

Metalworking defects in surgery screws as a possible cause of post-surgical infections

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In the first phase of this work, surface defects (metalworking) in stainless steel implantable prostheses and their possible relation to infections that can be generated after surgery was studied. In a second phase, the results obtained in the aforementioned stage were applied to knee cruciate ligaments surgery screws, considering the fact that a substantial number of Mucormycetes infections have been reported after arthroscopic surgery in Argentina since the year 2005. Two types of screws, transverse and interference screws, were analyzed. The Allen heads presented defects such as burrs and metalworking bending as a result of the machining process. These defects allow the accumulation of machining oil, which could be contaminated with fungal spores. When this is the case, the gaseous sterilization by ethylene oxide may be jeopardized. Cortical screws were also analyzed and were found to present serious metalworking defects inside their heads. To reduce the risk of infection in surgery, the use of screws with metalworking defects on the outer surface, analyzed with stereomicroscope and considering the inside part of the Allen as an outer surface, should be avoided altogether.

1. Introduction

There are many cases of bacterial and fungal infections which come as a result of contaminated surgical implants in clinics and hospitals. Infectious agents in steel prostheses are particularly difficult to eliminate, especially when the bacteria have completely colonized the piece [1]. In some cases, patients have to undergo a new surgery to remove the prosthesis because the effect of antibiotic (ATB) disinfection is not effective enough. Not only do these infections bring economic losses to health institutions but they may place a patient's life at risk of death, especially when the individual is an elderly person.

Since 2005, in Argentina there have been over 40 cases of mucormycosis fungal infections in cruciate ligaments surgery patients [2], many of which were detected in the city of Paraná (Entre Ríos, Argentina), with serious health implications.



The clinical forms of mucormycosis are varied and severe in all the cases. The one isolated in the aforementioned context was the *Rhizopus microsporus var. rhizopodiformis*. This fungus is an environmental opportunistic human pathogenic fungus and as such it requires special conditions to produce an infection. One of these conditions is its accidental inoculation in traumatological surgery owing to the use of contaminated material. Bone mucormycosis produces catastrophic effects on bones and the affected surrounding tissue, causing necrosis manifested by severe pain, need for surgical bone resection, prosthetic bone replacement, temporary or permanent disability and severe death cases.

Several authors have delved into bacterial adherence to biomaterials and its characteristics. Na and Friedman [3] studied the physical and chemical characteristics of adhesion. The importance of a smooth surface finish of stainless steel pieces was reported by Arnold and Bailey [4] in the use of pipes for the food industry. Katsikogianni and Missirlis [5] studied the mechanisms of bacterial adhesion to biomaterial surfaces, factors affecting adherence, techniques used in estimating bacteria-material interactions and the models that have been developed to predict the adherence.

The first part of this work was performed on stainless steel prostheses with and without metal mechanical defects. Colonization of the surface by microorganisms and subsequent disinfection was studied. Even though some of these results have been reported elsewhere [6], it was thought of utmost importance to present them here as well to provide the basis for the hypothesis proposed for the second part of the present work.

Two titanium screws, one transverse and another one interferential, used in knee cruciate ligaments surgery and later on drawn from the patient were analyzed in the second phase of the study. The analysis consisted in finding metalworking defects that may foster microorganism adherence. Similarly, a cortical screw used in osteosynthesis was studied. Tests were also performed on machining oil employed in the manufacture of screws in order to delve into and its properties as means to allow bacteria and fungi growth [7, 8].

2. Materials and methods

2.1 First phase: steel prostheses

In the first part of the work, the specimens studied were pieces of steel prostheses that had been used and then removed from patients. These pieces were cut so that they could fit in a test tube. Pieces with some fold defects, produced by the placement of the prosthesis in the patient, and similar pieces without defects were selected. In some cases flawed specimens were polished to remove imperfections completely. Depending on the trial, the specimens were of the same surface and the same shape or they presented different surface or shape.

Once the pieces were carefully chosen, the test samples were cleaned and sterilized by dry heat in a test tube at 270 °C for 2 hours. The next step was the inoculation of the samples, which entails the colonization of the metallic sample with a microorganism.

