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INFLUENCE OF RETRIEVED HIP- AND KNEE-PROSTHESIS BIOMATERIALS ON MICROBIAL DETECTION BY SONICATION

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Abstract

Microorganisms' ability to adhere and form a biofilm differs among biomaterials; however, clinical data are conflicting. Microbial adherence and biofilm formation on different biomaterials of explanted joint prosthesis components were investigated. Consecutive patients with explanted joint prosthesis were prospectively included. The bacterial load dislodged from retrieved prosthetic components was evaluated qualitatively and quantitatively in sonication-fluid cultures. For comparison between groups, one-way ANOVA and Wilcoxon signed-rank test were used. A total of 112 components originating from 58 knee and 54 hip prostheses were retrieved from 40 patients. Components were made of titanium alloy in 42 cases, cobalt-chromium alloy in 38 and polyethylene in 32. Bacteria in sonication-fluid cultures grew in all polyethylene components (100 %). Larger bacterial counts were found on polyethylene than on titanium ($p < 0.013$) or cobalt-chromium alloy ($p = 0.028$). Coagulase-negative Staphylococci, *Staphylococcus aureus* and *Streptococcus* species were most commonly isolated. In conclusion, polyethylene showed larger biofilm burden than metal alloys, indicating their higher microbial adhesion affinity *in vivo*.
Moreover, bacterial counts were larger after sonication of polyethylene liners than of metal alloys, suggesting intrinsic differences in the ability of microorganisms to form biofilms on various biomaterials. Polyethylene liners allowed the diagnosis of prosthetic joint infections (PJIs) in all investigated cases, suggesting that sonication of polyethylene liners rather than of the complete prosthesis was sufficient for pathogen detection in PJIs.

Keywords: Biofilm, arthroplasty, polyethylene, metal, sonication, biomaterials.

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Introduction

The key phenomenon in the pathogenesis of a prosthetic joint infection (PJI) is the attachment of microorganisms to the implant surface and the subsequent biofilm formation. In this situation, conventional diagnostic methods (such as synovial fluid and periprosthetic tissue culture) have a limited sensitivity, reported at 60-70 % (Evangelopoulos *et al.*, 2013; Trampuz *et al.*, 2007). The culture sensitivity is especially limited in chronic infections, typically associated with low microbial burden, and in patients previously receiving antibiotics (Puig-Verdie *et al.*, 2013).

Modern diagnostic technologies increase the sensitivity of peri-implant tissue and synovial fluid cultures. For example, culturing specimens in blood culture bottles demonstrates superior diagnostic performance as compared to conventional agar plate cultures (Font-Vizcarra *et al.*, 2012; Peel *et al.*, 2016). However, most microbiology laboratories use the agar plate method, with limited sensitivity ranging around 60-82 % (Evangelopoulos *et al.*, 2013; Larsen *et al.*, 2012; Trampuz *et al.*, 2007). As the

key phenomenon in the pathogenesis of implant-associated infection is the biofilm formation on the implant surface, sonication of explanted prostheses is superior when compared to periprosthetic tissue culture (Larsen *et al.*, 2012; Trampuz *et al.*, 2007).

The influence of the biomaterial type on the biofilm formation is controversial. Experimental animal studies suggest that microbial adherence and biofilm formation depend on the type of material (Cordero *et al.*, 1994; Cordero *et al.*, 1996; Petty *et al.*, 1985). However, clinical studies show conflicting data regarding microbial adherence to materials. Lass *et al.* (2014), in a study including hip prosthesis components, show that polyethylene liners and prosthesis heads have larger bacterial loads than other components. Gomez-Barrena *et al.* (2012), in a study investigating retrieved hip and knee components, observe no differences in bacterial adherence to individual biomaterials. Bacterial adherence depends on the type of microorganism rather than the type of biomaterial.

To provide insights into the role of biomaterials, such as polyethylene, titanium and cobalt-chromium alloy, the influence of the material type on the biofilm formation was investigated. For this purpose, the biofilm was investigated by sonication-fluid culture of retrieved hip and knee prostheses. Such findings might help further optimising microbiological diagnosis from retrieved implants using the sonication procedure.

