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Antibacterial Iodine-Supported Titanium Implants

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Abstract

Deep infection remains a serious complication in orthopedic implant surgery. In order to reduce the incidence of implant-associated infections, several biomaterial surface treatments have been proposed. This study focused on evaluating the antibacterial activity of iodine-supported titanium (Ti-I_2) and impact on post-implant infection, as well as determining the potential suitability of Ti-I_2 as a biomaterial. External fixation pins were used in this experiment as trial implants because it was easy to make the septic models.

The antibacterial activity of the metal was measured using a modification of the Japanese Industrial Standards method. Activity was evaluated by exposing the implants to *Staphylococcus aureus* or *Escherichia coli* and comparing reaction of pathogens to the Ti-I_2 versus the stainless steel and titanium controls. The Ti-I_2 clearly inhibited bacterial colonization more than the control metals. In addition, cytocompatibility was assessed by counting the number of colonies that formed on the metals. The three metals showed the same amount of fibroblast colony formation.

Japanese white rabbits were used as an *in vivo* model. Three pins were inserted into both femora of six rabbits for histological analysis. Pin sites were inspected and graded for infection and inflammation. Fewer signs of infection and inflammatory

changes were observed in conjunction with the Ti-I_2 pins. Furthermore, osteoconductivity of the implant was evaluated with osteoid formation surface of the pin. Consecutive bone formation was observed around the Ti-I_2 and titanium pins, while little osteoid formation was found around the stainless steel pins. These findings suggest that Ti-I_2 has antimicrobial activity and cytocompatibility. Therefore, Ti-I_2 substantially reduces the incidence of implant infection and shows particular promise as a biomaterial.

Key words: Iodine-supported titanium; Antibacterial implant; Biocompatibility; Infection; Cytotoxicity

1. **Introduction**

Bacterial infection has become a significant complication following implant placement. The infection rate ranges between 0.5% and 3.0% after primary total hip arthroplasty despite strict antiseptic operative procedures, including systemic prophylaxis [1-6]. Infection rates between 5% and 35% have been described for endoprosthetic replacement of large bone defects after tumor resection [7-12], while external fixation produced infection in 2-30% of cases found during a literature review [13-17]. Several biomaterial surface treatments have been proposed as a means of reducing the incidence of implant-associated infections. There has been investigation into the covalent attachment of polycationic groups [18,19]; ion implantation, such as F+ [20]; impregnating or loading chitosan nanoparticles with antimicrobial agents [21, 22]; coating implant surfaces with polymers drug-loaded [23, 24]; and coating implant surfaces with either quaternary ammonium compounds, human serum albumin, or silver ions [25-30]. However, there are several shortcomings of these proposed techniques including limited chemical stability, local inflammatory reactions due to material composition, and a lack of controlled release kinetics from the coatings.

In this work, titanium (Ti) surfaces were modified using anodization. Ti is the implant material of choice for use in orthopedic and dental applications. Its excellent

biocompatibility is reportedly attributable to the stable oxide that readily forms on Ti surfaces [31]. The biocompatibility of metal-oxides is well established as evidenced by their current clinical applications in orthopedic and dental implants [32]. Highly adhesive anodic oxides can be formed through anodization, and the composition of these anodic films is dependent on electrolyte composition [33]. Electrolytes containing calcium and phosphorus have been explored as a means of forming anodic films [33-35]. Here we describe the novel use of povidone-iodine as the electrolyte. The use of a povidone-iodine electrolyte resulted in the formation of an adhesive porous anodic oxide with the antiseptic properties of iodine. In addition, iodine is the heaviest essential element known to be needed by all living organisms and a component of thyroid hormones.

This present study aimed to evaluate the antibacterial activity of iodine-supported titanium (Ti-I_2) and its impact on implant infection, and to determine the potential use of Ti-I_2 as a biomaterial.

2. Materials and methods

2.1. Implants

External fixation pins were used in this experiment as trial implants because of the

ease of making the septic models. All iodine-supported titanium was produced by the Chiba Institute of Technology. Circular implant Ti-I_2, pure titanium or stainless steel disks (diameter: 20 mm; thickness: 2 mm) were used for *in vitro* antimicrobial tests. Semidisks, 50 mm in diameter and 2 mm thick, of these metals were used for *in vitro* cytocompatibility tests. External fixation pins of Ti-I_2, pure titanium or stainless steel (diameter: 2 mm; length: 45 mm) were used *in vivo*. The stainless steel material used in this study was SUS316. The titanium was commercially pure titanium. Ti-I_2 was produced by the Chiba Institute of Technology, (Narashino, Japan) using a technique described by Hashimoto [36]. The thickness of the anodic oxide film was between 5 and 7 μm, with more than 1400 pores/mm^2 capacity to support 10-12 μg/cm^2 iodine. All the metals were processed by Koshiya Medical Instruments Company (Kanazawa, Japan).

2.2. *In Vitro* antimicrobial properties

The antibacterial activity of the Ti-I_2 was measured using the method approved by Japanese Industrial Standards. The implants were exposed to Gram positive *Staphylococcus aureus* (*S. aureus*) strain 25923 (ATCC, Manassas, VA) or Gram negative *Escherichia coli* (*E. coli*) strain MG1455. Approximately one million colony forming units were inoculated on the autoclaved circular implants before they were covered by glass in a sterile dish and incubated at 37°C for 2, 6, or 24 h. Each implant

was washed using 5 mL phosphate-buffered saline (PBS). The wash eluate was diluted 1:50 with PBS and 100 μL was spread on the following media: *S. aureus* was grown in Brain Heart Infusion broth and *E. coli* was grown in LB broth (1% w/v tryptone, 0.5% w/v yeast extract, 0.5% w/v NaCl) at 37°C. The colonies were counted after 24 h. If all the pathogens were viable, 2000 colonies were counted (Figure 1). This method was repeated 15 times for both *S. aureus* and *E. coli*. The reaction of pathogens to the Ti-I_2 was compared with their reaction to pure titanium and stainless steel (controls). The differences in the number of bacteria on each metal were statistically analyzed.