Commercial yeasts (*Saccharomyces* spp) and the *Staphylococcus aureus* bacterium were used in this study, following the next procedure. A sterile atmosphere was guaranteed by working between burners and using glassware sterilized by dry heat for 120 minutes at 270 °C. Four grams of commercial yeast were placed in an Erlenmeyer flask and 200 mL of culture medium (pasteurized grape juice) were added. Previously sterilized specimens were placed in an oven and incubated at 28 °C for 96 hours. The specimens were then removed and rinsed in sterile distilled water and allowed to dry under sterile conditions. Then the specimens were disinfected by introducing them into a commercial disinfectant solution (composition: 5.91% Triethylene, 0.29% alkyl dimethyl benzyl ammonium chloride saccharinate) for 1 hour, rinsed in sterile distilled water for 2 hours to remove residual disinfectant and finally each sample placed in a test tube with sterile culture medium, incubated at 28 °C to observe occurrence of development.

All assays were performed by incubating a contaminated specimen that was not disinfected and a negative control tube with culture medium only.

2.1.1 Tests with *Staphylococcus aureus*

S. aureus was used because it has the following advantages: i) unlike yeasts, its smaller size allows it to access and colonize smaller cavities, and ii) most hospital-acquired infections are caused by this bacterium, which highlights the importance of the present research. On the other hand, it is difficult to see *S. aureus* in the metallographic microscope unless it is dyed. Also, more biosecurity measures must be taken since it is a potentially pathogenic microorganism.

Some colonies were taken from a Petri dish, in which the bacteria had developed in Tryptic Soy Agar (Britania), and placed in a test tube containing 1 mL tryptic soy broth with 1 % of glucose, to promote biofilm formation. The bacteria were incubated in a culture oven for 24 hours at 37 °C.

Once the inoculum developed, it was poured into 100 mL of tryptic soy broth with 1 % of glucose and sterile prostheses (with defects and perfectly polished samples) were introduced. They were incubated in a culture oven for 24 hours at 37 °C.

After 24 hours of incubation, the culture medium became turbid, indicating that there was bacteria development. The samples were taken and rinsed in sterile distilled water for 10 minutes with mild agitation. Washing was repeated 3 times and the samples were allowed to dry in a sterile atmosphere. Specimens were placed in individual Erlenmeyers containing the antibiotic cefazolin at a concentration of 1.28 mg of antibiotic per milliliter of sterile saline and allowed to react for 6 hours. After this time period, the specimens were rinsed in sterile distilled water for 15 minutes in individual Erlenmeyers to remove the antibiotic and allowed to dry.

The specimens were placed in test tubes containing 3 mL of sterile Tryptic Soy Broth (Britania) and incubated in a culture oven at 37 °C. The incubation was followed by means of a camera connected to a computer which took pictures every 20 minutes during the period of the trial.

2.1.2 External validation

The results obtained in this study were validated at the Laboratory of Microbiology, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral by means of additional tests.

In order to evaluate the prostheses two samples, with and without metalworking defects were used and inoculated with *S. aureus*. In the same way it was described above. Upon inoculation they were kept for 7 hours in an antibiotic solution of cefazoline at the same concentration the antibiotic would have in the in an infected patient's bloodstream (32 µg/mL). Following this procedure, the samples were removed and rinsed three times. The bacteria developed were counted in the residue of each rinse and reported as colony forming units (CFU) per mL.

2.2 Second phase: surgical screws

In a second phase of the study, two set screws (Figure 1) extracted from infected patients who had undergone knee cruciate ligaments surgery were used. Then, four sets of new screws available on the market were analyzed. Cortical screws were also evaluated.

2.2.1 Eye inspection of screws

An inspection was carried out with a magnifying glass at different magnifications with a maximum of x80 to determine the metalworking conditions of screws, both in terms of their external and internal structure.



Figure1. Transverse screw (left); interfacial screw (right).

2.2.2 Feasibility of machining oil as a source of infection

Sterile metalworking oil was inoculated with different amounts of *R. microsporus* var. *rhizopodiformis* spores (reaching final concentrations of 10^2 , 10^3 , 10^4 , 10^5 and 10^6 spores mL oil⁻¹). The inoculated oil samples were left at room temperature for 24 hours and then covered with Potato Dextrose Agar (PDA, Sigma Aldrich). The plates were incubated at 35°C for 7 days and observed daily.