Materials and Methods

Study design

The study was conducted at the Federal Centre of Traumatology, Orthopaedics and Arthroplasty in Barnaul (Russia), providing advanced specialty care to a population of 7.3 million inhabitants. Data were extracted from electronic medical charts. The study protocol was submitted to the local Ethics Committee, which considered the microbial investigation of retrieved prosthetic components as a part of a routine diagnostic procedure and waived the need for informed consent.

Study population

Consecutive patients with complete or partial prosthesis explantation due to chronic PJI (see definition below), in which at least one component of the prosthesis showed bacterial growth, were included in the present prospective cohort study, performed from January 2015 until December 2016. Patients with acute PJI – *i.e.* symptom duration less than 4 weeks or infection manifesting within 1 month after implantation, receiving antibiotics prior to surgery, in whom sonication of the retrieved implant was not performed or obvious contamination of the explanted material occurred during surgery, transport to or processing in the microbiology laboratory – were excluded.

Definitions

PJI was diagnosed according to the working criteria of the European Bone and Joint Infection Society (EBJIS), as done in several studies (Akgun *et al.*, 2018; Akgun *et al.*, 2017; Morgenstern *et al.*, 2018; Renz *et al.*, 2017; Renz *et al.*, 2018a; Renz *et al.*, 2018b; Sigmund *et al.*, 2018). Accordingly, PJI is diagnosed when one or more of the following criteria are met: (i) presence of sinus tract or macroscopic purulence; (ii) positive-inflammation histopathology of periprosthetic tissue, defined as ≥ 23 granulocytes per 10 high-power fields (*i.e.* type II or III according to Krenn *et al.*, 2014); (iii) increased synovial fluid leukocyte count, defined as > 2000 leukocytes/μL or > 70 % granulocytes; (iv) positive synovial fluid, periprosthetic tissue or sonication-fluid culture. Sonication culture was considered positive if ≥ 50 colony-forming units (CFU)/mL were detected, except for *Staphylococcus aureus* (*S. aureus*), streptococci and gram-negative rods, for which any growth (*i.e.* ≥ 1 CFU/mL) was considered positive (Trampuz *et al.*, 2007). Of note, synovial fluid leukocyte count was not considered to be a diagnostic criterion within the first 6 weeks after surgery in inflammatory joint disease and in case of periprosthetic fracture or luxation. In these situations, the leukocyte count can be increased also in the absence of an infection (Renz *et al.*, 2018b).

Prosthesis retrieval

Prosthetic components retrieved at revision surgery were separately placed into sterile containers. Then, the explanted material was transported within 4 h to the microbiology laboratory for sonication.

Sonication of prosthesis components

100 mL sterile normal saline solution was added to each container for sonication. The implants were totally submerged in the fluid. Then, the samples were vortexed and sonicated according to a previously described protocol, using 40 kHz and 0.2 W/cm^2 (Trampuz *et al.*, 2007). 0.5 mL aliquots were plated on to tryptic soy agar with 5% sheep blood, chocolate agar and Schaedler 5% sheep blood agar, respectively; 3 mL aliquots were inoculated into thioglycolate broth (all from BioMedia, Saint Petersburg, Russia). Inoculations were done before sonication (*i.e.* vortexing culture) and after sonication (*i.e.* sonication culture). Aerobic and anaerobic sheep-blood agar plates (BioMedia) were incubated for 14 d at 35 °C in 5 % CO_2 aerobically and anaerobically, respectively. Cultures were quantified by counting the number of colonies that grew on the plate and adjusting to the number of CFU (CFU/mL). Identification and susceptibility testing of isolated microorganisms was performed using a WalkAway 96 Plus automatic bacteriological analyser (Beckman Coulter).

