Research Article

The Efficacy of The Titanium Oxide-Coated Screws in The Prevention of Implant-Related Infections; an Experimental Animal Study

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Summary

Objective: The treatment of the implant related spinal infection requires long-term antibiotic use and causes prolonged hospital stay; even then in some cases the treatment goals cannot be achieved. This study aims to investigate the efficiency of TiO2-coated screws for the treatment of the implant-associated infections in rat spines.

Methods: 32 female white Sprague Dawley rats were randomized into 4 groups, each consisting of 8 animals. Under anesthesia, a 3-mm titanium micro screw was implanted in the thoracic spine. All 4 groups were inoculated with the same concentration of Staphylococcus aureus (10^6 CFU/10μl). Group 1 and 2 were assigned as titanium screw implanted groups and group 3 and 4 were assigned as TiO2-coated screw implanted groups.

Results: All animals were sacrificed after 21 days. The results were analyzed using “Mann Whitney-U” test. When the groups were compared, no statistically significant microbiological difference was found between the TiO2-coated screw groups and the titanium screw groups. However, the pathologic evaluation indicated that the inflammatory signs were milder in the animals implanted with TiO2-coated screws.

Conclusions: This study has shown that coating the titanium screws with TiO2 has not resulted in a significant difference in the prevention of implant-related infections.

Key words: Implant related infection, rat spine, titanium oxide, TiO2-coated screw

Özet

Amaç: İmplantla ilişkili spinal enfeksiyonlar, uzun süre antibiyotik kullanım gerekiyor ve hastanede yatış süresinin uzamasına neden olur; buna karşın bazı vakalarda tedavi amacına ulaşamayabilir. Bu çalışmada, sıçan omurgasında implantla ilişkili enfeksiyonların tedavisinde TiO2 kaplı vidaların etkinliği araştırılmaktadır.

Yöntem ve Gereçler: 32 dişi beyaz Sprague Dawley sıçan, her biri 8 hayvandan oluşan 4 gruba randomize edildi. Anestezi altında, torasik omurga 3 mm.lik bir titanyum mikrovida yerleştirildi. 4 grubun tümüne, aynı yoğunlukta (106 CFU/10μl) Staphylococcus aureus ile inokülasyon yapıldı. Titanyum vida yerleştirilen gruptlar grup 1 ve grup 2, TiO2 kaplı vida yerleştirilen gruplar grup 3 ve grup 4 olarak olarak belirlendi.

Sonuç: Bu çalışma, titanyum vidaların TiO2 ile kaplanmasının implant bağlı enfeksiyonlarının engellenmesinde anlamlı bir farka neden olmadığını göstermiştir.

Anahtar Kelimeler: İplant ilişkili enfeksiyon, sıcak omurgası, titanyum oksid, TiO2 kaplı vida

INTRODUCTION

The advances in the spine surgery that were obtained especially in the recent years have provided new opportunities in the treatment of a number of spine diseases such as trauma, tumor, infection and degenerative diseases(17). In addition to the advances in the surgical technique, important improvements were also achieved in the biomaterial technology and these advances resulted in a rapid increase in the number of biomaterials that are being used(12). Besides with all these improvements in the surgical technique, the increased use of implants inevitably resulted in an increase in the implant-associated complications. One of the most frequently observed complications in the spinal surgery is the implant-related infections, which can cause serious morbidities and even mortality(9,10). In the literature, this ratio ranges from 1% to 5% in the studies with broad series of patient(20). The treatment of implant-associated infections requires intense antibiotherapy and the removal of implant which necessitates additional surgical interventions, prolonged hospital stay and immobilization leading to impairment in patients' quality of life, severe distress, increased financial cost and mortality(6). In the fight against such infections, high doses of systemic antibiotics administered during the treatment provoke a severe risk of toxicity and the local application involves technical difficulties, therefore the use of prophylactic systemic antibiotics is generally not sufficient(9,10,19).

