester, PA, USA)—which were 4.0-mm in diameter and 30-mm in length—were coated with a FGF-2−apatite layer.[1−3] In brief, a supersaturated calcium phosphate solution (10 mL) with a calcium/phosphorus (Ca/P) molar ratio of 2.0 was prepared by mixing five infusion fluids clinically available in Japan at an optimised mixing ratio, using 8.137 mL of Ringer's solution (Otsuka Pharmaceutical, Tokyo, Japan), 36.85 μL of calcium chloride corrective injection 1 mEq/mL (Otsuka Pharmaceutical), 0.899 mL of Klinisalz (I'rom Pharmaceutical, Tokyo, Japan), 18.72 μL of dipotassium phosphate corrective injection 1 mEq/mL (Otsuka Pharmaceutical), and 0.909 mL of sodium bicarbonate substitution fluid for only BIFIL (Ajinomoto Pharmaceuticals, Tokyo, Japan). The supersaturated calcium phosphate solution was supplemented with FGF-2 (Fiblast; Kaken Pharmaceutical, Tokyo, Japan) at a FGF-2 concentration of 4 μg/mL. Each titanium cancellous screw was immersed in 10 mL of supersaturated calcium phosphate solution supplemented with FGF-2 at 37°C for 48 hours.

In vivo study

The screws were implanted percutaneously in the medial proximal tibia of 20 skeletally mature male Japanese white rabbits weighing approximately 3 kg. The surgical technique was the same as that reported in our previous studies.[1−3] In brief, each rabbit was administered an intravenous injection of barbiturate (40 mg/kg body weight). After shaving the legs, 10 mm incision was made in the skin on the medial proximal tibia, and a 2.5-mm diameter perforation was made in both tibial metaphyses by individual taps using sterile techniques. The screws were implanted in the bilateral medial proximal tibias of 10 rabbits in each group. After the implantation, the skin was sutured bilaterally to the screw. The group in which the screws were percutaneously implanted without the +CEZ pad was designated as the pad (−) group ($n = 20$; Fig. 2A), and the group in which the +CEZ pad was sutured to the skin
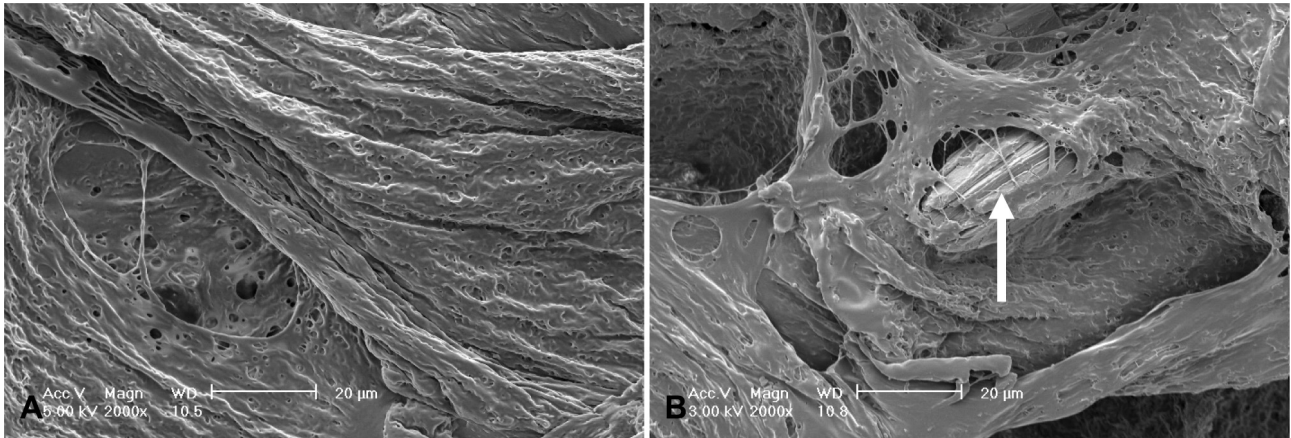
Fig. 3. Scanning electron microscopy images of (A) the −CEZ pad and (B) the +CEZ pad. Both pads have randomly folded and twisted surfaces with many holes. A CEZ granule protrudes from the PCL in the +CEZ pad (arrow). CEZ = cefazolin sodium; PCL = poly(ε-caprolactone).