Statistical analysis

The sample size was calculated on the assumption that differences in retrieved biofilm by sonication is

Table 1. Demographics and prostheses characteristics of 40 patients. [1] All made of polyethylene; [2] all made of cobalt-chromium; [3] 13 made of titanium alloy, 3 made of polyethylene; [4] all made of titanium alloy; [5] all made of cobalt-chromium; [6] 15 made of titanium alloy, 2 made of cobalt-chromium alloy.

Characteristics of the patients	All PJI (*n* = 40)	Knee PJI (*n* = 21)	Hip PJI (*n* = 19)
Patient age, median (range) – years	60 (43-75)	64 (56-75)	56 (43-74)
Male sex, number (%)	21 (53)	8 (38)	13 (68)
Number of components	112	58	54
Time from implantation until revision, median (range) – months	30.5 (1.5-108)	14.5 (1.5-48)	29 (2-108)
Type of component, number			
Liner[1]	29	21	8
Femoral head[2]	16	-	16
Acetabular cups[3]	16	-	16
Femoral stems[4]	14	-	14
Femoral knee components[5]	20	20	-
Tibial components[6]	17	17	-
Type of material, number			
Titanium alloy	42	15	27
Cobalt-chromium alloy	38	22	16
Polyethylene	32	21	11

15-20 % when different biomaterials are compared. To detect differences at a significance level of 0.05 (two-sided) with power of 80 %, a total of 87 components are required. Statistical analyses were performed using SigmaPlot (version 13.0; Systat Software, Chicago, IL, USA), graphics were plotted using Prism software (version 7.03; GraphPad). Qualitative data are expressed as number of subjects and percentage. Quantitative data are presented as median and interquartile range To compare different groups, one-way ANOVA and Wilcoxon signed-rank test for independent samples were used. The significance level in hypothesis testing was predetermined at $p < 0.05$.

Results

Patient demographic data and prosthesis characteristics

A total of 112 prosthetic components were retrieved from 40 patients with PJI affecting 21 knee and 19 hip arthroplasties (Table 1). 58 components (52 %) originated from knee prostheses and 54 components (48 %) from hip prostheses. The median time from primary implantation until revision of the prosthesis was 30.5 months (range: 1-120 months). The materials included 42 components made of titanium alloy, 38 made of cobalt-chromium alloy and 32 made of polyethylene. Not all components and/or materials were available for all patients.

Microbial detection in sonication- and vortexing-fluid culture

As required for inclusion in the study, prostheses retrieved from all 40 patients showed microbial growth in sonication- or vortexing-fluid culture from at least one prosthetic component. Out of 112 components, microorganisms were detected in 72 components (64 %) using the vortexing method and in 90 components (80 %) using the sonication method.

Fig. 1 shows the percentage of positive vortexing and sonication cultures according to the type of prosthesis components in knee and hip joints. Applying the sonication method, hip and knee polyethylene liners showed bacteria growth in 100 % of components, followed by femoral components of the knee (90 %) and acetabular cups (88 %), while femoral head and stem showed the lowest positivity rate of 50 %. Vortexing method showed an inferior positivity rate than sonication for all components except for femoral heads and stems.

Fig. 2 shows the percentage of positive culture using vortexing and sonication methods stratified according to the type of biomaterial. Bacteria in sonication-fluid cultures grew in all polyethylene components, followed by titanium alloy (79 %) and cobalt-chromium (71 %). Compared to the vortexing-only method, additional positive cultures were found by using the sonication method in 4 implants made of titanium alloy ($p = 0.457$), 6 made of cobalt-chromium ($p = 0.234$) and 8 made of polyethylene ($p = 0.005$).