3. Results and discussion

3.1 First phase: steel prostheses

3.1.1 Tests with yeast on stainless steel samples

In the first phase of the work, ten assays were performed, evaluating a different number of samples in each one. The obtained results are shown in Table 1. In 60% of the trials, yeast growth was observed in at least one sample, indicating that the disinfectant could not remove the total yeast inoculated (Figs. 2-3). Despite the disinfectant effect, there was colonization of samples in areas protected by mechanical defects. Where there were samples without growth inoculation, it could be attributed to yeasts not being able to enter protected sites (Tests 2, 4, 6 and 8). Therefore, if the stainless steel prosthesis presents some of the mentioned defects, there is a potential risk that a pathogen finds a place not reached by the antibiotic or antifungal and the patient's infection cannot be eradicated.

Table 1. Conduction tests with yeast

<i>Test</i>	<i>Number of Samples</i>	<i>Samples with growth</i>	<i>Time (days)</i>
<i>1</i>	6	1	10
<i>2</i>	7	0	-
<i>3</i>	9	1	5
<i>4</i>	9	0	-
<i>5</i>	9	3	5
<i>6</i>	7	0	-
<i>7</i>	7	1	8
<i>8</i>	7	0	-
<i>9</i>	7	1	6
<i>10</i>	7	1	5



Figure 2. Striped test piece and inoculated with yeast. Notice that the thicker stripes permit yeast housing.



Figure 3. Sample with metallurgical defect possibly induced during surgery. Yeasts survived disinfection and developed into a new culture medium.

3.1.2 Tests with *Staphylococcus aureus*

Table 2 shows the results of tests with *S. aureus*. In 100% of the cases, bacterial growth was observed in the samples with defects. These results suggest that defects protected the bacteria from the action of the ATB. Note that in colonized perfectly polished samples, the antibiotic acted without impediments and removed all bacteria (in 6 out of 7 tests) or significantly delayed its development (see test number 3). Figure 4 shows an illustrative image of one of the tests, where positive (turbid) and negative (crystalline) tubes can be observed.

Table 2. Conduction tests with *Staphylococcus aureus*

<i>Test</i>	<i>Samples with defects</i>				<i>Samples without defects</i>		<i>Positive control</i>
	1	2	3	4	5	6	
<i>I</i>	+	+	+	-	-	-	+
<i>II</i>	+	+	+	+	-	-	+
<i>III</i>	+	+	+	+	+	+	+
<i>IV</i>	+	+	+	+	-	-	+
<i>V</i>	+	+	+	+	-	-	+
<i>VI</i>	+	+	+	+	-	-	+
<i>VII</i>	+	+	+	+	-	-	+

Symbol "+" indicates development and "-" indicates the absence of development.



Figure 4. Conduction tests with *Staphylococcus aureus*. Tubes with development (turbid) contain samples with metalworking defects and tube with no development (crystalline) contains a sample without defects.

3.1.3 External Validation

The results of the validation tests carried out at Universidad Nacional del Litoral are presented in Figure 5. After a 7 hour contact of the samples with a solution of 32 $\mu\text{g/mL}$ of the cefazolin ATB *S. aureus* still persisted in spite of the different washing sessions carried out.

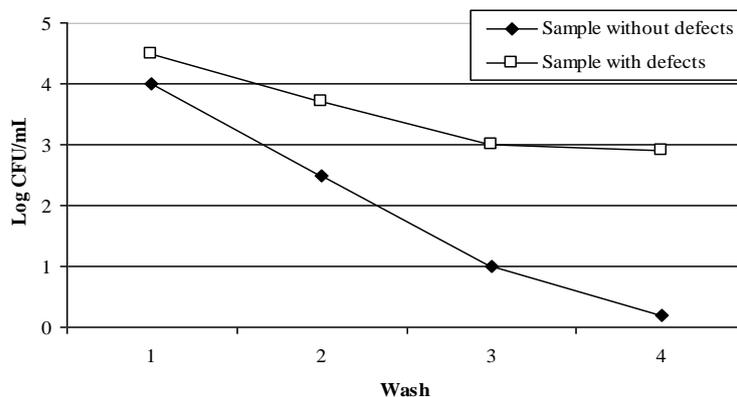


Figure 5. Persistence of *S. aureus* in samples with and without defects

By means of these validation tests previously obtained results were replicated. When a sample with defects was colonized by bacteria the ATB effect was reduced in comparison to a colonized sample without defects. In the case of the sample with defects, the persistence of viable cells of *S. aureus* reached a constant value and it was not possible to complete elimination, which was achieved in the case of the sample without defect after 4 washing sessions.