around the screw was designated as the pad (+) group ($n = 20$) (Fig. 2B). The four corners of the pads were sutured to the skin with 4-0 nylon to prevent their coming off. Each rabbit was postoperatively allowed to behave freely in its own cage. The pads were removed 1 week after the operations to avoid late infections. The rabbits did not receive any other antibiotics or treatment for their wounds and were sacrificed 4 weeks after the implantation.

All animal experiments were performed in accordance with the guidelines of the ethics committees of the University of Tsukuba (Ibaraki, Japan) and the National Institute of Advanced Industrial Science and Technology (Ibaraki, Japan) and the U.S. National Institutes of Health (Bethesda, MD) guidelines for the care and use of laboratory animals (NIH publication no. 85−23, revised 1985).

Classification of pin tract infections by visual inspection

Four weeks after the implantation and prior to when the rabbits were sacrificed, pin tract infections were evaluated by a modified Checketts classification method.[2,16] Grade 0 corresponds to "no redness" (i.e., no redness, discharge, or pin loosening is present). Grade 1 corresponds to infection only in the soft tissue, which is characterised by redness and discharge around the pin but without pin loosening. Grade 2 corresponds to infection in the soft and hard tissues, which is characterised by redness and discharge around the pin with pin loosening because of osteomyelitis. A third-party physician who was blind to the group identification evaluated the pin tract infections.

Histological analysis

The specimens around the screws coated with a FGF-2−apatite composite layer were fixed in 10% neutral-buffered formalin for 7 days, decalcified, and embedded in paraffin. The paraffin-embedded tissues were sliced in 5-μm sections parallel to the screw hole and stained with hematoxylin and eosin (H&E). All sections were examined by light microscopy (BX-51; Olympus Optical, Tokyo, Japan). Pin tract

inflammation of the soft tissue was histologically assessed in a blind manner by a single pathologist who classified it into one of three grades (i.e., Grade 0, Grade 1, or Grade 2). Grade 0 corresponded to "no inflammation with good wound healing"; Grade 1 corresponded to "slight inflammation"; and Grade 2 corresponded to "severe inflammation".[3] Pin tract inflammation of the hard tissue was also histologically assessed in a blind manner by a single pathologist who classified it into one of three grades (i.e., Grade 0, Grade 1, and Grade 2). Grade 0 corresponded to "no osteomyelitis"; Grade 1 corresponded to "slight osteomyelitis"; and Grade 2 corresponded to "severe osteomyelitis".

Bacteriological analysis

Bacterial detection was performed by SRL. Inc., Tachikawa, Tokyo, Japan. Exudates around the screws, collected by cotton swabs, were inoculated on three types of solid agar broth: sheep blood agar broth (Becton, Dickinson, Sparks, MD, USA), chocolate agar broth (Becton), and bromothymol blue lactose agar broth (Becton). The exudates were also inoculated into Gifu anaerobe-modified (GAM) semisolid agar broth (Nissui, Tokyo, Japan). The sheep blood agar broth and chocolate agar broth were aerobically incubated at 35°C for 48 hours, whereas the bromothymol blue lactose agar broth was anaerobically incubated at 35°C for 48 hours. The GAM semisolid agar broth was incubated 35°C for 72 hours. When no bacterial growth was detected visually on the solid agar broth after the 48-hour incubation period, some of the incubated GAM semisolid agar broth was subcultured again on the three solid agar broths. Isolated organisms were identified with the MicroScan WalkAway Plus system (Siemens, Munich, Germany). We also analyzed the number of bacterial species detected per screw in each group.