Fig. 3 shows the quantity of dislodged bacteria, stratified according to the type of prosthesis component of hip and knee prostheses. By the vortexing-only method, the median bacterial load was higher from hip and knee polyethylene liners (923 CFU/mL and 695 CFU/mL, respectively) than from other prostheses components, but not significantly different ($p > 0.05$). By the sonication method, significantly higher median CFU/mL were found on hip liners (1250 CFU/mL) than on femoral

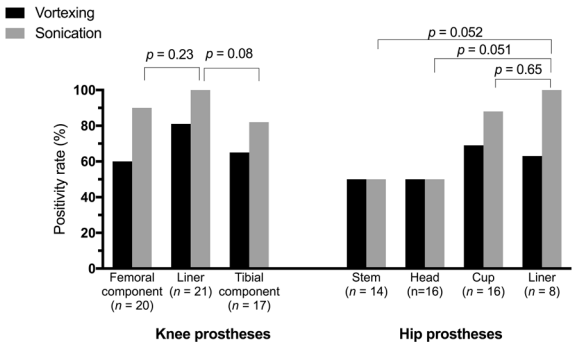

Fig. 1. Positivity rate of cultures after vortexing and sonication according to the type of component in knee and hip prostheses.

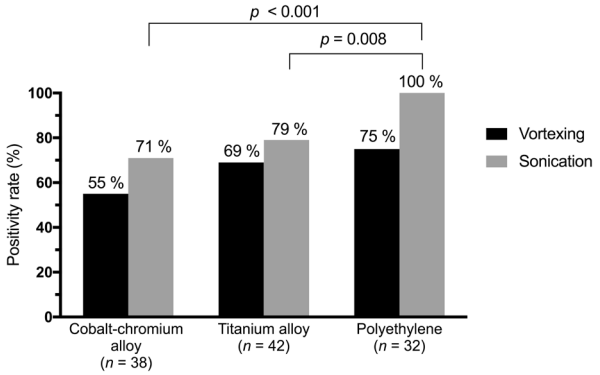

Fig. 2. Positivity rate of cultures after vortexing and sonication according to the type of material.

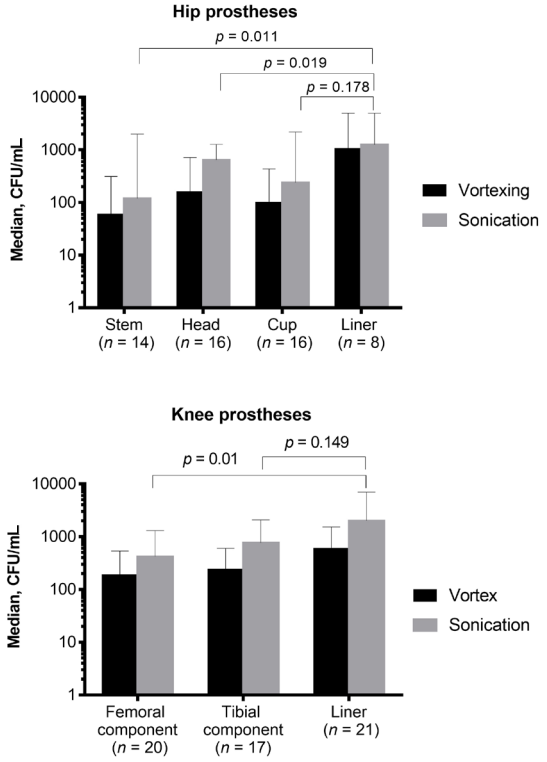

Fig. 3. Quantitative analysis of dislodged bacteria by vortexing and sonication (CFU/mL) in hip and knee prostheses according to the type of prosthesis component (median values are shown, error bars represent interquartile range).

stems (120 CFU/mL, $p = 0.011$) or heads (650 CFU/mL, $p < 0.019$), but not on cups (270 CFU/mL, $p = 0.178$). In knees, polyethylene liners showed significantly higher median CFU/mL (2000 CFU/mL) than on femoral knee components (330 CFU/mL, $p = 0.021$), but not on tibial components (800 CFU/mL, $p = 0.149$).

Fig. 4 shows the distribution of dislodged bacteria by vortexing and sonication methods stratified according to the different materials. The highest bacterial load was detected from polyethylene (by the sonication method), which was significantly higher than from any tested metal alloy (cobalt-chromium and titanium) by the sonication method ($p < 0.05$).

