Evaluation of Biofilm Formation on Spinal Fixation Materials

[1]Vadapalli, S; [2]Agostinho, A; [1]Harper, ML; [2]James, G
[1]Medtronic Spinal and Biologics, Memphis, TN; [2]Montana State University-Bozeman, Bozeman, MT.

Statement of Purpose: The ability of bacteria to form a biofilm is a key event in the pathogenesis of biomaterial-related infection. Surgical site infection is a common adverse event after any spinal procedure, often requiring prolonged hospitalization, revision surgery which can involve removal of instrumentation, and long term antibiotics.[1,2] Bacterial adherence and biofilm formation on the implant surface may vary depending on the species and the number of microorganisms, materials of implants, and physical and electrochemical characteristics of implant surfaces.[3] The objective of this study was to evaluate biofilm formation and attachment of two bacterial species on seven biomaterials commonly used in spinal procedures.

Methods: Seven biomaterials were evaluated along with a control of polycarbonate in a CDC biofilm reactor. The average surface roughness (Ra) measurements of the test coupons (Table 1) were characterized by white light interferometer (NewView 5000™, Zygo, Middlefield, CT). All metallic coupons were steam sterilized and the PEEK coupons were ETO sterilized prior to the start of the experiment.

Table 1: Roughness measurement of the test coupons

Coupon Material	Ra (µm)
	Mean ± St Dev
316L Stainless Steel	2.58 ± 0.26
Commercially pure Titanium (CP Ti)	3.93 ± 0.51
Titanium Alloy (Ti 6Al 4V)	3.10 ± 0.32
Cobalt Chromium (CoCr Warm Worked)	1.07 ± 0.13
Biodur 108 Stainless Steel	0.26 ± 0.08
Cobalt Chromium (CoCr Double Annealed)	2.97 ± 0.39
PEEK	1.01 ± 0.25

The reactor had with a working volume of ~350 ml and was operated with sterile 10% brain heart infusion medium (BHI) supplemented with 0.3% serum. The reactor was filled with medium, sterilized, and inoculated with 1mL of an overnight culture of either Staphylococcus epidermidis ATCC 35984 or a clinical strain of methicillin-resistant Staphylococcus aureus (MRSA strain M1). The reactor was incubated under continuously stirred batch conditions at 37°C. After 24 hours the test coupons were exposed to the media and the medium flow was initiated at 62 ml/hr. Following 2 and 24 hours of exposure, the coupons were removed from the reactor, rinsed in phosphate buffer solution (PBS) and analyzed. Three experiments were conducted for each bacterial species utilizing three coupons in each experiment for each of the biomaterials evaluated. Plate counts were performed on the test coupons by a sequence of vortex, sonicate, and vortex in PBS to produce a bacterial suspension. The bacteria suspensions were serially-diluted with PBS and plated in duplicate on tryptic soy agar (TSA) using the drop plate method. The plates were incubated at 37°C for 24-48 hours and the number of colony forming units (CFU) counted.

Results: Figures 1 and 2 indicate the average CFU for the biofilm forming on the seven biomaterials and control for S. epidermis and MRSA, respectively. The biomaterials had increased bacterial attachment at the 24 hour time point compared to the 2 hour time point. No relevant differences (> 1 log) in bacterial attachment and formation were observed among the different biomaterials at either 2 hour or 24 hours time points.

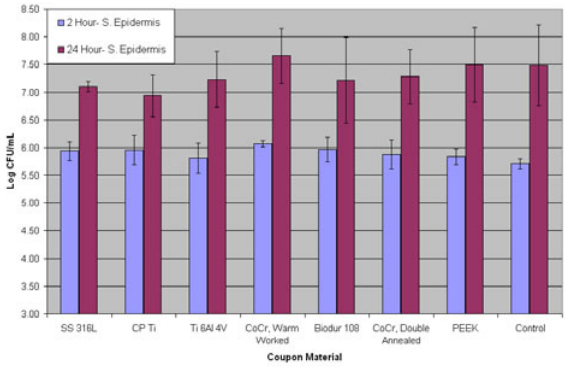

Figure 1: Adherence of S.Epidermis at 2 and 24 hours.

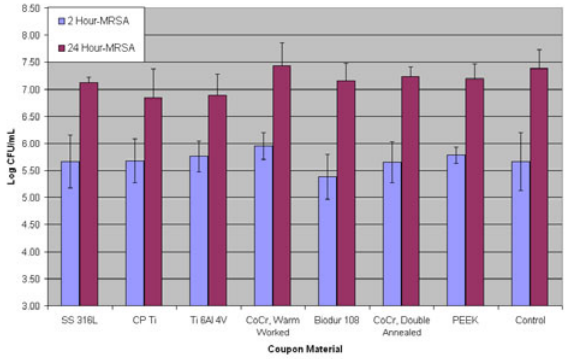

Figure 2: Adherence of MRSA at 2 and 24 hours.

Discussion: In the current study, no difference was present in the bacterial attachment and formation based on biomaterial for either S. epidermis or MRSA. On the contrary, Kee-Yong Ha et al[3] reported that S. epidermis showed significantly more CFU's on titanium than on stainless steel, regardless of the surface texture. Gracia et al[1] reported that no statistical differences were found between adherence of S. aureus strains to titanium and steel alloys. The conflicting results compared to previous studies is partly due to the different techniques and bacterial strains. Caution is needed in relating the in vitro findings to the in vivo situation, especially when considering differences in the inability to fully replicate the pathogenesis of biomaterial-related infection which involves complex interactions between the pathogen, biomaterial and the host defense.

References: 1. Gracia E, et al. *Int Orthop* 21: 46–51, 1997; 2. Sasso RC et al. *J of AAOS* 16: 330-337, 2008; 3. Kee-Yong Ha et al. *Spine* 30(1): 38–43, 2005.