Statistical analyses

Inhibition zones from the *in vitro* bactericidal activity test were compared against the criterion for clinical efficacy (i.e.,

18 mm) using the Student t test at a significance level of $p < 0.05$. The pin tract infection data (i.e., visual inspection and histological analysis) and the detection rate of each bacterium in the pad $(-)$ and pad $(+)$ groups were compared by the Chi-square test as the independence test at a significance level of $p < 0.05$. The number of bacterial species detected per screw in the pad $(-)$ and the pad $(+)$ groups were compared by the Student t test at a significance level $p < 0.05$.

Results

Characterization of the pad

The $-CEZ$ and $+CEZ$ pads had randomly rough surfaces with numerous micropores and macropores (Fig. 3A and B). These micro- and macropores formed as a result of the solidification and evaporation of 1,4-dioxane during the freeze-drying process. The resulting porous structure created a sponge-like, flexible quality that made the pads appropriate for a skin patch. In the SEM image of the $+CEZ$ pad, pillar-like granules were embedded within the PCL framework, and some granules protruded from the PCL (Fig. 3B). These granules were identified as CEZ because nitrogen, sodium, and sulphur—all of which are constituents of CEZ—were detected by EDX (data not shown). Except for the presence of the CEZ granules, there was no apparent difference in structure between the $-CEZ$ pads and the $+CEZ$ pads.

In vitro release of CEZ

The release of CEZ from the $+CEZ$ pad was sustained for at least 30 days (a slow-release stage, followed a rapid-release stage). The $+CEZ$ pad quickly released CEZ into HBSS during the first hour (Fig. 4), thereby increasing the CEZ concentration to more than the clinically effective concentration (i.e., 5 µg/mL). Within 1 day, the CEZ concentration had reached 18 µg/mL. During the following days, immersion
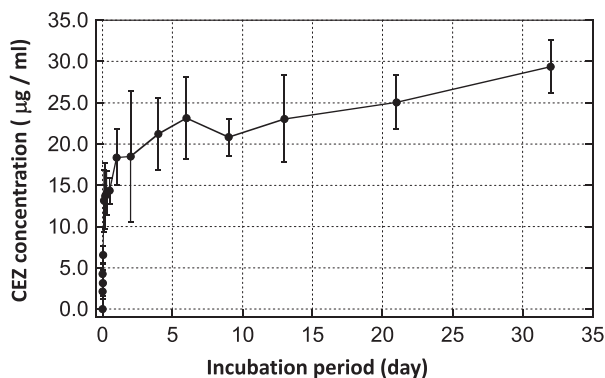
Fig. 4. The *in vitro* release profile of CEZ. Concentrations of total CEZ released from the $+CEZ$ pad after incubation in 10 mL of Hank's balance salt solution (HBSS) at 37°C for various periods up to 32 days ($n = 6$). Results are expressed as the average ± the SD. CEZ = cefazolin sodium; SD = standard deviation.
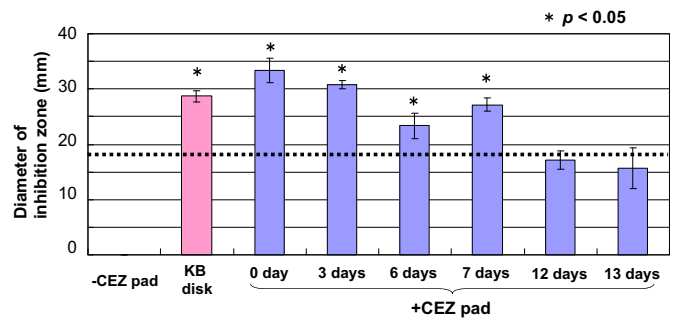
Fig. 5. The *in vitro* bactericidal activity against *Staphylococcus aureus* of the $+CEZ$ pads, $-CEZ$ pads (i.e., negative control), and KB disks (i.e., positive control). The inhibitory zone diameters for $+CEZ$ pads were measured after the elution of CEZ on agar plates for various periods up to 13 days. The broken line corresponds to the inhibitory zone diameter of 18 mm, above which indicates clinical efficacy. *$p < 0.05$, compared against the criterion for clinical efficacy (i.e., 18 mm). CEZ = cefazolin sodium; KB = Kirby–Bauer.

resulted in continued CEZ release, and the CEZ concentration increased gradually up to 29.4 µg/mL (Fig. 4).