Qualitative and quantitative analysis of microorganisms

Coagulase-negative staphylococci were the most commonly isolated microorganism (mainly *Staphylococcus epidermidis*), followed by *S. aureus* and *Streptococcus* species. Table 2 shows the qualitative and quantitative microbiological results in vortexing and sonication cultures stratified according to pathogen. In sonication cultures, bacteria were more commonly detected than in vortexing cultures and the number of CFU was consistently larger. By sonication, coagulase-negative staphylococci were isolated from 51 out of 68 components (75 %), followed by *S. aureus* (27 out of 30 components, 90 %). The largest median CFU quantity in sonication culture was found with *S. aureus* (1200 CFU/mL), followed by coagulase-negative staphylococci (700 CFU/mL), *Streptococcus* species (680 CFU/mL), gram-negative bacteria (165 CFU/mL) and *Enterococcus faecalis* (100 CFU/mL).

Discussion

Previous studies show that sonication of removed implants considerably improve the diagnosis of PJI, especially in chronic, low-grade infections (Evangelopoulos *et al.*, 2013; Holinka *et al.*, 2011). In the present study, sonication detected additional microorganisms in 18 % of the implants made of titanium, 11 % of the implants made of cobalt-chromium and 4 % of the implants made of polyethylene, compared to the vortexing-only method. Large numbers of CFU grow in sonication-fluid cultures, as reported by Portillo *et al.* (2013).

Interestingly, the bacterial counts were larger after sonication of polyethylene liners than of metal alloys, as reported by Holinka *et al.* (2012). This observation demonstrated that the biomaterials had diverse intrinsic affinity for bacterial adhesion and biofilm formation. The removed biofilm

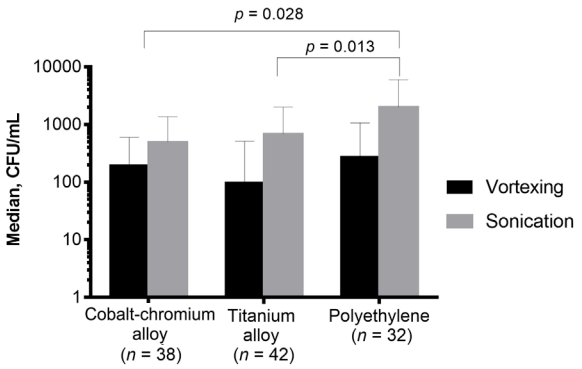

Fig. 4. Quantitative analysis of dislodged bacteria by vortexing and sonication (CFU/mL) according to the type of material (median values are shown, error bars represent interquartile range).

from retrieved implants was quantitatively and qualitatively evaluated, from which the total biofilm biomass originally present on the prosthesis surface was estimated, assuming equal sonication efficiency from different biomaterial types. However, differences in removed biofilms also reflected differences in removal efficiency by sonication from various types of materials. The inability to reliably determine actual biofilm biomass on the prosthetic component *in situ* before removal was an important limitation of the study (Holinka *et al.*, 2012). All types of *ex vivo* investigations depend on several factors, including destruction of the biofilm during removal of the prosthetic components, fixation method used (cemented and non-cemented) and biomaterial surface type (*i.e.* roughness) (Gomez-Barrena *et al.*, 2012). A further explanation for the inferior recovery of bacteria from sonicated cobalt-chromium alloys as compared to polyethylene might be the toxic properties of metal-wear particles. Cobalt and chromium are found in toxicologically relevant concentrations in the tissue adjacent to metal-on-metal prostheses and their cytotoxic effect is confirmed *in vitro*. *In vivo* exposure to cobalt and chromium interferes with the capacity for osteogenic differentiation of mesenchymal stromal cells residing in the bone marrow (Rakow *et al.*, 2016). The release of cobalt ions into the body is a key trigger of cell death, DNA damage and, subsequently, has been listed as a reasonably anticipated carcinogen (Schoon *et al.*, 2017). However, the effect on bacterial viability on the prosthesis surface remains unclear.