2.3. *In Vitro* Cytocompatibility Properties

The V79 cell line (Chinese hamster fibroblasts), provided by the RIKEN BioResource Center Cell Bank (Tsukuba, Japan), was used for the cytotoxicity tests. Culture medium consisted of alpha-minimum essential medium (α-MEM) supplemented with 10% fetal calf serum (FBS), 100 U/mL penicillin and 100 μg/mL streptomycin sulfate. Experiments were conducted in an incubator at 37°C with a humidified atmosphere of 95% air and 5% CO_2 for 24 h. Semidisks made of stainless steel, titanium or Ti-I_2, sterilized by heating at 180°C for 1 h, were placed in plastic 60 mm-petri dishes. A cell suspension of trypsinized subcultured V79 cells was diluted from 10^6 cells/mL to 10^2 cells/mL. Next, 6 mL of medium and 2 mL of the cell suspension were seeded on

the semidisks in dishes so as to provide 300 cells per dish. Control dishes without metals were also made. After seeding, the dishes were gently shaken and cultured in the incubator. After 1 week, the medium was extracted, and the cells were fixed with 5 mL 10% formalin for 30 min, stained with 8 mL of 0.15% methylene blue for an additional 30 min, washed thoroughly, and dried. Differences in colony formation between areas covered by the metal disks and plastic areas of the dishes were first qualitatively examined. Subsequently, colony formation in the dishes was compared with control dishes by counting the number of colonies [37].

2.4. *In Vivo* Effects

Pins were inserted into the femora of six mature female Japanese white rabbits weighing from 2.5 to 3.0 kg. The rabbits were anesthetized with an intramuscular injection of ketamine hydrochloride (50 mg/kg body weight; Warner-Lambert, Morris Plains, NJ) and an intravenous injection of pentobarbital sodium (40–50 mg/kg body-weight; Abbott Laboratories, North Chicago, IL). A longitudinal skin incision was made on the lateral side of the right thigh, and the muscle and fascia were carefully split. Half pins made of each of the three metals, 2 mm in diameter (Howmedica, Geneva, Switzerland), were inserted randomly into the lateral aspect of both femora in six rabbits. The 12 of each type of half-pins were inserted.

On postoperative day 14, the animals were euthanized and the histology of the pin tract was studied. Heparinized physiologic saline was perfused through the aorta, followed by perfusion with 4% paraformaldehyde in phosphate buffer (pH 7.4). The femurs were fixed for 48 h in the same solution. Next, all pins were removed and the femurs were decalcified with 10% EDTA and embedded in paraffin. A representative section was chosen for each pin tract site. The specimens were sectioned at 5 mm-thickness parallel to the bone axis and stained with hematoxylin-eosin stain. The tracts were inspected and graded for the presence of inflammation, abscesses, osteomyelitis, and inflammation around the tip. Inflammation of the pin tract and around the tip were scored from 0 to 2, where 0 = none, 1 = mild, 2 = severe. Pin tract abscesses were scored from 0 to 2, where 0 = none, 1 = surface, 2 = deep. Pyogenic osteomyelitis was scored from 0 to 2, where 0 = none, 1 = mild infection, 2 = abscess formation (Table 1). For the Ti-I_2, stainless steel, and pure titanium, the average score of each category and total scores were calculated. Severe inflammation and infection resulted in a higher score. Each metal was evaluated for a total of 12 pins.

2.5. *In Vivo* Biocompatibility

The biocompatibility of the titanium-supported iodine was evaluated by comparing osteoid formation on the surface of the external fixation pin with a pin made of pure

titanium. Pure titanium is highly osteoconductive [38]. Therefore, bone conduction was classified as normal if the osteoid formation was similar to that observed for pure titanium.

2.6. Statistical analysis

Statistical analyses were performed using StatView 5.0. The difference in the number of bacilli between each metal was analyzed by repeated measured ANOVA. Inflammation and infection scores were compared using Fisher exact tests.

3. Results

The iodine-supported titanium inhibited colony formation of both *S. aureus* and *E. coli* compared with stainless steel and titanium. Figures 2 and 3 show the colonization of each bacterium at 6 and 24 h. Fewer colonies formed on Ti-I_2 at all time points ($P<0.05$) (Fig. 4 and 5).

Cytotoxicity tests showed that about 300 cells were equally and uniformly distributed on the surface of each dish. Stainless steel, titanium and Ti-I_2 showed no differences in the number of colonies formed in each dish, nor were there differences in colony formation between the metal and plastic areas (Fig. 6).

The reactive tissues around the pin were evaluated macroscopically and 12 metal

pins were scored. The average total score showed that Ti-I_2 accumulated the least number of points, which was indicative of minimal inflammation and infection around the Ti-I_2. Statistical analysis showed that Ti-I_2 significantly inhibited inflammation and infection ($P < 0.01$) (Table 2).

All inserted pins were evaluated histologically for osteoid formation. There were excellent osteoid formations on the surface of the Ti-I_2 pins as well as the titanium pins, suggesting that Ti-I_2 is a good osteoconductive material. The bone grew into the pitch of the screw and the osteoid formations continued to the opposite cortical layer from the front cortical layer, all signs of osteoconduction. Conversely, osteoid formation was diminished on stainless steel, with only partial osteoid formation (Fig. 7). Bone conduction was not possible on stainless steel.

4. Discussion

A procedure was developed for the anodization of iodine-containing surfaces that can be directly supported to existing titanium implants. The results indicate that iodine-supported titanium has antibacterial activity, biocompatibility, and no cytotoxicity. There was no conflict of interest of any authors with the Ti-I_2 coated implants. The limitation in this study is to be able to coat with iodine only the implant

made of titanium at present.

