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"In vitro" evaluation of bacterial biofilm formation on different cerclage systems

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Abstract

Cerclage wiring may be used for fracture fixation or osteotomy stabilization in revision arthroplasty. There is a lack of evidence regarding the potential risk of bacterial colonization for the different types of cerclages. The objective of our research is to study the adhesion and biofilm formation of *S. epidermidis*, *S. aureus*, and *P. aeruginosa* on two different cerclage cable models, comparing a polymer cable and a stainless steel metal cable. A two-cm cerclage piece of each material was submerged in 2 mL of tryptic soy broth (TSB) inoculated with 10 μL of a 0.5 McFarland bacterial culture, and incubated at 37°C during 2 h for adhesion and 48 h for biofilm formation. The cerclages were washed with 1xPBS and sonicated in a new culture medium. Aliquots of several dilutions of each sonicated culture were spread in TSB agar and incubated at 37°C for 24 h. The number of colonies was counted. The colony-forming units per ml (CFU/mL) and the percentage of reduction were calculated. Experiments were triplicated. For *P. aeruginosa*, a statistically significant reduction in biofilm formation was found on the polymer cerclage cable, compared to the metal cerclage cable. Reductions of 59% and 88%, after 2 h and 48 h, respectively, were observed. For S. epidermis and *S. aureus*, there was a trend towards lower bacterial adhesion and biofilm formation for the polymer cerclage cable. In summary, these results demonstrate that the braided polymer cerclage cable may be less prone to bacterial adherence and biofilm formation compared to the braided metal cerclage cable.

Keywords

Bacteria, biofilm, adherence, surface character, infection, implant

Introduction

Prosthetic joint infection (PJI) is reported in 0.5-2.2% of cases after primary arthroplasty, with a higher incidence after revision surgery where it has been reported to contribute to up to 30% of failures.[1,2]

The most common causative microorganisms for PJI are Gram-positive staphylococcal species (*Staphylococcus aureus*) and coagulase-negative staphylococci (*S. epidermidis*). Nonetheless, other microorganisms have also been isolated in cases of PJI, such as gram-negative bacilli or polymicrobial cases in which more than one microorganism is isolated.[3]

A biofilm is an organized aggregate of microorganisms living within a self-produced matrix of extracellular polymeric substances that is attached to a biotic or abiotic surface. Bacterial adhesion to surfaces is controlled by physicochemical factors, such as surface chemistry, composition, topography and roughness, bacterial properties, such as bacterial hydrophobicity, surface charge, and cell size and also by environmental parameters.[4] Bacterial adhesion to a material surface is defined as a two-phase process. The initial physicalphase is instantaneous and reversible, and then comes an irreversible molecular and cellular phase.

Surface roughness plays an essential role in the adhesion of bacteria to material surfaces. The impact of surface roughness has been studied on different material surfaces. Irregularities, such as grooves, gaps, and cracks present a favorable environment for bacteria because it protects them from external forces.[5]

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Treatment for PJI requires a combination of antibiotics and surgery. Surgical procedures in chronic PJI generally require prosthesis resection. This treatment option is performed under one stage or two-stage procedure. Two-stage revision is currently considered the gold standard for the treatment of chronic PJI. The first stage involves radical debridement and prosthesis resection. The goal of this debridement is to eradicate all sources of bacteria and biofilm in the joint cavity. Following the first stage, in which a provisional antibiotic-loaded cement spacer is implanted, and after adequate systemic antibiotic treatment is completed, a second stage surgery for the definitive prosthesis is performed.

In some cases, an extended femoral osteotomy may be required during femoral stem extraction, followed by an osteotomy stabilization employing cerclage wire or cables (Figure 1). In turn, this results in the dilemma of introducing new hardware into a septic situs.[6] These cerclages, as all commonly used orthopaedic materials, may be potentially colonized by biofilm-forming bacteria. For this reason, most of the current research on biomaterials is focused on finding new alternatives to modify the implant surface to reduce bacterial adhesion, impede biofilm formation and provide effective bactericidal action.[7,8] Our study aimed to evaluate the adhesion and biofilm formation of *S. epidermidis*, *S. aureus*, and *P. aeruginosa* on two different cerclage cable models, comparing an polymer cable and a stainless-steel metal cable.

