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Laboratory studies

A comparison of *Staphylococcus aureus* biofilm formation on cobalt-chrome and titanium-alloy spinal implants

Shalin S. Patel [a], Wilson Aruni [b], Serkan Inceoglu [a], Yusuf T. Akpolat [a], Gary D. Botimer [a], Wayne K. Cheng [a], Olumide A. Danisa [a,*]

[a] Department of Orthopedic Surgery, Loma Linda University Medical Center, 11406 Loma Linda Drive, Suite 218, Loma Linda, CA 92354, USA
[b] Division of Microbiology and Molecular Genetics, Loma Linda University School of Medicine, Loma Linda, CA 92354, USA

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ABSTRACT

The use of cobalt chrome (CoCr) implants in spinal surgery has become increasingly popular. However, there have been no studies specifically comparing biofilm formation on CoCr with that of titanium-alloy spinal implants. The objective of this study was to compare the difference in propensity for biofilm formation between these two materials, as it specifically relates to spinal rods. *Staphylococcus aureus subsp. Aureus* (ATCC 6538) were incubated with two different types of spinal rods composed of either CoCr or titanium-alloy. The spinal rods were then subject to a trypsin wash to allow for isolation of the colonized organism and associated biofilms. The associated optical density values (OD) from the bacterial isolates were obtained and the bacterial solutions were plated on brain-heart infusion agar plates and the resultant colony-forming units (CFU) were counted. The OD values for the titanium-alloy rods were 1.105 ± 0.096 nm (mean ± SD) and 1.040 ± 0.026 nm at 48 hours and 96 hours, respectively. In contrast, the OD values for the CoCr rods were 1.332 ± 0.161 nm and 1.115 ± 0.207 nm at 48 and 96 hours, respectively ($p < 0.05$). The CFU values were 1481 ± 417/100 mm^2 and 745 ± 159/100 mm^2 at 48 and 96 hours, respectively for the titanium-alloy group. These values were significantly lower than the CFU values obtained from the CoCr group which were 2721 ± 605/100 mm^2 and 928 ± 88/100 mm^2 ($p < 0.001$) at both 48 and 96 hours respectively. Our findings, evaluating both the OD and CFU values, indicate that implants composed of CoCr had a higher proclivity towards biofilm formation compared to titanium-alloy implants.

1. Introduction

Biofilm formation after implantation of spinal hardware is a serious complication associated with spine surgery. The initiation of biofilm formation begins with bacterial adherence onto the surface of implanted hardware. If favorable environmental conditions exist, bacteria have the ability to bind to implanted hardware and multiply, thus forming a resistant coating [1–3]. Given the resistance of this biofilm layer to host immune defenses, it is often necessary to undertake explantation of hardware to fully eliminate the infectious process [4].

The intrinsic characteristics of implant material are implicated in the formation of biofilms [5]. *Staphylococcus aureus* is one of the most heavily implicated organisms in post-surgical hardware infections [6]. As a relatively new material, cobalt chrome (CoCr) is imaging friendly (MRI), has greater fatigue life [7], and has higher correction forces then titanium-alloys (Ti-alloy) [8], yet literature on biofilm formation on CoCr implants is limited. To our knowledge there are no published studies comparing biofilm formation on CoCr and Ti-alloy spinal implants. We hypothesized that there was no difference in biofilm formation properties between CoCr and Ti-alloy implants. This study involves a direct comparison of the degree of biofilm formation between CoCr and Ti-alloy spinal rods by examining both the difference in optical density (OD) values of bacterial isolates and the resultant number of colony-forming units (CFU); thus enabling us to investigate which material had a higher tendency towards biofilm formation.

2. Materials and methods

2.1. Preparation of bacteria

Lyophilized pellets of *Staphylococcus aureus subsp. Aureus* (ATCC 6538) were obtained and incubated in brain-heart infusion medium for a period of 24 hours to allow for sufficient rehydration.

* Corresponding author. Tel.: +1 909 558 6444; fax: +1 909 558 6118.
E-mail address: Odanisa@yahoo.com (O.A. Danisa).

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Twelve well plates were prepared (Fig. 1) in which 12 Ti-alloy (10 mm × 5.5 mm) (Depuy Synthes, Raynham, MA, USA) and 12 CoCr rods (10 mm × 5.5 mm) (Depuy Synthes) were placed.

2.2. Spinal implant preparation

Each rod was machine cut to allow for uniform diameter and length, and thus allow for identical surface area. With regard to rod surface treatment during the post-production process, each rod underwent identical treatment first involving glass bead blasting to allow for a matte finish. This was performed to remove any tool marks that were incurred during the production process. Furthermore, during the post-production process, both rods underwent an identical passivation process allowing for removal of any free ions and residual oil. Both rods were autoclaved at 121°C (249°F). Each rod was then immersed in 5 ml of brain-heart infusion medium (Fig. 1). To each of these well plates, 1 ml of overnight culture of *Staphylococcus aureus subsp. Aureus* (ATCC 6538) was added. The implants were then divided into two groups and incubated at 37°C for a period of either 48 (n = 4) or 96 hours (n = 8).