Implant methods are frequently used in almost all fields of modern medicine and are associated with a definitive risk of bacterial infection. Staphylococci account for the majority of infections of both temporarily and permanently implanted orthopedic devices [39]. Because systemic antibiotics often do not provide effective treatment for implant infections due to the phenomenon of drug resistance, it is important that the coating of the implant exhibit local antibacterial activity. In order to reduce the incidence of implant-associated infections, several biomaterial surface treatments have been proposed [18-30]. In particular, silver has raised the interest of many investigators because of its good antimicrobial action and low toxicity [30, 40-43]. On the other hand, silver has been found to have toxic effects towards human cells [44,45]. Other studies have shown that the hydroxyapatite can decrease infection by improving the compatibility of the bone [46]. However, hydroxyapatite does not have antimicrobial activity. Some antiseptically-coated implants, such as chlorhexidine, have been reported [47-49]. As shown in Table 3, the antibacterial spectrum of iodine is very wide. The antimicrobial effect acts on not only general bacteria but also viruses, tubercle bacilli and fungi. In addition, unlike antibiotics, resistant bacteria are not generated in iodine. Moreover, iodine is a trace metal and an essential component of the thyroid hormone. If

iodine is released from the implant, it is biologically safe for the human body because iodine can be excreted by the kidneys.

Mechanical strength is necessary for the implant. There is no problem for mechanical strength of Ti-I_2 because Ti-I_2 has just only anodized titanium. Titanium has already been used clinically for implant. However, when Ti-I_2 is actually used for biomaterial, the mechanical strength test will be needed.

Significant differences in bacterial adhesion on stainless steel, titanium and Ti-I_2 surfaces were observed. The Ti-I_2 surfaces have significantly less adhesion of *S. aureus* and *E. coli*, suggesting that Ti-I_2 would be very effective against postoperative infections. In this study, the implants were exposed to Gram positive *S. aureus* or Gram negative *E. coli* based on Japanese Industrial Standards. The antibacterial activity to Pseudomonas aeruginosa and Staphylococcus epidermidis will be evaluated in the future.

The present toxicological evaluation method for biomaterials, colony formation of V79 cells, is suitable as a screening test for biomaterials. It has the advantages of: (1) yielding accurate and reproducible survival rates; (2) allowing direct contact between materials and cells, even with solid opaque materials; (3) allowing a general assessment of whether cytotoxicity is caused by chemical or physical factors; and (4) being easy to

perform and to evaluate [37]. Stainless steel and titanium have clinical applications in the field of orthopedic surgery. In this study, these materials were no different than the controls in colony formation and cytotoxicity. The Ti-I_2 also had good biocompatibility because colony formation of normal fibroblasts was observed in the semi-disk metal area and the plastic area of the dishes. An absence of colonies from areas would have signified the release of a cytotoxic chemical substance. If physical properties such as roughness or surface energy of the materials affect colony formation, there would be no colonies on the material itself, only on the plastic part of the dishes. Ti-I_2 can be an excellent biomaterial as it exhibits low biological toxicity and shows excellent antibacterial activity.

In the present animal experiment, the Ti-I_2 resulted in a significantly reduced infection and inflammation rate. The pin sites were histologically inspected and graded for inflammation and infection (Table 1). If inflammation and infection were most severe, the score would be 8 points. The average score for the Ti-I_2 was 2.92, lower than that of stainless steel or pure titanium (Table 2). In most evaluation categories, Ti-I_2 indicated a low score. Inflammation score of titanium is also low point. That means titanium has biocompatibility. Therefore, we think it was reflected in few of the aseptic inflammation that iodine supported-titanium was made of titanium.

In biomaterials science, osteoconduction means growth of bone on the surface of a foreign material. Osteoconduction depends not only on biological factors, but also on the response to a foreign material, and the osteoconductive response is necessary for successful osteointegration [38]. The biocompatibility of the implant was evaluated by osteoconduction because bone conduction is often observed with biocompatible materials such as titanium. We found that while titanium had good osteoid formation (i.e., good osteoconduction), Ti-I_2 produced excellent consecutive osteoid formation around the pins.

5. Conclusion

The findings of this study suggest that iodine-supported titanium has antimicrobial activity and substantially reduces the incidence of pin tract infection. Therefore, iodine-supported titanium shows particular promise as an antibacterial biomaterial.

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Figure Captions

Figure 1. Antibacterial assessment using a modified version of the Japanese Industrial Standards method.

Figure 2. Representative plates of *S. aureus* colonization at 6 and 24 h.

Figure 3. Representative plates of *E. coli* colonization at 6 and 24 h.

Figure 4. Changes in the number of *S. aureus* colonies.

Figure 5. Changes in the number of *E. coli* colonies.

Figure 6. Colony formation on metal semidisks. Stainless steel, titanium and Ti-I_2 showed no difference in the amount of colony formation.

Figure 7. Osteoid formation on the surface of Ti-I_2. There were excellent osteoid formations on the Ti-I_2 pin and poor formations on the stainless steel pin.