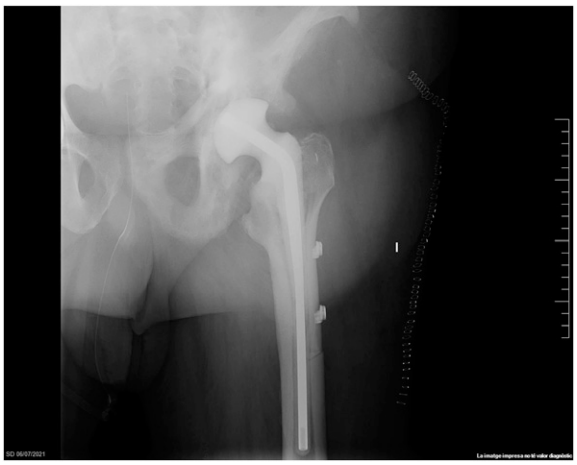

Figure 1. osteotomy stabilization employing cerclage cables.

Material and methods

Cerclage samples

Samples of 2 cm in length of each type of cable, a metal cerclage Cable-Ready 1.8 mm Stainless Steel Bone Plate Cable, Zimmer-Biomet (REF 00-2232-003-18, 1.8 mm Stainless Steel Bone Plate Cable, 610 mm LOT 63384029) and the SuperCable® Iso-Elastic™ Cerclage System (KINAMED, ref. 35-100-1010), were used in the experiments. The different experiments were carried out by triplicate for each strain. They were cut under sterile conditions.

Bacteria strains

Three different strains of *Staphylococcus aureus* (SH100, BH100, SA908), *S. epidermidis* (RPG2A, FG011, FG012), and *Pseudomonas aeruginosa* (PAO1, AG, AS) with high capacity to form biofilm were used in the study.

Adherence studies

The cerclage samples were embedded in Tryptic Soy Broth (TSB) or Mueller Hinton (MH) liquid medium for Gram-positive bacteria and Gram-negative bacteria, respectively, with a bacterial concentration of 0.5 McFarland corresponding to 1.5 × 108 cfu/mL and incubated at 37°C for 2 h. After 2 h, they were washed with 1 × phosphate-buffered saline (PBS) and introduced into a new tube with a fresh culture medium for being submitted to sonication using a needle sonicator and vortex. Serial dilutions of the medium were spread onto LB (Condalab, Spain) or blood agar plates (Oxoid, Spain) and incubated for 24 h at 37°C. After this time, colony counting will be carried out.

Counts of microorganisms were converted to CFU/mL by the following formula

CFU/mL= (No. of colonies x dilution factor)/Volume of culture plate*

* The volume of the culture plate was 0.01 mL (except from P. Aeruginosa at 2 h in which the volume was 0.1 mL, due to the type of growth observed in these strains).

Biofilm formation

To determine biofilm formation, the cerclage samples were embedded in TSB or MH liquid medium for Gram-positive bacteria and Gram-negative bacteria, respectively, containing a bacterial concentration of 0.5 McFarland corresponding to 1.5 × 108 cfu/mL, and incubated at 37°C for 48 h. After this time, they will be washed with 1x PBS and introduced into a new tube with a fresh culture medium for being submitted to sonications in a needle sonicator and vortex. Different dilutions of the medium were spread onto LB (Condalab, Spain) or blood agar plates (Oxoid, Spain) and incubated for 24 h at 37°C. After 24 h, the colony count was performed.

Counts of microorganisms were converted to CFU/mL by the same formula as section 2.3.

Scanning electron microscopy

The cerclage samples with or without bacteria were fixed with 2.5% glutaraldehyde in PBS (0.2 M; pH 7.4) and visualized by SEM microscopy at the Scientific Technological Service of the University of Barcelona (Barcelona, Spain).

Statistical analysis

Data obtained on colony counting were analyzed using ordinary one-way ANOVA and Tukey's multiple comparisons tests. A $p < 0.05$ was considered statistically significant.

For log10 comparison, the relationship between log reduction and percent reduction of *1 log reduction = 90% reduction and ** 2 log reduction = 99% reduction (https://microchemlab.com/information/log-and-percent-reductions-microbiology-and-antimicrobial-testing).

Results

Bacterial adherence

The cerclage samples were immersed in a bacterial culture solution for 2 h. Once the samples were washed and sonicated in a new culture media, aliquots were spread on agar plates for colony growth. The resulting colonies were counted and results interpreted considering the culture dilution. Log10 of each result was calculated (Table 1, Figure 2). Small differences in colony number have been found among differences of the same species. However, *P. aeruginosa* has a higher adherence capacity in comparison with the Gram-positive bacteria studied. No significant differences in bacterial adhesion among the strains were observed between the two types of cerclages.