The hypothesis of this work suggests that the samples or prostheses containing manufacture related or surgery induced defects could provide sites of bacteria protection if there is prosthesis infection. According to results, it can be confirmed that defects in prostheses will protect bacteria and antibiotic treatment will yield poor results [8]. However, if there is an infection in a perfectly polished prosthesis, free of defects, antibiotic treatment will be considerably more effective.

3.2 Second phase: surgical screws

3.2.1 Eye inspection on screws infected by *Rhizopus microsporus* var. *rhizopodiformis*

After visual inspection of the screws at x60 magnification, it was found that the external surfaces of screws showed no deformities. Conversely, metalworking defects were found within the screw head (Allen hexagon, Figure 6). Using a metallographic microscope, the metalworking defects were more evident (Figure 7).

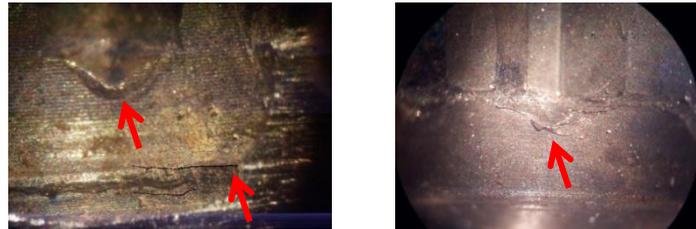


Figure 6. Axial section of the interference screw (left). Axial cut of the hexagon corner (right). Arrows indicate metalworking deformations.

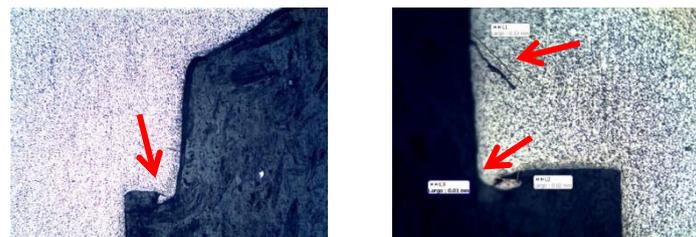


Figure 7. Metallography of a longitudinal cut of the screw heads (left); metalworking defects, bending and cracking (right).

Sections 3.1.1, 3.1.2 and 3.1.3 which report the results in the first phase of this study show that the metalworking defects such as folds, burrs and embossing become areas in which bacteria can develop and survive the effect of antibiotics, even when high concentrations are used. The application of these concepts to the occurrences on the screw heads infected with *Rhizopus microsporus* var. *Rhizopodiformis* leads to a new hypothesis: “if these metalworking defects on prostheses can protect bacteria from the antibiotic effect, they could also harbor machining oil used in the manufacture of screws. The oil could protect fungal spores from the sterilizing effect of the ethylene oxide”.

3.2.2 Eye inspection of cortical screws

If the aforementioned concept of metalworking defects as a bacteria harboring area is extended to the cortical screws, it could be assumed that the defects observed in the latter may also protect bacteria from the ATB effect.

In Figure 8 metalworking defects are observed at the bottom of the screw head, which may perfectly harbor bacteria and protect them from the ATB effect.