Figure 1

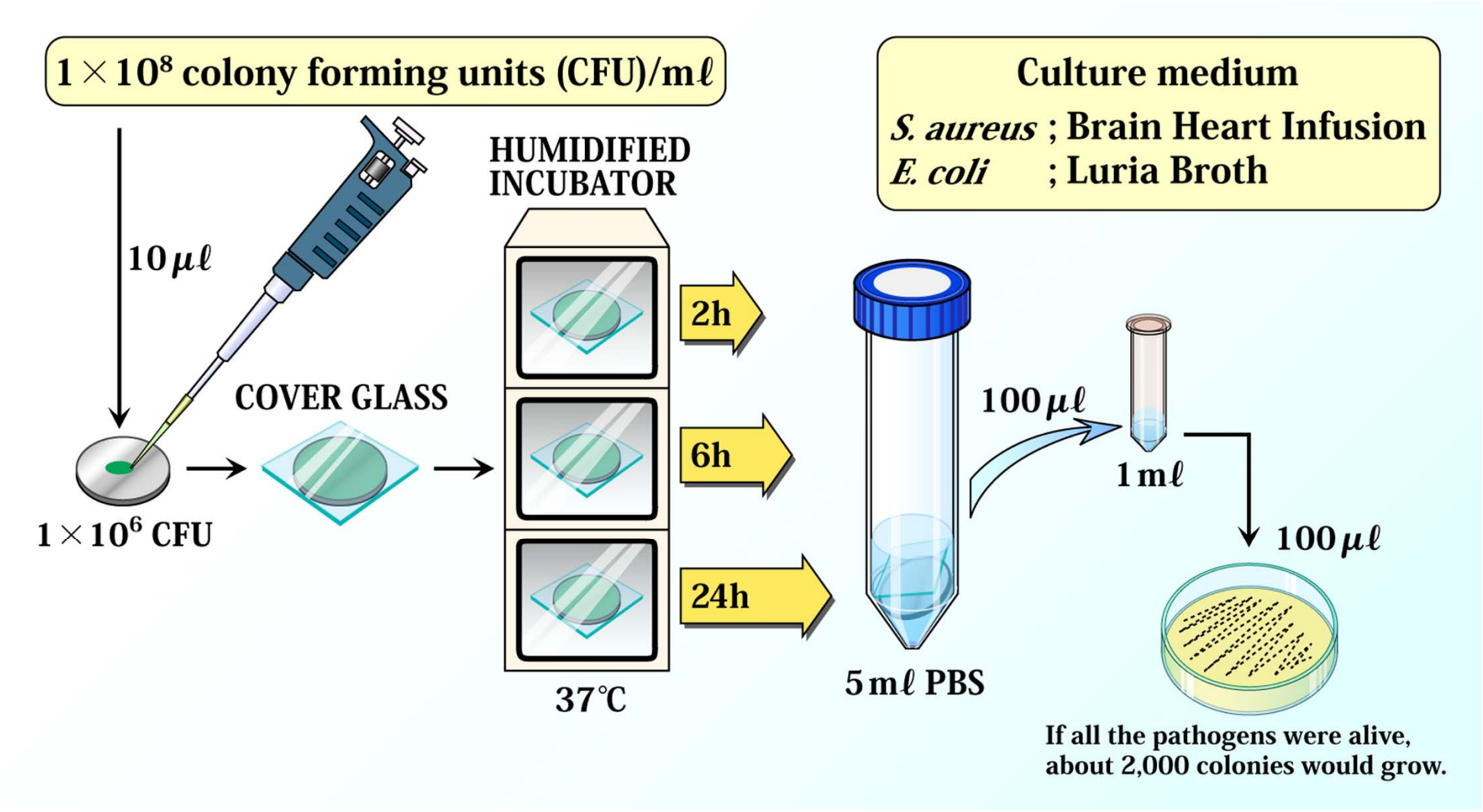

1 × 10⁸ colony forming units (CFU)/mℓ
Culture medium
S. aureus ; Brain Heart Infusion
E. coli ; Luria Broth
HUMIDIFIED INCUBATOR
10 μℓ
COVER GLASS
1 × 10⁶ CFU
2h
6h
24h
37℃
5 mℓ PBS
100 μℓ
1 mℓ
100 μℓ
If all the pathogens were alive, about 2,000 colonies would grow.