In vitro bactericidal activity test

The $+CEZ$ pad demonstrated sustained *in vitro* bactericidal activity against *S. aureus* (Fig. 5). An inhibitory zone diameter greater than 18 mm against *S. aureus* indicated that the strain is sensitive to CEZ and that the $+CEZ$ pad has clinical efficacy for that strain. In this study, the inhibitory zone diameter of the $+CEZ$ pads against *S. aureus* was statistically greater than 18 mm up to 7 days of CEZ elution on the agar plate (Day 0, $p < 0.001$; Day 3, $p < 0.001$; Day 6, $p = 0.003$; and Day 7, $p < 0.001$). The $-CEZ$ pads (i.e., negative control) had no inhibitory zone, whereas the KB disks (i.e., positive control) had a 28.7-mm inhibitory zone diameter (which was significantly greater than 18 mm).

Classification of pin tract infections by visual inspection

One rabbit in the pad $(-)$ group died prior to when it could be sacrificed. Therefore, the number of specimens were 18 rabbits in the pad $(-)$ group and 20 rabbits in the pad $(+)$ group. A Grade 1 infection (i.e., soft tissue infection) was observed in both groups. There was no Grade 2 infection (i.e., osteomyelitis) in either group. The infection rate (corresponding to the Grade 1 infection rate) was significantly lower in the pad $(+)$ group (15.0%) than in the pad $(-)$ group (72.2%; $p = 0.0004$).

Histological analysis

In the pad $(+)$ group, the percentages of Grade 0, Grade 1, and Grade 2 histological inflammation of the soft tissues were 30.0%, 55.0%, and 15.0%, respectively. However, in the pad $(-)$ group, the percentages of Grade 0, Grade 1, and Grade 2 inflammation of the soft tissues were 22.2%, 38.9%, and 38.9%, respectively. The histological inflammation rate of the soft tissues was significantly lower in the pad $(+)$ group than
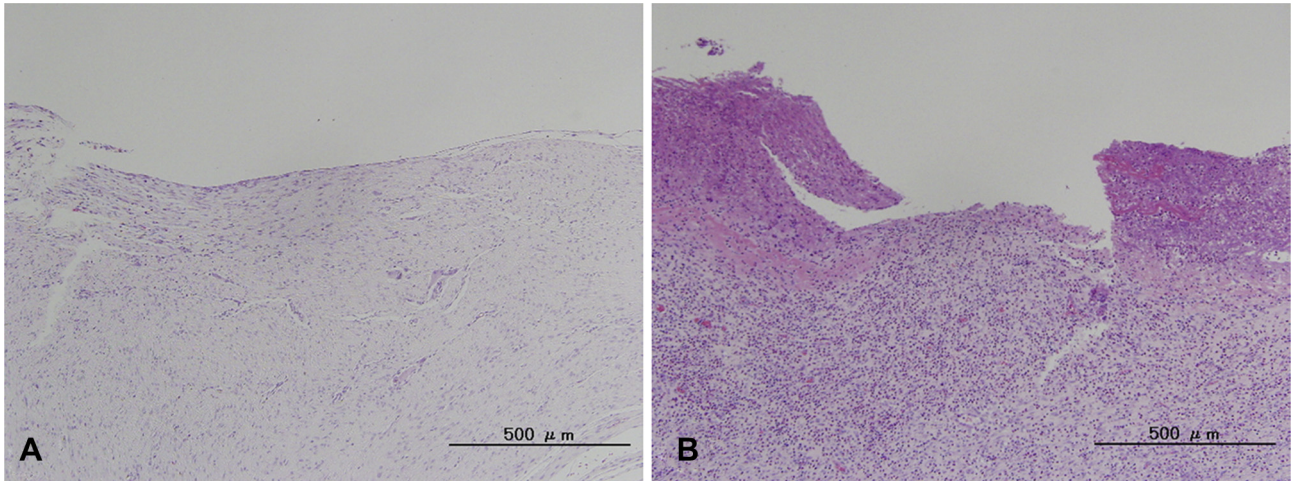
Fig. 6. Histological sections of specimens with (A) Grade 0 infection and (B) Grade 1 infection at 4 weeks after implantation [hematoxylin and eosin stain]. (A) No inflammation or very mild inflammation of the soft tissues is present around the screws in Grade 0 infection. (B) Soft tissue inflammation is present around the screws in Grade 1 infection.