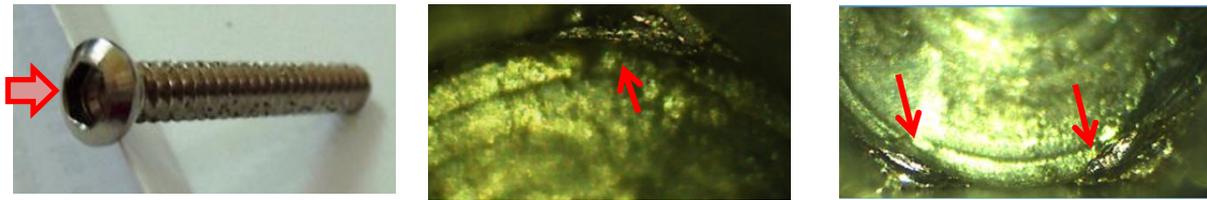


Figure 8. Bends and burrs on the bottom of the hexagon of a cortical screw.

3.2.3 Feasibility of machining oil as the medium for *Rhizopus microsporus* var. *rhizopodiformis* to survive in the screws

When the machining oil was studied as a possible source of screw contamination, it was found that the fungicidal capacity of the oil is low. It can inhibit growth of less than 10^3 spores per mL of oil (Figure 9). These results demonstrate that the metalworking defects could act as a suitable environment for the *R. microsporus* var. *rhizopodiformis* to develop in the machining oil accumulated in the burrs and cracks.

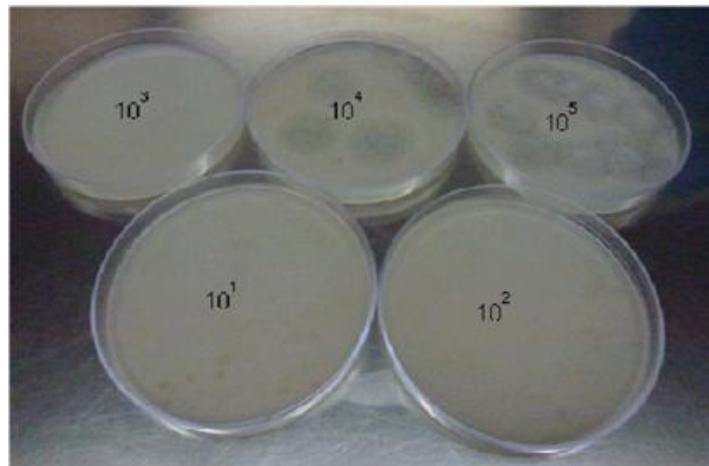


Figure 9: PDA Petri dishes where contaminated machining oil was contaminated with known inocula. Oil can inhibit growth of less than 10^3 spores per mL of oil.

4. Conclusions

This experimental work delved into the influence of surface defects (metalworking) in stainless steel implantable prostheses and their relationship with infections that are generated after surgery and generally lead to their removal.

In the first part of this work, the experiments were carried out according to the protocol here proposed and validated externally (sections 2.1, 2.1.1 and 3.1.3). Since the tests coincided with the hypothesis proposed, especially when it is a random event such as the colonization of bacteria and the place of the test piece which would be inoculated, it can be regarded as a scientific fact that if prostheses with metal mechanical defects become infected for any reason, they will carry bacteria, which places an implanted person's life at risk.

The concepts of these results were applied retrospectively, in a second phase, to knee cruciate ligaments surgery screws, in order to elaborate on the most probable theory that produced serious postsurgical fungal infections. Two types of screws, transverse and interference screws, were analyzed and they were found to contain defects such as metalworking burrs and bending inside the Allen

heads. These defects come as a result of the machining process and are invisible to the naked eye. In this case, metalworking defects would house machining oil that may be contaminated with fungal spores, and the action of sterilizing (ethylene oxide) gas would be affected. As well as this, tests on machining oil properties as a medium capable of allowing the viability of fungi were positive.

Consistent with these results, two practical measures during surgery are necessary in order to reduce the risk of infection:

- 1) Do not allow the use of screws with metalworking defects on the outer surface, analyzed with a stereomicroscope, with the inside part of the Allen screw regarded as part of the exterior surface.
- 2) Use screws that were sterilized by radiation only, as opposed to the gaseous sterilization by ethylene oxide.

Upon the application of these preventive measures in nearly 200 interventions, the percentage of infections in the context aforementioned has fallen to 0 % to date.

This research project points to the need to improve quality control standards in relation to prostheses, especially as far as screws are concerned, requiring that surface control is increased up to 80 and that the inside part of the screw head is considered as an outer surface.

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