Table 1. Adherence results from the two types of cerclage system under study.

	Metal cerclage		Polymer cerclage	
Microorganism/strain	CFU/mL	Log10	CFU/mL	Log10
S. epidermidis RPG2A	2.41E + 05	5.38	1.79E + 05	5.25
S. epidermidis FG011	6.12E + 04	4.79	4.50E + 04	4.65
S. epidermidis FG012	5.82E + 04	4.77	7.00E + 03	3.85
S. aureus SH1000	4.94E + 05	5.69	2.14E + 05	5.33
S. aureus BH100	1.39E + 05	5.14	2.60E + 04	4.41
S. aureus SA908	2.51E + 05	5.40	9.97E + 04	5.00
P. aeruginosa PAO1	6.93E + 06	6.84	2.64E + 06	6.42
P. aeruginosa AG	1.75E + 06	6.24	1.30E + 06	6.11
P. aeruginosa AS	2.99E + 06	6.47	8.69E + 05	5.94

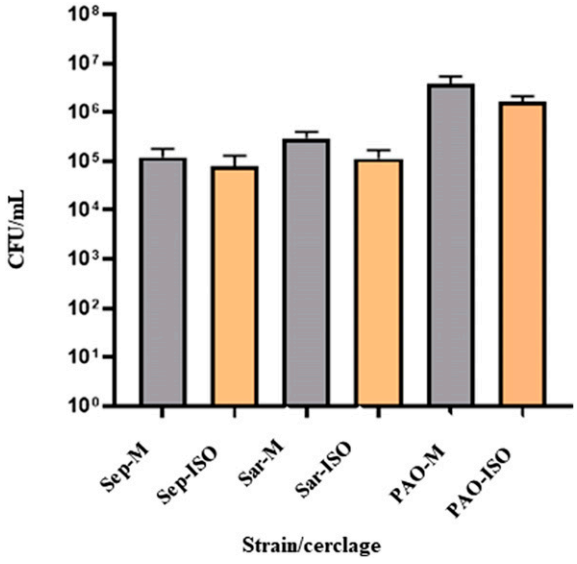

Figure 2. Sep: S. epidermidis; Sar: S. aureus; PAO: P. Aeruginosa; M: metal cerclage; ISO: Polymer cerclage.

Bacterial biofilm

After 48 h of incubation in a bacterial solution, cerclage samples were washed and sonicated in a new culture media. Aliquots were spread on agar plates for colony growth. The resulting colonies were counted and results interpreted considering the culture dilution. Log10 of each result was calculated (Table 2, Figure 3). Small differences in colony number have been found among differences of the same species. However, *P. aeruginosa* has a higher adherence capacity in comparison with the Gram-positive bacteria studied. For *P. aeruginosa*, a statistically significant reduction ($p = 0.024$) in biofilm formation at 48 h was found on the braided polymer cerclage cable as compared to the metal cerclage cable. In the other cases, the reduction was lower than 25%.

SEM images showed that in the case of metal cerclage, biofilms were also found in the places between each cable that form the cerclage (Figure 4).

When the percentage of biofilm reduction of braided polymer cerclage compared to metal cerclage was carried out taking into account the guidelines of Log and Percent Reductions in Microbiology and Antimicrobial Testing | Microchem Laboratory, in which the relationship between log reduction and percent reduction of *1 log reduction = 90% reduction and ** 2 log reduction = 99% reduction was used (Table 3). It was observed a reduction in adherence and biofilm formation in the case of polymer cerclage cable in all the studied bacterial species, being this reduction of 45% and 75% on biofilm formation in the case of *S. epidermidis* and *P. aeruginosa*, respectively.

Table 2. Biofilm results from the two types of cerclage system under study after 48 h.

	Metal cerclage		Polymer cerclage	
Microorganism/strain	CFU/mL	Log10	CFU/mL	Log10
S. epidermidis RPG21	7.17E + 06	6.86	1.73E + 05	5.2
S. epidermidis FG011	1.28E + 07	7.11	2.20E + 06	6.3
S. epidermidis FG012	3.43E + 07	7.54	1.57E + 07	7.2
S. aureus SH1000	2.40E + 07	7.38	1.10E + 07	7.0
S. aureus BH100	4.04E + 05	5.61	4.30E + 04	4.6
S. aureus SA908	1.82E + 05	5.26	1.74E + 05	5.2
P. aeruginosa PAO1	6.30E + 10	10.80	9.96E + 09	10.0
P. aeruginosa AG	1.33E + 10	10.12	5.63E + 07	7.8*
P. aeruginosa AS	2.10E + 10	10.32	8.53E + 08	8.9*

*Statistically significance ($p < 0.05$).