The development of the implant-related infections, involve a different mechanism originating from biomaterials. Implants not only disturb the host immune response but also provide an interface where bacteria can elude the host defenses and hold onto(6). The most important step in the implant-associated infections is the bacterial adhesion(9). The implant surface stimulates and catalyzes bacterial adhesion, but reduce the oxidative combustion of macrophages and the local inflammatory response(2,11). Furthermore, the fact that the metal implant surface is a physiochemical active surface, plays an important part in the regulation of molecular events(8). The active role of the implants on these molecular events include cellular adhesion, integration, proliferation, bacterial extra capsular exopolysaccharide biofilm layer formation, forms the basis for the events affecting the formation of the inflammation and the immunologic response(4,5). The biofilm layer formed by microorganisms on this surface provides a convenient medium for the survival of the bacteria as well as forming an obstacle for the host defense and the bacteria(9). Based on the available findings, it was thought that implants made of biomaterials, which interfere with bacterial attachment, would decrease the implant-associated infection(11). In this regard, photocatalytic titanium-dioxide (TiO2) material with anatase structure may have its benefits for antimicrobial purposes(7).

In this study, we compared the affectivity of the regular titanium alloy screws coated with TiO2 during the rutile phase on the bacteria by establishing an experimental model on the rat spine.

MATERIAL AND METHODS

All experiments were approved by the Ethics Committee of Bakırköy Research and Training Hospital for Neurology, Neurosurgery and Psychiatry of Istanbul (No.132/2011). In the study, 32 6-month-old, female, white Sprague Dawley rats of weights from 300 to 350 grams were used. The animals were divided into four groups,
each including eight rats and each group was kept in separate cages.

Group 1: Titanium screw + S. aureus (for microbiological sampling)
Group 2: Titanium screw + S. aureus (for pathological sampling)
Group 3: TiO2-coated screw + S. aureus (for microbiological sampling)
Group 4: TiO2-coated screw + S. aureus (for pathological sampling)

The rats were allowed to spontaneous respiration initially by xylazine, and then ketamine anesthesia was applied. They were placed on the operation table lying facedown. After local cleaning with Povidone iodine, an incision of approximately 2 cm was made in the thoracolumbar region. In Groups 1 and 2, the titanium alloy (Ti6Al4V) screws were implanted and 1 million S. aureus strain ATCC 29213 bacteria per 10 micro liters were inoculated (Figure 1). In Groups 3 and 4, the TiO2-coated screws were implanted and inoculated with the same strain and dose of bacteria. 21 days later, all rats were sacrificed by high-dose anesthesia. The previous operation site was incised under sterile conditions and anatomic layers were passed through.

**Microbiologic evaluation**

From Groups 1 and 3, samples from the screws and the bone tissue around the screw entry hole of the implanted vertebral segment were collected into falcon tubes. The bone samples were weighed on microbalances (Shimadzu, Libror AEG-120, Japan) and the bones were mechanically homogenized in a sterile mortar. Following the homogenization, serial dilutions were carried out and spread onto the tryptic soy agar and after a 24-hour incubation under 37°C, the bacterial count was quantitatively measured (CFU/g). For the evaluation of the bacterial growth on the screws, the removed screws were vortexted, and then inoculated in TSA to determine bacterial growth.

**Histopathological evaluation**

From the animals in Group 2 and 4, the vertebral segment subjected to implantation was totally removed including the adjacent upper and lower vertebrae. After the tissue parts were fixed in 10% buffered neutral formalin, they were taken to the decalcification in Gooding-Steward solution containing 10% formic acid in %5 formalin. When the appropriate softness was reached, the tissues were processed and embedded in paraffin, and sliced transversely (perpendicular to the vertebral column axis) and the parts including the lesion regions were selected. In the control tissues, attention was paid on obtaining sections from the respective level of the study group's lesions. The sections were thoroughly rinsed with running water to remove the acid, and then were processed to obtain paraffin blocks. Sections of 5 microns were obtained by a microtome, deparaffinized and stained with hematoxylin–eosin and Gomori's trichrome. The slides were evaluated under light microscope by a pathologist blind to the groups.