Figure 2

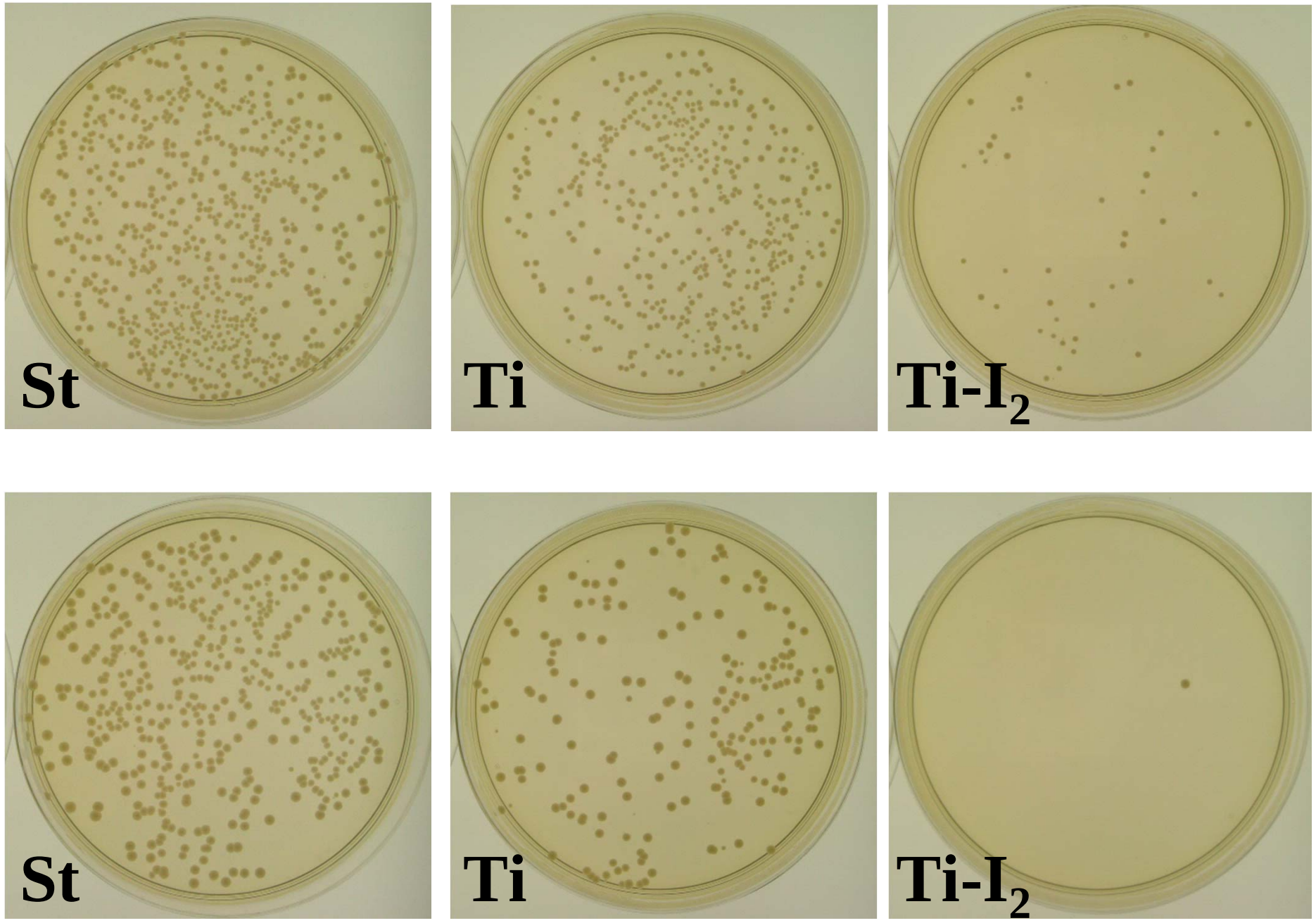


Figure 3

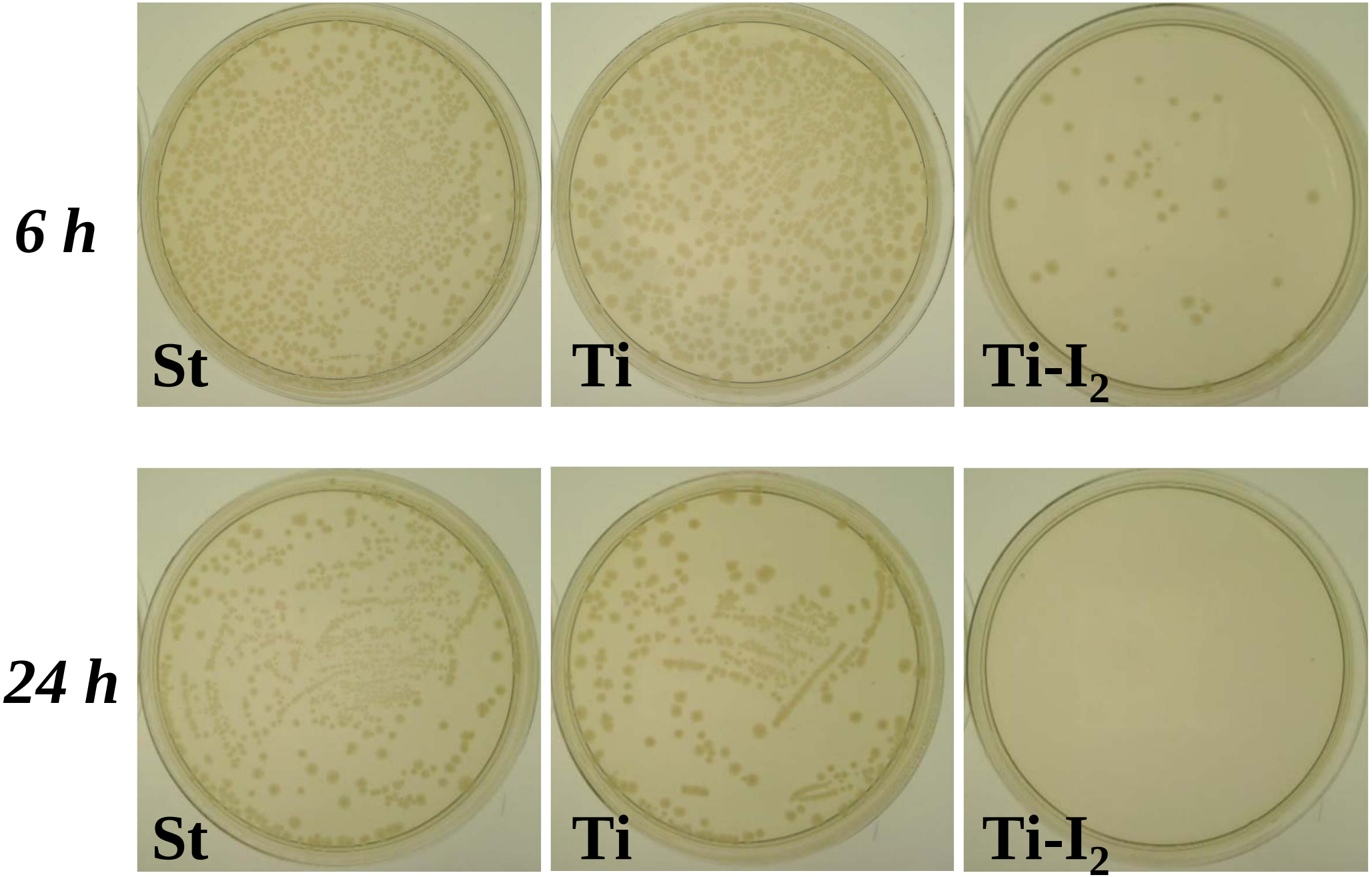


Figure 4

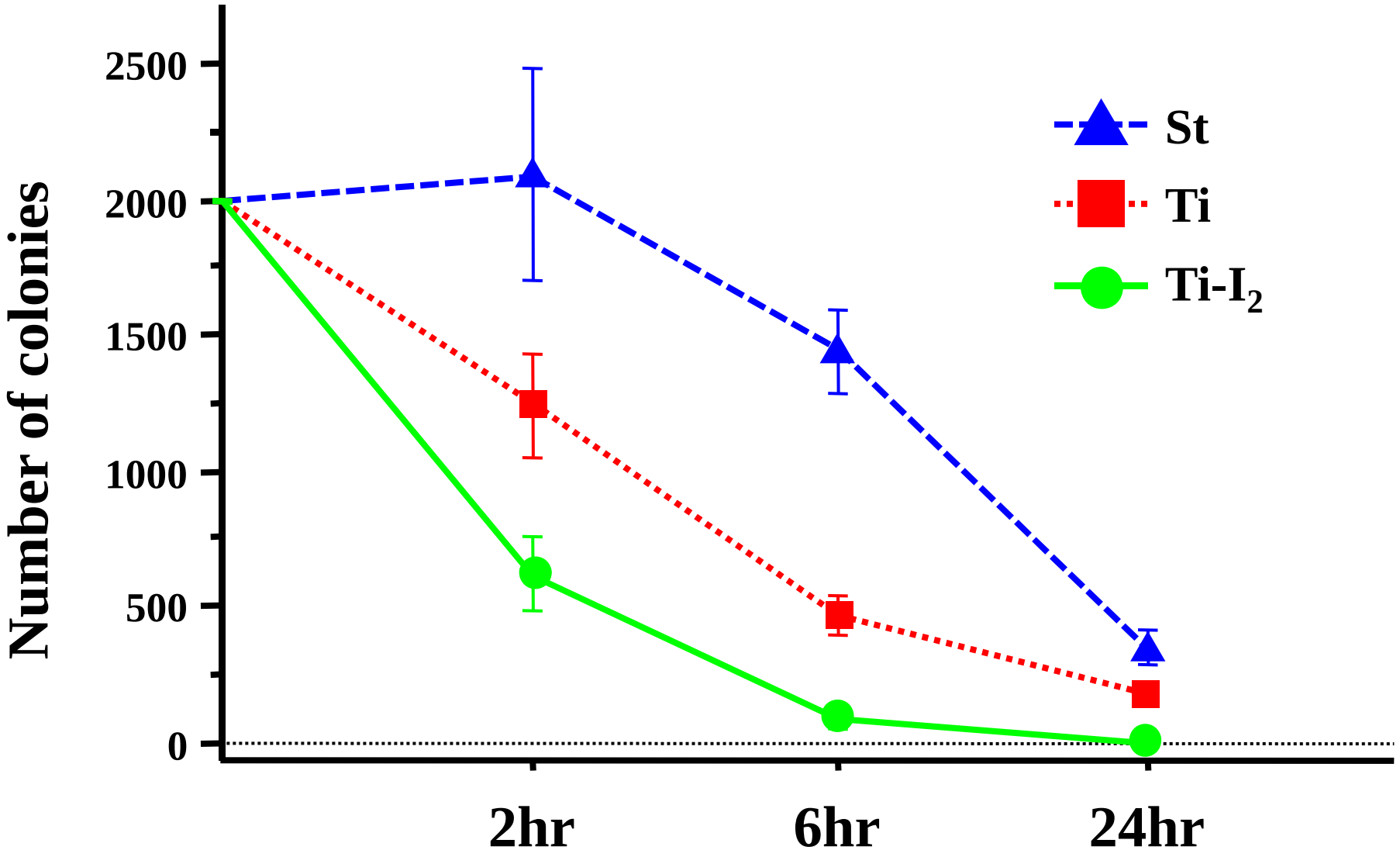