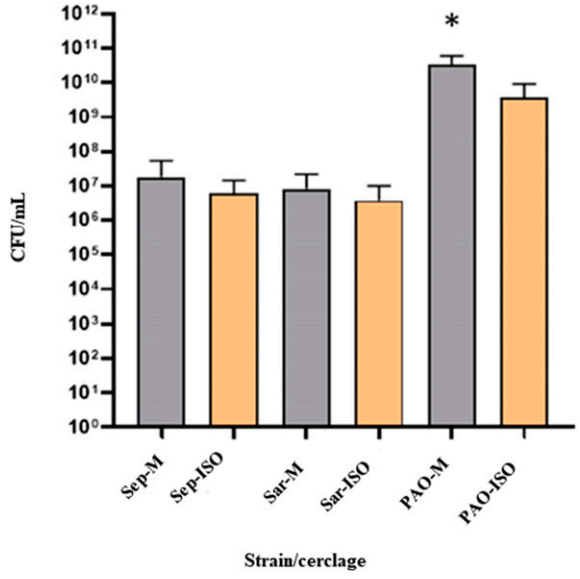

Figure 3. Sep: S. epidermidis; Sar: S. aureus; PAO: P. Aeruginosa; M: metal cerclage; ISO: Polymer cerclage; * For P. aeruginosa, a statistically significant reduction ($p = 0.024$).

Discussion

Bacterial biofilms are present in nature and are recognized to form rapidly on the surfaces of medical devices.[9] Although the exact physiopathology of medical device infections remains unclear, it is well known that infection is preceded by (1) contamination of the surgical bed, (2) adhesion of bacteria to the implant surface and (3) the formation of a biofilm. Based on a series of factors including bacterial load, the immunological state of the host, and the capacity of bacteria to adhere to the implant's surface, an infection may develop. Our study aimed to determine if there were *in vitro* differences between

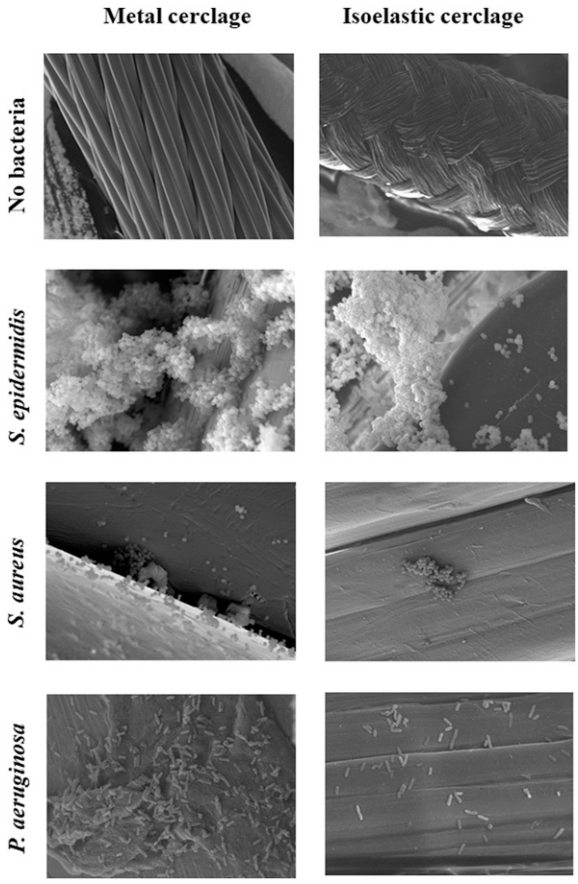

Figure 4. SEM images.

bacterial adhesion and biofilm formation of *S. epidermidis*, *S. aureus*, and *P. aeruginosa* on two different cerclage cable materials, which are commonly used in orthopedic surgery. Despite the macroscopic different structure between both models (Figure 5), a key difference was the material of each one: metallic versus polymer. Although there are several *in vitro* studies that evaluate *in vitro* bacterial adherence to different metals [10] as far as we can tell, there are no previous studies that compare these two materials. Malhotra et al.[11]compared bacterial adhesion and biofilm formation on five types of biomaterials (including highly cross-linked polyethylene). They tested four different microorganisms and concluded that Cobalt-chromium was detected to have the lowest tendency towards bacterial adherence, while highly cross-linked polyethylene showed the highest level of adherence. The combination of bacterial species could also play an antagonist or agonist effect. Slullitel et al. describe an antagonist effect between *E. coli* and *S. epidermidis* while the combination of *P. aeruginosa* and *S. aureus* presented a trend to increased adherence of *P. aeruginosa* independently.[12]

Table 3. Comparison between polymer cerclage and metal cerclage.