**Statistics**

For all statistical evaluations, the Windows SPSS program (13.0) was used. Data on the bacterial count colonized on the two different screws were assessed in two groups using the “Mann Whitney-U” test. The data were expressed as mean ± SD and P>0.05 (P:0.1) was considered insignificant. The data on the bacterial count colonized on the bone tissues of the animals on which two different screws were used were evaluated in two groups with the “Mann Whitney-U” test. The data were expressed as mean ± SD and P>0.05 (P:0.4) was considered insignificant.
RESULTS

Microbiological findings
Bacterial reproduction was observed in all the samples obtained from all the groups. There were no contaminations on the cultures, and except the S. aureus strains, bacterial reproduction was not observed. The bacteria counts are as presented in Table 1 and the counts obtained from the screws were markedly lower than those of the samples obtained from the bone tissues. However, there was no difference between the titanium and the TiO2 group in terms of the bacterial count (Table 2).

Pathological findings
In all groups, lesion site was identified as an area where the bone and muscle integrity was lost and defect caused by the loss of bone, muscle and connective tissue was filled with young granulation tissue characterized by edema, vascular and fibroblastic proliferation, infiltration of lymphocytes, histiocytes, multinuclear cells and limited amount of collagen deposition (Figure 2a). New bone matrix and cartilage deposition was also noted along the bony surfaces. In addition to findings just mentioned above, foci of polymorphonuclear leucocytes surrounding necrotic bone fragments were also present (Figure 2b). Comparing the inflammation degrees among the groups, markedly less inflammation was observed in the samples in which the TiO2-coated screws were implanted.

There was no statistically significant difference between the bacterial counts obtained from the culture of the screw samples from the Group 1 and 3, subject to the microbiological evaluation. Likewise, no statistically significant difference was found, comparing the bacterial count obtained from the bone tissue samples.
Table 1. Staphylococcus aureus bacteria counts obtained from the animals with titanium screws. (Group 1)

<table>
<thead>
<tr>
<th></th>
<th>Bacterial counts in screws</th>
<th>Bacterial counts in bone samples</th>
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<tbody>
<tr>
<td>I</td>
<td>2100</td>
<td>66877</td>
</tr>
<tr>
<td>II</td>
<td>235</td>
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<tr>
<td>III</td>
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</tr>
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<td>IV</td>
<td>1280</td>
<td>175291</td>
</tr>
<tr>
<td>V</td>
<td>3193</td>
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</tr>
<tr>
<td>VI</td>
<td>3365</td>
<td>171755</td>
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<tr>
<td>VII</td>
<td>5497</td>
<td>287577</td>
</tr>
<tr>
<td>VIII</td>
<td>2480</td>
<td>250555</td>
</tr>
</tbody>
</table>

Figure 2: Panoramic image reconstruction from the experimental groups: a) titanium oxide group and b) titanium control group. Abundant granulation tissue (GR) is seen in the former screw placement areas. New appositional bone deposition (*) is evident in both groups, areas of necrotic bone (**) is also present in b). Hematoxylin & eosin sections.
Table 2. Staphylococcus aureus bacteria counts obtained from the animals with titanium oxide coated screws. (Group 3)

<table>
<thead>
<tr>
<th></th>
<th>Bacterial counts in screws</th>
<th>Bacterial counts in bone samples</th>
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<tbody>
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<td>3800</td>
<td>251312</td>
</tr>
<tr>
<td>II</td>
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<td>VIII</td>
<td>8360</td>
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</tbody>
</table>

**DISCUSSION**

Titanium is a chemical element with the atomic number 22 and symbol Ti. It can form alloys with elements such as iron, aluminum, vanadium, and molybdenum. These alloys are light metals resistant to heat and corrosion\(^6\). Moreover, they do not react in organic liquids and are resistant to corrosion in acidic media accounting for their good biocompatibility. Ti6Al4V is a common alloy used in the spinal surgery instrumentation. The most important titanium minerals are rutile, ilmenite and anatase. The titanium chemical compound at rutile phase is TiO\(_2\)\(^16\). TiO\(_2\) is also biocompatible coating Ti alloy screws with TiO\(_2\) does not increase the cost.