2.3. Isolation of biofilm from spinal implant & evaluation of OD

After the respectively designated incubation periods had elapsed the implants were washed with a 0.25% trypsin solution to extract the biofilms from the rods. This bacterial solution was then collected and the overall turbidity of the solution was evaluated by determining the OD measurement via a spectrophotometer.

2.4. Evaluation of CFU

After the OD values were obtained the bacterial solution was centrifuged and the residual trypsin solution was decanted off and a bacterial pellet was isolated. This pellet was then dissolved in 200 ug of phosphate buffer and plated on brain-heart infusion agar plates and incubated for 24 hours (Fig. 2). The resulting number of CFU for each plate was evaluated, using a CFU counter.

3. Results

Two-way ANOVA analysis showed both OD and CFU were significantly influenced by duration of incubation and implant material. The CoCr rods yielded both a higher OD and CFU values compared to Ti-alloy ($p < 0.05$). Higher OD and CFU values were encountered at both 4-day incubation and 2-day incubation ($p < 0.05$).

The mean (± SD) OD values for the bacterial solution obtained from the Ti-alloy implants were 1.105 ± 0.096 nm and 1.040 ± 0.026 nm at 48 hours and 96 hours, respectively. In contrast, the OD values for the bacterial solution obtained from the CoCr rods were 1.332 ± 0.161 nm and 1.115 ± 0.207 nm at 48 and 96 hours, respectively, which were significantly higher than the Ti-alloy implants ($p = 0.025$) (Fig. 3).

Similarly, the CFU values were 1481 ± 417/100 mm^2 and 745 ± 159/100 mm^2 at 48 and 96 hours, respectively for the Ti-alloy group. These values were significantly lower than the CFU values obtained from the CoCr group which were 2721 ± 605/100 mm^2 and 928 ± 88/100 mm^2 ($p < 0.001$) at both 48 and 96 hours respectively (Fig. 4).

4. Discussion

Implant associated infections are a major source of morbidity and mortality after spine surgery. The characteristic features of different metal implants are a contributory factor in the development of biofilm on spinal implants. The formation of biofilms makes implant associated infections difficult to treat and often requires

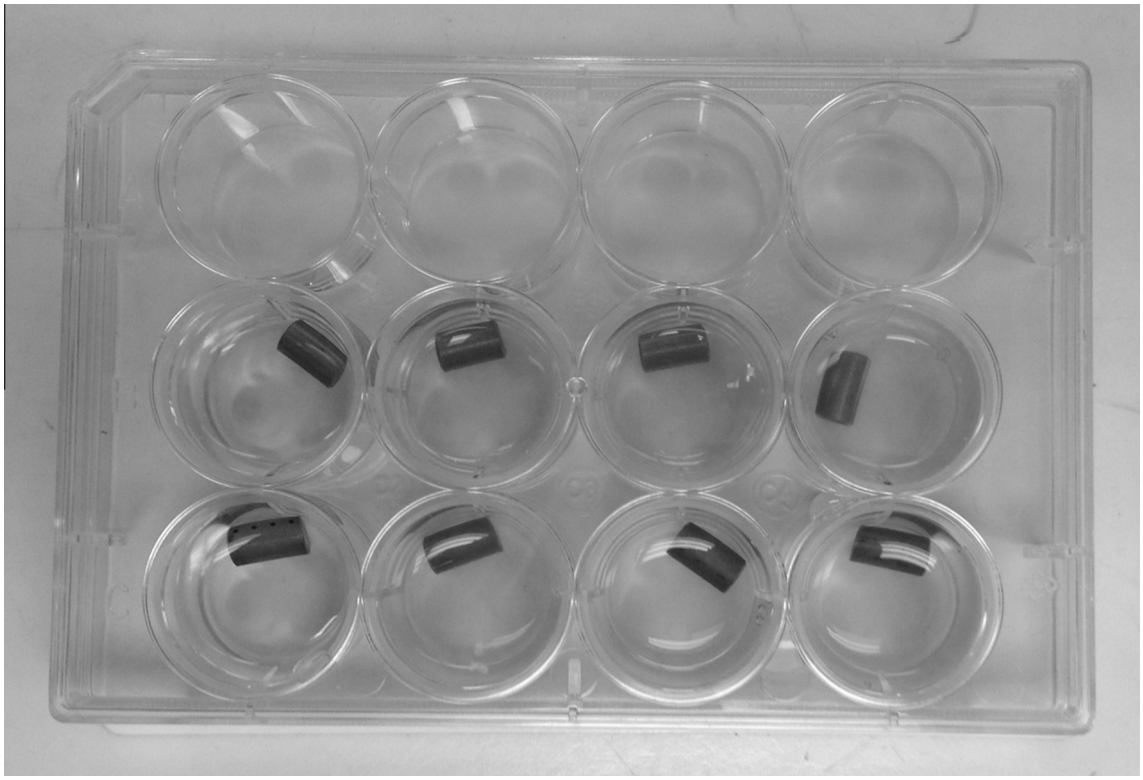

Fig. 1. Autoclaved titanium-alloy and cobalt chrome rods in 5 cc brain-heart infusion medium prior to incubation with *Staphylococcus aureus subsp. Aureus* (ATCC 6538).