	Metal cerclage		Polymer cerclage			Percentage of biofilm reduction of polymer cerclage compared to metal cerclage	
	Average CFU/mL	Average Log10 CFU/mL	Average CFU/mL	Average Log10 CFU/mL	Log10 reduction of CFU/mL	*1 log Reduction %	**2 log Reduction %
Results 2h							
S. epidermidis	1.20E + 05	4.98	7.71E + 04	4.58	−0.39	35.5	19.5
S. aureus	2.94E + 05	5.41	1.13E + 05	4.91	−0.50	44.7	24.6
P. aeruginosa	3.89E + 06	6.52	1.60E + 06	6.16	−0.36	33.7	16.8*
Results 48h							
S. epidermidis	1.81E + 07	7.17	6.01E + 06	6	−0.91	81.8	45.0
S. aureus	8.18E + 06	6.08	3.75E + 06	5.64	−0.44	39.8	21.9
P. aeruginosa	3.24E + 10	10.42	3.62E + 09	8.89	−1.52	137.0	75.3*

*Statistically significance ($p < 0.05$).

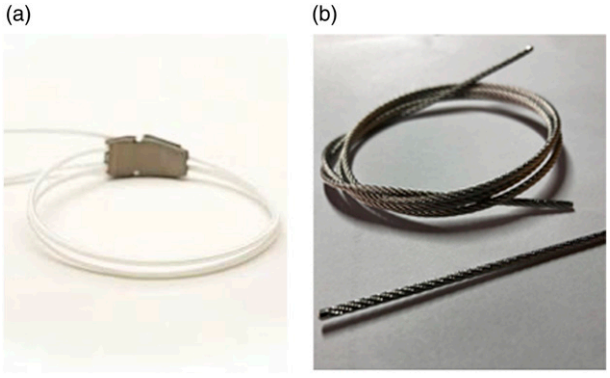

Figure 5. (a) Polymer cerclage, (b) Metal cerclage.

In our study, we found a reduction in biofilm formation of *P. aeruginosa* at 48 h in polymer cerclage ($p < 0.05$), when analyzed under an Electron Microscope, we observed that biofilms were especially prone to adhere and form in between the metallic filament intersections of the metallic cerclage cable. This finding suggests that a smoother surface, such as the polymer one, would prevent bacteria from adhering and reduce biofilm formation. On the other hand, the rougher surface on the metallic cable filaments seemed to favor adherence and biofilm formation. The variation in microscopic surface structure could explain the differences between adhesion and biofilm formation found in our results. Taylor et al.[13]reported that a tiny increase in surface roughness resulted in a pronounced increase in bacterial adhesion and the surface configuration also impacts bacterial adhesion. Bacterial cells preferentially adhere to surface irregularities that maximize the bacteria-surface area in contact.[14] However, we are wary of these results because many other factors are key for adhesion and biofilm formation in an *in vivo* scenario.

The main limitation to our study is that it is an *in vitro* study and the results of *in vitro* biofilm studies have not been reproduced in vivo. In vivo adherence to inorganic surfaces is also influenced by the microenvironment, for example, plasma proteins promoting biofilm formation.[15]

Conclusion

In conclusion, adhesion and biofilm formation studies for different strains of *P. Aeruginosa*, *S. aureus*, *S. epidermidis*, and were performed on two different cerclage cable products, including a polymer cable and a stainless-steel metal cable. For *P. aeruginosa*, a statistically significant reduction in biofilm formation at 48 h was observed on polymer cerclage cable in comparison to metallic cerclage cable. For *S. epidermidis* and *S. aureus*, there was a trend towards less bacterial adhesion and biofilm formation with the polymer cerclage cable although differences were not statistically significant. Additional studies with larger sample sizes are needed to determine if *S. epidermis* and *S. aureus* biofilm formation is reduced on the polymer cable versus the metal cable to a statistically significant degree.

Our results suggest the braided polymer cerclage cable may be less prone to bacterial adherence and biofilm formation *in vitro* as compared to the braided metal cerclage cable and could be considered for osteotomy stabilization in cases of PJI revision surgery. However, *in vivo* studies are necessary to evaluate the clinical applicability of these in-vitro results.

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Declaration of conflicting interests

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