Number of colonies
2500
2000
1500
1000
500
0
2hr
6hr
24hr
St
Ti
Ti-I2

Figure 5

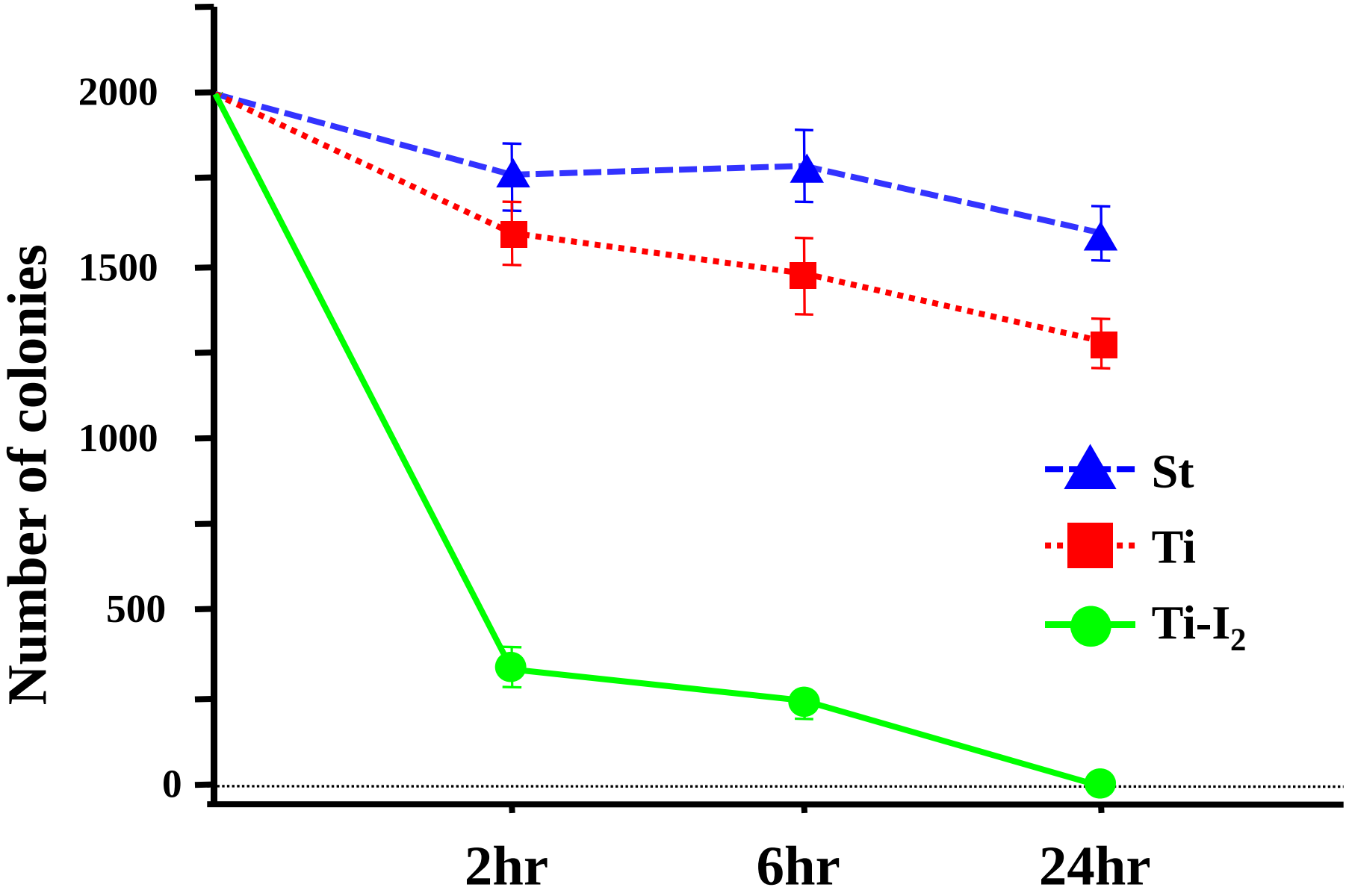

Number of colonies