in the pad (−) group ($p = 0.046$). In the pad (+) group, the percentages of Grade 0, Grade 1, and Grade 2 histological osteomyelitis of the hard tissues were 85.0%, 15.0%, and 0%, respectively. However, in the pad (−) group, the percentages of Grade 0, Grade 1, and Grade 2 osteomyelitis of the hard tissues were 72.2%, 27.8%, and 0%, respectively. There was no statistically significant difference between the two groups ($p = 0.335$). No inflammation or only very mild inflammation of scar tissue was observed in the soft tissues around the screws in rabbits with Grade 0 infection (Fig. 6A). By contrast, necrotic tissue associated with mild or severe inflammation was observed in the soft tissues around the screws in rabbits with Grade 1 infection (Fig. 6B).

Fig. 7. The bacterial detection rates in the pad (−) and pad (+) groups ($n = 18–20$). The + CEZ pad shows a reduced number of *Staphylococcus aureus*. The rate of no bacterial detection is 3.3 times higher in the pad (+) group than in the pad (−) group, and the difference is statistically significant. CEZ = cefazolin sodium.

Bacteriological examination

The +CEZ pad reduced the detection rate of *S. aureus* (Fig. 7). The rate of *S. aureus* detection was three times lower in the pad (+) group than in the pad (−) group, and the difference was statistically significant ($p = 0.021$). By contrast, the rate of no bacterial detection was 3.3 times higher in the pad (+) group than in the pad (−) group. The difference was statistically significant ($p = 0.031$). The differences in the detection rate of other bacteria were not significant between the pad (+) and pad (−) groups. The number of bacterial species detected per screw was significantly smaller in the pad (+) group (0.6 ± 0.7) than in the pad (−) group (1.1 ± 0.8; $p = 0.018$).

Discussion

The infection rate during percutaneous implantation using screws coated with a FGF-2–apatite composite layer was reduced to approximately one-fifth when using the +CEZ pad, based on visual inspection. The histological inflammation rate of the soft tissues was also significantly lower with the +CEZ pad than without the pad. According to our previous results on uncoated titanium screws, most pin tract infections occurred within 12 days and one-third of them occurred within 4 days.[3] Once a pin tract infection occurred prior to wound closure around the pin, the infection remained for the entire period of external fixation. Thus, preventing an infection during the early postoperative period is important for preventing pin tract infection altogether. This study confirmed that the bactericidal activity of the +CEZ pad starts immediately and is sustained for as long as 7 days under conditions similar to placing the pad on skin tissue (Fig. 5). This result suggests that the +CEZ pad retains its bactericidal efficacy during the early postoperative period, when the risk of pin tract infection is increased. The bactericidal efficacy of the +CEZ pad during the early postoperative period reduces the infection rate during external fixation by screws coated with FGF-2–apatite.
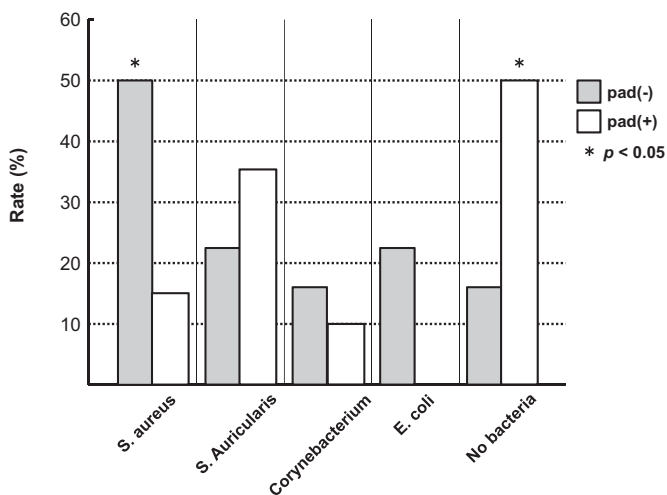
The immediate and sustained bactericidal action of the +CEZ pad results from the two-stage release of CEZ from the +CEZ pad on contact with a liquid medium. The two-stage release consists of an initial burst during the first day, followed by sustained release of CEZ lasting a few weeks (Fig. 4). The initial release of CEZ from the +CEZ pad is attributed to the rapid dissolution of CEZ on the PCL surface (Fig. 3B) because CEZ is highly soluble in an aqueous medium. The later stage of slow release is attributable to the hydrolytic degradation of the PCL matrix and the resultant exposure and dissolution of CEZ embedded in the matrix. The two-stage release of CEZ from the +CEZ pad is likely induced, even on skin tissue around the screw, by bleeding and fluid discharge from the wound.