The antimicrobial effects of TiO\(_2\) are activated by its photocatalytic behavior\(^7\). Photocatalytic titanium-dioxide (TiO\(_2\)) material with anatase structure may have its benefits for antimicrobial purposes. This has led to tremendous research on the mechanism and improvement of microstructure and photocatalytic performance\(^13\). The photocatalytic process of TiO\(_2\) involves the generation of electron-hole pairs when exposed to light\(^18\). Aggressive oxygen radicals are generated by the electron attack, and the hole accelerates hydroxyl radical formation. These radicals eventually attack bacteria or viruses in terms of inhibiting DNA clonal processing\(^21\).

TiO\(_2\) coating does not induce corrosion. In the in vitro studies conducted, the antibacterial activity of TiO\(_2\) on different levels was shown\(^1,11,15\). However, there are no in vivo studies in this subject. There are in vitro studies on the viral and antifungal efficacy of TiO\(_2\) nanoparticles\(^5\). Heidenau et. al. have demonstrated that there is a significant difference in terms of toxicity between the TiO\(_2\)-coated titanium alloy and the Ti6Al4V alloy in their study\(^2\). These were considered as non-toxic in the body. Also, infections are thought to increase metallic corrosion\(^3\).

In our study, Ti6Al4V-alloy screws and alloy screws coated with TiO\(_2\) during the rutile phase were used. As a result of the microbiological and pathological evaluation conducted in this study, no significant difference was found between
the control group and the study group in terms of the number of microbiological agents on the screw and tissue and the osteomyelitis in the bony tissue. Although in the microbiological analysis, the growth of microorganisms on the whole screw and tissue indicates that there is not a full antibacterial activity, there were some statistically insignificant differences in terms of the microorganism counts. The microorganism count was calculated based on the number per gram on the screw and the tissue. The mean microorganism count on the bony tissue and its distribution among the subjects were similar in the control and study groups. However, the distribution of microbe counts on the screw indicated differences between the groups. While there were 4 subjects in the TiO2 group with a bacterial count below 100, there was only 1 subject in the TiO2 group. The total and mean microorganism counts did not result in a statistical difference. Similarly, during the pathological evaluation, in all the subjects osteomyelitis was found, whereas considering the inflammation rates, the TiO2 group was observed to have relatively less inflammation.

In the light of these results, the TiO2-coated screws were found to possess no evident antibacterial activity. It should be kept in mind that in our experimental design, not only the screws, but also the tissue was also purposefully contaminated with bacteria. We have used a bacterial dosage which we have previously optimalized in an rat vertebral osteomyelitis model\(^{14}\). It is plausible that experimental contamination of both the screw and the tissues may obscure the potential bacterial and/or bacteriostatic effects TiO2 coating by providing an exaggerated bacterial contamination, unlikely to happen in modern surgical procedures. Animal studies where only the screws are contaminated may provide better understanding of the potential benefits of TiO2 coating. However, we think that the level of effect with a larger TiO2 surface area will be the focus of further studies.

**CONCLUSION**

In this study, the TiO2-coated screws were shown to have no marked antibacterial activity. The use of the TiO2-coated screws in the screw sizes used spinal surgery and/or with different coating methods may cause different effects on the bacterial colonization. Further studies are required to assess the optimal experimental model and these should include optimization “the screw contamination model” as well as understanding the effects of the sizes and the surgical procedure's details.

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