2000
1500
1000
500
0
2hr
6hr
24hr
St
Ti
Ti-I2

Figure 6

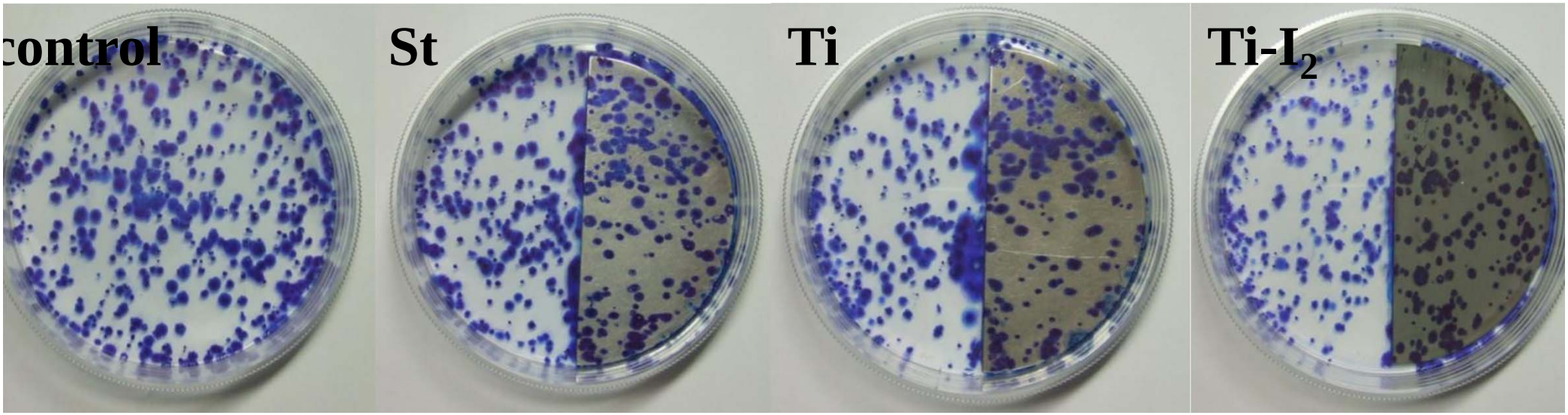
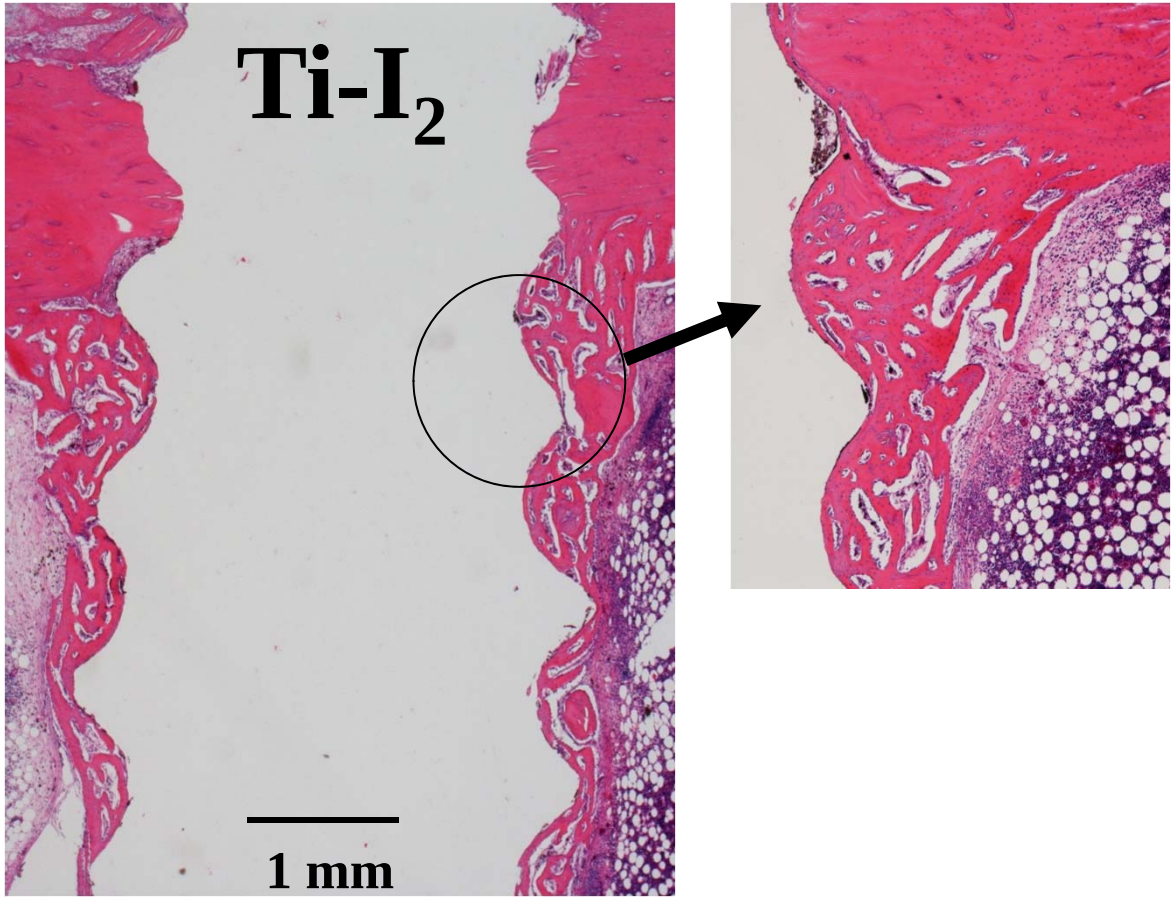