Our previous study showed that titanium screws coated with FGF-2−apatite reduced pin tract infection by promoting wound healing.[2,3] Wound healing requires time, even with the aid of FGF-2−apatite. This composite layer, however, has no direct bactericidal activity to prevent early postoperative bacterial invasion. The +CEZ pad developed in this study complements FGF-2−apatite by the initial burst release of CEZ, followed by a sustained release. Thus, using the +CEZ pad in combination with the screws coated with a FGF-2−apatite composite layer effectively improved infection resistance around the pin tract.

The present screws were prepared under the same conditions as that of previous titanium screws coated with the FGF-2−apatite layer[2]; however, the present infection rate for the pad (−) group (72.2%) was higher than the rate of the previous results (43.8%). The increased infection rate is tentatively ascribed to enforced changes in the animal experimental facility, which included changes in the operating room, cages of rabbits, and breeding environment. These changes eventually can influence the bacterial density in the atmosphere.

Various methods have been attempted to prevent pin tract infections. The standard approach employed clinically is the oral or parenteral administration of antibiotics. However, the continuous use of a large amount of antibiotics has the secondary risk of antibiotic-resistant bacteria. Alternative methods using silver, titanium-copper, iodine, chlorhexidine, and a tobramycin-impregnated polymethylmethacrylate pin sleeve have recently been reported.[17−21] Our method using the +CEZ pad has the advantages of reducing the amount of antibiotics, simple methodology, and safety. The amount of CEZ applied by the +CEZ pad was a 7-day total of 0.8 mg/kg, which was two orders of magnitude smaller than the amount systemically applied by conventional drip infusion (20−100 mg/kg per day).[22,23] The efficacy of the +CEZ pad was moreover retained for as long as 7 days under the conditions of use. The +CEZ pad can be easily used independently of the screws. Therefore, the +CEZ pad can easily be positioned, removed, and changed, depending on the symptomatic state of the wound during the implantation. For example, it can be used when pin tract infection is diagnosed during external fixation early postoperatively and long-term after an operation.

Reducing the incidence of pin tract infections is clinically important during the course of treatment by percutaneous implants such as external fixation. The + CEZ pad developed in this study may also decrease the risk of inadequate healing of a fractured bone, including nonunion.

A limitation of this study is that the data for long-term implantation and implantation under loaded conditions remain unclear. Further studies entailing long-term implantation and implantation under loaded conditions are required. It is notable that a pad containing other antibiotics can be created for any bacteria.

In conclusion, a +CEZ pad was prepared by a freeze-drying process. Antibiotic activity of the +CEZ pad was detected, even after 7 days of preincubation on an agar plate. The infection rate was reduced to approximately one-fifth when using the +CEZ pad. Use of the +CEZ pad thus effectively improves resistance to infection during percutaneous implantation.

Conflicts of interest

All authors declare no conflicts of interest.

Acknowledgements

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