Microbial characteristics – such as microorganism species, size of initial inoculum, interaction with the host defences, time elapsed from onset of adhesion to removal of the implants – further influence bacterial adhesion and biofilm formation (Gomez-Barrena *et al.*, 2012). Also, microorganism species, maturity of biofilm, size and morphology of implants will affect sonication efficiency. However, sonication consistently demonstrated removal of > 99.9 % of adherent bacteria from different materials, as demonstrated by scanning electron microscopy or confocal laser scanning imaging (data not shown). More reliable methods for biofilm quantification may be used to confirm the results of the study, including biofilm staining or metabolic detection assays (Magana *et al.*, 2018).

Another limitation of the study was the impossibility of comparing biofilm quantity or quality on components in direct and forceful contact with bone and on those exposed to synovial fluid or soft tissue. In addition, significantly different surface features, such as treated, microrough or smooth surfaces, influence microbial adhesion (Cordero *et al.*, 1994; Hussain *et al.*, 2016), which was not analysed in the present study. Moreover, the polyethylene liners occupied a different space from the metals. If the liner were made of another material, they might have been more highly colonised because of the location rather than the material. This influence was not investigated in the study. A further limitation was the inability to calculate the exact component surface in relation to the fluid used for sonication. To standardise the study, a volume of 100 mL was chosen, as this represents the average of the volumes (50-200 mL) used by Gomez-Barrena *et al.* (2012) and Portillo *et al.* (2013). Finally, the indwelling time of the components was quite different and might further influence the microbial adhesion.

Despite the limitations, the study had important practical implications for future research and diagnostic routine praxis. If intrinsic differences in the ability for biofilm formation exist between various biomaterials, the present study might stimulate development of novel processes to use biomaterials with better microbial colonisation 'resistance' or to modify their surface accordingly. Modified surfaces might resist microbial colonisation and promote healing.

Conclusions

The bacterial counts were larger after sonication of polyethylene liners than of metal alloys, suggesting that intrinsic differences in the ability for biofilm formation existed among various biomaterials. Polyethylene components from hip and knee prostheses allowed the diagnosis of PJI in all investigated cases. These results suggested that sonication of retrieved polyethylene liners might be sufficient for detection of infecting pathogen in patients with chronic PJI, rather than submitting the whole prosthesis for sonication.

Acknowledgements

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Table 2. Microbiological results of vortexing and sonication cultures of 112 retrieved components. Only significantly positive results were considered. The sum of vortexing and sonication cultures from the components exceed the total number of 112 due to polymicrobial infections. IQR: interquartile range.

Type of microorganism	Number of components	Vortexing culture		Sonication culture	
		Number of components with positive culture (%)	Median, IQR (CFU/mL)	Number of components with positive culture (%)	Median, IQR (CFU/mL)
Coagulase-negative staphylococci	68	38 (56)	195 (100-606)	51 (75)	700 (240-2000)
S. aureus	30	25 (83)	400 (58-670)	27 (90)	1200 (300-3400)
Streptococcus species	9	7 (78)	400 (350-600)	9 (100)	680 (233-1600)
Enterococcus faecalis	5	2 (40)	5 (5-25)	3 (60)	100 (100-110)
Gram-negative bacteria	6	6 (100)	111 (5-223)	6 (100)	165 (13-614)
All pathogens	118	78 (66)	-	96 (81)	-

org), a no-profit organisation supporting research, education, global networking and care of patients with bone, joint or implant-associated infection, providing an unrestricted educational grant.

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Discussion with Reviewer

David Grainger: *In vitro-in vivo* correlations are a significant problem in this field. Your data would have allowed you to make the correlations with published *in vitro* data. Is this not a missed opportunity?
Authors: It is difficult to correlate *in vitro* with *in vivo* study results since the experimental conditions considerably influence the test result; therefore, the *in vivo* data were compared only with animal experimental studies (Cordero *et al.*, 1994; Cordero *et al.*, 1996; Petty *et al.*, 1985).

Editor's note: The Scientific Editor responsible for this paper was Fintan Moriarty.