Figure 7



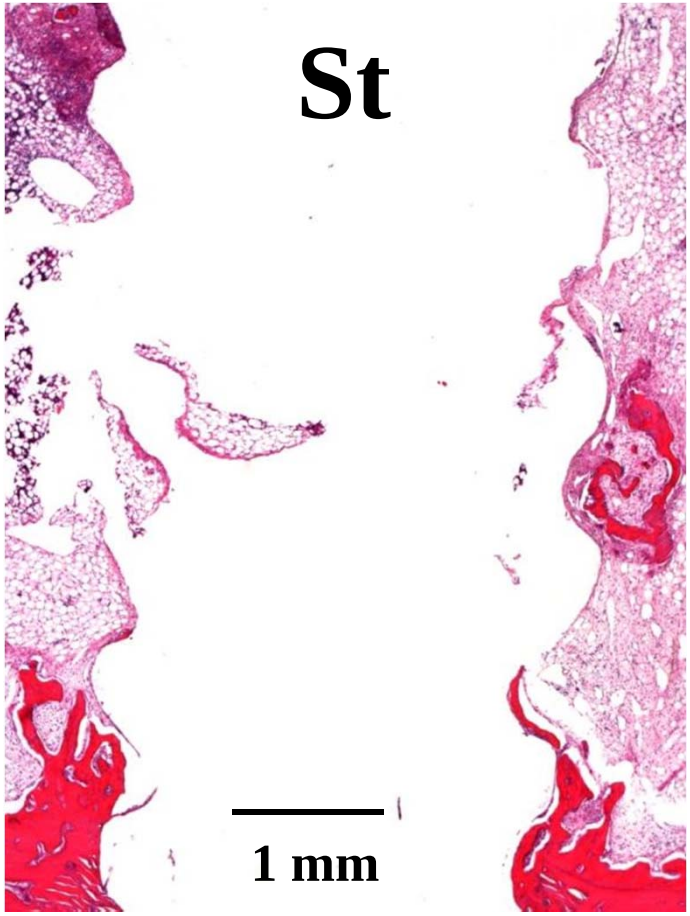


Table 1

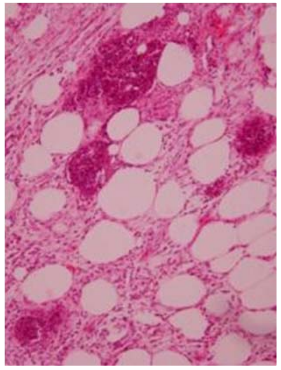
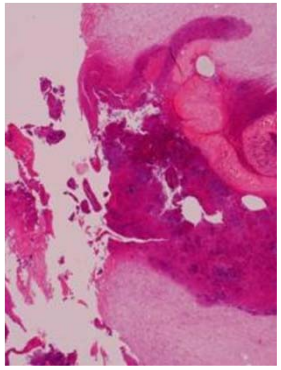
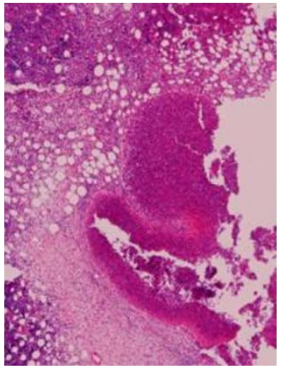
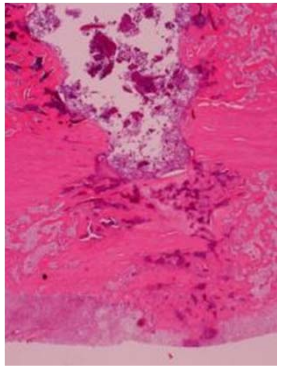
Score of Inflammation and Infection by Histological Analysis

	Pin tract inflammation	Abscess of pin tract	Osteo-myelitis	Inflammation around the tip
+	Severe; 2	Deep; 2	Abscess; 2	Severe; 2
	Slight ; 1	Surface; 1	Slight ; 1	Slight ; 1
—	0	0	0	0

The values 0,1,and 2 are the score points.

Table 2

Average score of inflammation and infection (n=12)

	Pin tract inflammation	Abscess of pin tract	Osteo myelitis	Inflammation around the tip	Total Score
					n=12
St	1.50	1.16	1.42	0.83	4.92±1.73
Ti	1.33	1.83	1.17	0.08	4.42±0.90
Ti-I_2	0.75	1.00	1.00	0.17	2.92±1.16

* *

Asterisk (*) indicates significant difference at P<0.01

Table 3

Antibacterial spectrum of iodine

	Microorganism									
	General bacillus	*Syphilis treponema*	*Pseudomonas aeruginosa*	*M R S A*	*Tubercle*	*Spore*	*Fungus*	*General virus*	*H B V , H C V*	*H I V*
Iodine	○	○	○	○	○	×	○	○	○	○